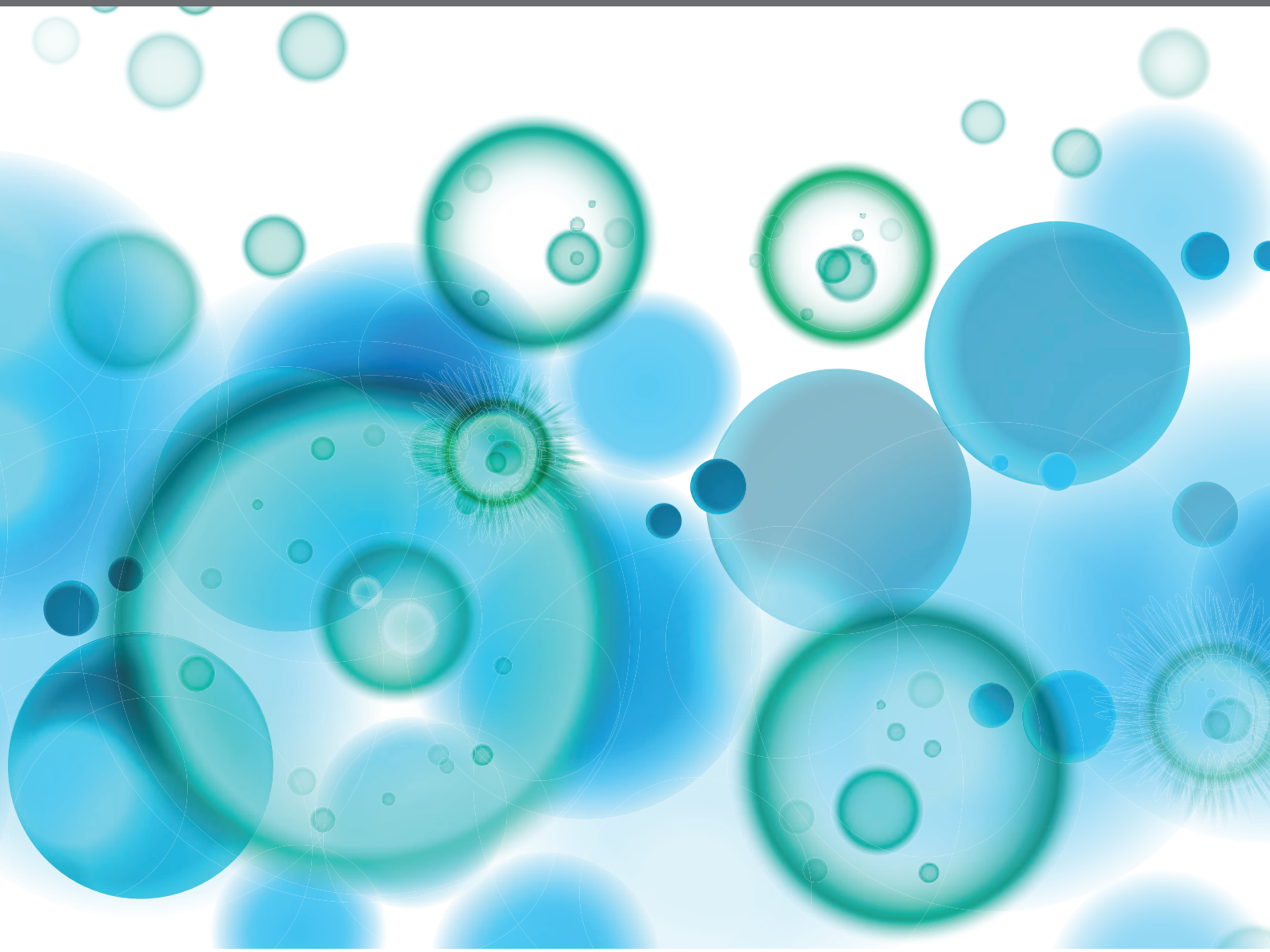
INNATE CELLS IN THE PATHOGENESIS OF FOOD ALLERGY

EDITED BY: Pamela Guerrerio, Simon Patrick Hogan, Ana Olivera and Karen Laky

PUBLISHED IN: Frontiers in Immunology



frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88971-180-2
DOI 10.3389/978-2-88971-180-2

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

INNATE CELLS IN THE PATHOGENESIS OF FOOD ALLERGY

Topic Editors:
Pamela Guerrerio, National Institutes of Health (NIH), United States
Simon Patrick Hogan, University of Michigan, United States
Ana Olivera, National Institutes of Health (NIH), United States
Karen Laky, National Institutes of Health (NIH), United States

Prof. Simon Hogan is the co-inventor on patent PCT/US2018/018618 Inhibition of Unfolded Protein response for Suppressing or preventing allergic reaction to food. All other Topic Editors declare no potential conflicts of interest with regard to the topic theme.

Citation: Guerrerio, P., Hogan, S. P., Olivera, A., Laky, K., eds. (2021). Innate Cells in the Pathogenesis of Food Allergy. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-88971-180-2

Table of Contents

frontiers in Immunology

EDITORIAL
published: 10 June 2021
doi: 10.3389/fimmu.2021.709991

Editorial: Innate Cells in the Pathogenesis of Food Allergy

Ana Olivera[1]*, *Karen Laky*[2], *Simon Patrick Hogan*[3] and *Pamela Frischmeyer-Guerrerio*[2]

[1] Mast Cell Biology Section, Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH), Bethesda, MD, United States, [2] Food Allergy Research Section, Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH), Bethesda, MD, United States, [3] Mary H Weiser Food Allergy Center, Department of Pathology, Michigan Medicine, University of Michigan, Ann Arbor, MI, United States

Keywords: food allergy, eosinophilic esophagitis, innate cells, sensitization, cofactors, oral tolerance, microbiota

Editorial on the Research Topic

Innate Cells in the Pathogenesis of Food Allergy

OPEN ACCESS

Edited and reviewed by:
Francesca Granucci, University of Milano-Bicocca, Italy

***Correspondence:**
Ana Olivera
ana.olivera@nih.gov

Specialty section:
This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology

Received: *14 May 2021*
Accepted: *27 May 2021*
Published: *10 June 2021*

Citation:
Olivera A, Laky K, Hogan SP and Frischmeyer-Guerrerio P (2021) Editorial: Innate Cells in the Pathogenesis of Food Allergy. Front. Immunol. 12:709991. doi: 10.3389/fimmu.2021.709991

Food allergy results from a failure to establish or maintain oral tolerance to certain food proteins and is manifested by adverse responses following ingestion of these foods that range from mild to life-threatening. The increasing prevalence in pediatric and adult food allergy in recent decades and the potentially life-threatening nature of reactions make food allergy a significant public health concern (1). Clinical data and experimental studies establish a role for the adaptive immune system, particularly food-specific IgE, in food allergy. Emerging evidence indicates that innate cells, non-adaptive immune processes, and environmental factors including diet, drugs, and exercise also contribute to abnormal responses to food (2, 3). However, precisely how innate cells, non-adaptive immune processes and factors impact the food allergy diathesis remains poorly understood.

This Frontiers Research Topic represents a collection of articles from worldwide experts that collectively present a current overview of mechanisms in food allergy, diagnostics, and treatments, with particular emphasis on the roles played by innate immune cells. We received a total of 16 articles, including 8 Reviews and 1 Perspective on central topics to food allergy and eosinophilic esophagitis (EoE); and 7 Original Research articles that describe various mechanistic aspects and involvement of innate cells, mediators, microbiota, and factors that alter responses to food in mouse models and humans.

MICROBIOTA, PASSAGE OF ANTIGENS AND TH2 RESPONSES IN THE SENSITIZATION TO FOOD ANTIGENS

The intestinal epithelium regulates passage of molecules across the gut wall to the underlying immune compartment and is critical for homeostatic tolerance to food and microbial antigens (3). A tolerogenic response is thought to be primarily mediated by antigen specific, peripherally derived $CD4^{+}FoxP3^{+}$ T regulatory cells (Tregs) which are fortified by additional supportive regulatory mechanisms including $MHCII^{+}CX3CR1^{Hi}$IL-10 producing macrophages and commensal microbes and their metabolites (4). Ali et al. discuss how damage to or alteration of intestinal epithelial cells

compromises active tolerogenic processes that normally limit innate immune cell induced type 2 (Th2) immune responses and oral antigen sensitization. Furthermore, they discuss the cellular and molecular mechanisms by which food and microbial antigens cross the intestinal epithelial barrier to activate innate immune pathways and immune tolerance mechanisms. Much of our current understanding of mechanisms responsible for oral tolerance and food sensitization have been derived from animal-based studies. Bruton et al. discuss the advantages and challenges of models of food allergy to study tolerance and sensitization mechanisms and summarize evidence supporting the prominent roles of innate cell types in the elicitation of allergic sensitization.

Smeekens et al. demonstrate the importance of intestinal epithelial barrier in preventing food sensitization. The authors describe a mouse strain (CC027/GeniUnc) genetically susceptible to food allergy, in the absence of adjuvants. These mice had reduced fecal IgA and alterations in certain bacterial phyla upon exposure to antigens, which promoted increased antigen absorption, and this was associated with increased food-specific IgE. Mennini et al. also highlight a possible role for the microbiota-innate immune axis in food sensitization describing increased bacterial load and microbial dysbiosis in the esophagus of patients with EoE, although a causal relationship remains to be established.

A study by Noah et al. demonstrated that antigen passage across the intestinal epithelium results in sensitization and allergy to food. They show that housing mice at thermoneutrality (26–30°C), a temperature of metabolic homeostasis, enhanced food allergy responses and this was associated with a switch in the mechanism of passage of luminal antigens across the small intestine epithelium from goblet cell antigen passages to secretory antigen passages (SAPs). In further support of the concept that SAPs promote oral food sensitization, a similar process favored development of food allergy in *Il4ra*^{F709} mice housed at standard temperatures. An elegant study by Farazuddin et al. describes the development and utilization of a nanoscale oil-in-water emulsion vaccine to suppress food allergy. The vaccine induced long-lasting suppression of oral allergen-induced anaphylaxis. Protection was associated with strong IFNγ-mediated suppression of Th2-cytokines, alarmins and ILC2. Intriguingly, the Smeekens and Farazuddin studies also point toward the existence of mechanisms that dissociate antigen-specific IgE from food-induced anaphylaxis: in CC027/GeniUnc mice, elevated IgE to some food allergens, unlike IgE to peanut and walnut, did not trigger anaphylaxis (Smeekens et al.); furthermore, the suppressive effects of the vaccine were achieved despite a persistence of allergen-specific IgE (Farazuddin et al.). Further investigation is warranted to elucidate these regulatory mechanisms.

ANTIGEN PRESENTING CELLS, TREGS, MONOCYTES AND EOSINOPHILS IN FOOD ALLERGY

Several articles in this Research Topic underscore the contributions of various immune cells to food allergy following alterations in barrier permeability, as well as the importance of early life "immune education". Key players in the regulation of oral tolerance versus allergy are antigen-presenting cells (APC), including dendritic cells, monocytes and macrophages. APC present food antigens to $CD4^+$ T cells and depending on tissue cues either drive expansion of Th2 cells and promote IgE-specific B cell responses, or expansion of Tregs and tolerance. Based on human and animal model data, Liu et al. review evidence supporting a role for APC populations in the regulation of oral tolerance or sensitization to foods.

Microbiota drive the differentiation of a population of $ROR\gamma t^+$ Tregs in the gut that is crucial for tolerogenic homeostasis in early life (5). Notably, these Treg populations are reduced in children with food allergies (6). Knoop et al. demonstrate that $ROR\gamma t^+$ Tregs developed at weaning, but not before, are long-lived and required to suppress Th2 responses and maintain tolerance to antigens later in life. This study supports the importance of immune education early in life for the proper expansion of this population of peri-weaning Tregs that cannot be substituted by post-weaning T cells.

Further supporting a role for dysregulation of early life immune education in food allergy, Neeland et al. demonstrate that infants with food allergy present an altered innate immune signature characterized by increased frequency of a monocytic population that is hyper-responsive to endotoxin stimulation and posit that in early life aberrant reprograming of innate cells is associated with the development of food allergy.

Eosinophils are an innate immune cell lineage that define EoE and are associated with food allergy. Doyle et al. review literature on the potential roles of eosinophils in EoE and food allergy. Based on cumulative observations, they propose a dual role for eosinophils in these disorders where eosinophils 1) are initially protective and actively regulate local tissue immunity and/or 2) if persistently activated drive tissue remodeling and fibrosis.

THE ROLE OF BASOPHILS AND MAST CELLS

Basophils and mast cells often take the center stage in food allergy as predominant effector cells in IgE-mediated responses. In addition, their rapid responses to bacterial products and mediators released by damaged epithelium position them at the crossroads between the innate and adaptive arms of the immune system, as innate stimulation can alter their responses to antibody cross-linking (7–9). Several articles in this Research Topic summarize critical knowledge on aspects of basophil/mast cell activation, roles in food allergy, utility as diagnostic tools, and targets for pharmacological intervention. Paranjape et al. review the associations of basophil responsiveness and surface marker expression with clinical outcomes in food allergy and blood levels of specific IgG and IgE antibodies in humans. These associations have made ex-vivo basophil activation tests a useful diagnostic tool for diagnosing food allergy and for monitoring progress during immunotherapy. Kanagaratham et al. discuss how IgE and IgG, acting through activating or inhibitory

receptors on mast cells, basophils, and other innate cells, regulate acute effector cell reactions and the induction of Th2-immunity in food allergy. Knowledge on the mechanisms involved in such regulation by Ig receptors can be harnessed for therapeutic applications. Complementary to this review, Dispenza et al. summarize therapeutic strategies to target IgE and IgE-receptor signaling in food-induced anaphylaxis.

The role played by gastrointestinal mast cells, which are located in proximity with IgE^+ plasma cells, in the manifestations of food allergies is discussed by Paranjape et al. and Kanagaratham et al. Ptaschinski et al. implicate soluble stem cell factor (sSCF) in the accumulation of small intestine mast cells in a food allergy model. They show that neutralization of sSCF diminished intestinal mast cell numbers and mediator release after challenge. Reduced mast cell frequency and activation correlated with reduced gut permeability and manifestation of food anaphylaxis reactions. Krajewski et al. show that food-derived components such as curcumin, known for its anti-allergic and anti-inflammatory properties, suppressed mast cell activation and survival by inhibiting protein disulfide isomerase (PDI), a thiol reductase expressed on the surface of mast cells. In a food allergy model, blockade of PDI reduced small intestine mast cell numbers and activation and attenuated food allergy reactions. Both studies add to our understanding of how intestinal mast cells can be regulated and provide rationale for novel treatments for food allergy.

The severity of an allergic reaction to food, even to a particular food allergen in the same individual, can vary substantially for reasons that are poorly understood. Muñoz-Cano et al., review evidence that implicates exogenous cofactors such as non-steroidal anti-inflammatory drugs, exercise, and alcohol, as contributors to this heterogeneity. Mechanisms by which these exogenous factors may potentiate allergic responses by enhancement of mast cell and basophil activation are discussed.

Taken together, this Research Topic on the role of innate cells in the pathogenesis of food allergy provides a valuable collection that gives insight into the many exciting avenues of research that continue to enhance our understanding of food allergy and EoE.

AUTHOR CONTRIBUTIONS

All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the Intramural Research Program of the National Institutes of Health (NIH), National Institute of Allergy and Infectious Diseases (NIAID) (AO, KL and PF-G) and by the National Institutes of Health grants; AI138177, AI140133, AI112626; the Mary H. Weiser Food Allergy Center (MHWFAC) and the Ask with Endowment of the MHWFAC (SH).

ACKNOWLEDGMENTS

We thank all authors that contributed to this Research Topic, and the reviewers for their time and constructive comments.

REFERENCES

1. Sicherer SH, Sampson HA. Food Allergy: A Review and Update on Epidemiology, Pathogenesis, Diagnosis, Prevention, and Management. *J Allergy Clin Immunol* (2018) 141(1):41–58. doi: 10.1016/j.jaci.2017.11.003
2. Johnston LK, Chien KB, Bryce PJ. The Immunology of Food Allergy. *J Immunol* (2014) 192(6):2529–34. doi: 10.4049/jimmunol.1303026
3. Sampson HA, O'Mahony L, Burks AW, Plaut M, Lack G, Akdis CA. Mechanisms of Food Allergy. *J Allergy Clin Immunol* (2018) 141(1):11–19. doi: 10.1016/j.jaci.2017.11.005
4. Tomar S, Hogan SP. Recent Advances in Mechanisms of Food Allergy and Anaphylaxis. *F1000Res* (2020) 9:F1000 Faculty Rev–863. doi: 10.12688/f1000research.25638.1
5. Ohnmacht C, Park JH, Cording S, Wing JB, Atarashi K, Obata Y, et al. MUCOSAL IMMUNOLOGY. The Microbiota Regulates Type 2 Immunity Through RORgammat (+) T Cells. *Science* (2015) 349(6251):989–93. doi: 10.1126/science.aac4263
6. Abdel-Gadir A, Stephen-Victor E, Gerber GK, Noval Rivas M, Wang S, Harb H, et al. Microbiota Therapy Acts Via a Regulatory T Cell MyD88/RORgammat Pathway to Suppress Food Allergy. *Nat Med* (2019) 25(7):1164–74. doi: 10.1038/s41591-019-0461-z
7. Falduto GH, Pfeiffer A, Luker A, Metcalfe DD, Olivera A. Emerging Mechanisms Contributing to Mast Cell-Mediated Pathophysiology With Therapeutic Implications. *Pharmacol Ther* (2021) 220:107718. doi: 10.1016/j.pharmthera.2020.107718
8. Galli SJ, Gaudenzio N, Tsai M. Mast Cells in Inflammation and Disease: Recent Progress and Ongoing Concerns. *Annu Rev Immunol* (2020) 38:49–77. doi: 10.1146/annurev-immunol-071719-094903
9. Marshall JS, Jawdat DM. Mast Cells in Innate Immunity. *J Allergy Clin Immunol* (2004) 114(1):21–7. doi: 10.1016/j.jaci.2004.04.045

Conflict of Interest: SH receives research grant support from Regeneron Pharmaceuticals.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

ORIGINAL RESEARCH
published: 24 September 2020
doi: 10.3389/fimmu.2020.567981

Hyper-Inflammatory Monocyte Activation Following Endotoxin Exposure in Food Allergic Infants

Melanie R. Neeland[1,2]*, *Boris Novakovic*[1,2], *Thanh D. Dang*[1,2], *Kirsten P. Perrett*[1,2,3], *Jennifer J. Koplin*[1,2] *and Richard Saffery*[1,2]

[1] Murdoch Children's Research Institute, Royal Children's Hospital, Parkville, VIC, Australia, [2] Department of Paediatrics, The University of Melbourne, Parkville, VIC, Australia, [3] Department of Allergy and Immunology, Royal Children's Hospital, Parkville, VIC, Australia

Several recent studies have reported a key role for innate cell hyper-responsiveness in food allergy. This has predominantly been observed in early life, with evidence that innate immune function may return to baseline if food allergy resolves in later childhood. Hallmarks of hyper-responsiveness include increased circulating frequency of monocytes and altered innate cell cytokine responses to *in vitro* exposure with bacterial endotoxin. These features mirror the defining signatures of trained innate immunity, seen in other complex diseases. In this study, detailed immune cell and cytokine profiling was performed on peripheral blood mononuclear cells at baseline from 27 1 year old infants in the HealthNuts cohort ($n = 16$ egg allergic and $n = 11$ non-allergic healthy controls) and following monocyte stimulation. We show that egg allergic infants have increased frequency of circulating monocytes, reduced numbers of regulatory CD4 T cells and increased monocyte: CD4 T cell ratios relative to healthy controls. Monocytes from both egg allergic and non-allergic infants responded to endotoxin stimulation with rapid cytokine production and downregulation of the surface receptor CD16, however monocytes from egg allergic infants were hyper-responsive, producing significantly more inflammatory cytokines (TNFα, IL-6, IL-1β, IL-8) and innate cell recruiting factors (MIP-1α) than healthy controls. This work indicates that monocytes of food allergic infants are programmed to a hyper-inflammatory phenotype and that the development of food allergy may be associated with trained immunity in early life.

Keywords: monocytes, food allergy, trained immunity, inflammatory response, regulatory T (Treg) cell

OPEN ACCESS

Edited by:
Simon Patrick Hogan, University of Michigan, United States

Reviewed by:
Paola Italiani, National Research Council (CNR), Italy
Carina Venter, University of Colorado, United States

***Correspondence:**
Melanie R. Neeland
melanie.neeland@mcri.edu.au

Specialty section:
This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology

Received: *31 May 2020*
Accepted: *25 August 2020*
Published: *24 September 2020*

Citation:
Neeland MR, Novakovic B, Dang TD, Perrett KP, Koplin JJ and Saffery R (2020) Hyper-Inflammatory Monocyte Activation Following Endotoxin Exposure in Food Allergic Infants. Front. Immunol. 11:567981. doi: 10.3389/fimmu.2020.567981

INTRODUCTION

IgE-mediated food allergies are increasing globally and are more common in children than adults, with up to 10% of infants being peanut, raw egg, and/or sesame allergic (1, 2). Despite a high burden of disease, there are limited treatment options for food allergy and management is limited primarily to individualized dietary avoidance. As such, substantial research efforts are now being employed to understand the cause of food allergy at a mechanistic level.

The contribution of the innate immune system in the development of pediatric food allergy is becoming increasingly clear. A hyper-inflammatory cord blood environment following *in vitro* microbial stimulation has been associated with the development of food allergy and other allergic diseases in early life (3, 4). We have also recently reported that the non-T cell fraction from infants

with egg allergy produce higher levels of inflammatory cytokines following *in vitro* endotoxin stimulation relative to non-allergic infants (5). Similar findings have been reported in asthma, where children who practice traditional farming, and are exposed to a microbe-rich environment, not only show lower rates of asthma but also a more anti-inflammatory monocyte-driven response following *in vitro* exposure to endotoxin (6). Combined, this work suggests an altered trajectory of myeloid inflammatory responses in childhood that has implications for the development of allergic disease (7).

Using samples collected from a population-based cohort of challenge-confirmed egg allergic infants and aged-matched healthy controls, we characterized the monocyte and CD4 T cell immune signatures associated with the development of food allergy and investigated the monocyte-specific functional responses to bacterial stimulation in the first year of life.

TABLE 1 | Demographics and clinical characteristics of selected cohort.

Characteristic	Non-allergic	Egg-allergic	*p*-value^
Total number	11	16	
Sex: male, *n* (%)	5 (45)	8 (50)	
Age at blood collection (months), median (min–max)	14 (13–16)	14 (13–18)	
Both parents born in Australia, *n* (%)	7 (64)	9 (56)	$p = 0.6$
Current eczema*, *n* (%)	3 (27)	6 (37.5)	$p = 0.5$
Asthma at age four#, *n* (%)	4 (36)	6 (37.5)	$p = 0.9$
Any siblings, *n* (%)	6 (54.5)	7 (44)	$p = 0.59$
Positive OFC to egg (%)	0	100	
Sensitized to peanut or sesame (%)	0	0	
Egg SPT (mm), median (min–max)	0 (0–1)	5 (3–10.5)	
Egg sIgE (kUA/L), median (min–max)	0.11 (0.02–0.32)	1.55 (0.11–18.7)	

**Doctor diagnosed eczema requiring topical steroid treatment or eczema observed by a trained nurse.*

#Doctor diagnosed asthma at age four.

^p-value for proportions by Chi square-test.

METHODS

Subjects and Study Design

PBMC samples from 27 1 year-old infants in the HealthNuts cohort (8) were used in this study ($n = 16$ egg allergic and $n = 11$ non-allergic 1 year-old infants). **Table 1** describes the demographics and clinical characteristics of the selected cohort. Egg allergic infants had a positive oral egg challenge and an SPT wheal size of ≥3 mm or a specific IgE level of ≥0.35 kUA/L at age 1 year. Egg allergic infants also had a negative skin prick test (<2 mm) or negative specific IgE (<0.35 kUA/L) to both peanut and sesame. Non-allergic controls were not sensitized to any foods and had a negative oral food challenge to egg or peanut at age 1 year. Oral food challenges were performed as described previously (9) and serum-specific IgE levels were measured using the ImmunoCAP System FEIA.

Preparation of Cells for Fluorescence Activated Cell Sorting (FACS)

Blood was collected during clinic appointments 2 h following oral food challenge. PBMCs were isolated by density gradient and cryopreserved in liquid nitrogen as previously described (10). PBMCs were thawed in 10 mL thaw media (complete RPMI supplemented with 10% heat-inactivated FCS with 25 U/ML benzonase) at 37°C, centrifuged at 300 × g for 10 min and washed in media before viability count. Mean viability as determined by trypan blue exclusion was 81%. Following cell count, PBMCs were washed in PBS at 300 × g for 10 min and resuspended in PBS at 1×10^6/mL. Fixable viability stain 510 (BD Biosciences) was added at 0.5 μl per mL of cell suspension. Cells were incubated at room temperature for 15 min protected from light, washed in FACS buffer (2% FCS, 2 mM EDTA in PBS) and resuspended in 50 μl FACS buffer containing human FC-block for 5 min. The antibody cocktail (CD3 APC-H7, CD19 PerCpCy5.5, HLA-DR BB515, CD4 A700, CD25 PE, CD127 V450, CCR7 PE-CF594, CD45RA BV711, CD11c PE-Cy6, CD14 BV786) made up at 2X was added 1:1 to the resuspended cells and incubated on ice for 30 min. Cells were then washed and resuspended in 300 μl FACS buffer for cell sorting. Monocytes were sorted using a BD Influx Cell Sorter according to the gating strategy outline in **Figure 1** and 2×10^5 events per sample were recorded for immune phenotyping analysis. Compensation was performed at the time of sample acquisition using compensation beads (BD Biosciences).

Stimulation of Purified Monocytes and Detection of Cytokines in Cell Culture Supernatant

Monocytes were resuspended at 5×10^4/100 μl in cell culture media (complete RPMI supplemented with 10% FCS and penicillin streptomycin) and seeded in 96-well round bottom culture plates. An additional 100 μl of cell culture media (unstimulated) or an additional 100 μl of cell culture media containing 2 ng/mL lipopolysaccharide (LPS) (stimulated—final 1 ng/mL) were added prior to incubation for 24 h at 37°C, 5% $C0_2$ (**Figure 1**). Cell culture supernatants were then harvested and frozen at −80°C for later quantification of inflammatory cytokines (TNF, IL-10, IL-6, IL-1β, IL-8, GMCSF, MIP-1α) using the Human Soluble Protein Flex Set Cytometric Bead Arrray (BD Biosciences) according to manufacturer's instruction. Cytometric bead array data were acquired on a BD LSR II X-20 Fortessa and analyzed using the FCAP Array Software. Unstimulated samples below the detection range were arbitrarily reported as half the lower limit of detection for each cytokine and included in the analysis.

Analysis of Monocyte Surface Receptors Post Stimulation by Flow Cytometry

Monocytes were assessed for expression of cell surface markers CD14 and CD16 after cell culture by flow cytometry. Adherent monocytes were removed from the 96-well culture plate using cold PBS 2 mM EDTA for 15 min. Monocytes were centrifuged

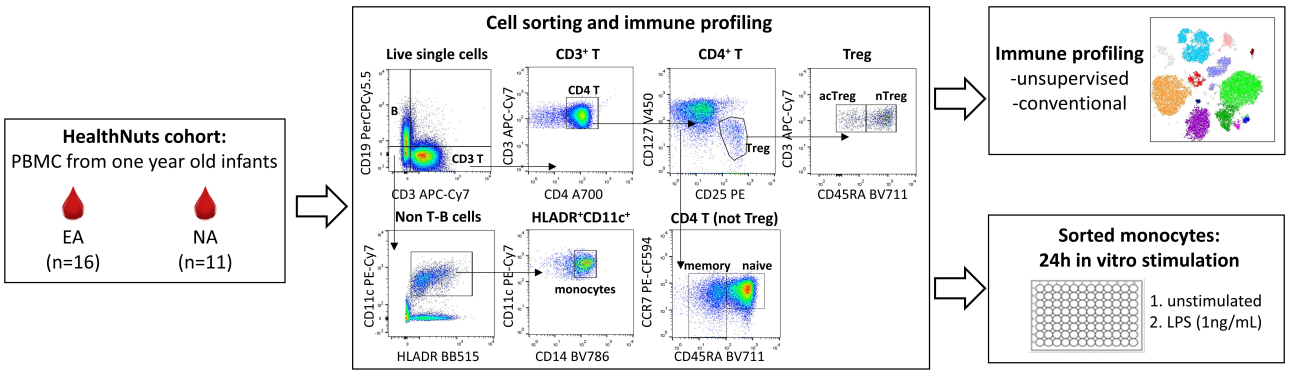


FIGURE 1 | Experimental workflow for immune profiling and monocyte stimulations in 1 year old healthy and food allergic infants. Cryopreserved PBMC from $n = 16$ egg allergic (EA) and $n = 11$ non-allergic (NA) 1 year-old infants were thawed and used for multi-parameter fluorescence activated cell sorting (FACS) to explore circulating monocyte and CD4 T cell immune cell profiles (analyzed by unsupervised and traditional manual gating), and to purify monocytes for culture experiments. Sorted monocytes underwent a 24 h stimulation with media (control) or LPS (1 ng/mL).

at 600 × g for 5 min and resuspended in 50 µl FACS buffer containing human FC-block for 5 min. The functional monocyte antibody cocktail (CD11c PE-Cy7, HLADR BB515, CD14 BV786, CD16 BUV395) made up at 2X was added 1:1 to the resuspended cells and incubated on ice for 30 min. Cells were washed and resuspended in 150 µl FACS buffer for acquisition using a BD LSR II X-20 Fortessa. Compensation was performed at the time of sample acquisition using compensation beads (BD Biosciences).

Data Analysis

Flow cytometry files underwent standard pre-processing to remove debris, doublets and to select for live cells. Live single cells were analyzed by manual gating as outlined in **Figure 1** using FlowJo V10.6. All cell populations were expressed as proportion of live single cells. To verify the manual gating approach and to visualize these data in two dimensions, we ran a t-distributed stochastic neighbor embedding (tSNE) analysis. This analysis was performed on 270,000 randomly selected live single cells (10,000 cells per participant) using CD3, CD19, CD4, CD25, CD127, CCR7, CD45RA, HLA-DR, CD11c, and CD14 for clustering. The default parameters within the tSNE plugin in FlowJo v10.6 were used. Monocyte: CD4 T cell ratios were calculated for each individual by dividing the frequency of monocytes by their respective CD4 T cell subpopulation frequency. Cytokine data is expressed as fold change from unstimulated for each individual. Log transformed values were used to create a heatmap using the Morpheus heatmap tool (https://software.broadinstitute.org/morpheus). For immune cell profiling and cytokine data, *p*-values were determined by Mann-Whitney *U*-test (2-tailed) and adjusted for multiple comparisons using the Benjamani and Hochberg approach to control the false discovery rate (FDR) (11). FDR-adjusted $p < 0.1$ were considered significant. Both raw and adjusted *p*-values are reported. The statistical analysis was performed in R (version 3.5.2) and figures were generated using GraphPad Prism version 6.01. Results are presented as box and whisker plots (where the box represents the 25th−75th percentiles, the line represents the median and the whiskers extend to minimum and maximum values).

Ethics

Approval to conduct the HealthNuts study was obtained from the Victorian State Government Office for Children (reference no. CDF/07/492), the Victorian State Government Department of Human Services (reference no. 10/07), and the Royal Children's Hospital Human Research Ethics Committee (reference no. 27047).

RESULTS

Egg Allergic Infants Show Altered Monocyte and CD4 T Cell Profiles in Peripheral Blood

We comprehensively analyzed the circulating monocyte and CD4 T cell profiles in egg allergic 1 year-old infants relative to healthy controls using both traditional manual gating and unsupervised analyses. To complement and validate our traditional manual gating approach, we used the dimensionality reduction technique tSNE, that visualizes cytometry data by giving each datapoint a location on a two-dimensional map (12). Based on the expression of lineage markers, the generated tSNE clusters were classified into eight cell types with the following average frequencies: B cells (26.9%), naïve CD4 T (28.8%), memory CD4 T (5.04%), naïve Treg (1.9%), memory Treg (1.1%), monocytes (6.18%), $CD11c^+$ DC (2.53%), and $CD3^+CD4^-$ T cells (22.8%) (**Figure 2A**). Approximately 5% of cells were negative for all markers. The phenotypes and frequencies identified by this unsupervised analysis were comparable to those obtained by manual gating (**Figure 2B**).

When comparing cell populations between clinical groups, we show that egg allergic infants have increased median frequency of circulating monocytes relative to non-allergic controls (4.6 vs. 1.8% of total live cells, $p = 0.062$, FDR-adjusted $p = 0.07$, **Figure 2B**), and reduced median frequency of circulating

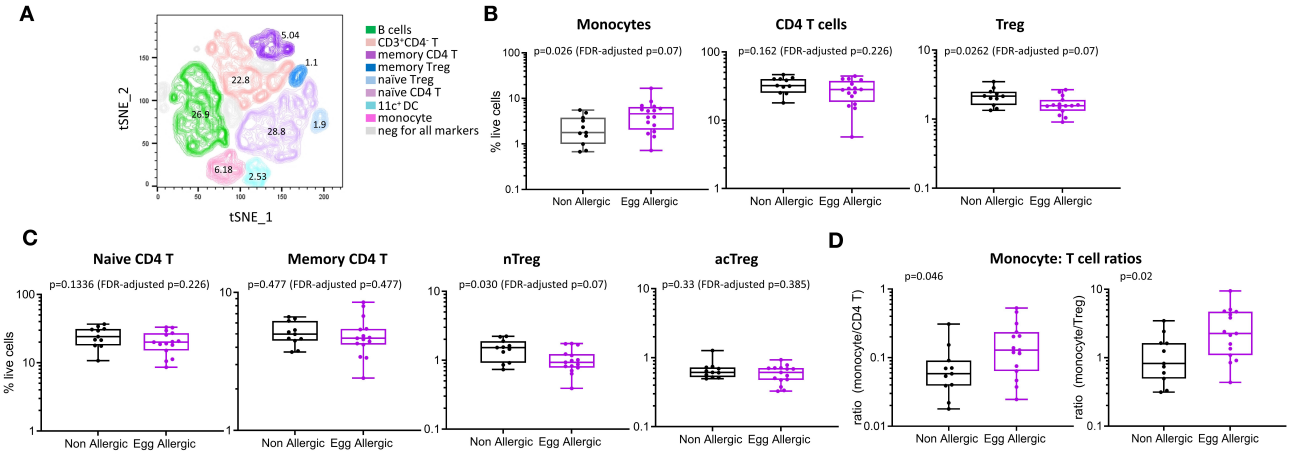


FIGURE 2 | Immune cell profiling shows altered circulating monocyte and CD4 T cell profiles in egg allergic infants. **(A)** tSNE representation of live single cells [270,000 randomly selected cells (10,000 cells per participant)]. The generated tSNE clusters were classified into the following cell types based on expression of lineage markers: B cells (green), memory CD4 T cells (dark purple), naïve CD4 T (light purple), memory Treg (dark blue), naïve Treg (light blue), monocyte (pink), $CD11c^+$ dendritic cells (aqua), $CD3^+CD4^-$ T cells (orange). **(B)** Monocytes, CD4 T cells and Treg expressed as proportion of total live cells in non-allergic (black), and egg allergic (purple) infants. **(C)** Naïve CD4 T, memory CD4 T, naïve Treg (nTreg), and activated Treg (acTreg) expressed as proportion of total live cells in non-allergic (black) and egg allergic (purple) infants. **(D)** Monocyte: CD4 T cell ratios for total CD4 T cells and Tregs in non-allergic (black) and egg allergic (purple) infants.

regulatory CD4 T cells relative to non-allergic controls (1.6 vs. 2.2% of live cells, $p = 0.026$, FDR-adjusted $p = 0.07$, **Figure 2B**). When investigating if there was a difference in a particular subset of regulatory CD4 T cell, we found that the naïve ($CD45RA^+$) Treg population (nTreg) is significantly reduced in egg allergic infants (0.9 vs. 1.5% of live cells, $p = 0.03$, FDR-adjusted $p = 0.07$, **Figure 2C**), whilst no difference was found in the activated ($CD45RA^-$) Treg population (acTreg) (**Figure 2C**).

To investigate differences within the individual between these cell types, we calculated the monocyte: CD4 T cell ratio in each subject for CD4 T cells and Tregs. Egg allergic infants show increased median monocyte: CD4 T ratios for total CD4 T cells (0.13 vs. 0.05, $p = 0.046$) and Tregs (2.4 vs. 0.82, $p = 0.02$), (**Figure 2D**).

Hyper-Responsive Cytokine Production From Stimulated Monocytes in Egg Allergic Infants

Purified $CD14^+$ monocytes from egg allergic infants were found to produce significantly more TNFα, IL-10, IL-6, IL-1β, IL-8, and MIP-1α relative to healthy controls (**Figure 3**). Median fold change (stim/unstim) and *p*-values for each cytokine in egg allergic vs. non-allergic infants were: TNFα (11 vs. 1.8, $p = 0.059$, FDR-adjusted $p = 0.08$), IL-10 (7.2 vs. 2.0, $p = 0.056$, FDR-adjusted $p = 0.08$), IL-6 (69 vs. 10, $p = 0.0037$, FDR-adjusted $p = 0.012$), IL-1β (75 vs. 8.0, $p = 0.046$, FDR-adjusted $p = 0.08$), IL-8 (3.7 vs. 1.0, $p = 0.0037$, FDR-adjusted $p = 0.012$), GMCSF (253 vs. 36, $p = 0.109$, FDR-adjusted $p = 0.109$), and MIP-1α (190 vs. 11, $p = 0.07$, FDR-adjusted $p = 0.08$). The heightened inflammatory nature of monocytes following stimulation from food allergic infants can be visualized in the corresponding heatmap (**Figure 3B**). In order to visualize the overall cytokine response of every individual, data were displayed as a principal component analysis (PCA) plot based on all cytokine release levels (**Supplementary Figure 1**). Interestingly, we observed that while the control group is uniform and clusters together, the allergic individuals showed much more variability as a group (**Supplementary Figure 1**).

Following stimulation, monocytes downregulated CD16, a surface receptor involved in recognition of LPS and previously shown to be internalized following activation (13) (**Supplementary Figure 2**). In our study, monocytes from unstimulated cultures showed CD16 expression, however this was internalized following LPS stimulation in both non-allergic and egg allergic infants, highlighting LPS-induced monocyte activation in both groups.

DISCUSSION

This study demonstrated that 1 year-old infants with challenge-confirmed IgE mediated egg allergy have increased frequency of circulating monocytes, reduced numbers of regulatory CD4 T cells and increased individual monocyte: CD4 T cell ratios relative to healthy controls. Monocytes from egg allergic infants were also hyper-responsive following *in vitro* stimulation with LPS, producing significantly higher levels of a range of inflammatory cytokines and chemokines relative to monocytes from healthy controls.

Most work investigating the immune origins of food allergy have focused on T and B cells as mediators of the allergen-specific immune response. This has revealed a key role for Th2 cells in promoting the allergic immune response, and regulatory- CD4 T and -B cells in the induction of immune tolerance (14–16). The innate immune system, however, remains

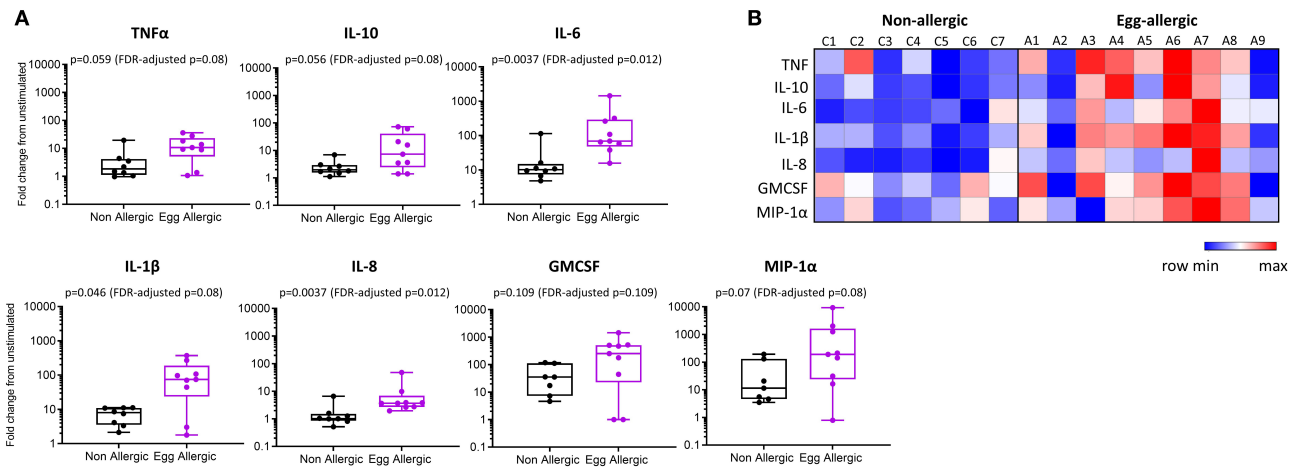


FIGURE 3 | Elevated cytokine production from LPS-stimulated monocytes in egg allergic infants. **(A)** Monocytes were cultured for 24 h with media alone or 1 ng/ml LPS. Cytokines were assessed in the cell culture supernatant by cytometric bead array and expressed as fold change from unstimulated in non-allergic ($n = 7$, black box plot) and egg allergic ($n = 9$, purple box plot) infants. **(B)** Heatmap showing log2-fold change in cytokine production for all cytokines in each individual using the Morpheus heatmap tool.

relatively unexplored in childhood food allergy. One of the first studies to demonstrate innate immune dysfunction showed elevated production of inflammatory cytokines in the cord blood from children who subsequently became allergic (food allergy, eczema, and/or asthma), which correlated with the propensity for Th2 responses at birth and during the first year of life (4). Similar findings were observed in cord-blood derived monocytes from children who develop food allergy at 1 year of age, with elevated production of inflammatory cytokines observed following stimulation with LPS. This study also reported an increased monocyte: CD4 T cell ratio in the cord blood of infants with food allergy (3). We have also previously reported that the non-T cell fraction from food allergic infants showed increased inflammatory cytokine responses (TNFα, IL-6, IL-1β, and IL-8) to LPS stimulation, and that this was more pronounced in infants with persistent food allergy outcomes (5). However, as this was a mixed population of immune cells—including dendritic cells, monocytes, and natural killer cells—it was unclear if this was due to the monocyte fraction alone. Here, we confirm that the global inflammatory response observed in our previous work is a monocyte-specific signature. In fact, monocytes from food allergic infants responded to stimulation with at least 3-fold more capacity in each inflammatory cytokine investigated relative to healthy controls. We have also expanded our previous work to show that stimulated monocytes from food allergic infants produce elevated granulocyte-macrophage colony-stimulating factor (GMCSF) and macrophage inflammatory protein 1-alpha (MIP-1α), key immune factors involved in the differentiation and recruitment of monocytes and other innate immune cells during an immune response (17, 18).

Our data also show an increased monocyte: CD4 T cell ratio in egg allergic 1 year old infants relative to healthy controls. This suggests an increase in monocytes is associated with a corresponding reduction in circulating CD4 T cells, including Tregs. Indeed, egg allergic infants also showed a reduction in the total proportion of circulating Tregs, further highlighting that food allergy is associated with both a skew toward enhanced inflammation and a corresponding reduction in regulatory immune responses in early life.

Collectively, the innate immune features emerging in food allergy closely resemble those of trained immunity. This response is underpinned by epigenetic reprogramming of innate immune cells that leads to altered inflammatory responses upon repeated microbial exposure (19). Trained immunity can enhance protection against infectious diseases but can be deleterious in non-communicable diseases characterized by inappropriate inflammation. Such effects have recently been described in autoimmune and autoinflammatory disease (20). Signatures of trained immunity include a skew toward higher myeloid cells in the circulation and a hyper-inflammatory phenotype following microbial stimulation, driven primarily by monocytes (21). In the context of food allergy, we and others have shown these monocyte-driven signatures originate early, either in the cord blood before allergy develops or within the first year of life at the time of food allergy diagnosis. It will be important to investigate if this monocyte hyper-responsiveness continues into later life in persistently food allergic children, and whether this signature returns to control levels if food allergy naturally resolves or tolerance is induced through immunotherapy in childhood. Evidence from recent work in hypercholesteremia patients suggests that trained immunity can persist even after several months of lipid lowering statin treatment (22). Future work in our laboratory will also focus on epigenetic reprogramming of monocytes in food allergy from infancy to adolescence, and across the spectrum of food allergy phenotypes.

As blood collection was performed following clinical testing in our study, it should be considered that a positive egg oral food challenge could influence some of the innate immune parameters investigated. As such, the results presented likely represent a snapshot of the innate immune response following *in vivo* allergen exposure. However, we have previously reported no differences in innate cell activation or inflammatory plasma cytokine production in food allergic infants who had a blood sample taken on a non-oral food challenge day vs. an active oral food challenge day (5, 23).

In summary, we have extended our previous findings to show that the altered innate immune signature observed in infants with food allergy is mediated by monocytes that not only circulate in higher frequency but are also hyper-responsive to endotoxin activation. This work suggests that monocytes from food allergic infants are programmed to a hyper-inflammatory phenotype and that the development of food allergy in the first year of life may be associated with aberrant trained innate immune responses.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Approval to conduct the HealthNuts study was obtained from the Victorian State Government Office for Children (reference no. CDF/07/492), the Victorian State Government Department of Human Services (reference no. 10/07), and the Royal Children's Hospital Human Research Ethics Committee (reference no. 27047). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

MN designed and performed the experiments, analyzed the data, and wrote the manuscript. BN analyzed the data and wrote the manuscript. TD contributed to experimental design and collected and processed the biospecimens. KP and JK contributed to experimental design. RS designed the experiments, supervised the work, and provided funding. All authors edited and approved the final manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2020.567981/full#supplementary-material

REFERENCES

1. Sicherer SH, Sampson HA. Food allergy: a review and update on epidemiology, pathogenesis, diagnosis, prevention, and management. *J Allergy Clin Immunol*. (2018) 141:41–58. doi: 10.1016/j.jaci.2017.11.003
2. Osborne NJ, Koplin JJ, Martin PE, Gurrin LC, Lowe AJ, Matheson MC, et al. HealthNuts: prevalence of challenge-proven IgE-mediated food allergy using population-based sampling and predetermined challenge criteria in infants. *J Allergy Clin Immunol*. (2011) 127:668–76. doi: 10.1016/j.jaci.2011.01.039
3. Zhang Y, Collier F, Naselli G, Saffery R, Tang ML, Allen KJ, et al. Cord blood monocyte-derived inflammatory cytokines suppress il-2 and induce nonclassic "T(H)2-type" immunity associated with development of food allergy. *Sci Transl Med*. (2016) 8:321ra8. doi: 10.1126/scitranslmed.aad4322
4. Tulic MK, Hodder M, Forsberg A, McCarthy S, Richman T, D'Vaz N, et al. Differences in innate immune function between allergic and nonallergic children: new insights into immune ontogeny. *J Allergy Clin Immunol*. (2011) 127:470–8. doi: 10.1016/j.jaci.2010.09.020
5. Neeland MR, Koplin JJ, Dang TD, Dharmage SC, Tang ML, Prescott SL, et al. Early life innate immune signatures of persistent food allergy. *J Allergy Clin Immunol*. (2018) 142:857–64. doi: 10.1016/j.jaci.2017.10.024
6. Stein MM, Hrusch CL, Gozdz J, Igartua C, Pivniouk V, Murray SE, et al. Innate immunity and asthma risk in amish and hutterite farm children. *N Engl J Med*. (2016) 375:411–21. doi: 10.1056/NEJMoa1508749
7. Dang A, Logsdon S, Hogan, SP. Investigating innate immune mechanisms in early-life development and outcomes of food allergy. *J Allergy Clin Immunol*. (2018) 142:790–2. doi: 10.1016/j.jaci.2018.08.001
8. Osborne NJ, Koplin JJ, Martin PE, Gurrin LC, Thiele L, Tang ML, et al. HealthNuts study: the healthnuts population-based study of paediatric food allergy: validity, safety and acceptability. *Clin Exp Allergy*. (2010) 40:1516–22. doi: 10.1111/j.1365-2222.2010.03562.x
9. Koplin JJ, Tang ML, Martin PE, Osborne NJ, Lowe AJ, Ponsonby AL, et al. HealthNuts: predetermined challenge eligibility and cessation criteria for oral food challenges in the healthnuts population-based study of infants. *J Allergy Clin Immunol*. (2012) 129:1145–7. doi: 10.1016/j.jaci.2011.09.044
10. Neeland MR, Andorf S, Manohar M, Dunham D, Lyu SC, Dang TD, et al. Mass cytometry reveals cellular fingerprint associated with ige+ peanut tolerance and allergy in early life. *Nat Commun*. (2020) 11:1091. doi: 10.1038/s41467-020-14919-4
11. Benjamini Y, Hochberg Y. Controlling the false discovery rate - a practical and powerful approach to multiple testing. *J Roy Stat Soc B Stat Meth*. (1995) 57:289–300. doi: 10.1111/j.2517-6161.1995.tb02031.x
12. Van der Maaten LJP, Hinton GE. Visualizing high-dimensional data using t-SNE. *J Mach Learn Res*. (2008) 9:2579–605.
13. Paniagua N, García C, Salgado A, Ventura-Ayala L, Navarro C, Torres M, et al. The IgG Fc receptor, CD16, interacts with LPS and is internalized during the *in vitro* stimulation of human monocytes. *Front Immunol*. (2015) 6:372. doi: 10.3389/conf.fimmu.2015.05.00372
14. Sampson HA, O'Mahony L, Burks AW, Plaut M, Lack G, Akdis CA. Mechanisms of food allergy. *J Allergy Clin Immunol*. (2018) 141:11–19. doi: 10.1016/j.jaci.2017.11.005
15. Shreffler WG, Wanich N, Moloney M, Nowak-Wegrzyn A, Sampson HA. Association of allergen-specific regulatory T cells with the onset of clinical tolerance to milk protein. *J Allergy Clin Immunol*. (2009) 123:43–52. doi: 10.1016/j.jaci.2008.09.051
16. Qamar N, Fishbein AB, Erickson KA, Cai M, Szychlinski C, Bryce PJ, et al. Naturally occurring tolerance acquisition to foods in previously allergic children is characterized by antigen specificity and associated with increased subsets of regulatory T cells. *Clin Exp Allergy*. (2015) 45:1663–72. doi: 10.1111/cea.12570
17. Ushach I, Zlotnik A. Biological role of granulocyte macrophage colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF) on cells of the myeloid lineage. *J Leukoc Biol*. (2016) 100:481–9. doi: 10.1189/jlb.3RU0316-144R

18. Menten P, Wuyts A, Van Damme J. Macrophage inflammatory protein-1. *Cytokine Growth Factor Rev*. (2002) 13:455–81. doi: 10.1016/S1359-6101(02)00045-X
19. Netea MG, Joosten LA, Latz E, Mills KH, Natoli G, Stunnenberg HG, et al. Trained immunity: a program of innate immune memory in health and disease. *Science*. (2016) 352:aaf1098. doi: 10.1126/science.aaf1098
20. Arts RJW, Joosten LAB, Netea MG. The potential role of trained immunity in autoimmune and autoinflammatory disorders. *Front Immunol*. (2018) 9:298. doi: 10.3389/fimmu.2018.00298
21. Netea MG, Dominguez-Andres J, Barreiro LB, Chavakis T, Divangahi M, Fuchs E, et al. Defining trained immunity and its role in health and disease. *Nat Rev Immunol*. (2020) 20:375–88. doi: 10.1038/s41577-020-0285-6
22. Bekkering S, Stiekema LCA, Bernelot Moens S, Verweij SL, Novakovic B, Prange K, et al. Treatment with statins does not revert trained immunity in patients with familial hypercholesterolemia. *Cell Metab*. (2019) 30:1–2. doi: 10.1016/j.cmet.2019.05.014
23. Dang TD, Tang ML, Koplin JJ, Licciardi PV, Eckert JK, Tan T, et al. HealthNuts: characterization of plasma cytokines in an infant population cohort of challenge-proven food allergy. *Allergy*. (2013) 68:1233–40. doi: 10.1111/all.12215

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

PERSPECTIVE
published: 01 December 2020
doi: 10.3389/fimmu.2020.603295

Eosinophils in Eosinophilic Esophagitis: The Road to Fibrostenosis is Paved With Good Intentions

Alfred D. Doyle[1]*, *Mia Y. Masuda*[1], *Hirohito Kita*[1,2] and *Benjamin L. Wright*[1,3]

[1] Division of Allergy, Asthma, and Clinical Immunology, Department of Medicine, Mayo Clinic Arizona, Scottsdale, AZ, United States, [2] Department of Immunology, Mayo Clinic Arizona, Scottsdale, AZ, United States, [3] Division of Pulmonology, Phoenix Children's Hospital, Phoenix, AZ, United States

OPEN ACCESS

Edited by:
Karen Laky,
National Institutes of Health (NIH), United States

Reviewed by:
Melanie Anne Ruffner,
Children's Hospital of Philadelphia, United States
Min Wu,
University of North Dakota, United States

***Correspondence:**
Alfred D. Doyle
doyle.alfred@mayo.edu

Specialty section:
This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology

Received: 06 September 2020
Accepted: 03 November 2020
Published: 01 December 2020

Citation:
Doyle AD, Masuda MY, Kita H and Wright BL (2020) Eosinophils in Eosinophilic Esophagitis: The Road to Fibrostenosis is Paved With Good Intentions. Front. Immunol. 11:603295. doi: 10.3389/fimmu.2020.603295

Eosinophilic esophagitis (EoE) is an antigen-driven disease associated with epithelial barrier dysfunction and chronic type 2 inflammation. Eosinophils are the defining feature of EoE histopathology but relatively little is known about their role in disease onset and progression. Classically defined as destructive, end-stage effector cells, eosinophils (a resident leukocyte in most of the GI tract) are increasingly understood to play roles in local immunity, tissue homeostasis, remodeling, and repair. Indeed, asymptomatic esophageal eosinophilia is observed in IgE-mediated food allergy. Interestingly, EoE is a potential complication of oral immunotherapy (OIT) for food allergy. However, we recently found that patients with peanut allergy may have asymptomatic esophageal eosinophilia at baseline and that peanut OIT induces transient esophageal eosinophilia in most subjects. This is seemingly at odds with multiple studies which have shown that EoE disease severity correlates with tissue eosinophilia. Herein, we review the potential role of eosinophils in EoE at different stages of disease pathogenesis. Based on current literature we suggest the following: (1) eosinophils are recruited to the esophagus as a homeostatic response to epithelial barrier disruption; (2) eosinophils mediate barrier-protective activities including local antibody production, mucus production and epithelial turnover; and (3) when type 2 inflammation persists, eosinophils promote fibrosis.

Keywords: eosinophilic esophagitis, eosinophil, food allergy, oral immunotherapy, epithelial barrier, fibrosis, esophagus

INTRODUCTION

Eosinophilic esophagitis (EoE) is an increasingly prevalent disease entity clinically characterized by symptoms of esophageal dysfunction (1). Endoscopically, EoE is defined by the presence of edema, longitudinal furrows, exudates, and rings and esophageal narrowing in more advanced disease (2). Histopathologic diagnosis requires the presence of esophageal eosinophilia with a tissue eosinophil

density ≥15 eos/hpf (1). Patients with EoE are often atopic and up to 70% may have IgE-mediated food allergy (3–5). Treatments for EoE include high-dose proton-pump inhibitors, swallowed topical steroids, dietary elimination, and esophageal dilation (6). While there are no FDA-approved therapies for EoE, a number of clinical trials investigating biologic agents are ongoing (7). In terms of its pathogenesis, EoE is driven primarily by food antigens (8, 9) and less commonly environmental allergens (10, 11); however, EoE does not appear to be IgE-mediated (12). Recent literature suggests that EoE is associated with impaired epithelial barrier function of the esophageal mucosa (13–15). Barrier disruption may alter local antigen processing leading to chronic type 2 inflammation and dysregulation of endogenous protease activity (16, 17). These inflammatory responses (including eosinophilia) may subsequently give rise to a perpetual cycle of remodeling and repair.

ORAL IMMUNOTHERAPY AND THE INITIATION OF EOE

OIT for IgE-mediated food allergy represents a unique vantage point from which to understand the pathogenesis of EoE. OIT is based on the principle that graduated antigen exposure desensitizes acute effector cells (e.g. mast cells, basophils) and modulates antigen-specific T- and B-cell responses allowing for ingestion of pre-defined doses of a triggering food protein (18). Importantly, approximately 50% of subjects receiving OIT develop gastrointestinal symptoms and up to 5% develop EoE (19, 20). Generally, EoE resolves with cessation of OIT; however, some subjects develop persistent disease (21, 22). The prevalence of EoE among patients with food allergy is substantially increased compared to the general population and subjects do not routinely undergo upper endoscopy before starting OIT; therefore, it is difficult to exclude the possibility that OIT subjects have pre-existing subclinical EoE (5).

To address this, we performed a study analyzing longitudinal endoscopic biopsies during a 2-year clinical OIT trial in adults with IgE-mediated peanut allergy (23, 24). We observed that some subjects did, indeed, have asymptomatic esophageal eosinophilia (≥ 15 eos/hpf) at baseline; eosinophils are not present in the normal esophagus. Tissue eosinophilia was associated with mild endoscopic abnormalities (rings, edema, linear furrows) as well as other histopathologic alterations (basal zone hyperplasia). Importantly, while a few subjects had tissue eosinophilia at baseline, all subjects had evidence of dilated intercellular spaces in at least one segment of the esophagus suggesting IgE-mediated food allergy is also associated with epithelial barrier disruption of the esophagus. When participants were followed longitudinally, OIT induced or exacerbated esophageal eosinophilia in almost all subjects. Intriguingly, esophageal eosinophilia was transient in most subjects despite the fact that antigen exposure with OIT was continued. For a majority, tissue eosinophilia was mild and asymptomatic, although one patient developed dysphagia and food impaction and was diagnosed with EoE. The only other subject with persistent esophageal eosinophilia failed the final desensitization challenge after two years of peanut OIT.

The esophageal eosinophilia observed in OIT subjects is usually asymptomatic and transient. However, it is unclear to what extent or when this may occur in EoE subjects. We hypothesize that patients diagnosed with EoE have more profound epithelial barrier impairment and/or dysfunctional wound healing and repair responses that perpetuate type 2 inflammatory responses. It remains an open question as to whether controlled, graduated antigen exposure can desensitize EoE subjects to trigger foods. This is the rationale for recent studies of epicutaneous therapy in EoE (25).

In addition to clinical and histologic features, OIT and EoE subjects share similar immunologic characteristics. For example, OIT induces food-specific IgA and IgG4 responses in saliva and peripheral blood (26, 27). These markers are also increased in the saliva, biopsy homogenates and peripheral blood of patients with EoE (28–30). Importantly, food-specific IgA and IgG4 levels are associated with the development of sustained unresponsiveness to food challenge following OIT (27).

Overlaps in the clinical and histopathologic features of OIT and EoE subjects suggest that food allergy and EoE exist on the same disease spectrum. Taken together, these observations suggest that: (1) IgE-mediated food allergy, like EoE, is associated with epithelial barrier dysfunction of the esophagus; (2) antigen exposure in this context promotes tissue eosinophilia; (3) esophageal eosinophilia during OIT is often asymptomatic; and (4) antigen-driven tissue eosinophilia can resolve or persist resulting in EoE. We hypothesize that eosinophils are recruited initially during OIT to restore homeostasis; however, when tissue inflammatory and remodeling responses become dysregulated they contribute to EoE pathogenesis (**Figure 1**).

EOE ENDOTYPES AND DISEASE PROGRESSION

Similar to asthma, EoE may be clustered into different endotypes using clinical, endoscopic, histopathologic, and molecular features. A single, multi-site cross-sectional study of endoscopic, histologic, and molecular data from esophageal biopsies identified three discrete EoE endotypes (31). EoE endotype 1 (EoEe1) has a

Abbreviations: EoE, Eosinophilic esophagitis; GI, Gastrointestinal; OIT, Oral immunotherapy; FDA, Food and Drug Administration; Ig, Immunoglobulin; EoEe, EoE endotype; IL, Interleukin; TSLP, Thymic stromal lymphopoietin; MBP, Major basic protein; EPX, Eosinophil peroxidase; TGF, Transforming growth factor; ILC, Innate lymphoid cell; LPS, Lipopolysaccharide; S. aureus, Staphylococcus aureus; C5a, Complement component 5a; EETs, Eosinophil extracellular traps; DNA, Deoxyribonucleic acid; EMT, epithelial-mesenchymal transition; MMP, Matrix metalloproteinase; MHC, Major histocompatibility complex; CD, Cluster of differentiation; GM-CSF, Granulocyte-macrophage colony-stimulating factor; PG, Prostaglandin; CRTH2, Prostaglandin D2 receptor; Th, T helper; VEGF, Vascular endothelial growth factor; NGF, Nerve growth factor; EDN, Eosinophil-derived neurotoxin; IFN, Interferon; TNF, Tumor necrosis factor; APRIL, A proliferation-inducing ligand; BAFF, B-cell activating factor; SCF, Stem cell factor; FGF, Fibroblast growth factor; HB-EGF, Heparin-binding EGF-like growth factor; PDGF, Platelet-derived growth factor; VIP, Vasoactive intestinal peptide; DAO, Diamine oxidase; ALOX, arachidonate lipoxygenase; IDO, Indoleamine 2,3-dioxygenase; ECP, Eosinophil cationic protein; PAF, Platelet activating factor; CCL, C-C motif chemokine; CXCL, C-X-C motif chemokine.

normal endoscopic appearance and is usually steroid-sensitive. Markers of inflammation and epithelial differentiation are relatively unchanged in this endotype. EoE endotype 2 (EoEe2) is usually pediatric-onset, associated with atopy, and steroid-refractory. Gene expression in EoEe2 is notable for marked upregulation of pro-inflammatory mediators (e.g. IL-4 and TSLP). EoE endotype 3 (EoEe3) tends to be adult-onset, non-atopic, and is associated with fibrostenosis and narrow-caliber esophagus. Gene expression in this group denotes loss of epithelial differentiation. Among the endotypes, EoEe1 is the most benign, while EoEe2 is associated with marked type 2 inflammation. Unsupervised clustering of cytokine gene expression suggests these endotypes may be further subdivided into additional subgroups (32). Of note, validation and verification of these endotypes requires further study and natural history studies have not followed EoE cohorts longitudinally in order to understand specific relationships between endotypes; however, these EoE endotypes may represent different points of progression across a continuum of disease. This same principle may be reflected in the fact that children have non-specific gastrointestinal symptoms; whereas, food impaction due to fibrostenosis is much more common in teens and adults.

LESSONS FROM TARGETING EOSINOPHILS AND EOE-LIKE DISEASE

Unsuccessful clinical trials targeting eosinophils and EoE-like disease provide two lines of evidence supporting the hypothesis that eosinophils may be dispensable in EoE pathology. Given their conspicuous presence in tissue pathology, early clinical trials of biologics in EoE focused on depletion of eosinophils. IL-5 promotes eosinophil maturation, proliferation, activation, and survival (33); therefore, targeting this cytokine pathway was a logical first step. Three anti-IL-5 agents, mepolizumab, reslizumab, and benralizumab, have been developed. Mepolizumab and reslizumab neutralize IL-5 by binding it directly preventing interaction with IL-5Rα. Benralizumab binds to IL-5Rα blocking interaction with IL-5 and promoting antibody-dependent cellular cytotoxicity (ADCC) and deletion of eosinophils. In an alternative strategy, lirentelimab targets siglec-8. Siglec-8 is a transmembrane protein shared by eosinophils and mast cells. Ligation of siglec-8 induces apoptosis of eosinophils and inhibits mast cell activity. Lirentelimab both mediates these activities and induces ADCC of eosinophils (34).

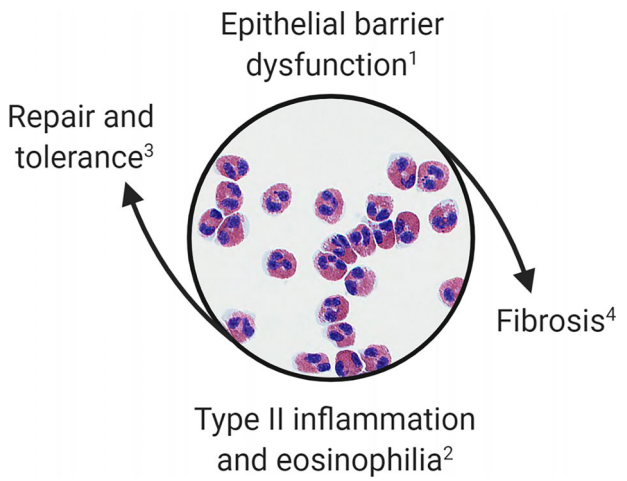


FIGURE 1 | Proposed model of esophageal eosinophilia. Eosinophils are initially recruited to the esophagus to restore barrier function. When inflammatory and remodeling responses become dysregulated eosinophils contribute to type 2 inflammation, worsening of barrier integrity, and fibrosis. This paradigm allows for categorization of (1) patients with IgE-mediated food allergy or subclinical barrier dysfunction; (2) OIT patients, EoEe1, and EoEe2 representing asymptomatic, mild or severe phases of the inflammatory response; (3) OIT patients who successfully develop sustained unresponsiveness or those who naturally outgrow a food allergy; and (4) EoEe3, which is characterized by fibrostenosis.

Promising results of an open label trial of mepolizumab in 4 patients with EoE led to the first randomized, double-blind, placebo-controlled (DBPC) trial of mepolizumab in 11 patients (35, 36). The results of this clinical trial were notable for reductions in tissue eosinophil counts but limited clinical improvement in EoE symptoms. A subsequent prospective trial randomized 59 pediatric subjects with EoE to three different dosing arms, using the lowest dose of mepolizumab as a comparator, as opposed to a placebo group (37). While the investigators demonstrated reductions in tissue eosinophilia, again there were no significant differences in clinical symptoms between treatment arms. In the largest randomized DBPC trial of anti-IL-5 therapy in EoE to date, 226 pediatric subjects received reslizumab and, again, significant improvements in clinical symptoms were not seen in the treatment arm compared to placebo (38). Importantly, despite overall reductions in tissue eosinophilia, in each of these trials a majority of subjects did not achieve histologic remission (peak tissue eosinophil counts <15 eos/hpf). Indeed, mepolizumab and reslizumab appear to have greater effects on peripheral blood eosinophils compared to esophageal tissue eosinophils [e.g. 90 vs 55% reduction respectively with mepolizumab (39)]. Together, the outcomes of these trials suggest that eosinophils do not exclusively mediate tissue pathology in EoE or incomplete eosinophil depletion accounts for the persistence of symptoms. Trials of benralizumab and lirentelimab, drugs more likely to completely deplete tissue eosinophils, may be more informative.

Additional evidence that eosinophils may not be the primary driver of clinical symptoms is the existence of EoE-like disease; an entity characterized by EoE symptoms responsive to swallowed topical corticosteroids, but without tissue eosinophilia (40). Kindred of patients with EoE-like disease often reveal multiple family members affected with EoE. Generally, these patients do not have advanced endoscopic findings and their histopathology reveals papillary elongation with increased T cells. These patients are distinguished from EoE patients by reduced eotaxin-3 expression. Finally, patients with EoE-like disease may evolve to develop classical EoE. It is tempting to speculate that EoE-like disease represents yet another endotype on the EoE spectrum. Molecular studies comparing patients with EoE-like disease, food allergy, and EoEe1 are needed to evaluate disease overlap.

ROLES OF EOSINOPHILS IN EOE

Eosinophils are often considered destructive end-stage effector cells defined by their ability to release toxic granule proteins that can damage surrounding tissue. However, comprehensive reviews of eosinophil activities suggest a much more complex cell with roles in health and disease (41–45). As shown in **Table 1**, eosinophils produce and release various mediators that are involved in inflammation, immunoregulation, and tissue remodeling and repair. In EoE, levels of tissue eosinophilia correlate with disease severity as well as response to treatments (156, 164). On the other hand, eosinophils clearly have a homeostatic or physiologic role as resident cells in the rest of the GI tract and findings from EoE-like disease along with the limited effectiveness of anti-IL-5 therapy suggest a minor role in clinical symptoms in EoE. Moreover, we have recently identified asymptomatic eosinophilia in the esophagus of OIT subjects at baseline. These seemingly divergent observations can be explained by considering the temporal effects of eosinophil activities throughout the progression of EoE. Specifically, we propose that the appearance of eosinophils in the esophagus begins as an extension to their homeostatic function in other GI tissues to enhance barrier function. These eosinophils may become activated to contribute to further protective activities and wound repair and, over time, contribute to disease pathology and fibrosis.

Eosinophil activities in allergic disease are well studied, particularly in asthma, with identified roles for inflammation (e.g. MBP, IL-13), mucus production (IL-13), epithelial damage (MBP, EPX), tissue remodeling/fibrosis (IL-13, TGF-β), and smooth muscle hyperresponsiveness (IL-13, leukotrienes) (165, 166). These pathways have also been observed in EoE by examination of patient biopsies, cell culture experiments, and mouse models. The many potential roles of eosinophils in EoE are well reviewed (67, 167–174). Below we highlight examples of eosinophil activities in barrier maintenance, defense, repair, and fibrosis that suggest esophageal eosinophilia is a protective response that becomes problematic over time.

EARLY PHASE/PROTECTIVE RESPONSE IN EOE

Recruitment of Eosinophils

The epithelial barrier has been implicated as central to the disease process in EoE (13, 175). While initiating events remain unclear in EoE, environmental insults to the epithelium (e.g. allergens) can trigger the release of inflammatory signaling molecules including TSLP, IL-25, and IL-33 [all shown to be elevated in EoE (79)] that promote a type 2 inflammatory response [e.g. IL-13 production by ILC2s (176, 177)] which leads to production of eosinophil chemotactic factors, particularly eotaxin-3 (178).

Eosinophils and Epithelial Barrier Maintenance

Eosinophils have been linked with a host of activities that help to protect/restore the epithelial barrier including antimicrobial defenses, remodeling and repair activities, and immune regulation. Mice deficient for eosinophils have shown that under homeostatic conditions eosinophils support mucus-secreting goblet cell numbers in the small intestine (50). Expression of certain mucins has been shown to be upregulated in biopsies of patients with EoE and EoE mouse models [e.g (51, 179).] but further investigation is needed to understand the activities of esophageal glands that are located beyond the reach of these biopsies (i.e. in the lamina propria and submucosa). Interestingly, in our experience with mouse and pig models of EoE, eosinophils tend to accumulate in the lamina propria similar to the rest of the GI tract (unpublished observations). Notably, IL-5 induced esophageal eosinophilia in a transgenic mouse model was not sufficient to induce pathology but with additional stimulus from a hapten increased epithelial layer eosinophilia was observed along with pathologies associated with EoE (180). In humans, a recent retrospective study of esophageal biopsies utilizing specialized forceps that enabled more reliable subepithelial sampling found that one-third of subjects demonstrated greater subepithelial eosinophil density as compared to the epithelium (181, 182). These observations suggest a likely unappreciated level of eosinophils in the esophageal lamina propria. Further investigation is needed to understand the role of subepithelial esophageal eosinophilia in disease pathogenesis.

Eosinophils and Epithelial Barrier Defense

Eosinophils have been shown to directly mediate host anti-microbial defense activities in the gut. For example, in response to activation with LPS, S. aureus, C5a, or TSLP, eosinophils release eosinophil extracellular traps (EETs). EETs are mitochondrial DNA laced with toxic eosinophil granule proteins (i.e. MBP, EPX, EDN, ECP) that are released into the extracellular space and can bind and kill bacteria. Indeed, hypereosinophilic mice exhibited local extracellular DNA deposition and were protected against sepsis after cecal ligation and puncture (183). Notably, EETs were detected in the esophagus of active EoE subjects (79), suggesting a role for this mechanism in protection against microbes in EoE. In addition, MBP and IL-13 in particular induce epithelial turnover, an effective mechanism for expulsion of organisms/substances and replacement of damaged epithelium (126, 184).

Eosinophils and Epithelial Barrier Repair/ Immune Tolerance

As shown in **Table 1**, eosinophils can produce factors that help to restore the barrier by promoting epithelial to mesenchymal transition (EMT) which facilitates wound repair. These factors may include, but are not limited to, TGF-β (92), MMP-9 (114), IL-4 (185), IL-13 (185), EPX (127), and MBP (92, 93, 186). IL-13 in particular is considered a central mediator in EoE (68, 69) and IL-13 expressing eosinophils have been identified in the esophageal tissue of EoE subjects (185). Notably, IL-33 promotes IL-13 production by eosinophils (70, 187–190). Recently, IL-13 has been shown to upregulate synaptopodin, an actin-associated protein associated with wound healing, and barrier integrity, in the epithelium of EoE subjects (71). Eosinophils can also modulate the immune response to

TABLE 1 | Protective and pathologic effects of eosinophil-derived mediators.

Eosinophil-derived Mediator	Protective effects	Pathologic effects	References*
IL-1α		Fibrosis	(46–49)
IL-1β	Barrier function: mucosal IgA production wound repair: EMT	Fibrosis	(46, 49–52),
IL-1Ra	Immune tolerance: inhibits IL-1α, IL-1β		(53)
IL-3		Inflammation	(54)
IL-4	Wound repair: EMT	Fibrosis	(46, 49, 55)
IL-5	Eosinophil survival	Eosinophil survival	(49, 56, 57) 32197970
IL-6	Barrier function: mucosal IgA production	Fibrosis	(49, 58, 59)
IL-8		Inflammation angiogenesis	(49, 51, 55, 60–62)
IL-9		Inflammation: mast cell survival and activation Barrier function: decreases adherens and tight junction expression	(63, 64)
IL-10	Immune tolerance: IgG4 production, Treg induction		(65, 66)
IL-13	Wound repair: EMT, synaptopodin barrier function: mucus production, synaptopodin intracellular pH regulation expulsion: epithelial turnover	Inflammation: promotes TARC, MDC, eotaxin barrier dysfunction: synaptopodin, ↓ filaggrin, vimentin, desmoglein, ↑calpain-14 epithelial hyperplasia dilated intercellular space formation fibrosis: activates fibroblasts, stimulates production of TGF-β	(32, 49, 67–73)
IL-17	Antimicrobial	Inflammation	(74)
IL-18		Inflammation	(75–78)
IL-25		Inflammation	(79, 80)
IFNγ	Antimicrobial	Inflammation	(81–83)
TNF-α	Wound repair: EMT	Inflammation angiogenesis	(52, 81, 84, 85)
Osteopontin		Angiogenesis fibrosis	(86)
Amphiregulin	Wound repair immune tolerance: Treg activity	Fibrosis	(87)
APRIL	Plasma cell survival	Plasma cell survival	(59, 88)
BAFF	Plasma cell survival	Plasma cell survival	(88)
SCF		Inflammation: mast cell survival	(89, 90)
TGF-α	Wound repair: EMT	Fibrosis epithelial hyperplasia angiogenesis	(91)
TGF-β	Wound repair: EMT barrier function: mucosal IgA production immune tolerance: Treg induction	Fibrosis: activates fibroblasts, promotes collagen production smooth muscle proliferation/activation epithelial hyperplasia	(46, 92–95)
GM-CSF	Wound repair	Inflammation	(54, 56, 96–98)
VEGF		Angiogenesis Tissue remodeling	(99–101)
FGF-2	Epithelial turnover wound repair	Fibrosis Epithelial hyperplasia smooth muscle activation angiogenesis	(102, 103)
NGF		Nerve growth fibrosis angiogenesis	(104, 105)
HB-EGF	Wound repair	Smooth muscle activation	(106)
PDGF-bb	Wound repair	Angiogenesis smooth muscle activation fibrosis	(107)

(Continued)

TABLE 1 | Continued

Eosinophil-derived Mediator	Protective effects	Pathologic effects	References*
Substance P	Wound repair	Pain inflammation angiogenesis	(108)
VIP	Smooth muscle relaxation		(108, 109)
α-defensin	Antimicrobial	Inflammation: innate immune activation	(110, 111)
Angiogenin		Angiogenesis	(99, 112, 113)
MMP-9	Wound repair IL-1β, TGF-β activation	IL-1β, TGF-β activation	(114, 115)
Heparanase	Wound repair	Inflammation angiogenesis	(116–118)
DAO (histaminase)	Resolution of inflammation		(119)
15-lipoxygenase derivatives (e.g ALOX15).	Resolution of inflammation arachidonic acid metabolism		(120–123)
IDO	Immune tolerance	Inflammation angiogenesis	(124, 125)
Superoxide (O_2^-)	Antimicrobial	Inflammation	(61)
MBP-1	Antimicrobial epithelial hyperplasia/proliferation (FGF-9)	Cytotoxic barrier dysfunction smooth muscle activation inflammation: mast cell/basophil degranulation fibrosis	(126–132)
EPX	Antimicrobial	Cytotoxic inflammation: mast cell activation fibrosis	(127, 130, 132–134)
ECP	Antimicrobial	Inflammation: mast cell activation neurotoxic cytotoxic	(132, 135)
EDN	Antimicrobial	Inflammation: dendritic cell activation neurotoxic	(132, 136–138)
CLC	Antimicrobial	Inflammation: carrier for other eosinophil granule cationic RNases	(32, 132, 139, 140)
EET's	Antimicrobial	Contain toxic granules - see above	(79)
PAF		Inflammation	(141)
Thromboxane B2		Smooth muscle activation	(142)
Leukotriene C4	Barrier function: mucus production	Smooth muscle activation inflammation	(143, 144)
PGD2		Inflammation	(145)
PGE2	Resolution of inflammation	Inflammation pain	(142)
PGF2α		Smooth muscle activation inflammation	(146, 147)
Protectin D1	Resolution of inflammation		(148, 149)
CCL17 (TARC)		Inflammation	(150, 151)
CCL22 (MDC)		Inflammation	(150, 151)
CCL5 (RANTES)		Inflammation	(152, 153)
CCL11 (eotaxin-1)		Inflammation	(154)
CXCL5 (ENA-78)		Inflammation angiogenesis	(155)
CXCL1 (GRO-α)	Wound repair	Inflammation angiogenesis	(49, 62, 156–158)
CCL2 (MCP-1)		Inflammation	(159, 160)
CCL23 (MIP-1α)		Inflammation	(49, 84, 161)
CCL4 (MIP-1β)		Inflammation	(62, 159, 162)
CXCL9 (MIG)		Inflammation	(163)
CXCL10 (IP10)		Inflammation	(163)

**Each mediator has references listed that support production by eosinophils. Additional references implicate certain mediators in EoE, though the source may not be identified.*

facilitate barrier repair. Indeed, mice deficient for eosinophils have established that eosinophils support IgA production (50, 88), which in turn, is secreted to the lumen to facilitate barrier function. Notably, food-specific IgA is increased in EoE (30). Finally, eosinophils expressing MHCII and CD80 have been identified in EoE subjects (191, 192) and may present antigen to T cells. TGF-β and IL-10 can influence the production of IgA, IgG4, and T regulatory cell responses. Both cytokines are produced by eosinophils in EoE (92, 185) but a mechanistic link remains to be established.

CHRONIC PHASE/PATHOLOGIC ACTIVITIES IN EOE

During the chronic phase of the disease, eosinophil activities may contribute to inflammation, tissue remodeling, and fibrosis. Eosinophil-derived mediators that are helpful in barrier defense and repair can, over time, contribute to these activities.

Eosinophils and Inflammation

Eosinophils may promote sustained eosinophilic inflammation by production of eosinophil survival factors GM-CSF and IL-5, expression of which has been observed in tissue eosinophils from EoE subjects (185). The eosinophil microenvironment may become problematic for the epithelium with chronic inflammation—for example, eosinophil oxygen metabolism may induce tissue hypoxia resulting in barrier impairment (193). Eosinophils are also a source of IL-9 and have been linked with mast cell numbers in the esophagus (63) which are, in turn, linked with disease severity (194). Interestingly, mast cell numbers are increased in EoE as compared to EoE-like disease (40). In addition, eosinophils produce eicosanoids including PGD2. PGD2 signals through CRTH2, which has been shown to support ILC2 accumulation (195). CRTH2 has also been shown to be expressed by a subset of IL-5 and IL-13 producing Th2 cells in EoE (196). Interestingly, elevated numbers of CRTH2+CD4+ T cells are observed in EoE as compared to EoE-like disease (40). Finally, eosinophil granule proteins including MBP can damage epithelium resulting in increased pro-inflammatory mediators (197) and have been shown to reduce barrier integrity in the colonic epithelium (128). MBP also can induce mast cell and basophil degranulation as well as smooth muscle and fibroblast activation (129, 198–200) thereby contributing to inflammation and fibrosis.

Eosinophils and Remodeling/Fibrosis

Chronic IL-13-mediated wound healing activities may become problematic. For example, IL-13 induced synaptopodin overexpression has been shown to impair barrier integrity and reduce epithelial differentiation (71). IL-13 is also linked with epithelial barrier disruption by downregulation of epithelial junction molecules and upregulation of the protease calpain-14 (201). Phase 2 trials of biologics targeting IL-13 pathways have demonstrated improvement in endoscopic and histologic findings in EoE (202–204). Data from mouse models of EoE crossed with eosinophil deficient lines suggest a role for eosinophils in hyperplasia and fibrosis in an allergen-driven model (179, 205) while no role was observed in an IL-13 overexpression model (206). Together these findings would be consistent with a role for eosinophil-derived IL-13 in these remodeling activities that are hallmark pathological features of human EoE (206). Notably, eosinophil-derived IL-13 caused extensive remodeling in the mouse lung by promoting MMP-12 production, a mediator identified as elevated in EoE (120, 207). The activities of MMP-12 in human EoE require investigation. Eosinophil-derived factors IL-13 and TGF-β (and others including IL-1β, and IL-4) induce fibroblast to myofibroblast differentiation and eosinophil-derived TGF-β in particular is linked with production of collagen (46, 208, 209). TGF-β also can induce smooth muscle proliferation, hyperplasia, and contraction (210) which may contribute to esophageal dysmotility. Finally, activated eosinophils produce angiogenic factors such as VEGF and nerve remodeling factors such as NGF and EDN which may contribute to nerve growth and cytotoxicity, respectively.

DISCUSSION

The role(s) of eosinophils remains unclear in EoE. The observations we and others have made show asymptomatic eosinophilia is likely to be a common occurrence. This suggests that, like other areas of the GI tract, eosinophils may promote tissue homeostasis. Eosinophil activities in EoE and other diseases suggest a role for protecting/restoring the barrier. However, if the barrier is not restored it is likely that eosinophils contribute to inflammation and remodeling/fibrosis. Notably, many of the eosinophil-derived mediators discussed herein have wound healing barrier restoring activities in addition to being linked with pathologies associated with chronic inflammation such as fibrosis. Thus, we suggest the road to fibrostenosis is paved with good intentions. These observations also suggest it may be important to target eosinophils based on EoE endotype. Conceivably targeting those with the fibrostenotic EoE (EoEe3) may result in reduced chronic remodeling pathology while sparing subjects in whom eosinophils may primarily benefit esophageal barrier function. In addition, our perspective suggests therapeutic strategies aimed at protecting, improving, or restoring barrier function by promoting homeostatic eosinophil pathways (e.g. mucus and antibody production) may be helpful.

AUTHOR CONTRIBUTIONS

AD and BW drafted the manuscript. MM contributed to the content of the table. AD, MM, HK, and BW reviewed and provided critical feedback on the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This study is supported by the Don and Kathy Levin Family Foundation (ADD, MYM, BLW), Mayo Clinic Foundation

(BLW, HK), Phoenix Children's Hospital Foundation (BLW), Arizona Biomedical Research Consortium (ADHS18-198880) (BLW). Dr. Wright is a scholar in the Consortium of Eosinophilic Gastrointestinal Disease Researchers (CEGIR). CEGIR (U54 AI117804) is part of the Rare Disease Clinical Research Network (RDCRN), an initiative of the Office of Rare Diseases Research (ORDR), NCATS, and is funded through collaboration between NIAID, NIDDK, and NCATS. CEGIR is also supported by patient advocacy groups including American Partnership for Eosinophilic Disorders (APFED), Campaign Urging Research for Eosinophilic Diseases (CURED), and Eosinophilic Family Coalition (EFC). As a member of the RDCRN, CEGIR is also supported by its Data Management and Coordinating Center (DMCC) (U2CTR002818).

ACKNOWLEDGMENTS

The authors would like to thank the Levin Family for their support of food allergy research at Mayo Clinic Arizona. This work is dedicated to our friend and colleague Michael "Mike" McGarry. Mike was an excellent mentor providing deep insight into eosinophil biology and broad expertise in hematology. We will miss Mike's mentorship, curiosity, good nature, and hearty laugh.

REFERENCES

1. Dellon ES, Liacouras CA, Molina-Infante J, Furuta GT, Spergel JM, Zevit N, et al. Updated International Consensus Diagnostic Criteria for Eosinophilic Esophagitis: Proceedings of the AGREE Conference. *Gastroenterology* (2018) 155(4):1022–33 e10. doi: 10.1053/j.gastro.2018.07.009
2. Hirano I, Moy N, Heckman MG, Thomas CS, Gonsalves N, Achem SR. Endoscopic assessment of the oesophageal features of eosinophilic oesophagitis: validation of a novel classification and grading system. *Gut* (2013) 62(4):489–95. doi: 10.1136/gutjnl-2011-301817
3. Chehade M, Jones SM, Pesek RD, Burks AW, Vickery BP, Wood RA, et al. Phenotypic Characterization of Eosinophilic Esophagitis in a Large Multicenter Patient Population from the Consortium for Food Allergy Research. *J Allergy Clin Immunol Pract* (2018) 6(5):1534–44 e5. doi: 10.1016/j.jaip.2018.05.038
4. Leigh LY, Spergel JM. An in-depth characterization of a large cohort of adult patients with eosinophilic esophagitis. *Ann Allergy Asthma Immunol* (2019) 122(1):65–72 e1. doi: 10.1016/j.anai.2018.09.452
5. Hill DA, Dudley JW, Spergel JM. The Prevalence of Eosinophilic Esophagitis in Pediatric Patients with IgE-Mediated Food Allergy. *J Allergy Clin Immunol Pract* (2017) 5(2):369–75. doi: 10.1016/j.jaip.2016.11.020
6. Rank MA, Sharaf RN, Furuta GT, Aceves SS, Greenhawt M, Spergel JM, et al. Technical Review on the Management of Eosinophilic Esophagitis: A Report From the AGA Institute and the Joint Task Force on Allergy-Immunology Practice Parameters. *Gastroenterology* (2020) 158(6):1789–810 e15. doi: 10.1016/j.anai.2020.03.021
7. Wechsler JB, Hirano I. Biological therapies for eosinophilic gastrointestinal diseases. *J Allergy Clin Immunol* (2018) 142(1):24–31 e2. doi: 10.1016/j.jaci.2018.05.018
8. Kelly KJ, Lazenby AJ, Rowe PC, Yardley JH, Perman JA, Sampson HA. Eosinophilic esophagitis attributed to gastroesophageal reflux: improvement with an amino acid-based formula. *Gastroenterology* (1995) 109(5):1503–12. doi: 10.1016/0016-5085(95)90637-1
9. Markowitz JE, Spergel JM, Ruchelli E, Liacouras CA. Elemental diet is an effective treatment for eosinophilic esophagitis in children and adolescents. *Am J Gastroenterol* (2003) 98(4):777–82. doi: 10.1111/j.1572-0241.2003.07390.x
10. Reed CC, Iglesia EGA, Commins SP, Dellon ES. Seasonal exacerbation of eosinophilic esophagitis histologic activity in adults and children implicates role of aeroallergens. *Ann Allergy Asthma Immunol* (2019) 122(3):296–301. doi: 10.1016/j.anai.2018.12.013
11. Ram G, Lee J, Ott M, Brown-Whitehorn TF, Cianferoni A, Shuker M, et al. Seasonal exacerbation of esophageal eosinophilia in children with eosinophilic esophagitis and allergic rhinitis. *Ann Allergy Asthma Immunol* (2015) 115(3):224–8 e1. doi: 10.1016/j.anai.2015.07.004
12. Clayton F, Fang JC, Gleich GJ, Lucendo AJ, Olalla JM, Vinson LA, et al. Eosinophilic esophagitis in adults is associated with IgG4 and not mediated by IgE. *Gastroenterology* (2014) 147(3):602–9. doi: 10.1053/j.gastro.2014.05.036
13. Rochman M, Azouz NP, Rothenberg ME. Epithelial origin of eosinophilic esophagitis. *J Allergy Clin Immunol* (2018) 142(1):10–23. doi: 10.1016/j.jaci.2018.05.008
14. Rothenberg ME, Spergel JM, Sherrill JD, Annaiah K, Martin LJ, Cianferoni A, et al. Common variants at 5q22 associate with pediatric eosinophilic esophagitis. *Nat Genet* (2010) 42(4):289–91. doi: 10.1038/ng.547
15. Kottyan LC, Davis BP, Sherrill JD, Liu K, Rochman M, Kaufman K, et al. Genome-wide association analysis of eosinophilic esophagitis provides insight into the tissue specificity of this allergic disease. *Nat Genet* (2014) 46(8):895–900. doi: 10.1038/ng.3033
16. O'Shea KM, Aceves SS, Dellon ES, Gupta SK, Spergel JM, Furuta GT, et al. Pathophysiology of Eosinophilic Esophagitis. *Gastroenterology* (2018) 154 (2):333–45. doi: 10.1053/j.gastro.2017.06.065
17. Azouz NP, Klingler AM, Pathre P, Besse JA, Baruch-Morgenstern NB, Ballaban AY, et al. Functional role of kallikrein 5 and proteinase-activated receptor 2 in eosinophilic esophagitis. *Sci Transl Med* (2020) 12(545): eaaz7773. doi: 10.1126/scitranslmed.aaz7773
18. Kulis MD, Patil SU, Wambre E, Vickery BP. Immune mechanisms of oral immunotherapy. *J Allergy Clin Immunol* (2018) 141(2):491–8. doi: 10.1016/j.jaci.2017.12.979
19. Investigators PGoC, Vickery BP, Vereda A, Casale TB, Beyer K, du Toit G, et al. AR101 Oral Immunotherapy for Peanut Allergy. *N Engl J Med* (2018) 379(21):1991–2001. doi: 10.1056/NEJMoa1812856
20. Petroni D, Spergel JM. Eosinophilic esophagitis and symptoms possibly related to eosinophilic esophagitis in oral immunotherapy. *Ann Allergy Asthma Immunol* (2018) 120(3):237–40 e4. doi: 10.1016/j.anai.2017.11.016
21. Lucendo AJ, Arias A, Tenias JM. Relation between eosinophilic esophagitis and oral immunotherapy for food allergy: a systematic review with meta-analysis. *Ann Allergy Asthma Immunol* (2014) 113(6):624–9. doi: 10.1016/j.anai.2014.08.004
22. Burk CM, Dellon ES, Steele PH, Virkud YV, Kulis M, Burks AW, et al. Eosinophilic esophagitis during peanut oral immunotherapy with omalizumab. *J Allergy Clin Immunol Pract* (2017) 5(2):498–501. doi: 10.1016/j.jaip.2016.11.010
23. Wright BL, Fernandez-Becker NQ, Kambham N, Purington N, Tupa D, Zhang W, et al. Baseline Gastrointestinal Eosinophilia Is Common in Oral Immunotherapy Subjects With IgE-Mediated Peanut Allergy. *Front Immunol* (2018) 9:2624. doi: 10.3389/fimmu.2018.02624
24. Wright BL, Fernandez-Becker NQ, Kambham N, Purington N, Cao S, Tupa D, et al. Gastrointestinal Eosinophil Responses in a Longitudinal, Randomized Trial of Peanut Oral Immunotherapy. *Clin Gastroenterol Hepatol* (2020). doi: 10.1016/j.cgh.2020.05.019
25. Spergel JM, Elci OU, Muir AB, Liacouras CA, Wilkins BJ, Burke D, et al. Efficacy of Epicutaneous Immunotherapy in Children With Milk-Induced Eosinophilic Esophagitis. *Clin Gastroenterol Hepatol* (2020) 18(2):328–36 e7. doi: 10.1016/j.cgh.2019.05.014
26. Kulis M, Saba K, Kim EH, Bird JA, Kamilaris N, Vickery BP, et al. Increased peanut-specific IgA levels in saliva correlate with food challenge outcomes after peanut sublingual immunotherapy. *J Allergy Clin Immunol* (2012) 129 (4):1159–62. doi: 10.1016/j.jaci.2011.11.045
27. Wright BL, Kulis M, Orgel KA, Burks AW, Dawson P, Henning AK, et al. Component-resolved analysis of IgA, IgE, and IgG4 during egg OIT identifies markers associated with sustained unresponsiveness. *Allergy* (2016) 71(11):1552–60. doi: 10.1111/all.12895

28. Wright BL, Kulis M, Guo R, Orgel KA, Wolf WA, Burks AW, et al. Food-specific IgG4 is associated with eosinophilic esophagitis. *J Allergy Clin Immunol* (2016) 138(4):1190–2 e3. doi: 10.1016/j.jaci.2016.02.024
29. Dellon ES, Guo R, McGee SJ, Hamilton DK, Nicolai E, Covington J, et al. A Novel Allergen-Specific Immune Signature-Directed Approach to Dietary Elimination in Eosinophilic Esophagitis. *Clin Transl Gastroenterol* (2019) 10 (12):e00099. doi: 10.14309/ctg.0000000000000099
30. Peterson K, Lin E, Saffari H, Qeadan F, Pyne A, Firszt R, et al. Food-specific antibodies in oesophageal secretions: association with trigger foods in eosinophilic esophagitis. *Aliment Pharmacol Ther* (2020) 52:997–1007. doi: 10.1111/apt.15879
31. Shoda T, Wen T, Aceves SS, Abonia JP, Atkins D, Bonis PA, et al. Eosinophilic oesophagitis endotype classification by molecular, clinical, and histopathological analyses: a cross-sectional study. *Lancet Gastroenterol Hepatol* (2018) 3(7):477–88. doi: 10.1016/S2468-1253(18) 30096-7
32. Dunn JLM, Shoda T, Caldwell JM, Wen T, Aceves SS, Collins MH, et al. Esophageal type 2 cytokine expression heterogeneity in eosinophilic esophagitis in a multisite cohort. *J Allergy Clin Immunol* (2020) 145 (6):1629–40 e4. doi: 10.1016/j.jaci.2020.01.051
33. Rothenberg ME, Hogan SP. The eosinophil. *Annu Rev Immunol* (2006) 24:147–74. doi: 10.1146/annurev.immunol.24.021605.090720
34. Legrand F, Cao Y, Wechsler JB, Zhu X, Zimmermann N, Rampertaap S, et al. Sialic acid-binding immunoglobulin-like lectin (Siglec) 8 in patients with eosinophilic disorders: Receptor expression and targeting using chimeric antibodies. *J Allergy Clin Immunol* (2019) 143(6):2227–37 e10. doi: 10.1016/j.jaci.2018.10.066
35. Stein ML, Collins MH, Villanueva JM, Kushner JP, Putnam PE, Buckmeier BK, et al. Anti-IL-5 (mepolizumab) therapy for eosinophilic esophagitis. *J Allergy Clin Immunol* (2006) 118(6):1312–9. doi: 10.1016/j.jaci.2006.09.007
36. Straumann A, Conus S, Grzonka P, Kita H, Kephart G, Bussmann C, et al. Anti-interleukin-5 antibody treatment (mepolizumab) in active eosinophilic oesophagitis: a randomised, placebo-controlled, double-blind trial. *Gut* (2010) 59(1):21–30. doi: 10.1136/gut.2009.178558
37. Assa'ad AH, Gupta SK, Collins MH, Thomson M, Heath AT, Smith DA, et al. An antibody against IL-5 reduces numbers of esophageal intraepithelial eosinophils in children with eosinophilic esophagitis. *Gastroenterology* (2011) 141(5):1593–604. doi: 10.1053/j.gastro.2011.07.044
38. Spergel JM, Rothenberg ME, Collins MH, Furuta GT, Markowitz JE, Fuchs G,3, et al. Reslizumab in children and adolescents with eosinophilic esophagitis: results of a double-blind, randomized, placebo-controlled trial. *J Allergy Clin Immunol* (2012) 129(2):456–63, 63 e1-3. doi: 10.1016/j.jaci.2011.11.044
39. Conus S, Straumann A, Bettler E, Simon HU. Mepolizumab does not alter levels of eosinophils, T cells, and mast cells in the duodenal mucosa in eosinophilic esophagitis. *J Allergy Clin Immunol* (2010) 126(1):175–7. doi: 10.1016/j.jaci.2010.04.029
40. Straumann A, Blanchard C, Radonjic-Hoesli S, Bussmann C, Hruz P, Safroneeva E, et al. A new eosinophilic esophagitis (EoE)-like disease without tissue eosinophilia found in EoE families. *Allergy* (2016) 71 (6):889–900. doi: 10.1111/all.12879
41. Lee JJ, Jacobsen EA, McGarry MP, Schleimer RP, Lee NA. Eosinophils in health and disease: the LIAR hypothesis. *Clin Exp Allergy* (2010) 40(4):563–75. doi: 10.1111/j.1365-2222.2010.03484.x
42. Lee JJ, Jacobsen EA, Ochkur SI, McGarry MP, Condjella RM, Doyle AD, et al. Human versus mouse eosinophils: "that which we call an eosinophil, by any other name would stain as red". *J Allergy Clin Immunol* (2012) 130 (3):572–84. doi: 10.1016/j.jaci.2012.07.025
43. Hogan SP, Rosenberg HF, Moqbel R, Phipps S, Foster PS, Lacy P, et al. Eosinophils: biological properties and role in health and disease. *Clin Exp Allergy* (2008) 38(5):709–50. doi: 10.1111/j.1365-2222.2008.02958.x
44. Jacobsen EA, Helmers RA, Lee JJ, Lee NA. The expanding role(s) of eosinophils in health and disease. *Blood* (2012) 120(19):3882–90. doi: 10.1182/blood-2012-06-330845
45. Lee J, Rosenberg HF. *Eosinophils in health and disease. 1st ed.* London ; Waltham, MA: Elsevier/Academic Press (2013). p. 654. xxiii.
46. Gomes I, Mathur SK, Espenshade BM, Mori Y, Varga J, Ackerman SJ. Eosinophil-fibroblast interactions induce fibroblast IL-6 secretion and extracellular matrix gene expression: implications in fibrogenesis. *J Allergy Clin Immunol* (2005) 116(4):796–804. doi: 10.1016/j.jaci.2005.06.031
47. Del Pozo V, De Andres B, Martin E, Maruri N, Zubeldia JM, Palomino P, et al. Murine eosinophils and IL-1: alpha IL-1 mRNA detection by in situ hybridization. Production and release of IL-1 from peritoneal eosinophils. *J Immunol* (1990) 144(8):3117–22.
48. Weller PF, Rand TH, Barrett T, Elovic A, Wong DT, Finberg RW. Accessory cell function of human eosinophils. HLA-DR-dependent, MHC-restricted antigen-presentation and IL-1 alpha expression. *J Immunol* (1993) 150 (6):2554–62.
49. Blanchard C, Stucke EM, Rodriguez-Jimenez B, Burwinkel K, Collins MH, Ahrens A, et al. A striking local esophageal cytokine expression profile in eosinophilic esophagitis. *J Allergy Clin Immunol* (2011) 127(1):208–17, 17 e1-7. doi: 10.1016/j.jaci.2010.10.039
50. Jung Y, Wen T, Mingler MK, Caldwell JM, Wang YH, Chaplin DD, et al. IL-1beta in eosinophil-mediated small intestinal homeostasis and IgA production. *Mucosal Immunol* (2015) 8(4):930–42. doi: 10.1038/mi.2014.123
51. Arias A, Vicario M, Bernardo D, Olalla JM, Fortea M, Montalban-Arques A, et al. Toll-like receptors-mediated pathways activate inflammatory responses in the esophageal mucosa of adult eosinophilic esophagitis. *Clin Transl Gastroenterol* (2018) 9(4):147. doi: 10.1038/s41424-018-0017-4
52. Muir AB, Lim DM, Benitez AJ, Modayur Chandramouleeswaran P, Lee AJ, Ruchelli ED, et al. Esophageal epithelial and mesenchymal cross-talk leads to features of epithelial to mesenchymal transition in vitro. *Exp Cell Res* (2013) 319(6):850–9. doi: 10.1016/j.yexcr.2012.12.002
53. Sugawara R, Lee EJ, Jang MS, Jeun EJ, Hong CP, Kim JH, et al. Small intestinal eosinophils regulate Th17 cells by producing IL-1 receptor antagonist. *J Exp Med* (2016) 213(4):555–67. doi: 10.1084/jem.20141388
54. Kita H, Ohnishi T, Okubo Y, Weiler D, Abrams JS, Gleich GJ. Granulocyte/macrophage colony-stimulating factor and interleukin 3 release from human peripheral blood eosinophils and neutrophils. *J Exp Med* (1991) 174(3):745–8. doi: 10.1084/jem.174.3.745
55. Nakajima H, Gleich GJ, Kita H. Constitutive production of IL-4 and IL-10 and stimulated production of IL-8 by normal peripheral blood eosinophils. *J Immunol* (1996) 156(12):4859–66.
56. Broide DH, Paine MM, Firestein GS. Eosinophils express interleukin 5 and granulocyte macrophage-colony-stimulating factor mRNA at sites of allergic inflammation in asthmatics. *J Clin Invest* (1992) 90(4):1414–24. doi: 10.1172/JCI116008
57. Desreumaux P, Janin A, Colombel JF, Prin L, Plumas J, Emilie D, et al. Interleukin 5 messenger RNA expression by eosinophils in the intestinal mucosa of patients with coeliac disease. *J Exp Med* (1992) 175(1):293–6. doi: 10.1084/jem.175.1.293
58. Hamid Q, Barkans J, Meng Q, Ying S, Abrams JS, Kay AB, et al. Human eosinophils synthesize and secrete interleukin-6, in vitro. *Blood* (1992) 80 (6):1496–501. doi: 10.1182/blood.V80.6.1496.1496
59. Chu VT, Frohlich A, Steinhauser G, Scheel T, Roch T, Fillatreau S, et al. Eosinophils are required for the maintenance of plasma cells in the bone marrow. *Nat Immunol* (2011) 12(2):151–9. doi: 10.1038/ni.1981
60. Braun RK, Franchini M, Erard F, Rihs S, De Vries IJ, Blaser K, et al. Human peripheral blood eosinophils produce and release interleukin-8 on stimulation with calcium ionophore. *Eur J Immunol* (1993) 23(4):956–60. doi: 10.1002/eji.1830230429
61. Cherry WB, Yoon J, Bartemes KR, Iijima K, Kita H. A novel IL-1 family cytokine, IL-33, potently activates human eosinophils. *J Allergy Clin Immunol* (2008) 121(6):1484–90. doi: 10.1016/j.jaci.2008.04.005
62. Cheung PF, Wong CK, Lam CW. Molecular mechanisms of cytokine and chemokine release from eosinophils activated by IL-17A, IL-17F, and IL-23: implication for Th17 lymphocytes-mediated allergic inflammation. *J Immunol* (2008) 180(8):5625–35. doi: 10.4049/jimmunol.180.8.5625
63. Otani IM, Anilkumar AA, Newbury RO, Bhagat M, Beppu LY, Dohil R, et al. Anti-IL-5 therapy reduces mast cell and IL-9 cell numbers in pediatric patients with eosinophilic esophagitis. *J Allergy Clin Immunol* (2013) 131 (6):1576–82. doi: 10.1016/j.jaci.2013.02.042
64. Doshi A, Khamishon R, Rawson R, Duong L, Dohil L, Myers SJ, et al. Interleukin 9 Alters Epithelial Barrier and E-cadherin in Eosinophilic Esophagitis. *J Pediatr Gastroenterol Nutr* (2019) 68(2):225–31. doi: 10.1097/MPG.0000000000002144

65. Takeda K, Shiraishi Y, Ashino S, Han J, Jia Y, Wang M, et al. Eosinophils contribute to the resolution of lung-allergic responses following repeated allergen challenge. *J Allergy Clin Immunol* (2015) 135(2):451–60. doi: 10.1016/j.jaci.2014.08.014
66. Lu S, Herzlinger M, Cao W, Noble L, Yang D, Shapiro J, et al. Utility of 15 (S)-HETE as a Serological Marker for Eosinophilic Esophagitis. *Sci Rep* (2018) 8(1):14498. doi: 10.1038/s41598-018-32944-8
67. Aceves SS, Ackerman SJ. Relationships between eosinophilic inflammation, tissue remodeling, and fibrosis in eosinophilic esophagitis. *Immunol Allergy Clin North Am* (2009) 29(1):197–211, xiii-xiv. doi: 10.1016/j.iac.2008.10.003
68. Mishra A, Rothenberg ME. Intratracheal IL-13 induces eosinophilic esophagitis by an IL-5, eotaxin-1, and STAT6-dependent mechanism. *Gastroenterology* (2003) 125(5):1419–27. doi: 10.1016/j.gastro.2003.07.007
69. Blanchard C, Mingler MK, Vicario M, Abonia JP, Wu YY, Lu TX, et al. IL-13 involvement in eosinophilic esophagitis: transcriptome analysis and reversibility with glucocorticoids. *J Allergy Clin Immunol* (2007) 120 (6):1292–300. doi: 10.1016/j.jaci.2007.10.024
70. Jacobsen EA, Doyle AD, Colbert DC, Zellner KR, Protheroe CA, LeSuer WE, et al. Differential activation of airway eosinophils induces IL-13-mediated allergic Th2 pulmonary responses in mice. *Allergy* (2015) 70(9):1148–59. doi: 10.1111/all.12655
71. Rochman M, Travers J, Abonia JP, Caldwell JM, Rothenberg ME. Synaptopodin is upregulated by IL-13 in eosinophilic esophagitis and regulates esophageal epithelial cell motility and barrier integrity. *JCI Insight* (2017) 2(20):e96789. doi: 10.1172/jci.insight.96789
72. Sherrill JD, Kc K, Wu D, Djukic Z, Caldwell JM, Stucke EM, et al. Desmoglein-1 regulates esophageal epithelial barrier function and immune responses in eosinophilic esophagitis. *Mucosal Immunol* (2014) 7(3):718–29. doi: 10.1038/mi.2013.90
73. Zeng C, Vanoni S, Wu D, Caldwell JM, Wheeler JC, Arora K, et al. Solute carrier family 9, subfamily A, member 3 (SLC9A3)/sodium-hydrogen exchanger member 3 (NHE3) dysregulation and dilated intercellular spaces in patients with eosinophilic esophagitis. *J Allergy Clin Immunol* (2018) 142(6):1843–55. doi: 10.1016/j.jaci.2018.03.017
74. Guerra ES, Lee CK, Specht CA, Yadav B, Huang H, Akalin A, et al. Central Role of IL-23 and IL-17 Producing Eosinophils as Immunomodulatory Effector Cells in Acute Pulmonary Aspergillosis and Allergic Asthma. *PloS Pathog* (2017) 13(1):e1006175. doi: 10.1371/journal.ppat.1006175
75. Niranjan R, Rajavelu P, Ventateshaiah SU, Shukla JS, Zaidi A, Mariswamy SJ, et al. Involvement of interleukin-18 in the pathogenesis of human eosinophilic esophagitis. *Clin Immunol* (2015) 157(2):103–13. doi: 10.1016/j.clim.2015.01.007
76. Dutt P, Shukla JS, Ventateshaiah SU, Mariswamy SJ, Mattner J, Shukla A, et al. Allergen-induced interleukin-18 promotes experimental eosinophilic oesophagitis in mice. *Immunol Cell Biol* (2015) 93(10):849–57. doi: 10.1038/icb.2015.30
77. Verma AK, Kandikattu HK, Manohar M, Shukla A, Upparahalli Venkateshaiah S, Zhu X, et al. Intestinal overexpression of IL-18 promotes eosinophils-mediated allergic disorders. *Immunology* (2019) 157 (2):110–21. doi: 10.1111/imm.13051
78. Gatault S, Delbeke M, Driss V, Sarazin A, Dendooven A, Kahn JE, et al. IL-18 Is Involved in Eosinophil-Mediated Tumoricidal Activity against a Colon Carcinoma Cell Line by Upregulating LFA-1 and ICAM-1. *J Immunol* (2015) 195(5):2483–92. doi: 10.4049/jimmunol.1402914
79. Simon D, Radonjic-Hosli S, Straumann A, Yousefi S, Simon HU. Active eosinophilic esophagitis is characterized by epithelial barrier defects and eosinophil extracellular trap formation. *Allergy* (2015) 70(4):443–52. doi: 10.1111/all.12570
80. Terrier B, Bieche I, Maisonobe T, Laurendeau I, Rosenzwajg M, Kahn JE, et al. Interleukin-25: a cytokine linking eosinophils and adaptive immunity in Churg-Strauss syndrome. *Blood* (2010) 116(22):4523–31. doi: 10.1182/blood-2010-02-267542
81. Spencer LA, Szela CT, Perez SA, Kirchhoffer CL, Neves JS, Radke AL, et al. Human eosinophils constitutively express multiple Th1, Th2, and immunoregulatory cytokines that are secreted rapidly and differentially. *J Leukoc Biol* (2009) 85(1):117–23. doi: 10.1189/jlb.0108058
82. Gupta SK, Fitzgerald JF, Kondratyuk T, HogenEsch H. Cytokine expression in normal and inflamed esophageal mucosa: a study into the pathogenesis of allergic eosinophilic esophagitis. *J Pediatr Gastroenterol Nutr* (2006) 42 (1):22–6. doi: 10.1097/01.mpg.0000188740.38757.d2
83. Mulder DJ, Pooni A, Mak N, Hurlbut DJ, Basta S, Justinich CJ. Antigen presentation and MHC class II expression by human esophageal epithelial cells: role in eosinophilic esophagitis. *Am J Pathol* (2011) 178(2):744–53. doi: 10.1016/j.ajpath.2010.10.027
84. Costa JJ, Matossian K, Resnick MB, Beil WJ, Wong DT, Gordon JR, et al. Human eosinophils can express the cytokines tumor necrosis factor-alpha and macrophage inflammatory protein-1 alpha. *J Clin Invest* (1993) 91 (6):2673–84. doi: 10.1172/JCI116506
85. Finotto S, Ohno I, Marshall JS, Gauldie J, Denburg JA, Dolovich J, et al. TNF-alpha production by eosinophils in upper airways inflammation (nasal polyposis). *J Immunol* (1994) 153(5):2278–89.
86. Puxeddu I, Berkman N, Ribatti D, Bader R, Haitchi HM, Davies DE, et al. Osteopontin is expressed and functional in human eosinophils. *Allergy* (2010) 65(2):168–74. doi: 10.1111/j.1398-9995.2009.02148.x
87. Matsumoto K, Fukuda S, Nakamura Y, Saito H. Amphiregulin production by human eosinophils. *Int Arch Allergy Immunol* (2009) 149 Suppl 1:39–44. doi: 10.1159/000210652
88. Chu VT, Beller A, Rausch S, Strandmark J, Zanker M, Arbach O, et al. Eosinophils promote generation and maintenance of immunoglobulin-A-expressing plasma cells and contribute to gut immune homeostasis. *Immunity* (2014) 40(4):582–93. doi: 10.1016/j.immuni.2014.02.014
89. Hartman M, Piliponsky AM, Temkin V, Levi-Schaffer F. Human peripheral blood eosinophils express stem cell factor. *Blood* (2001) 97(4):1086–91. doi: 10.1182/blood.V97.4.1086
90. Arias A, Lucendo AJ, Martinez-Fernandez P, Gonzalez-Castro AM, Fortea M, Gonzalez-Cervera J, et al. Dietary treatment modulates mast cell phenotype, density, and activity in adult eosinophilic oesophagitis. *Clin Exp Allergy* (2016) 46(1):78–91. doi: 10.1111/cea.12504
91. Wong DT, Weller PF, Galli SJ, Elovic A, Rand TH, Gallagher GT, et al. Human eosinophils express transforming growth factor alpha. *J Exp Med* (1990) 172(3):673–81. doi: 10.1084/jem.172.3.673
92. Aceves SS, Newbury RO, Dohil R, Bastian JF, Broide DH. Esophageal remodeling in pediatric eosinophilic esophagitis. *J Allergy Clin Immunol* (2007) 119(1):206–12. doi: 10.1016/j.jaci.2006.10.016
93. Kagalwalla AF, Akhtar N, Woodruff SA, Rea BA, Masterson JC, Mukkada V, et al. Eosinophilic esophagitis: epithelial mesenchymal transition contributes to esophageal remodeling and reverses with treatment. *J Allergy Clin Immunol* (2012) 129(5):1387–96 e7. doi: 10.1016/j.jaci.2012.03.005
94. Wong DT, Elovic A, Matossian K, Nagura N, McBride J, Chou MY, et al. Eosinophils from patients with blood eosinophilia express transforming growth factor beta 1. *Blood* (1991) 78(10):2702–7. doi: 10.1182/blood.V78.10.2702.2702
95. Levi-Schaffer F, Garbuzenko E, Rubin A, Reich R, Pickholz D, Gillery P, et al. Human eosinophils regulate human lung- and skin-derived fibroblast properties in vitro: a role for transforming growth factor beta (TGF-beta). *Proc Natl Acad Sci U.S.A.* (1999) 96(17):9660–5. doi: 10.1073/pnas.96.17.9660
96. Moqbel R, Hamid Q, Ying S, Barkans J, Hartnell A, Tsicopoulos A, et al. Expression of mRNA and immunoreactivity for the granulocyte/macrophage colony-stimulating factor in activated human eosinophils. *J Exp Med* (1991) 174(3):749–52. doi: 10.1084/jem.174.3.749
97. Mann A, Breuhahn K, Schirmacher P, Blessing M. Keratinocyte-derived granulocyte-macrophage colony stimulating factor accelerates wound healing: Stimulation of keratinocyte proliferation, granulation tissue formation, and vascularization. *J Invest Dermatol* (2001) 117(6):1382–90. doi: 10.1046/j.0022-202x.2001.01600.x
98. McNamee EN, Biette KA, Hammer J, Harris R, Miyazawa H, Lee JJ, et al. Targeting granulocyte-macrophage colony-stimulating factor in epithelial and vascular remodeling in experimental eosinophilic esophagitis. *Allergy* (2017) 72(8):1232–42. doi: 10.1111/all.13105
99. Persad R, Huynh HQ, Hao L, Ha JR, Sergi C, Srivastava R, et al. Angiogenic remodeling in pediatric EoE is associated with increased levels of VEGF-A, angiogenin, IL-8, and activation of the TNF-alpha-NFkappaB pathway. *J Pediatr Gastroenterol Nutr* (2012) 55(3):251–60. doi: 10.1097/MPG.0b013e31824b6391
100. Horiuchi T, Weller PF. Expression of vascular endothelial growth factor by human eosinophils: upregulation by granulocyte macrophage colony-stimulating factor and interleukin-5. *Am J Respir Cell Mol Biol* (1997) 17 (1):70–7. doi: 10.1165/ajrcmb.17.1.2796
101. Hoshino M, Nakamura Y, Hamid QA. Gene expression of vascular endothelial growth factor and its receptors and angiogenesis in bronchial

asthma. *J Allergy Clin Immunol* (2001) 107(6):1034–8. doi: 10.1067/mai.2001.115626
102. Huang JJ, Joh JW, Fuentebella J, Patel A, Nguyen T, Seki S, et al. Eotaxin and FGF enhance signaling through an extracellular signal-related kinase (ERK)-dependent pathway in the pathogenesis of Eosinophilic esophagitis. *Allergy Asthma Clin Immunol* (2010) 6(1):25. doi: 10.1186/1710-1492-6-25
103. Stenfeldt AL, Wenneras C. Danger signals derived from stressed and necrotic epithelial cells activate human eosinophils. *Immunology* (2004) 112(4):605–14. doi: 10.1111/j.1365-2567.2004.01906.x
104. Micera A, Vigneti E, Pickholtz D, Reich R, Pappo O, Bonini S, et al. Nerve growth factor displays stimulatory effects on human skin and lung fibroblasts, demonstrating a direct role for this factor in tissue repair. *Proc Natl Acad Sci U.S.A.* (2001) 98(11):6162–7. doi: 10.1073/pnas.101130898
105. Solomon A, Aloe L, Pe'er J, Frucht-Pery J, Bonini S, Bonini S, et al. Nerve growth factor is preformed in and activates human peripheral blood eosinophils. *J Allergy Clin Immunol* (1998) 102(3):454–60. doi: 10.1016/S0091-6749(98)70135-6
106. Powell PP, Klagsbrun M, Abraham JA, Jones RC. Eosinophils expressing heparin-binding EGF-like growth factor mRNA localize around lung microvessels in pulmonary hypertension. *Am J Pathol* (1993) 143(3):784–93.
107. Dyer KD, Percopo CM, Xie Z, Yang Z, Kim JD, Davoine F, et al. Mouse and human eosinophils degranulate in response to platelet-activating factor (PAF) and lysoPAF via a PAF-receptor-independent mechanism: evidence for a novel receptor. *J Immunol* (2010) 184(11):6327–34. doi: 10.4049/jimmunol.0904043
108. Aliakbari J, Sreedharan SP, Turck CW, Goetzl EJ. Selective localization of vasoactive intestinal peptide and substance P in human eosinophils. *Biochem Biophys Res Commun* (1987) 148(3):1440–5. doi: 10.1016/S0006-291X(87)80293-0
109. Verma AK, Manohar M, Venkateshaiah SU, Blecker U, Collins MH, Mishra A. Role of Vasoactive Intestinal Peptide in Promoting the Pathogenesis of Eosinophilic Esophagitis (EoE). *Cell Mol Gastroenterol Hepatol* (2018) 5(1):99–100 e7. doi: 10.1016/j.jcmgh.2017.09.006
110. Driss V, Legrand F, Hermann E, Loiseau S, Guerardel Y, Kremer L, et al. TLR2-dependent eosinophil interactions with mycobacteria: role of alpha-defensins. *Blood* (2009) 113(14):3235–44. doi: 10.1182/blood-2008-07-166595
111. Khatun A, Sakurai M, Okada K, Sakai Y, Morimoto M. Detection of alpha-defensin in eosinophils in helminth-infected mouse model. *J Vet Med Sci* (2018) 80(12):1887–94. doi: 10.1292/jvms.18-0601
112. Hoshino M, Takahashi M, Aoike N. Expression of vascular endothelial growth factor, basic fibroblast growth factor, and angiogenin immunoreactivity in asthmatic airways and its relationship to angiogenesis. *J Allergy Clin Immunol* (2001) 107(2):295–301. doi: 10.1067/mai.2001.111928
113. Kishimoto K, Liu S, Tsuji T, Olson KA, Hu GF. Endogenous angiogenin in endothelial cells is a general requirement for cell proliferation and angiogenesis. *Oncogene* (2005) 24(3):445–56. doi: 10.1038/sj.onc.1208223
114. Okada S, Kita H, George TJ, Gleich GJ, Leiferman KM. Migration of eosinophils through basement membrane components in vitro: role of matrix metalloproteinase-9. *Am J Respir Cell Mol Biol* (1997) 17(4):519–28. doi: 10.1165/ajrcmb.17.4.2877
115. Esnault S, Kelly EA, Johnson SH, DeLain LP, Haedt MJ, Noll AL, et al. Matrix Metalloproteinase-9-Dependent Release of IL-1beta by Human Eosinophils. *Mediators Inflammation* (2019) 2019:7479107. doi: 10.1155/2019/7479107
116. Kobayashi M, Naomoto Y, Nobuhisa T, Okawa T, Takaoka M, Shirakawa Y, et al. Heparanase regulates esophageal keratinocyte differentiation through nuclear translocation and heparan sulfate cleavage. *Differentiation* (2006) 74(5):235–43. doi: 10.1111/j.1432-0436.2006.00072.x
117. Zcharia E, Zilka R, Yaar A, Yacoby-Zeevi O, Zetser A, Metzger S, et al. Heparanase accelerates wound angiogenesis and wound healing in mouse and rat models. *FASEB J* (2005) 19(2):211–21. doi: 10.1096/fj.04-1970com
118. Temkin V, Aingorn H, Puxeddu I, Goldshmidt O, Zcharia E, Gleich GJ, et al. Eosinophil major basic protein: first identified natural heparanase-inhibiting protein. *J Allergy Clin Immunol* (2004) 113(4):703–9. doi: 10.1016/j.jaci.2003.11.038
119. Zeiger RS, Colten HR. Histaminase release from human eosinophils. *J Immunol* (1977) 118(2):540–3.
120. Wen T, Stucke EM, Grotjan TM, Kemme KA, Abonia JP, Putnam PE, et al. Molecular diagnosis of eosinophilic esophagitis by gene expression profiling. *Gastroenterology* (2013) 145(6):1289–99. doi: 10.1053/j.gastro.2013.08.046
121. MacMillan DK, Hill E, Sala A, Sigal E, Shuman T, Henson PM, et al. Eosinophil 15-lipoxygenase is a leukotriene A4 synthase. *J Biol Chem* (1994) 269(43):26663–8.
122. Isobe Y, Arita M, Matsueda S, Iwamoto R, Fujihara T, Nakanishi H, et al. Identification and structure determination of novel anti-inflammatory mediator resolvin E3, 17,18-dihydroxyeicosapentaenoic acid. *J Biol Chem* (2012) 287(13):10525–34. doi: 10.1074/jbc.M112.340612
123. Hui Y, Chen S, Lombardo KA, Resnick MB, Mangray S, Matoso A. ALOX15 Immunohistochemistry Aids in the Diagnosis of Eosinophilic Esophagitis on Pauci-eosinophilic Biopsies in Children. *Pediatr Dev Pathol* (2017) 20(5):375–80. doi: 10.1177/1093526617693106
124. Odemuyiwa SO, Ghahary A, Li Y, Puttagunta L, Lee JE, Musat-Marcu S, et al. Cutting edge: human eosinophils regulate T cell subset selection through indoleamine 2,3-dioxygenase. *J Immunol* (2004) 173(10):5909–13. doi: 10.4049/jimmunol.173.10.5909
125. Prendergast GC, Smith C, Thomas S, Mandik-Nayak L, Laury-Kleintop L, Metz R, et al. Indoleamine 2,3-dioxygenase pathways of pathogenic inflammation and immune escape in cancer. *Cancer Immunol Immunother* (2014) 63(7):721–35. doi: 10.1007/s00262-014-1549-4
126. Mulder DJ, Pacheco I, Hurlbut DJ, Mak N, Furuta GT, MacLeod RJ, et al. FGF9-induced proliferative response to eosinophilic inflammation in oesophagitis. *Gut* (2009) 58(2):166–73. doi: 10.1136/gut.2008.157628
127. Pegorier S, Wagner LA, Gleich GJ, Pretolani M. Eosinophil-derived cationic proteins activate the synthesis of remodeling factors by airway epithelial cells. *J Immunol* (2006) 177(7):4861–9. doi: 10.4049/jimmunol.177.7.4861
128. Furuta GT, Nieuwenhuis EE, Karhausen J, Gleich G, Blumberg RS, Lee JJ, et al. Eosinophils alter colonic epithelial barrier function: role for major basic protein. *Am J Physiol Gastrointest Liver Physiol* (2005) 289(5):G890–7. doi: 10.1152/ajpgi.00015.2005
129. O'Donnell MC, Ackerman SJ, Gleich GJ, Thomas LL. Activation of basophil and mast cell histamine release by eosinophil granule major basic protein. *J Exp Med* (1983) 157(6):1981–91. doi: 10.1084/jem.157.6.1981
130. Gleich GJ, Adolphson CR, Leiferman KM. The biology of the eosinophilic leukocyte. *Annu Rev Med* (1993) 44:85–101. doi: 10.1146/annurev.me.44.020193.000505
131. Dellon ES, Speck O, Woodward K, Covey S, Rusin S, Gebhart JH, et al. Markers of eosinophilic inflammation for diagnosis of eosinophilic esophagitis and proton pump inhibitor-responsive esophageal eosinophilia: a prospective study. *Clin Gastroenterol Hepatol* (2014) 12(12):2015–22. doi: 10.1016/j.cgh.2014.06.019
132. Furuta GT, Kagalwalla AF, Lee JJ, Alumkal P, Maybruck BT, Fillon S, et al. The oesophageal string test: a novel, minimally invasive method measures mucosal inflammation in eosinophilic oesophagitis. *Gut* (2013) 62(10):1395–405. doi: 10.1136/gutjnl-2012-303171
133. Martin LJ, Franciosi JP, Collins MH, Abonia JP, Lee JJ, Hommel KA, et al. Pediatric Eosinophilic Esophagitis Symptom Scores (PEESS v2.0) identify histologic and molecular correlates of the key clinical features of disease. *J Allergy Clin Immunol* (2015) 135(6):1519–28 e8. doi: 10.1016/j.jaci.2015.03.004
134. Wright BL, Doyle AD, Shim KP, Pai RK, Barshow SM, Horsley-Silva JL, et al. Image Analysis of Eosinophil Peroxidase Immunohistochemistry for Diagnosis of Eosinophilic Esophagitis. *Dig Dis Sci* (2020). doi: 10.1007/s10620-020-06230-5
135. Bystrom J, Amin K, Bishop-Bailey D. Analysing the eosinophil cationic protein–a clue to the function of the eosinophil granulocyte. *Respir Res* (2011) 12:10. doi: 10.1186/1465-9921-12-10
136. Yang D, Chen Q, Su SB, Zhang P, Kurosaka K, Caspi RR, et al. Eosinophil-derived neurotoxin acts as an alarmin to activate the TLR2-MyD88 signal pathway in dendritic cells and enhances Th2 immune responses. *J Exp Med* (2008) 205(1):79–90. doi: 10.1084/jem.20062027
137. Yang D, Rosenberg HF, Chen Q, Dyer KD, Kurosaka K, Oppenheim JJ. Eosinophil-derived neurotoxin (EDN), an antimicrobial protein with chemotactic activities for dendritic cells. *Blood* (2003) 102(9):3396–403. doi: 10.1182/blood-2003-01-0151
138. Acharya KR, Ackerman SJ. Eosinophil granule proteins: form and function. *J Biol Chem* (2014) 289(25):17406–15. doi: 10.1074/jbc.R113.546218
139. Grozdanovic MM, Doyle CB, Liu L, Maybruck BT, Kwatia MA, Thiyagarajan N, et al. Charcot-Leyden crystal protein/galectin-10 interacts with cationic ribonucleases and is required for eosinophil granulogenesis. *J Allergy Clin Immunol* (2020) 146(2):377–89 e10. doi: 10.1016/j.jaci.2020.01.013

140. Persson EK, Verstraete K, Heyndrickx I, Gevaert E, Aegerter H, Percier JM, et al. Protein crystallization promotes type 2 immunity and is reversible by antibody treatment. *Science* (2019) 364(6442):eaaw4295. doi: 10.1126/science.aaw4295
141. Lee T, Lenihan DJ, Malone B, Roddy LL, Wasserman SI. Increased biosynthesis of platelet-activating factor in activated human eosinophils. *J Biol Chem* (1984) 259(9):5526–30.
142. Foegh ML, Maddox YT, Ramwell PW. Human peritoneal eosinophils and formation of arachidonate cyclooxygenase products. *Scand J Immunol* (1986) 23(5):599–603. doi: 10.1111/j.1365-3083.1986.tb01993.x
143. Lexmond WS, Pardo M, Rooney K, Goettel JA, Snapper SB, Yen EH, et al. Elevated levels of leukotriene C4 synthase mRNA distinguish a subpopulation of eosinophilic oesophagitis patients. *Clin Exp Allergy* (2013) 43(8):902–13. doi: 10.1111/cea.12146
144. Walsh GM, Hartnell A, Moqbel R, Cromwell O, Nagy L, Bradley B, et al. Receptor expression and functional status of cultured human eosinophils derived from umbilical cord blood mononuclear cells. *Blood* (1990) 76 (1):105–11. doi: 10.1182/blood.V76.1.105.105
145. Luna-Gomes T, Magalhaes KG, Mesquita-Santos FP, Bakker-Abreu I, Samico RF, Molinaro R, et al. Eosinophils as a novel cell source of prostaglandin D2: autocrine role in allergic inflammation. *J Immunol* (2011) 187(12):6518–26. doi: 10.4049/jimmunol.1101806
146. Parsons WG,3, Roberts LJ,2. Transformation of prostaglandin D2 to isomeric prostaglandin F2 compounds by human eosinophils: a potential mast cell-eosinophil interaction. *Adv Prostaglandin Thromboxane Leukot Res* (1989) 19:499–502.
147. Zhang S, Wu X, Yu S. Prostaglandin D2 receptor D-type prostanoid receptor 2 mediates eosinophil trafficking into the esophagus. *Dis Esophagus* (2014) 27(6):601–6. doi: 10.1111/dote.12118
148. Yamada T, Tani Y, Nakanishi H, Taguchi R, Arita M, Arai H. Eosinophils promote resolution of acute peritonitis by producing proresolving mediators in mice. *FASEB J* (2011) 25(2):561–8. doi: 10.1096/fj.10-170027
149. Schwab JM, Chiang N, Arita M, Serhan CN. Resolvin E1 and protectin D1 activate inflammation-resolution programmes. *Nature* (2007) 447 (7146):869–74. doi: 10.1038/nature05877
150. Jacobsen EA, Ochkur SI, Pero RS, Taranova AG, Protheroe CA, Colbert DC, et al. Allergic pulmonary inflammation in mice is dependent on eosinophil-induced recruitment of effector T cells. *J Exp Med* (2008) 205(3):699–710. doi: 10.1084/jem.20071840
151. Liu LY, Bates ME, Jarjour NN, Busse WW, Bertics PJ, Kelly EA. Generation of Th1 and Th2 chemokines by human eosinophils: evidence for a critical role of TNF-alpha. *J Immunol* (2007) 179(7):4840–8. doi: 10.4049/jimmunol.179.7.4840
152. Lim KG, Wan HC, Resnick M, Wong DT, Cruikshank WW, Kornfeld H, et al. Human eosinophils release the lymphocyte and eosinophil active cytokines, RANTES and lymphocyte chemoattractant factor. *Int Arch Allergy Immunol* (1995) 107(1-3):342. doi: 10.1159/000237023
153. Johnsson M, Bove M, Bergquist H, Olsson M, Fornwall S, Hassel K, et al. Distinctive blood eosinophilic phenotypes and cytokine patterns in eosinophilic esophagitis, inflammatory bowel disease and airway allergy. *J Innate Immun* (2011) 3(6):594–604. doi: 10.1159/000331326
154. Nakajima T, Yamada H, Iikura M, Miyamasu M, Izumi S, Shida H, et al. Intracellular localization and release of eotaxin from normal eosinophils. *FEBS Lett* (1998) 434(3):226–30. doi: 10.1016/S0014-5793(98)00863-1
155. Persson T, Monsef N, Andersson P, Bjartell A, Malm J, Calafat J, et al. Expression of the neutrophil-activating CXC chemokine ENA-78/CXCL5 by human eosinophils. *Clin Exp Allergy* (2003) 33(4):531–7. doi: 10.1046/j.1365-2222.2003.01609.x
156. O'Shea KM, Rochman M, Shoda T, Zimmermann N, Caldwell J, Rothenberg ME. Eosinophilic esophagitis with extremely high esophageal eosinophil counts. *J Allergy Clin Immunol* (2020). doi: 10.1016/j.jaci.2020.05.045
157. Lee PJ, Zhang X, Shan P, Ma B, Lee CG, Homer RJ, et al. ERK1/2 mitogen-activated protein kinase selectively mediates IL-13-induced lung inflammation and remodeling in vivo. *J Clin Invest* (2006) 116(1):163–73. doi: 10.1172/JCI25711
158. Devalaraja RM, Nanney LB, Du J, Qian Q, Yu Y, Devalaraja MN, et al. Delayed wound healing in CXCR2 knockout mice. *J Invest Dermatol* (2000) 115(2):234–44. doi: 10.1046/j.1523-1747.2000.00034.x
159. Sayej WN, Menoret A, Maharjan AS, Fernandez M, Wang Z, Balarezo F, et al. Characterizing the inflammatory response in esophageal mucosal biopsies in children with eosinophilic esophagitis. *Clin Transl Immunol* (2016) 5(7):e88. doi: 10.1038/cti.2016.30
160. Izumi S, Hirai K, Miyamasu M, Takahashi Y, Misaki Y, Takaishi T, et al. Expression and regulation of monocyte chemoattractant protein-1 by human eosinophils. *Eur J Immunol* (1997) 27(4):816–24. doi: 10.1002/eji.1830270404
161. Matsumoto K, Fukuda S, Hashimoto N, Saito H. Human eosinophils produce and release a novel chemokine, CCL23, in vitro. *Int Arch Allergy Immunol* (2011) 155 Suppl 1:34–9. doi: 10.1159/000327263
162. Oliveira SH, Taub DD, Nagel J, Smith R, Hogaboam CM, Berlin A, et al. Stem cell factor induces eosinophil activation and degranulation: mediator release and gene array analysis. *Blood* (2002) 100(13):4291–7. doi: 10.1182/blood.V100.13.4291
163. Dajotoy T, Andersson P, Bjartell A, Lofdahl CG, Tapper H, Egesten A. Human eosinophils produce the T cell-attracting chemokines MIG and IP-10 upon stimulation with IFN-gamma. *J Leukoc Biol* (2004) 76(3):685–91. doi: 10.1189/jlb.0803379
164. Reed CC, Wolf WA, Cotton CC, Rusin S, Perjar I, Hollyfield J, et al. Optimal Histologic Cutpoints for Treatment Response in Patients With Eosinophilic Esophagitis: Analysis of Data From a Prospective Cohort Study. *Clin Gastroenterol Hepatol* (2018) 16(2):226–33 e2. doi: 10.1016/j.cgh.2017.09.046
165. McBrien CN, Menzies-Gow A. The Biology of Eosinophils and Their Role in Asthma. *Front Med (Lausanne)* (2017) 4:93. doi: 10.3389/fmed.2017.00093
166. Jacobsen EA, Lee NA, Lee JJ. Re-defining the unique roles for eosinophils in allergic respiratory inflammation. *Clin Exp Allergy* (2014) 44(9):1119–36. doi: 10.1111/cea.12358
167. Cheng E, Souza RF, Spechler SJ. Tissue remodeling in eosinophilic esophagitis. *Am J Physiol Gastrointest Liver Physiol* (2012) 303(11):G1175–87. doi: 10.1152/ajpgi.00313.2012
168. Aceves SS. Remodeling and fibrosis in chronic eosinophil inflammation. *Dig Dis* (2014) 32(1-2):15–21. doi: 10.1159/000357004
169. Simon D, Straumann A, Schoepfer AM, Simon HU. Current concepts in eosinophilic esophagitis. *Allergo J Int* (2017) 26(7):258–66. doi: 10.1007/s40629-017-0037-8
170. Clayton F, Peterson K. Eosinophilic Esophagitis: Pathophysiology and Definition. *Gastrointest Endosc Clin N Am* (2018) 28(1):1–14. doi: 10.1016/j.giec.2017.07.011
171. Rothenberg ME. Biology and treatment of eosinophilic esophagitis. *Gastroenterology* (2009) 137(4):1238–49. doi: 10.1053/j.gastro.2009.07.007
172. Davis BP, Rothenberg ME. Mechanisms of Disease of Eosinophilic Esophagitis. *Annu Rev Pathol* (2016) 11:365–93. doi: 10.1146/annurev-pathol-012615-044241
173. Davis BP. Pathophysiology of Eosinophilic Esophagitis. *Clin Rev Allergy Immunol* (2018) 55(1):19–42. doi: 10.1007/s12016-017-8665-9
174. Blanchard C, Simon D, Schoepfer A, Straumann A, Simon HU. Eosinophilic esophagitis: unclear roles of IgE and eosinophils. *J Intern Med* (2017) 281 (5):448–57. doi: 10.1111/joim.12568
175. Muir AB, Wang JX, Nakagawa H. Epithelial-stromal crosstalk and fibrosis in eosinophilic esophagitis. *J Gastroenterol* (2019) 54(1):10–8. doi: 10.1007/s00535-018-1498-3
176. Mjosberg JM, Trifari S, Crellin NK, Peters CP, van Drunen CM, Piet B, et al. Human IL-25- and IL-33-responsive type 2 innate lymphoid cells are defined by expression of CRTH2 and CD161. *Nat Immunol* (2011) 12(11):1055–62. doi: 10.1038/ni.2104
177. Doherty TA, Baum R, Newbury RO, Yang T, Dohil R, Aquino M, et al. Group 2 innate lymphocytes (ILC2) are enriched in active eosinophilic esophagitis. *J Allergy Clin Immunol* (2015) 136(3):792–4 e3. doi: 10.1016/j.jaci.2015.05.048
178. Blanchard C, Wang N, Stringer KF, Mishra A, Fulkerson PC, Abonia JP, et al. Eotaxin-3 and a uniquely conserved gene-expression profile in eosinophilic esophagitis. *J Clin Invest* (2006) 116(2):536–47. doi: 10.1172/JCI26679
179. Mishra A, Wang M, Pemmaraju VR, Collins MH, Fulkerson PC, Abonia JP, et al. Esophageal remodeling develops as a consequence of tissue specific IL-5-induced eosinophilia. *Gastroenterology* (2008) 134(1):204–14. doi: 10.1053/j.gastro.2007.10.002
180. Masterson JC, McNamee EN, Hosford L, Capocelli KE, Ruybal J, Fillon SA, et al. Local hypersensitivity reaction in transgenic mice with squamous

epithelial IL-5 overexpression provides a novel model of eosinophilic oesophagitis. *Gut* (2014) 63(1):43–53. doi: 10.1136/gutjnl-2012-303631
181. Schoepfer AM, Simko A, Bussmann C, Safroneeva E, Zwahlen M, Greuter T, et al. Eosinophilic Esophagitis: Relationship of Subepithelial Eosinophilic Inflammation With Epithelial Histology, Endoscopy, Blood Eosinophils, and Symptoms. *Am J Gastroenterol* (2018) 113(3):348–57. doi: 10.1038/ajg.2017.493
182. Hirano I. Clinical relevance of esophageal subepithelial activity in eosinophilic esophagitis. *J Gastroenterol* (2020) 55(3):249–60. doi: 10.1007/s00535-019-01624-3
183. Yousefi S, Gold JA, Andina N, Lee JJ, Kelly AM, Kozlowski E, et al. Catapult-like release of mitochondrial DNA by eosinophils contributes to antibacterial defense. *Nat Med* (2008) 14(9):949–53. doi: 10.1038/nm.1855
184. Cliffe LJ, Humphreys NE, Lane TE, Potten CS, Booth C, Grencis RK. Accelerated intestinal epithelial cell turnover: a new mechanism of parasite expulsion. *Science* (5727) 2005) 308:1463–5. doi: 10.1126/science.1108661
185. Straumann A, Kristl J, Conus S, Vassina E, Spichtin HP, Beglinger C, et al. Cytokine expression in healthy and inflamed mucosa: probing the role of eosinophils in the digestive tract. *Inflammation Bowel Dis* (2005) 11(8):720–6. doi: 10.1097/01.MIB.0000172557.39767.53
186. Mueller S, Aigner T, Neureiter D, Stolte M. Eosinophil infiltration and degranulation in oesophageal mucosa from adult patients with eosinophilic oesophagitis: a retrospective and comparative study on pathological biopsy. *J Clin Pathol* (2006) 59(11):1175–80. doi: 10.1136/jcp.2005.031922
187. Stolarski B, Kurowska-Stolarska M, Kewin P, Xu D, Liew FY. IL-33 exacerbates eosinophil-mediated airway inflammation. *J Immunol* (2010) 185(6):3472–80. doi: 10.4049/jimmunol.1000730
188. Kurowska-Stolarska M, Stolarski B, Kewin P, Murphy G, Corrigan CJ, Ying S, et al. IL-33 amplifies the polarization of alternatively activated macrophages that contribute to airway inflammation. *J Immunol* (2009) 183(10):6469–77. doi: 10.4049/jimmunol.0901575
189. Bouffi C, Rochman M, Zust CB, Stucke EM, Kartashov A, Fulkerson PC, et al. IL-33 markedly activates murine eosinophils by an NF-kappaB-dependent mechanism differentially dependent upon an IL-4-driven autoinflammatory loop. *J Immunol* (2013) 191(8):4317–25. doi: 10.4049/jimmunol.1301465
190. Shik D, Moshkovits I, Karo-Atar D, Reichman H, Munitz A. Interleukin-33 requires CMRF35-like molecule-1 expression for induction of myeloid cell activation. *Allergy* (2014) 69(6):719–29. doi: 10.1111/all.12388
191. Patel AJ, Fuentebella J, Gernez Y, Nguyen T, Bass D, Berquist W, et al. Increased HLA-DR expression on tissue eosinophils in eosinophilic esophagitis. *J Pediatr Gastroenterol Nutr* (2010) 51(3):290–4. doi: 10.1097/MPG.0b013e3181e083e7
192. Le-Carlson M, Seki S, Abarbanel D, Quiros A, Cox K, Nadeau KC. Markers of antigen presentation and activation on eosinophils and T cells in the esophageal tissue of patients with eosinophilic esophagitis. *J Pediatr Gastroenterol Nutr* (2013) 56(3):257–62. doi: 10.1097/MPG.0b013e3182758d49
193. Masterson JC, Biette KA, Hammer JA, Nguyen N, Capocelli KE, Saeedi BJ, et al. Epithelial HIF-1alpha/claudin-1 axis regulates barrier dysfunction in eosinophilic esophagitis. *J Clin Invest* (2019) 129(8):3224–35. doi: 10.1172/JCI126744
194. Bolton SM, Kagalwalla AF, Arva NC, Wang MY, Amsden K, Melin-Aldana H, et al. Mast Cell Infiltration Is Associated With Persistent Symptoms and Endoscopic Abnormalities Despite Resolution of Eosinophilia in Pediatric Eosinophilic Esophagitis. *Am J Gastroenterol* (2020) 115(2):224–33. doi: 10.14309/ajg.0000000000000474
195. Oyesola OO, Duque C, Huang LC, Larson EM, Fruh SP, Webb LM, et al. The Prostaglandin D2 Receptor CRTH2 Promotes IL-33-Induced ILC2 Accumulation in the Lung. *J Immunol* (2020) 204(4):1001–11. doi: 10.4049/jimmunol.1900745
196. Wen T, Aronow BJ, Rochman Y, Rochman M, Kc K, Dexheimer PJ, et al. Single-cell RNA sequencing identifies inflammatory tissue T cells in eosinophilic esophagitis. *J Clin Invest* (2019) 129(5):2014–28. doi: 10.1172/JCI125917
197. Gleich GJ, Frigas E, Loegering DA, Wassom DL, Steinmuller D. Cytotoxic properties of the eosinophil major basic protein. *J Immunol* (1979) 123 (6):2925–7.
198. Flavahan NA, Slifman NR, Gleich GJ, Vanhoutte PM. Human eosinophil major basic protein causes hyperreactivity of respiratory smooth muscle. Role of the epithelium. *Am Rev Respir Dis* (1988) 138(3):685–8. doi: 10.1164/ajrccm/138.3.685
199. Jacoby DB, Gleich GJ, Fryer AD. Human eosinophil major basic protein is an endogenous allosteric antagonist at the inhibitory muscarinic M2 receptor. *J Clin Invest* (1993) 91(4):1314–8. doi: 10.1172/JCI116331
200. Rochester CL, Ackerman SJ, Zheng T, Elias JA. Eosinophil-fibroblast interactions. Granule major basic protein interacts with IL-1 and transforming growth factor-beta in the stimulation of lung fibroblast IL-6-type cytokine production. *J Immunol* (1996) 156(11):4449–56.
201. Davis BP, Stucke EM, Khorki ME, Litosh VA, Rymer JK, Rochman M, et al. Eosinophilic esophagitis-linked calpain 14 is an IL-13-induced protease that mediates esophageal epithelial barrier impairment. *JCI Insight* (2016) 1(4): e86355. doi: 10.1172/jci.insight.86355
202. Rothenberg ME, Wen T, Greenberg A, Alpan O, Enav B, Hirano I, et al. Intravenous anti-IL-13 mAb QAX576 for the treatment of eosinophilic esophagitis. *J Allergy Clin Immunol* (2015) 135(2):500–7. doi: 10.1016/j.jaci.2014.07.049
203. Hirano I, Collins MH, Assouline-Dayan Y, Evans L, Gupta S, Schoepfer AM, et al. RPC4046, a Monoclonal Antibody Against IL13, Reduces Histologic and Endoscopic Activity in Patients With Eosinophilic Esophagitis. *Gastroenterology* (2019) 156(3):592–603 e10. doi: 10.1053/j.gastro.2018.10.051
204. Hirano I, Dellon ES, Hamilton JD, Collins MH, Peterson K, Chehade M, et al. Efficacy of Dupilumab in a Phase 2 Randomized Trial of Adults With Active Eosinophilic Esophagitis. *Gastroenterology* (2020) 158(1):111–22 e10. doi: 10.1053/j.gastro.2019.09.042
205. Mishra A, Hogan SP, Brandt EB, Rothenberg ME. An etiological role for aeroallergens and eosinophils in experimental esophagitis. *J Clin Invest* (2001) 107(1):83–90. doi: 10.1172/JCI10224
206. Zuo L, Fulkerson PC, Finkelman FD, Mingler M, Fischetti CA, Blanchard C, et al. IL-13 induces esophageal remodeling and gene expression by an eosinophil-independent, IL-13R alpha 2-inhibited pathway. *J Immunol* (2010) 185(1):660–9. doi: 10.4049/jimmunol.1000471
207. Doyle AD, Mukherjee M, LeSuer WE, Bittner TB, Pasha SM, Frere JJ, et al. Eosinophil-derived IL-13 promotes emphysema. *Eur Respir J* (2019) 53 (5):1801291. doi: 10.1183/13993003.01291-2018
208. Hashimoto S, Gon Y, Takeshita I, Maruoka S, Horie T. IL-4 and IL-13 induce myofibroblastic phenotype of human lung fibroblasts through c-Jun NH2-terminal kinase-dependent pathway. *J Allergy Clin Immunol* (2001) 107 (6):1001–8. doi: 10.1067/mai.2001.114702
209. Verrecchia F, Mauviel A. Transforming growth factor-beta and fibrosis. *World J Gastroenterol* (2007) 13(22):3056–62. doi: 10.3748/wjg.v13.i22.3056
210. Beppu LY, Anilkumar AA, Newbury RO, Dohil R, Broide DH, Aceves SS. TGF-beta1-induced phospholamban expression alters esophageal smooth muscle cell contraction in patients with eosinophilic esophagitis. *J Allergy Clin Immunol* (2014) 134(5):1100–7 e4. doi: 10.1016/j.jaci.2014.04.004

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 07 December 2020
doi: 10.3389/fimmu.2020.604054

Role of the Intestinal Epithelium and Its Interaction With the Microbiota in Food Allergy

Ayesha Ali[1,2†], *HuiYing Tan*[1,2†] *and Gerard E. Kaiko*[1,2*]

[1] School of Biomedical Sciences and Pharmacy, University of Newcastle, Newcastle, NSW, Australia, [2] Hunter Medical Research Institute, Newcastle, NSW, Australia

The intestinal epithelial tract forms a dynamic lining of the digestive system consisting of a range of epithelial cell sub-types with diverse functions fulfilling specific niches. The intestinal epithelium is more than just a physical barrier regulating nutrient uptake, rather it plays a critical role in homeostasis through its intrinsic innate immune function, pivotal regulation of antigen sensitization, and a bi-directional interplay with the microbiota that evolves with age. In this review we will discuss these functions of the epithelium in the context of food allergy.

Keywords: epithelium, microbiota, allergy, anaphylaxis, intestinal immunity

OPEN ACCESS

Edited by:
Simon Patrick Hogan, University of Michigan, United States

Reviewed by:
Barbara Wróblewska, Institute of Animal Reproduction and Food Research (PAN), Poland
Kathryn A. Knoop, Mayo Clinic, United States

***Correspondence:**
Gerard E. Kaiko
Gerard.kaiko@newcastle.edu.au

[†]*These authors have contributed equally to this work*

Specialty section:
This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology

Received: *08 September 2020*
Accepted: *10 November 2020*
Published: *07 December 2020*

Citation:
Ali A, Tan H and Kaiko GE (2020) Role of the Intestinal Epithelium and Its Interaction With the Microbiota in Food Allergy. Front. Immunol. 11:604054. doi: 10.3389/fimmu.2020.604054

INTRODUCTION TO THE INTESTINAL EPITHELIAL BARRIER

The intestinal epithelium lining forms the luminal surface to the external environment of both the small and large intestines. The intestinal epithelium provides a controlled homeostatic system for molecular transit in order to mediate the balance of the multiple functions of the intestinal tract; digestion, immunity and tolerance, and a repository for the various known and unknown symbiotic functions of the microbiota. In order to achieve this fine balance the intestinal epithelium acts as mucosal barrier to micro-organisms while permitting a pathway to protein antigens and small molecule metabolites.

The first layer of physical defense, the mucus layer, plays an important role in reducing adherence of pathogenic microbes while providing a rich-layer of sustenance for slow-growing anaerobic commensal organisms (1, 2). It also acts to shield the host from digestive enzymes and microorganism epithelial penetration. The renewal of the mucus layer (colon consists of an inner more viscous and outer less viscous layer, small intestine a single layer only) is achieved through the secretion of mucus by goblet cells (primarily *via* the *MUC2* gene) (3). The underlying layer of intestinal epithelial cells (IECs) functions as a physical barrier facilitated by tight junctions (4, 5). The intercellular junctions consist of desmosomes, gap junctions, and adherent junctions made up of various integral proteins such as claudins, occludins, and zonula occludens (ZO-1, ZO-2) which operate in concert to maintain the integrity of the epithelial barrier by regulating the paracellular transport of ions, metabolites, and macromolecules (4–7). The tubular crypt and epithelial lining that defines the structure of both the small intestine and colon is continuously regenerated (every 3–5 days) by a population of long-lived Lgr5+ stem cells that reside at the base of the crypt (8). These stem cells give rise to progenitor cells (transit amplifying cells) and multiple differentiated epithelial cell subsets as they migrate through division up the crypt-villus axis before undergoing a program of apoptosis and luminal shedding. Each of these differentiated epithelial cell subtypes possess specific

functions in maintenance of the barrier's digestive, neuroendocrine, and immune functions. These include absorptive cells (colonocytes, enterocytes), goblet cells, enteroendocrine cells, Paneth cells (of the small intestine), tuft cells, and M cells (8). As the epithelium and associated mucus layer provide the first line of defense against microbes they also provide the first line of tolerance against commensal members of the microbiota. In order to achieve this the barrier maintains a hypo-reactivity to microbial ligands in stark contrast to the underlying immune-stromal-rich layer of lamina propria, which is home to the innate and adaptive immune cells with far greater reactivity to commensal and pathogenic microbial ligands (9, 10).

Disruption of the integrity of the intestinal barrier is often associated with inflammatory bowel disease, however, this process is also linked to numerous other local and systemic inflammatory diseases. For example, reduced expression of tight junction proteins leads to a rise in intestinal permeability associated with both local intestinal food allergies as well as systemic allergy in the form of asthma (11–14). In this review, we will discuss the role of the intestinal barrier and IECs in regulating food allergy, as well as specific factors (host and environmental/microbiome) that may create a susceptibility within the epithelial barrier to promote food allergy. Host factors may include changes in protein expression resulting in alterations to the epithelial barrier. This could be either intrinsically programmed in the IECs (genetic or epigenetic) or extrinsically regulated, however, this distinction of etiology remains an open hypothetical question as currently there is little research in this area. We will also discuss how the microbiome closely interacts with the intestinal epithelium and may act as a conduit for changes mediated by an array of exogenous environmental factors including diet.

IEC SPECIFICATION AND FUNCTION IN FOOD ALLERGY

The intestinal epithelial barrier consists of different IEC subtypes with diverse functional specification interspersed with non-epithelial cell types including intraepithelial lymphocytes. Food allergy is a highly heritable condition (~80%), however, GWAS studies in food allergy have suffered from heterogeneity of disease classification (15). In terms of genetic susceptibility in epithelial genes a recent GWAS demonstrated a strong association between the *SERPINB10* gene and susceptibility to multiple food allergies (16). This gene is known to be expressed by epithelial cells and involved in IL-13-induced transcriptional changes in bronchial epithelial cells, suggesting a possible role for epithelial SERPINB10 in mediating susceptibility to food allergy (17). More work is required to dissect the potential mechanism and interrogate larger GWAS populations pools. Any intrinsic epigenetic changes in the epithelium that promoted or suppressed susceptibility to food allergy would need to be programmed in the long-lived dividing stem/progenitor cells of the crypt base. To date this remains largely unexplored.

Mature enterocytes and colonocytes are polarized epithelial cells lined by tight junctions and responsible for maintaining the integrity of the barrier (4, 5, 18). The apical surfaces of the enterocytes are covered with an array of tiny microvilli increasing the cell surface for absorption and acting as sites of high digestive enzyme concentration (glycosidases, peptidases, and lipases) key to regulating the breakdown and uptake of protein and carbohydrate, food allergens (19). IECs are polarized such that the apical plasma membrane (brush border and microvilli) is distinctly different from the basolateral membrane with different sets of transport proteins at each (19, 20). Overlying the brush border are enzymes that catalyze the final stages in the digestion of proteins, lipids, and carbohydrates. The enzymes at this brush border are tethered to the membrane. One of the key brush border enzymes is enteropeptidase, which catalyzes the activation of trypsinogen (one of the major proteases secreted from the pancreas) into trypsin a key proteolytic enzyme to breakdown proteins into amino acids together with exopeptidases and endopeptidases (19, 20). Amino acids and glucose (from carbohydrate metabolism) are transported across the epithelium against their concentration gradient from the intestinal lumen across IECs by a two-Na^+/one-glucose (or amino acid) symporter located in the microvilli membranes (19, 20). The Na^+ ions that are pumped into the cell are pumped out across the basolateral membrane by the Na^+/K^+ ATPase with energy provided by ATP hydrolysis. Intracellular glucose and amino acids are pumped across the basolateral membrane into the blood by specific transporters, including GLUT2 (19, 20). In the case of lipid and fat aggregates bile acids facilitate their absorption across the intestinal epithelium. Bile acids contain both hydrophobic and hydrophilic faces. This property allows emulsification of fat into microscopic droplets of micelles, ensuring fat is more easily digested by lipases produced by the pancreas and IECs, while enhancing transport across the epithelium (19, 20). The negative charge imparted on lipids by bile acid adsorption alters electrostatic interaction in the negatively charged mucus, thus facilitating movement of digested lipids through the mucus layer (19, 20).

IECs can express class I and II MHC molecules, thus facilitating a role in non-professional antigen presentation (21), and potentially enabling the direct presentation of protein antigens to lymphocytes that may regulate an allergic response in the intestinal tract. Furthermore, evidence across multiple cell lines demonstrates IEC secreted exosomes contain MHC-II/peptide complexes capable of generating antigen-specific responses in mice (22–24). This suggests a paracrine mechanism by which absorptive IECs may also mediate indirect presentation of food allergens to the immune cells. However, the nature of the induced immune response and the type of T cell polarization generated to IEC-derived MHC-II/peptide complexes is not yet clear as *in vivo* studies have found conflicting results for immunogenic versus tolerogenic T cell responses to ovalbumin in experimental models of food allergy (22, 24). It is likely that the nature of the immune response is dependent on the microenvironment of the lamina propria and

inflammatory context of the initial antigen exposure. Whether such a process provides signals for oral tolerance or food allergy in humans remains unclear.

Goblet cells are found scattered in between enterocytes and secrete high-molecular-weight glycoprotein mucins (e.g. MUC2). The dynamic mucus layer formed over the epithelial surface provides a physical and chemical protective barrier for the host against enteric bacteria by acting like a molecular sieve limiting bacterial penetration to the mucosa (25). The small intestine possesses a more porous single layer of mucus compared to the dual colonic layers (1). Mucus often works in concert with secreted IgA through specific binding moieties to immobilize bacteria in the intestinal lumen and allow the removal of microbes through subsequent degradation and renewal of the mucus layers *via* intestinal peristalsis (25, 26). Goblet cells play a critical role in luminal to lamina propria immune cell antigen transfer regulating the balance between tolerance and food allergy and this will be briefly overviewed below (27, 28).

Microfold (M) cells are a rare specialized epithelial subtype that are found overlying intestinal lymphoid tissue, such as Peyer's patches. These cells play a role in transepithelial antigen transport, delivering foreign antigens and microbes to organized lymphoid tissues within the mucosa of the small intestine and colon (29). Initiation of the mucosal immune response occurs when M cells take up luminal microbes and antigens through the process of phagocytosis, endocytosis, or transcytosis and deliver their payload to dendritic cells located in the lymphoid follicle in the lamina propria (30, 31). Studies have also reported that M cells can express IgA receptor on the apical surface to bind and retro-transport luminal secretory IgA-bound antigens back into Peyer's Patches, thus creating the capacity for sampling, sensing, and tonal control of foreign antigen load (32, 33).

Paneth cells at the base of intestinal crypt are specialist secretors of antimicrobial peptides (AMPs) including lysozyme, secretory phospholipase A2 (sPLA2), C-type lectin regenerating islet-derived protein IIIγ (RegIIIγ), angiogenin4, cathelicidins, and alpha defensins that help to protect the epithelial barrier and maintain a homeostatic balance with the microbiota (34–38). AMPs are generated when pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), are stimulated by microbial ligands (such as LPS) and nucleotide-binding oligomerisation domain containing molecules (NODs) are activated by muramyl dipeptides (39). The myeloid differentiation primary response gene MyD88 in Paneth cells plays a critical role in translating this microbial ligand sensing into an AMP response to regulate the balance of abundance of both commensal and pathogenic intestinal microbes (40). Despite this key role in intestinal homeostasis and sensing of foreign antigens a role for Paneth cells in relation to food allergy remains unknown. More studies are needed to address this, in particular as to how indirect regulation of the microbiota by Paneth cells as evolves with age may be a key interface in the relationship between the microbiota and food allergy.

Embedded within the epithelium throughout the gastrointestinal tract are a diverse array of enteroendocrine cells (EECs) (41). EECs, of which there are at least 10 subsets, are essential in regulating physiological processes including appetite, stomach emptying, serotonin controls, and glucose levels by secretion of subset-specific peptide hormones and neuropeptides, such as somatostatin, motilin, cholecystokinin, neurotensin, vasoactive intestinal peptide, and enteroglucagon in response to food ingestion stimuli (42, 43). The arrangement of EECs varies in different parts of the gastrointestinal tract and can be distinguished by "closed cells" and "open cells" (41). EECs in the stomach epithelium are "closed cells" due to lack of direct contact with luminal contents, whereas EECs in the small and large intestine have an "open type" cell arrangement as it has microvilli with direct luminal contact. The secretion of hormones by EECs in the small intestine are tightly regulated by the dietary nutrients absorbed, while EECs in the colon may have a greater propensity to respond to a range of microbial products (41). The abundance of EECs in food allergy has to our knowledge not yet been characterized. There are also no studies on a functional link between EECs and IgE-mediated food allergy, however, lower numbers of EECs have been associated with irritable bowel syndrome (IBS) patients with food hypersensitivities, and dietary intervention studies to remove food triggers and reduce symptoms induce a normalization in Chromogranin A-positive EECs in colonic biopsies of IBS compared to healthy controls (44–46). Given the increasing recognition that a significant proportion of IBS may represent a type of non-IgE localized atypical food allergy there may be a key role for factors released by EECs in this type of non-classical food allergy. In a seminal prospective study of 108 IBS patients, 70% of those endoscopically challenged with four specific food components demonstrated immediate disruption of the intestinal barrier characterized by fluid extravasation, changes in permeability and tight junctions, as well as increased eosinophil degranulation (47). Although an emerging area with more evidence and interventional studies required, a role for EECs in localized atypical non-IgE food allergies is an important avenue for future research.

Tuft cells are a rare chemosensory epithelial subtype known for their importance in expulsion of parasitic helminths and generation of a strong Type 2 immune response as the exclusive source of IL-25 in the intestine (48–50). These cells promote the outgrowth of Type 2 innate lymphoid cells (ILC2s) and drive eosinophilia in the intestinal tract, and are induced to proliferate from stem/progenitor cells in the crypt through an IL-13-dependent feed-forward mechanism (48–50). Despite Tuft cells being a clear mediator of Type 2 immunity in the gut there has been minimal evidence so far for their role in food allergy. A recent study from *Leyva-Castillo et al.* was able to demonstrate that mechanical skin injury-induced IL-33 release triggered a concomitant increase in small intestinal mast cells and this drove enhanced anaphylaxis following oral challenge (51). This work convincingly showed that the increase in small intestinal mast cell numbers was elicited by an expansion of IL-25-producing

intestinal Tuft cells driving the proliferation of IL-4/IL-13-secreting ILC2s in the intestine. The direct effect of Tuft cells (through genetic deletion) on food-induced anaphylaxis was not assessed. However, this study represents the first evidence for a role of Tuft cells in food allergy.

Detailed studies focusing on the contribution of individual IEC subtypes and their secreted products to the pathogenesis of food allergy are likely to emerge over coming years due to the advent of new technologies. The use of intestinal organoids from primary human tissue along with single cell sequencing tools will enable a greater resolution for the functional dissection of IEC subsets (52). Such findings can then be translated into gene knockout studies in mouse models of food allergy, and even orthotopic transplantation of organoids into immune-deficient mice. Most *in vitro* studies to date have relied on colorectal cancer cell lines in two-dimensional monolayers and although this has provided important and valuable insights many of the characteristics of immortalized monolayers are distinctly different from primary human epithelial cells generated from intestinal stem cells, including their metabolism and ability to generate differentiated cell subsets (53–56).

ROLE OF THE INTESTINAL EPITHELIAL BARRIER IN REGULATING PROTEIN ANTIGEN PASSAGE, AND ALLERGIC RESPONSES TO FOOD ANTIGENS

It is important for the intestinal epithelial barrier to control different types of luminal antigens being translocated in order to maintain homeostatic control and to guide mucosal immune responses in the fine balance between tolerance and sensitization (57). There are several specialized pathways involved in the transport of antigen from the lumen across epithelium to underlying lamina propria immune cells (28, 58–60). The route through which antigens cross the epithelial barrier may have a profound influence on the type and scale of immune response generated (**Figure 1**).

IECs exist in close contact with T cells and dendritic cells in the underlying lamina propria to facilitate an antigen flow through the professional antigen presenting cells (APCs), and as outlined the IECs can themselves function as non-professional APCs. The epithelial layer plays an important role in protecting

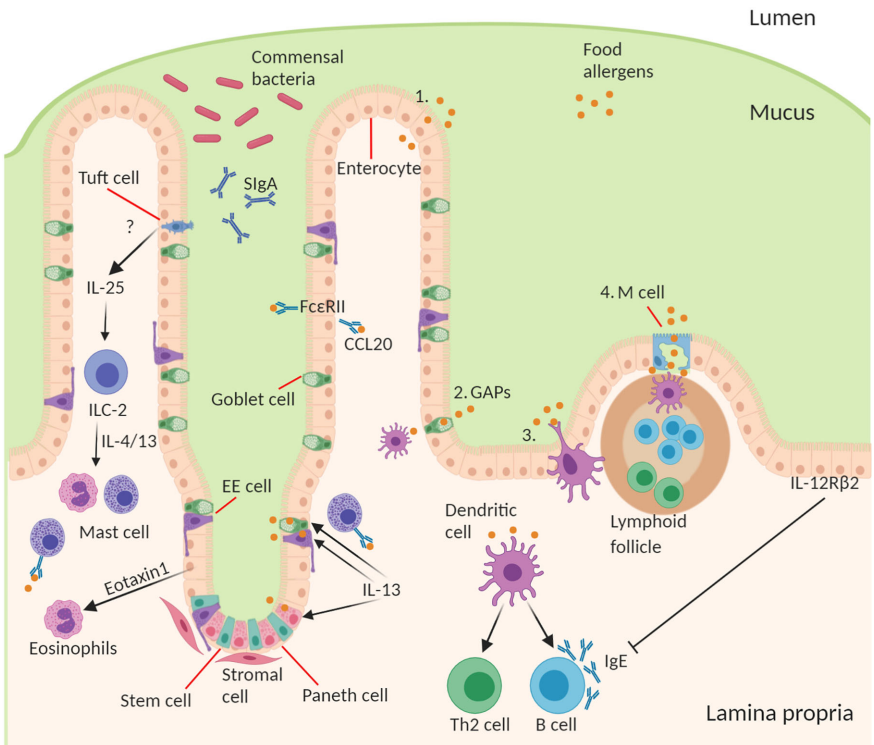


FIGURE 1 | Structure and cell type specification of intestinal epithelium and function in antigen uptake. The intestinal epithelial barrier consists of stem cells, Paneth cells (small intestine), goblet cells, tuft cells, enteroendocrine (EE) cells, enterocytes (small intestine)/colonocytes (colon), and microfold (M) cells overlying lymphoid follicles. *Route of antigen uptake:* (1) paracellular transport of food allergen, (2) goblet associated antigen passages, (3) direct antigen uptake by dendritic cells (DCs) stretching a dendrite into the lumen between the epithelial intercellular space, (4) M cell mediated antigen transcytosis. DC present food antigen to lymphocytes to T cell differentiation into Th2 cells and B cell isotype class switching to produce allergen-specific IgE. *Contribution of IECs to amplifying food allergy*: IgE complexed with food antigen can transcytose across intestinal epithelial cells *via* FcεRII, this process stimulates CCL20 release. Tuft cell produce IL-25 driving the expansion of ILC2s and their production of IL-4 and IL-13, which in turn drive mast cell and eosinophil activation. Epithelial cells produce Eotaxin-1, a chemoattractant for eosinophils. During an allergic response IL-13 acts on secretory cells like Paneth cells, EE cells, and goblet cells to increase antigen uptake and presentation to IgE-bearing mast cells. Mechanism of inhibition of allergic response by IECs through activation of basolateral expressed IL-12Rβ2.

the mucosal immune system from excessive exposure to immunogenic proteins. This protection of the epithelium is maintained by the formation of tight junctions (TJs) between cells (61, 62). TJs are a multi-protein complex that forms a selectively permeable seal between adjacent epithelial cells near the apical surface (61, 62). Metabolites, ions, and water can pass through the intercellular space between cells using a paracellular pathway, which is regulated by TJ proteins such as claudins, occludins, and ZO-1. However, any alteration in this passive paracellular transport in the form of leaks can allow macromolecules like food antigens to cross the epithelial barrier increasing the exposure to antigen that could lead to allergic sensitization. Several *in-vitro* studies using IECs have shown that specific dietary components can alter TJ proteins and overall barrier integrity (61, 62). Gliadin, a wheat protein, can interfere with the interaction between occludins and ZO-1, leading to increased monolayer permeability (63). Other dietary components, such as capsinoids in chili, have also demonstrated capacity, *in vitro* at least, to alter paracellular flux and decrease TJ barrier integrity (64, 65). This process of IEC paracellular transport is dysregulated *in vivo* in the small intestine during oral antigen challenge in pre-sensitized mice, leading to the development of secretory diarrhea (66). The leaky jejunoileal epithelium is dependent on a rapid intestinal epithelial Cftr-dependent Chloride ion secretory response associated with increased paracellular permeability from loss of intercellular claudin-1, 2, 3 and 5, E-cadherin, and desmosomal cadherins. Similarly, the loss of water channel expression (Aquaporin 4 and 8) in IECs during an oral challenge in a pre-sensitized setting also likely contributes to secretory diarrhea (67). In this way the intestinal epithelium also creates a feed-forward loop where a leaky barrier to antigen likely amplifies the allergic response.

IECs constitutively express the CD23 (Fc epsilon RII) receptor for IgE on their membranes, which enables transcytosis of IgE and capture of IgE/antigen complexes to transport it across the epithelium from the lumen to lamina propria (68, 69). This IgE/antigen transport process is increased in a pre-sensitized setting of food allergic patients and can trigger IECs to secrete CCL20, thus acting to amplify the allergic response to food antigens (68–70). The intestinal epithelium can also release cytokines and chemokines to both amplify or suppress the allergic response. For example, IECs are a major source of eotaxin-1 that drives eosinophil recruitment to the gut in food allergy (71). Epithelial Type 2-inducing cytokines, such as IL-25, IL-33, and TSLP, are important drivers of inflammation in food allergy, however, most experimental studies have relied on whole body knockouts or systemic antibody-mediated neutralization in mice (72–75). While IECs can certainly produce IL-25 and TSLP, the importance of IECs as the cellular source of these cytokines in food allergy remains unclear because IEC-specific conditional-deletion studies remain limited, and some food allergy models rely on skin keratinocytes to produce these cytokines through epicutaneous sensitization (73, 74). IECs also express the IL-12Rβ2 chain on their basolateral surface in proximity to underlying immune cells, and activation of this receptor by IL-12 (a Th1 immune response-inducing cytokine) leads to a reduction in serum IgE and food anaphylactic responses (76).

M cells on the apical surface of intestinal lymphoid follicles mediate antigen uptake and transcytosis across the intestinal epithelium (58). These cells are specialized for uptake of particulate antigens and intact microorganisms from the lumen facilitating transcellular transport to the underlying dendritic cell rich sub-epithelial dome where they are can initiate or amplify an immune response (77). In addition to non-specific uptake of antigens there is also a receptor-mediated transport that occurs through M cells. Various enteric pathogens such as *Salmonella enterica* can enter into the host through M cells by the means of GP2-FimH interactions (78). IECs transport IgA into the intestinal lumen where these antibodies contribute to barrier function by excluding uptake of antigens as well as microbes. M cells are important in the production of these IgA antibodies given their antigen sampling, intimate proximity to lymphoid follicle B cells, and co-dependency of M cells and IgA induction on RANKL (79–81). Thus, M cell-mediated antigen uptake may help to maintain control over gut immune homeostasis and microbial diversity through an IgA-dependent feedback loop. Moreover, this function of M cells may play an important role in the genesis of food allergy. Using a peanut mouse model of food allergy *Chambers et al.*, showed that in the pre-sensitized gut epithelium ingested peanut proteins were present inside the M cell cytoplasm with negligible presence in absorptive enterocytes (82). These antigen sampling M cells allowed passage of the protein to the mucosal immune cells of the sub-epithelial dome.

Intestinal goblet cells also perform a critical role in the uptake of luminal antigens and provision to the immune cells (28). Goblet cells are present throughout the intestinal crypts and villi (aside from the follicular associated epithelium) and their capacity to sample the lumen provides a key sentinel function. In seminal work McDole et al., used *in-vivo* two-photon imaging experiments to demonstrate that goblet cell-associated antigen passages (GAPs) were able to deliver a diverse set of antigens from the intestinal lumen to $CD103^+$ lamina propria dendritic cells (DCs) (28). The role of GAPs in transferring protein antigens to tolerogenic DCs, that migrate to mesenteric lymph nodes and promote the development of T-regulatory (T regs) cells, may help facilitate oral tolerance. Aside from this active control by GAPs over antigen passage to DCs, the epithelium can also release TGF-β, and metabolize vitamin A into retinoic acid to induce the development of $CD103^+$ tolerogenic DCs (83–85). A high frequency of GAPs are observed in the small intestine in mice and the formation of these passages has also been noted in human small intestinal tissue with responsiveness to acetylcholine. In contrast, at steady state in the colon goblet cells have a dramatically diminished capacity to form GAPs due to inhibitory stimuli from a high microbial load (27). However, antibiotic-mediated acute disruption of the microbiota facilitates the formation of colonic GAPs enabling delivery of luminal antigens to colonic DCs (86). In the context of established food allergy IL-13 can act to dramatically increase the number of these small intestinal GAPs, and expand the type of secretory cells that

form these passages to include Paneth cells and EECs (87). This process drives anaphylaxis through antigen transfer to mucosal mast cells.

The fourth mechanism by which luminal antigen can be sampled involves direct action of DCs. Aside from uptake of antigen indirectly provided by epithelial cells, DCs can also directly sample the gut lumen through extending trans-epithelial dendrites (TEDs) (60, 88). DCs on the basolateral side of the epithelium can extend these TEDs across the TJ complexes to sample antigen and microbes (60, 88). Moreover, DCs can express their own TJ proteins, such as occludin, claudin, and ZO-1, to interact with epithelial cells to permit sampling of luminal antigens without disruption of the epithelial layers (60, 88). The ability of DCs to sample the small intestinal lumen for antigen through TEDs is driven by CX_3CR1/fractalkine, a transmembrane chemokine expressed at the surface of IECs (59). CX3/CR1-deficient mice show impaired ability of DCs to sample bacteria from the lumen. $CX3/CR1^+$ DC TEDs act to drive inflammatory responses to pathogens but it remains less clear whether this process is involved in food antigen sensitization or oral tolerance (89, 90).

The sampling and sensitization of food allergens occurs predominately in the small intestine and similarly the localized inflammatory responses, intestinal permeability, and feedback amplification of this process are manifested primarily at the jejunal-ileal region. However, it remains entirely possible given the migratory nature of immune cells that tolerogenic or pro-inflammatory changes, such as those orchestrated by the microbiota, in the colon may impact the small intestinal tract and vice versa.

INTERPLAY BETWEEN IECS AND THE MICROBIOME IN FOOD ALLERGY

The importance of the microbes in food allergy is a concept that first arose in the context of the "hygiene hypothesis" which posited that a loss of microbial exposure was responsible for a skewing of the immune response and increased susceptibility to allergies in "westernized" countries (91). This exposure may be altered by lifestyle changes including antibiotics, public hygiene, dietary changes, migration from rural/farming to urban communities, and higher rates of Caesarean birth and formula feeding. Since the advent of sequencing technologies to study the microbiome and gnotobiotic tools for its manipulation in experimental systems this area has received renewed attention with a reductionist approach to explore this relationship. Researchers have begun to examine how microbes and their molecules interact with the host, which has led to the development of a new iteration of the "hygiene hypothesis," which suggests that a balanced intestinal microbiota characterized by diverse Firmicutes is necessary for intestinal health and loss of this balance increases susceptibility to food allergy (**Figure 2**). Although there is a dominant heritable component to food allergy, there is obviously a significant role for environmental factors, including "westernized" lifestyle changes, that predispose to disease. All these lifestyle factors from antibiotics, to diet, to urbanized living converge to have an impact on the composition and function of the intestinal microbiota. Moreover, the intestinal microbiota is itself highly heritable and so likely plays a role in the substantial heritability of food allergy. Indeed the microbiome may help to explain the significant "heritability gap" so commonly observed for many diseases, including food allergy, where the predicted heritability derived from twin and sibling studies is not matched by that predicted by GWAS of host genetics. The microbiome heritability in early life is derived from the impact of familial genetics on microbiome composition, sharing a common environment especially during development and childhood, and the strong maternal influence on the microbiota from birth to breastfeeding (92). The large TwinsUK population study revealed the strong influence of shared host genetics on microbiota taxa including with specific microbial genera that play a role in basic human traits, such as BMI (93). Furthermore, host gene variants such as those in *APOA5*, *LCT*, *NOD2*, and *FUT2* have also been shown to profoundly impact the composition of the microbiome (92, 94). But perhaps the most important influence on the early childhood microbiota, and its strongest source of heritability, are maternal factors in vertical transmission including diet, delivery mode, and breastfeeding (95–97). In the following section we will discuss how the microbiota contributes to the predisposition to food allergy.

One of the most widely studied mechanisms for microbiota to host signaling is *via* the TLR ligand-PRR recognition system. Of the TLR ligands analyzed the strongest data functionally linking to allergy appears to be attributed to TLR4 and TLR9. Studies in T84 colorectal cell line monolayer cultures have shown that stimulation of TLR9 reduces inflammatory cytokine production in co-cultures with PBMCs from food allergy patients (98). The location of TLR9 activation at the apical surface, rather than basolateral, may be key to driving this more tolerogenic response (99). Unmethylated versus methylated CpG/TLR9 signaling as the dominant signal to IECs may play a role in distinguishing a healthy microbiota *versus* an invading bacteria and driving the generation of tolerogenic DCs and Tregs (100, 101). Furthermore, TLR2 and TLR9 on IECs have been shown to protect against barrier disruption by enhancing ZO-1 expression (102, 103). In contrast, activation of TLR4 had an opposing effect. Interestingly, wheat α-amylase trypsin inhibitors, a recognized plant-derived food allergen, activates TLR4 (104).

A large Twins cohort study (>2,700 individuals) recently revealed that the top three diseases most strongly associated with changes in the microbiota are inflammatory bowel disease, Type 2 diabetes, and food allergy (105). The most prominent association in this disease analysis indicated a negative association of food allergy with the abundance of Prevotellaceae. In mice a reduction in *Firmicutes* spp. and increase in *Proteobacteria* spp. (an observation common to many chronic inflammatory diseases) has been associated with an aggravated response to food antigen, and colonization of germ-free (GF) mice with the fecal microbiota of healthy infants, rich in *Bifidobacterium* spp. and *Bacteroides* spp., protected

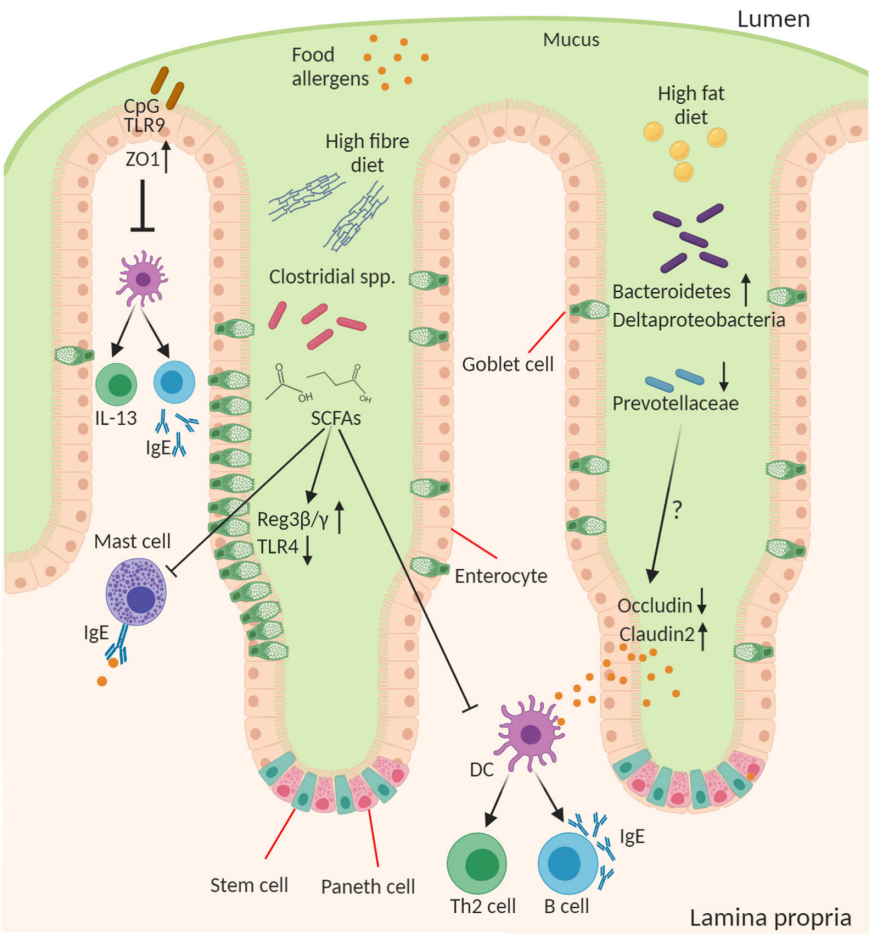


FIGURE 2 | Role of the microbiota in epithelial regulation of food allergy. Diet and the intestinal microbiota interact to produce the outgrowth or loss of certain bacterial genera in food allergy this can result in altered concentration of microbial ligands, such as TLR ligands, and small molecule metabolites that stimulate host epithelial and immune responses. CpG oligonucleotides from bacterial DNA bind epithelial TLR9 to increase expression of ZO-1 improving epithelial tight junction barrier integrity to antigen and reducing cytokine production from immune cells. A high-fiber diet leads to outgrowth of many fermenting Clostridial species and enable their production of short chain fatty acids (SCFAs), in particular butyrate, that act on the epithelium to induce goblet cell hyperplasia, increase antimicrobial proteins (Reg3β and γ), and reduce TLR4 expression. SCFAs also inhibit mast cell activation and favor the development of tolerogenic dendritic cells (DCs) and regulatory T cells and suppress the inflammatory DC-Th2-IgE pathway. A high-fat diet leads to increase in Bacteroidetes and Deltaproteobacteria and reduction in abundance of Prevoltellaceae. This microbial dysbiosis drives increased barrier permeability and antigen transfer to lamina propria cells by increasing Claudin2 and decreasing Occludin tight junction expression. The soluble microbial factors responsible for this epithelial change remain to be discovered.

against the development of cow's milk allergy (CMA) (106, 107). There is evidence that human milk oligosaccharides, which increase the abundance of *Lactobacillus* and *Bifidobacterium* strains may act to suppress the pro-allergic cytokine release by IECs, suggesting a possible mechanistic role of IECs underlying the negative correlation between breast feeding and development of food allergy (108, 109).

Work from *Stefka et al.*, demonstrated that treatment of mice with antibiotics led to enhanced IgE responses to peanut allergen with similar observations in GF mice (110). The authors showed the key bacterial genera mediating the microbiota-driven allergy suppression were the Clostridium clusters XIVa, XIVb, and IV. These clusters are primarily fiber-fermenting bacteria that produce short chain fatty acids (SCFAs), such as butyrate, propionate, and acetate. Mono-colonization of GF mice with these bacteria increased Foxp3+ Tregs, IgA, and IL-22 and reduced mast cell activation. In the epithelium these bacteria enhanced levels of AMPs (Reg3β and γ) and increased numbers of goblet cells. In follow-up work the authors extended these observations to show that GF mice colonized with fecal bacteria from healthy, but not CMA, infants were protected against anaphylactic responses to a cow's milk allergen (111). By correlating taxonomic and host ileal signatures from colonized GF mice, the Clostridial species *Anaerostipes caccae* was identified as a key candidate. Gnotobiotic experiments revealed that the butyrate-producer *A. caccae*, which was diminished in CMA, was able to protect against the allergic response to food. It was previously demonstrated that dietary supplementation with fiber or butyrate was able to induce similar oral tolerance (112). Butyrate is of particular interest among the fiber fermentation

SCFA products because it is known to act directly to suppress many inflammatory responses including mast cell activation, induce AMPs, IL-22, and goblet cell hyperplasia, as well as induce Treg proliferation through histone deacetylase inhibition and GPR43/GPR109A agonism (112–116). Butyrate is also an important metabolic energy source for differentiated colonocytes (56). Further support of these findings for a protective effect of butyrate-producing species is provided by the observation that *Clostridium butyricum*, which produces high levels of butyrate from granulose starch, can suppress TLR4 expression by colonic IECs, potentially improving barrier integrity (117). Despite these discoveries we are likely only now just scratching the surface of this complex interaction with the host, and getting to a point where it's clear there are many more unknowns than knowns in regards to microbiota-derived molecular signals.

If a "healthy" high-fiber diet can reduce susceptibility, then recent evidence suggests the converse might also be true with a "westernized" diet. *Hussain et al.* recently demonstrated that a high fat diet (independent of obesity) induces changes in the mouse intestinal microbiota that lead to enhanced susceptibility to food allergy (118). Not surprisingly the diet led to profound changes in bacterial communities with increases in *Desulfovibrionaceae* (Deltaproteobacteria), and *Rikenellaceae* (Bacteroidetes) and a reduction in the abundance of the *Muribaculaceae* and *Prevotellaceae*. Interestingly, the latter correlates with the microbiome results of the food allergy analysis in the twins cohort study (105) and likewise in a separate birth cohort study with maternal carriage of *Prevotella* being protective against infant risk of food allergy (119). This microbial dysbiosis altered the intestinal epithelial barrier to facilitate increased passage of protein allergen correlating with reduced expression of occludin and increases in the channel forming TJ protein claudin-2 (118). However, the mechanism and molecular factors responsible for this microbiota-induced change in IECs remains unclear.

The microbiota has the potential to produce an enormous array of products with bioactivity on the human epithelium and immune system, most of which remains to be discovered. Many such compounds may impact susceptibility to food allergy. For example, the microbiota can metabolize tryptophan into ligands for aryl hydrocarbon receptors expressed on epithelial and immune cells, transform primary into secondary bile acids with effects on IECs and Tregs, produce monohydroxy fatty acid 12,13-diHOME linked to asthma, and the intestinal microbiota can even produce histamine in asthma patients (120–123). The reasons why some bacterial species are more closely correlated with food allergy than others are of interest for the development of prevention strategies, especially probiotics. However, on the flip-side some bacteria are also capable of producing proteins that themselves act as allergens. In patients suffering from allergic disorders such as asthma, atopic dermatitis, or nasal polyposis, *Staphylococcus aureus* colonization appears more frequently (87, 90, 87%, respectively), in contrast to (20–50%) colonization of healthy adults (124–126). Furthermore, cell culture supernatants of *S. aureus* induce mast cell degranulation *via* δ-toxin (127). Many asthma, nasal polyposis, rhinitis, and atopic dermatitis patients demonstrate Staphylococcal enterotoxin and fibronectin binding protein-specific IgE in their serum, and so the ability of this group of bacterial proteins to act as allergens has been well characterized (127–129). Interestingly, skin colonization with *S. aureus* is also correlated with the onset of food allergy (peanut and egg) in infants, independently of the severity of atopic dermatitis (130, 131). Furthermore, as breast feeding is negatively associated with risk of food allergy it is of interest that cessation of this feeding has recently been found to diminish the abundance of *Staphylococcus* species in the infant fecal microbiome (132). Despite these findings there is currently very little evidence for a direct role of specific bacterial proteins, including Staphylococcal enterotoxins, in IgE-mediated food allergy. This is primarily due to a lack of studies to directly address this possibility. Given the strong associations of food allergy with changes in the intestinal microbiome this is an area that requires further investigation. Such future studies would necessitate a combination of *in silico* prediction tools to explore the microbiome based on algorithms using the primary and secondary structure of known allergens. This would need to be followed by empirical validation through sera bacterial protein-specific IgE assessment using ELISA, immunoblot, or peptide arrays.

Future identification followed by functional analysis of bacterial products and specific probiotic organisms will be critical to furthering our understanding of the role and therapeutic potential of the microbiota in regulating the intestinal epithelium in food allergy.

POTENTIAL TREATMENTS TARGETING THE INTESTINAL EPITHELIUM

Any new therapies for food allergy are likely to be combinatorial with oral immunotherapy (OIT) either to a specific food, such as peanuts, or to multiple-food OIT protocols. Monoclonal antibodies targeting IL-25 and TSLP are in various stages of clinical development. Tezepelumab (anti-TSLP) has shown promise in treating asthma and is now being trialed in other allergic diseases, including atopic dermatitis (133) (NCT03809663). Changes in IL-25-producing Tuft cells in patients with food allergy remain unclear and require further clinical analysis before a clinical target would be valid. Given the efficacy of anti-TSLP in other allergic diseases, the link to food allergy through the skin and atopic march, and promising evidence from experimental mouse models, this therapeutic option would seem to be a future target worth considering. By combining this with OIT it may maximize efficacy while reducing treatment length of costly biologics. Targeting TLR9 using synthetic analogues with OIT may provide another avenue to harness the epithelium for the treatment of food allergy. TLR9 agonism showed promise in reducing allergic asthma symptoms in Phase 2 trials, however, this approach failed to produce efficacy when used in the more uncontrolled population of moderate-to-severe asthmatics (134, 135). Acute targeting of GAPs during early stages of life through localized delivery of a muscarinic acetylcholine receptor 4 agonist may provide another avenue for induction of oral tolerance or treatment of children at

risk of heritable food allergies (90). Evidence from mouse models also suggests that proper synchronization of maternal breastmilk with infant development may also act to prevent an imbalanced immune response to dietary antigens (136). Potentially this an important consideration for infants unable to receive maternal breastmilk and provided instead with a donor source. The use of a prebiotic starch diet, or defined anaerobic Clostridial probiotic consortia, or metabolites such as butyrate may better harness the microbiota to promote oral tolerance to allergen. Perhaps a combinatorial treatment consisting of a high-fiber substrate with a butyrate-producing taxa, and butyrate metabolite may act to feed-forward amplify this effect. Such a treatment would likely need to be applied within the first 3 years of life before the microbiota reaches maturation.

CONCLUSION

The intestinal epithelial barrier plays a critical role in maintaining a state of guarded tolerance to the luminal milieu of dietary and microbial antigens. IECs are key to regulating food allergen uptake and presentation to the immune system. The bi-directional interplay between IECs and the microbiota likely plays a critical role in setting the tonal control over allergic sensitization *versus* tolerance. Greater molecular mechanistic understanding of this process, in particular on the microbiome, will be key to finding potential new combinatorial therapies for food allergy.

AUTHOR CONTRIBUTIONS

HT and AA contributed equally to the research and writing of the manuscript. GK conceived, co-wrote, and edited manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

Funding provided by NHMRC Australia APP1162666 and APP1146524.

REFERENCES

1. Johansson ME, Phillipson M, Petersson J, Velcich A, Holm L, Hansson GC. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Proc Natl Acad Sci U S A* (2008) 105(39):15064–9. doi: 10.1073/pnas.0803124105
2. Turner JR. Intestinal mucosal barrier function in health and disease. *Nat Rev Immunol* (2009) 9(11):799–809. doi: 10.1038/nri2653
3. Atuma C, Strugala V, Allen A, Holm L. The adherent gastrointestinal mucus gel layer: thickness and physical state *in vivo*. *Am J Physiol Gastrointest Liver Physiol* (2001) 280(5):G922–9. doi: 10.1152/ajpgi.2001.280.5.G922
4. Koch S, Nusrat A. Dynamic regulation of epithelial cell fate and barrier function by intercellular junctions. *Ann N Y Acad Sci* (2009) 1165:220–7. doi: 10.1111/j.1749-6632.2009.04025.x
5. Slifer ZM, Blikslager AT. The Integral Role of Tight Junction Proteins in the Repair of Injured Intestinal Epithelium. *Int J Mol Sci* (2020) 21(3). doi: 10.3390/ijms21030972
6. Gumbiner B, Lowenkopf T, Apatira D. Identification of a 160-kDa polypeptide that binds to the tight junction protein ZO-1. *Proc Natl Acad Sci U S A* (1991) 88(8):3460–4. doi: 10.1073/pnas.88.8.3460
7. Stevenson BR, Siliciano JD, Mooseker MS, Goodenough DA. Identification of ZO-1: a high molecular weight polypeptide associated with the tight junction (zonula occludens) in a variety of epithelia. *J Cell Biol* (1986) 103 (3):755–66. doi: 10.1083/jcb.103.3.755
8. Clevers H. The intestinal crypt, a prototype stem cell compartment. *Cell* (2013) 154(2):274–84. doi: 10.1016/j.cell.2013.07.004
9. Iwasaki A, Kelsall BL. Localization of distinct Peyer's patch dendritic cell subsets and their recruitment by chemokines macrophage inflammatory protein (MIP)-3alpha, MIP-3beta, and secondary lymphoid organ chemokine. *J Exp Med* (2000) 191(8):1381–94. doi: 10.1084/jem.191.8.1381
10. He B, Xu W, Santini PA, Polydorides AD, Chiu A, Estrella J, et al. Intestinal bacteria trigger T cell-independent immunoglobulin A(2) class switching by inducing epithelial-cell secretion of the cytokine APRIL. *Immunity* (2007) 26 (6):812–26. doi: 10.1016/j.immuni.2007.04.014
11. Chen T, Liu X, Ma L, He W, Li W, Cao Y, et al. Food allergens affect the intestinal tight junction permeability in inducing intestinal food allergy in rats. *Asian Pac J Allergy Immunol* (2014) 32(4):345–53. doi: 10.12932/AP0443.32.4.2014
12. Pizzuti D, Senzolo M, Buda A, Chiarelli S, Giacomelli L, Mazzon E, et al. In vitro model for IgE mediated food allergy. *Scand J Gastroenterol* (2011) 46 (2):177–87. doi: 10.3109/00365521.2010.525716
13. Liu Z, Li N, Neu J. Tight junctions, leaky intestines, and pediatric diseases. *Acta Paediatr* (2005) 94(4):386–93. doi: 10.1111/j.1651-2227.2005.tb01904.x
14. Perrier C, Corthésy B. Gut permeability and food allergies. *Clin Exp Allergy* (2011) 41(1):20–8. doi: 10.1111/j.1365-2222.2010.03639.x
15. Sicherer SH, Furlong TJ, Maes HH, Desnick RJ, Sampson HA, Gelb BD. Genetics of peanut allergy: a twin study. *J Allergy Clin Immunol* (2000) 106(1 Pt 1):53–6. doi: 10.1067/mai.2000.108105
16. Marenholz I, Grosche S, Kalb B, Rüschendorf F, Blümchen K, Schlags R, et al. Genome-wide association study identifies the SERPINB gene cluster as a susceptibility locus for food allergy. *Nat Commun* (2017) 8(1):1056. doi: 10.1038/s41467-017-01220-0
17. Mo Y, Zhang K, Feng Y, Yi L, Liang Y, Wu W, et al. Epithelial SERPINB10, a novel marker of airway eosinophilia in asthma, contributes to allergic airway inflammation. *Am J Physiol Lung Cell Mol Physiol* (2019) 316(1):L245–l54. doi: 10.1152/ajplung.00362.2017
18. Maury J, Nicoletti C, Guzzo-Chambraud L, Maroux S. The filamentous brush border glycocalyx, a mucin-like marker of enterocyte hyper-polarization. *Eur J Biochem* (1995) 228(2):323–31. doi: 10.1111/j.1432-1033.1995.tb20267.x
19. Hooton D, Lentle R, Monro J, Wickham M, Simpson R. The Secretion and Action of Brush Border Enzymes in the Mammalian Small Intestine. *Rev Physiol Biochem Pharmacol* (2015) 168:59–118. doi: 10.1007/112_2015_24
20. Lodish H, Berk A, Zipursky SL. Transport across Epithelia. In: *Molecular Cell Biology*, *4th edition*. New York: W.H. Freeman (2000).
21. Wosen JE, Mukhopadhyay D, Macaubas C, Mellins ED. Epithelial MHC Class II Expression and Its Role in Antigen Presentation in the Gastrointestinal and Respiratory Tracts. *Front Immunol* (2018) 9:2144. doi: 10.3389/fimmu.2018.02144
22. Van Niel G, Mallegol J, Bevilacqua C, Candalh C, Brugière S, Tomaskovic-Crook E, et al. Intestinal epithelial exosomes carry MHC class II/peptides able to inform the immune system in mice. *Gut* (2003) 52(12):1690–7. doi: 10.1136/gut.52.12.1690
23. van Niel G, Raposo G, Candalh C, Boussac M, Hershberg R, Cerf-Bensussan N, et al. Intestinal epithelial cells secrete exosome-like vesicles. *Gastroenterology* (2001) 121(2):337–49. doi: 10.1053/gast.2001.26263

24. Zeng HT, Liu JQ, Zhao M, Yu D, Yang G, Mo LH, et al. Exosomes carry IL-10 and antigen/MHC II complexes to induce antigen-specific oral tolerance. *Cytokine* (2020) 133:155176. doi: 10.1016/j.cyto.2020.155176
25. Johansson ME, Ambort D, Pelaseyed T, Schutte A, Gustafsson JK, Ermund A, et al. Composition and functional role of the mucus layers in the intestine. *Cell Mol Life Sci* (2011) 68(22):3635–41. doi: 10.1007/s00018-011-0822-3
26. Sajjan SU, Forstner JF. Role of the putative "link" glycopeptide of intestinal mucin in binding of piliated Escherichia coli serotype O157:H7 strain CL-49. *Infect Immun* (1990) 58(4):868–73. doi: 10.1128/IAI.58.4.868-873.1990
27. Knoop KA, McDonald KG, McCrate S, McDole JR, Newberry RD. Microbial sensing by goblet cells controls immune surveillance of luminal antigens in the colon. *Mucosal Immunol* (2015) 8(1):198–210. doi: 10.1038/mi.2014.58
28. McDole JR, Wheeler LW, McDonald KG, Wang B, Konjufca V, Knoop KA, et al. Goblet cells deliver luminal antigen to $CD103^+$ dendritic cells in the small intestine. *Nature* (2012) 483(7389):345–9. doi: 10.1038/nature10863
29. Kobayashi N, Takahashi D, Takano S, Kimura S, Hase K. The Roles of Peyer's Patches and Microfold Cells in the Gut Immune System: Relevance to Autoimmune Diseases. *Front Immunol* (2019) 10:2345. doi: 10.3389/fimmu.2019.02345
30. Trier JS. Structure and function of intestinal M cells. *Gastroenterol Clin North Am* (1991) 20(3):531–47.
31. Liang E, Kabcenell AK, Coleman JR, Robson J, Ruffles R, Yazdanian M. Permeability measurement of macromolecules and assessment of mucosal antigen sampling using *in vitro* converted M cells. *J Pharmacol Toxicol Methods* (2001) 46(2):93–101. doi: 10.1016/S1056-8719(02)00163-6
32. Rey J, Garin N, Spertini F, Corthesy B. Targeting of secretory IgA to Peyer's patch dendritic and T cells after transport by intestinal M cells. *J Immunol* (2004) 172(5):3026–33. doi: 10.4049/jimmunol.172.5.3026
33. Rol N, Favre L, Benyacoub J, Corthesy B. The role of secretory immunoglobulin A in the natural sensing of commensal bacteria by mouse Peyer's patch dendritic cells. *J Biol Chem* (2012) 287(47):40074–82. doi: 10.1074/jbc.M112.405001
34. Yu S, Balasubramanian I, Laubitz D, Tong K, Bandyopadhyay S, Lin X, et al. Paneth cell-derived lysozyme defines the composition of mucolytic microbiota and the inflammatory tone of the intestine. *Immunity* (2020) 53(2):398–416.e8. doi: 10.1016/j.immuni.2020.07.010
35. Qu XD, Lloyd KC, Walsh JH, Lehrer RI. Secretion of type II phospholipase A2 and cryptdin by rat small intestinal Paneth cells. *Infect Immun* (1996) 64 (12):5161–5. doi: 10.1128/IAI.64.12.5161-5165.1996
36. Cash HL, Whitham CV, Behrendt CL, Hooper LV. Symbiotic bacteria direct expression of an intestinal bactericidal lectin. *Science* (2006) 313 (5790):1126–30. doi: 10.1126/science.1127119
37. Walker CR, Hautefort I, Dalton JE, Overweg K, Egan CE, Bongaerts RJ, et al. Intestinal intraepithelial lymphocyte-enterocyte crosstalk regulates production of bactericidal angiogenin 4 by Paneth cells upon microbial challenge. *PLoS One* (2013) 8(12):e84553. doi: 10.1371/journal.pone.0084553
38. Ayabe T, Wulff H, Darmoul D, Cahalan MD, Chandy KG, Ouellette AJ. Modulation of mouse Paneth cell alpha-defensin secretion by mIKCa1, a Ca2+-activated, intermediate conductance potassium channel. *J Biol Chem* (2002) 277(5):3793–800. doi: 10.1074/jbc.M107507200
39. Rumio C, Sommariva M, Sfondrini L, Palazzo M, Morelli D, Vigano L, et al. Induction of Paneth cell degranulation by orally administered Toll-like receptor ligands. *J Cell Physiol* (2012) 227(3):1107–13. doi: 10.1002/jcp.22830
40. Vaishnava S, Behrendt CL, Ismail AS, Eckmann L, Hooper LV. Paneth cells directly sense gut commensals and maintain homeostasis at the intestinal host-microbial interface. *Proc Natl Acad Sci U S A* (2008) 105(52):20858–63. doi: 10.1073/pnas.0808723105
41. Gribble FM, Reimann F. Enteroendocrine Cells: Chemosensors in the Intestinal Epithelium. *Annu Rev Physiol* (2016) 78:277–99. doi: 10.1146/annurev-physiol-021115-105439
42. Dockray G. Gut endocrine secretions and their relevance to satiety. *Curr Opin Pharmacol* (2004) 4(6):557–60. doi: 10.1016/j.coph.2004.05.005
43. Rindi G, Leiter AB, Kopin AS, Bordi C, Solcia E. The "normal" endocrine cell of the gut: changing concepts and new evidences. *Ann N Y Acad Sci* (2004) 1014:1–12. doi: 10.1196/annals.1294.001
44. Mazzawi T, Eikrem Ø, Lied GA, Hausken T. Abnormal Uroguanylin Immunoreactive Cells Density in the Duodenum of Patients with Diarrhea-Predominant Irritable Bowel Syndrome Changes following Fecal Microbiota Transplantation. *Gastroenterol Res Pract* (2020) 2020:3520686. doi: 10.1155/2020/3520686
45. Mazzawi T, Gundersen D, Hausken T, El-Salhy M. Increased chromogranin a cell density in the large intestine of patients with irritable bowel syndrome after receiving dietary guidance. *Gastroenterol Res Pract* (2015) 2015:823897. doi: 10.1155/2015/823897
46. Mazzawi T, Hausken T, Gundersen D, El-Salhy M. Dietary guidance normalizes large intestinal endocrine cell densities in patients with irritable bowel syndrome. *Eur J Clin Nutr* (2016) 70(2):175–81. doi: 10.1038/ejcn.2015.191
47. Fritscher-Ravens A, Pflaum T, Mösinger M, Ruchay Z, Röcken C, Milla PJ, et al. Many Patients With Irritable Bowel Syndrome Have Atypical Food Allergies Not Associated With Immunoglobulin E. *Gastroenterology* (2019) 157(1):109–18.e5. doi: 10.1053/j.gastro.2019.03.046
48. Gerbe F, Sidot E, Smyth DJ, Ohmoto M, Matsumoto I, Dardalhon V, et al. Intestinal epithelial tuft cells initiate type 2 mucosal immunity to helminth parasites. *Nature* (2016) 529(7585):226–30. doi: 10.1038/nature16527
49. Howitt MR, Lavoie S, Michaud M, Blum AM, Tran SV, Weinstock JV, et al. Tuft cells, taste-chemosensory cells, orchestrate parasite type 2 immunity in the gut. *Science* (2016) 351(6279):1329–33. doi: 10.1126/science.aaf1648
50. von Moltke J, Ji M, Liang HE, Locksley RM. Tuft-cell-derived IL-25 regulates an intestinal ILC2-epithelial response circuit. *Nature* (2016) 529(7585):221–5. doi: 10.1038/nature16161
51. Leyva-Castillo JM, Galand C, Kam C, Burton O, Gurish M, Musser MA, et al. Mechanical Skin Injury Promotes Food Anaphylaxis by Driving Intestinal Mast Cell Expansion. *Immunity* (2019) 50(5):1262–75.e4. doi: 10.1016/j.immuni.2019.03.023
52. Haber AL, Biton M, Rogel N, Herbst RH, Shekhar K, Smillie C, et al. A single-cell survey of the small intestinal epithelium. *Nature* (2017) 551 (7680):333–9. doi: 10.1038/nature24489
53. Boonekamp KE, Dayton TL, Clevers H. Intestinal organoids as tools for enriching and studying specific and rare cell types: advances and future directions. *J Mol Cell Biol* (2020) 12(8):562–8. doi: 10.1093/jmcb/mjaa034
54. Gehart H, van Es JH, Hamer K, Beumer J, Kretzschmar K, Dekkers JF, et al. Identification of Enteroendocrine Regulators by Real-Time Single-Cell Differentiation Mapping. *Cell* (2019) 176(5):1158–73.e16. doi: 10.1016/j.cell.2018.12.029
55. Gracz AD, Samsa LA, Fordham MJ, Trotier DC, Zwarycz B, Lo YH, et al. Sox4 Promotes Atoh1-Independent Intestinal Secretory Differentiation Toward Tuft and Enteroendocrine Fates. *Gastroenterology* (2018) 155 (5):1508–23.e10. doi: 10.1053/j.gastro.2018.07.023
56. Kaiko GE, Ryu SH, Koues OI, Collins PL, Solnica-Krezel L, Pearce EJ, et al. The Colonic Crypt Protects Stem Cells from Microbiota-Derived Metabolites. *Cell* (2016) 165(7):1708–20. doi: 10.1016/j.cell.2016.05.018
57. Farré R, Fiorani M, Abdu Rahiman S, Matteoli G. Intestinal Permeability, Inflammation and the Role of Nutrients. *Nutrients* (2020) 12(4). doi: 10.3390/nu12041185
58. Mabbott NA, Donaldson DS, Ohno H, Williams IR, Mahajan A. Microfold (M) cells: important immunosurveillance posts in the intestinal epithelium. *Mucosal Immunol* (2013) 6(4):666–77. doi: 10.1038/mi.2013.30
59. Niess JH, Brand S, Gu X, Landsman L, Jung S, McCormick BA, et al. CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. *Science* (2005) 307(5707):254–8. doi: 10.1126/science.1102901
60. Rescigno M, Rotta G, Valzasina B, Ricciardi-Castagnoli P. Dendritic cells shuttle microbes across gut epithelial monolayers. *Immunobiology* (2001) 204(5):572–81. doi: 10.1078/0171-2985-00094
61. Ulluwishewa D, Anderson RC, McNabb WC, Moughan PJ, Wells JM, Roy NC. Regulation of tight junction permeability by intestinal bacteria and dietary components. *J Nutr* (2011) 141(5):769–76. doi: 10.3945/jn.110.135657
62. Samadi N, Klems M, Untersmayr E. The role of gastrointestinal permeability in food allergy. *Ann Allergy Asthma Immunol* (2018) 121(2):168–73. doi: 10.1016/j.anai.2018.05.010
63. Drago S, El Asmar R, Di Pierro M, Grazia Clemente M, Tripathi A, Sapone A, et al. Gliadin, zonulin and gut permeability: Effects on celiac and non-celiac intestinal mucosa and intestinal cell lines. *Scand J Gastroenterol* (2006) 41(4):408–19. doi: 10.1080/00365520500235334
64. Jensen-Jarolim E, Gajdzik L, Haberl I, Kraft D, Scheiner O, Graf J. Hot spices influence permeability of human intestinal epithelial monolayers. *J Nutr* (1998) 128(3):577–81. doi: 10.1093/jn/128.3.577

65. Hashimoto K, Kawagishi H, Nakayama T, Shimizu M. Effect of capsianoside, a diterpene glycoside, on tight-junctional permeability. *Biochim Biophys Acta* (1997) 1323(2):281–90. doi: 10.1016/S0005-2736(96)00196-4
66. Yamani A, Wu D, Ahrens R, Waggoner L, Noah TK, Garcia-Hernandez V, et al. Dysregulation of intestinal epithelial CFTR-dependent Cl(-) ion transport and paracellular barrier function drives gastrointestinal symptoms of food-induced anaphylaxis in mice. *Mucosal Immunol* (2020). doi: 10.1038/s41385-020-0306-6
67. Yamamoto T, Kuramoto H, Kadowaki M. Downregulation in aquaporin 4 and aquaporin 8 expression of the colon associated with the induction of allergic diarrhea in a mouse model of food allergy. *Life Sci* (2007) 81(2):115–20. doi: 10.1016/j.lfs.2007.04.036
68. Tu Y, Salim S, Bourgeois J, Di Leo V, Irvine EJ, Marshall JK, et al. CD23-mediated IgE transport across human intestinal epithelium: inhibition by blocking sites of translation or binding. *Gastroenterology* (2005) 129(3):928–40. doi: 10.1053/j.gastro.2005.06.014
69. Li H, Nowak-Wegrzyn A, Charlop-Powers Z, Shreffler W, Chehade M, Thomas S, et al. Transcytosis of IgE-antigen complexes by CD23a in human intestinal epithelial cells and its role in food allergy. *Gastroenterology* (2006) 131(1):47–58. doi: 10.1053/j.gastro.2006.03.044
70. Li H, Chehade M, Liu W, Xiong H, Mayer L, Berin MC. Allergen-IgE complexes trigger CD23-dependent CCL20 release from human intestinal epithelial cells. *Gastroenterology* (2007) 133(6):1905–15. doi: 10.1053/j.gastro.2007.09.024
71. Kim E, Lembert M, Fallata GM, Rowe JC, Martin TL, Satoskar AR, et al. Intestinal Epithelial Cells Regulate Gut Eotaxin Responses and Severity of Allergy. *Front Immunol* (2018) 9:1692. doi: 10.3389/fimmu.2018.01692
72. Frossard CP, Zimmerli SC, Rincon Garriz JM, Eigenmann PA. Food allergy in mice is modulated through the thymic stromal lymphopoietin pathway. *Clin Transl Allergy* (2015) 6:2. doi: 10.1186/s13601-016-0090-2
73. Han H, Roan F, Johnston LK, Smith DE, Bryce PJ, Ziegler SF. IL-33 promotes gastrointestinal allergy in a TSLP-independent manner. *Mucosal Immunol* (2018) 11(2):394–403. doi: 10.1038/mi.2017.61
74. Han H, Thelen TD, Comeau MR, Ziegler SF. Thymic stromal lymphopoietin-mediated epicutaneous inflammation promotes acute diarrhea and anaphylaxis. *J Clin Invest* (2014) 124(12):5442–52. doi: 10.1172/JCI77798
75. Khodoun MV, Tomar S, Tocker JE, Wang YH, Finkelman FD. Prevention of food allergy development and suppression of established food allergy by neutralization of thymic stromal lymphopoietin, IL-25, and IL-33. *J Allergy Clin Immunol* (2018) 141(1):171–9.e1. doi: 10.1016/j.jaci.2017.02.046
76. Regoli M, Man A, Gicheva N, Dumont A, Ivory K, Pacini A, et al. Morphological and Functional Characterization of IL-12Rβ2 Chain on Intestinal Epithelial Cells: Implications for Local and Systemic Immunoregulation. *Front Immunol* (2018) 9:1177. doi: 10.3389/fimmu.2018.01177
77. Dillon A, Lo DD. M Cells: Intelligent Engineering of Mucosal Immune Surveillance. *Front Immunol* (2019) 10:1499. doi: 10.3389/fimmu.2019.01499
78. Hase K, Kawano K, Nochi T, Pontes GS, Fukuda S, Ebisawa M, et al. Uptake through glycoprotein 2 of FimH(+) bacteria by M cells initiates mucosal immune response. *Nature* (2009) 462(7270):226–30. doi: 10.1038/nature08529
79. Tezuka H, Ohteki T. Regulation of IgA Production by Intestinal Dendritic Cells and Related Cells. *Front Immunol* (2019) 10:1891. doi: 10.3389/fimmu.2019.01891
80. Knoop KA, Kumar N, Butler BR, Sakthivel SK, Taylor RT, Nochi T, et al. RANKL is necessary and sufficient to initiate development of antigen-sampling M cells in the intestinal epithelium. *J Immunol* (2009) 183(9):5738–47. doi: 10.4049/jimmunol.0901563
81. Nagashima K, Sawa S, Nitta T, Tsutsumi M, Okamura T, Penninger JM, et al. Identification of subepithelial mesenchymal cells that induce IgA and diversify gut microbiota. *Nat Immunol* (2017) 18(6):675–82. doi: 10.1038/ni.3732
82. Chambers SJ, Wickham MS, Regoli M, Bertelli E, Gunning PA, Nicoletti C. Rapid *in vivo* transport of proteins from digested allergen across pre-sensitized gut. *Biochem Biophys Res Commun* (2004) 325(4):1258–63. doi: 10.1016/j.bbrc.2004.10.161
83. Butler M, Ng CY, van Heel DA, Lombardi G, Lechler R, Playford RJ, et al. Modulation of dendritic cell phenotype and function in an *in vitro* model of the intestinal epithelium. *Eur J Immunol* (2006) 36(4):864–74. doi: 10.1002/eji.200535497
84. Coombes JL, Siddiqui KR, Arancibia-Cárcamo CV, Hall J, Sun CM, Belkaid Y, et al. A functionally specialized population of mucosal $CD103^+$ DCs induces Foxp3+ regulatory T cells *via a* TGF-beta and retinoic acid-dependent mechanism. *J Exp Med* (2007) 204(8):1757–64. doi: 10.1084/jem.20070590
85. Iliev ID, Spadoni I, Mileti E, Matteoli G, Sonzogni A, Sampietro GM, et al. Human intestinal epithelial cells promote the differentiation of tolerogenic dendritic cells. *Gut* (2009) 58(11):1481–9. doi: 10.1136/gut.2008.175166
86. Knoop KA, McDonald KG, Kulkarni DH, Newberry RD. Antibiotics promote inflammation through the translocation of native commensal colonic bacteria. *Gut* (2016) 65(7):1100–9. doi: 10.1136/gutjnl-2014-309059
87. Noah TK, Knoop KA, McDonald KG, Gustafsson JK, Waggoner L, Vanoni S, et al. IL-13-induced intestinal secretory epithelial cell antigen passages are required for IgE-mediated food-induced anaphylaxis. *J Allergy Clin Immunol* (2019) 144(4):1058–73.e3. doi: 10.1016/j.jaci.2019.04.030
88. Rescigno M, Urbano M, Valzasina B, Francolini M, Rotta G, Bonasio R, et al. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat Immunol* (2001) 2(4):361–7. doi: 10.1038/86373
89. Nicoletti C, Arques JL, Bertelli E. CX_3CR1 is critical for Salmonella-induced migration of dendritic cells into the intestinal lumen. *Gut Microbes* (2010) 1(3):131–4. doi: 10.4161/gmic.1.3.11711
90. Kulkarni DH, Gustafsson JK, Knoop KA, McDonald KG, Bidani SS, Davis JE, et al. Goblet cell associated antigen passages support the induction and maintenance of oral tolerance. *Mucosal Immunol* (2020) 13(2):271–82. doi: 10.1038/s41385-019-0240-7
91. Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. *N Engl J Med* (2002) 347(12):911–20. doi: 10.1056/NEJMra020100
92. Hall AB, Tolonen AC, Xavier RJ. Human genetic variation and the gut microbiome in disease. *Nat Rev Genet* (2017) 18(11):690–9. doi: 10.1038/nrg.2017.63
93. Goodrich JK, Waters JL, Poole AC, Sutter JL, Koren O, Blekhman R, et al. Human genetics shape the gut microbiome. *Cell* (2014) 159(4):789–99. doi: 10.1016/j.cell.2014.09.053
94. Lim MY, You HJ, Yoon HS, Kwon B, Lee JY, Lee S, et al. The effect of heritability and host genetics on the gut microbiota and metabolic syndrome. *Gut* (2017) 66(6):1031–8. doi: 10.1136/gutjnl-2015-311326
95. Lundgren SN, Madan JC, Emond JA, Morrison HG, Christensen BC, Karagas MR, et al. Maternal diet during pregnancy is related with the infant stool microbiome in a delivery mode-dependent manner. *Microbiome* (2018) 6(1):109. doi: 10.1186/s40168-018-0490-8
96. Robertson RC, Manges AR, Finlay BB, Prendergast AJ. The Human Microbiome and Child Growth - First 1000 Days and Beyond. *Trends Microbiol* (2019) 27(2):131–47. doi: 10.1016/j.tim.2018.09.008
97. Tapiainen T, Paalanne N, Tejesvi MV, Koivusaari P, Korpela K, Pokka T, et al. Maternal influence on the fetal microbiome in a population-based study of the first-pass meconium. *Pediatr Res* (2018) 84(3):371–9. doi: 10.1038/pr.2018.29
98. de Kivit S, Tobin MC, DeMeo MT, Fox S, Garssen J, Forsyth CB, et al. In vitro evaluation of intestinal epithelial TLR activation in preventing food allergic responses. *Clin Immunol* (2014) 154(2):91–9. doi: 10.1016/j.clim.2014.07.002
99. Lee J, Mo JH, Katakura K, Alkalay I, Rucker AN, Liu YT, et al. Maintenance of colonic homeostasis by distinctive apical TLR9 signalling in intestinal epithelial cells. *Nat Cell Biol* (2006) 8(12):1327–36. doi: 10.1038/ncb1500
100. Bouladoux N, Hall JA, Grainger JR, dos Santos LM, Kann MG, Nagarajan V, et al. Regulatory role of suppressive motifs from commensal DNA. *Mucosal Immunol* (2012) 5(6):623–34. doi: 10.1038/mi.2012.36
101. Haller D, Bode C, Hammes WP, Pfeifer AM, Schiffrin EJ, Blum S. Non-pathogenic bacteria elicit a differential cytokine response by intestinal epithelial cell/leucocyte co-cultures. *Gut* (2000) 47(1):79–87. doi: 10.1136/gut.47.1.79
102. Cario E, Gerken G, Podolsky DK. Toll-like receptor 2 enhances ZO-1-associated intestinal epithelial barrier integrity *via* protein kinase C. *Gastroenterology* (2004) 127(1):224–38. doi: 10.1053/j.gastro.2004.04.015
103. Sodhi C, Levy R, Gill R, Neal MD, Richardson W, Branca M, et al. DNA attenuates enterocyte Toll-like receptor 4-mediated intestinal mucosal injury after remote trauma. *Am J Physiol Gastrointest Liver Physiol* (2011) 300(5):G862–73. doi: 10.1152/ajpgi.00373.2010
104. Breiteneder H, Radauer C. A classification of plant food allergens. *J Allergy Clin Immunol* (2004) 113(5):821–30; quiz 31. doi: 10.1016/j.jaci.2004.01.779

105. Jackson MA, Verdi S, Maxan ME, Shin CM, Zierer J, Bowyer RCE, et al. Gut microbiota associations with common diseases and prescription medications in a population-based cohort. *Nat Commun* (2018) 9(1):2655. doi: 10.1038/s41467-018-05184-7
106. Noval Rivas M, Burton OT, Wise P, Zhang YQ, Hobson SA, Garcia Lloret M, et al. A microbiota signature associated with experimental food allergy promotes allergic sensitization and anaphylaxis. *J Allergy Clin Immunol* (2013) 131(1):201–12. doi: 10.1016/j.jaci.2012.10.026
107. Rodriguez B, Prioult G, Hacini-Rachinel F, Moine D, Bruttin A, Ngom-Bru C, et al. Infant gut microbiota is protective against cow's milk allergy in mice despite immature ileal T-cell response. *FEMS Microbiol Ecol* (2012) 79 (1):192–202. doi: 10.1111/j.1574-6941.2011.01207.x
108. Zehra S, Khambati I, Vierhout M, Mian MF, Buck R, Forsythe P. Human Milk Oligosaccharides Attenuate Antigen-Antibody Complex Induced Chemokine Release from Human Intestinal Epithelial Cell Lines. *J Food Sci* (2018) 83(2):499–508. doi: 10.1111/1750-3841.14039
109. van Hoffen E, Ruiter B, Faber J, M'Rabet L, Knol EF, Stahl B, et al. A specific mixture of short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides induces a beneficial immunoglobulin profile in infants at high risk for allergy. *Allergy* (2009) 64(3):484–7. doi: 10.1111/j.1398-9995.2008.01765.x
110. Stefka AT, Feehley T, Tripathi P, Qiu J, McCoy K, Mazmanian SK, et al. Commensal bacteria protect against food allergen sensitization. *Proc Natl Acad Sci U S A* (2014) 111(36):13145–50. doi: 10.1073/pnas.1412008111
111. Feehley T, Plunkett CH, Bao R, Choi Hong SM, Culleen E, Belda-Ferre P, et al. Healthy infants harbor intestinal bacteria that protect against food allergy. *Nat Med* (2019) 25(3):448–53. doi: 10.1038/s41591-018-0324-z
112. Tan J, McKenzie C, Vuillermin PJ, Goverse G, Vinuesa CG, Mebius RE, et al. Dietary Fiber and Bacterial SCFA Enhance Oral Tolerance and Protect against Food Allergy through Diverse Cellular Pathways. *Cell Rep* (2016) 15 (12):2809–24. doi: 10.1016/j.celrep.2016.05.047
113. Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, deRoos P, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* (2013) 504(7480):451–5. doi: 10.1038/nature12726
114. Burger-van Paassen N, Vincent A, Puiman PJ, van der Sluis M, Bouma J, Boehm G, et al. The regulation of intestinal mucin MUC2 expression by short-chain fatty acids: implications for epithelial protection. *Biochem J* (2009) 420(2):211–9. doi: 10.1042/BJ20082222
115. Wang CC, Wu H, Lin FH, Gong R, Xie F, Peng Y, et al. Sodium butyrate enhances intestinal integrity, inhibits mast cell activation, inflammatory mediator production and JNK signaling pathway in weaned pigs. *Innate Immun* (2018) 24(1):40–6. doi: 10.1177/1753425917741970
116. Zhao Y, Chen F, Wu W, Sun M, Bilotta AJ, Yao S, et al. GPR43 mediates microbiota metabolite SCFA regulation of antimicrobial peptide expression in intestinal epithelial cells *via* activation of mTOR and STAT3. *Mucosal Immunol* (2018) 11(3):752–62. doi: 10.1038/mi.2017.118
117. Isono A, Katsuno T, Sato T, Nakagawa T, Kato Y, Sato N, et al. Clostridium butyricum TO-A culture supernatant downregulates TLR4 in human colonic epithelial cells. *Dig Dis Sci* (2007) 52(11):2963–71. doi: 10.1007/s10620-006-9593-3
118. Hussain M, Bonilla-Rosso G, Kwong Chung CKC, Bäriswyl L, Rodriguez MP, Kim BS, et al. High dietary fat intake induces a microbiota signature that promotes food allergy. *J Allergy Clin Immunol* (2019) 144(1):157–70.e8. doi: 10.1016/j.jaci.2019.01.043
119. Vuillermin PJ, O'Hely M, Collier F, Allen KJ, Tang MLK, Harrison LC, et al. Maternal carriage of Prevotella during pregnancy associates with protection against food allergy in the offspring. *Nat Commun* (2020) 11(1):1452. doi: 10.1038/s41467-020-14552-1
120. Agus A, Planchais J, Sokol H. Gut Microbiota Regulation of Tryptophan Metabolism in Health and Disease. *Cell Host Microbe* (2018) 23(6):716–24. doi: 10.1016/j.chom.2018.05.003
121. Barcik W, Pugin B, Westermann P, Perez NR, Ferstl R, Wawrzyniak M, et al. Histamine-secreting microbes are increased in the gut of adult asthma patients. *J Allergy Clin Immunol* (2016) 138(5):1491–4.e7. doi: 10.1016/j.jaci.2016.05.049
122. Levan SR, Stamnes KA, Lin DL, Panzer AR, Fukui E, McCauley K, et al. Elevated faecal 12,13-diHOME concentration in neonates at high risk for asthma is produced by gut bacteria and impedes immune tolerance. *Nat Microbiol* (2019) 4(11):1851–61. doi: 10.1038/s41564-019-0498-2
123. Song X, Sun X, Oh SF, Wu M, Zhang Y, Zheng W, et al. Microbial bile acid metabolites modulate gut RORγ(+) regulatory T cell homeostasis. *Nature* (2020) 577(7790):410–5. doi: 10.1038/s41586-019-1865-0
124. Nordengrün M, Michalik S, Völker U, Bröker BM, Gómez-Gascón L. The quest for bacterial allergens. *Int J Med Microbiol* (2018) 308(6):738–50. doi: 10.1016/j.ijmm.2018.04.003
125. Holtfreter S, Grumann D, Balau V, Barwich A, Kolata J, Goehler A, et al. Molecular Epidemiology of Staphylococcus aureus in the General Population in Northeast Germany: Results of the Study of Health in Pomerania (SHIP-TREND-0). *J Clin Microbiol* (2016) 54(11):2774–85. doi: 10.1128/JCM.00312-16
126. Krismer B, Weidenmaier C, Zipperer A, Peschel A. The commensal lifestyle of Staphylococcus aureus and its interactions with the nasal microbiota. *Nat Rev Microbiol* (2017) 15(11):675–87. doi: 10.1038/nrmicro.2017.104
127. Nakamura Y, Oscherwitz J, Cease KB, Chan SM, Muñoz-Planillo R, Hasegawa M, et al. Staphylococcus δ-toxin induces allergic skin disease by activating mast cells. *Nature* (2013) 503(7476):397–401. doi: 10.1038/nature12655
128. Liu JN, Shin YS, Yoo HS, Nam YH, Jin HJ, Ye YM, et al. The Prevalence of Serum Specific IgE to Superantigens in Asthma and Allergic Rhinitis Patients. *Allergy Asthma Immunol Res* (2014) 6(3):263–6. doi: 10.4168/aair.2014.6.3.263
129. Tripathi A, Conley DB, Grammer LC, Ditto AM, Lowery MM, Seiberling KA, et al. Immunoglobulin E to staphylococcal and streptococcal toxins in patients with chronic sinusitis/nasal polyposis. *Laryngoscope* (2004) 114 (10):1822–6. doi: 10.1097/00005537-200410000-00027
130. Cook-Mills JM, Kaplan MH, Turner MJ, Kloepfer KM, Kumar R. Exposure: Staphylococcus aureus skin colonization predisposes to food allergy in the Learning Early about Allergy to Peanut (LEAP) and LEAP-On studies. *J Allergy Clin Immunol* (2019) 144(2):404–6. doi: 10.1016/j.jaci.2019.06.014
131. Tsilochristou O, du Toit G, Sayre PH, Roberts G, Lawson K, Sever ML, et al. Association of Staphylococcus aureus colonization with food allergy occurs independently of eczema severity. *J Allergy Clin Immunol* (2019) 144(2):494–503. doi: 10.3410/f.735907550.793563272
132. Galazzo G, van Best N, Bervoets L, Dapaah IO, Savelkoul PH, Hornef MW, et al. Development of the Microbiota and Associations With Birth Mode, Diet, and Atopic Disorders in a Longitudinal Analysis of Stool Samples, Collected From Infancy Through Early Childhood. *Gastroenterology* (2020) 158(6):1584–96. doi: 10.1053/j.gastro.2020.01.024
133. Corren J, Parnes JR, Wang L, Mo M, Roseti SL, Griffiths JM, et al. Tezepelumab in Adults with Uncontrolled Asthma. *N Engl J Med* (2017) 377(10):936–46. doi: 10.1056/NEJMoa1704064
134. Beeh KM, Kanniess F, Wagner F, Schilder C, Naudts I, Hammann-Haenni A, et al. The novel TLR-9 agonist QbG10 shows clinical efficacy in persistent allergic asthma. *J Allergy Clin Immunol* (2013) 131(3):866–74. doi: 10.1016/j.jaci.2012.12.1561
135. Casale TB, Cole J, Beck E, Vogelmeier CF, Willers J, Lassen C, et al. CYT003, a TLR9 agonist, in persistent allergic asthma - a randomized placebo-controlled Phase 2b study. *Allergy* (2015) 70(9):1160–8. doi: 10.1111/all.12663
136. Knoop KA, McDonald KG, Coughlin PE, Kulkarni DH, Gustafsson JK, Rusconi B, et al. Synchronization of mothers and offspring promotes tolerance and limits allergy. *JCI Insight* (2020) 5(15). doi: 10.1172/jci.insight.137943

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

MINI REVIEW
published: 10 December 2020
doi: 10.3389/fimmu.2020.603272

Perturbations to Homeostasis in Experimental Models Revealed Innate Pathways Driving Food Allergy

Kelly Bruton, Joshua F. E. Koenig, Allyssa Phelps and Manel Jordana *

Department of Pathology and Molecular Medicine, McMaster Immunology Research Centre (MIRC), McMaster University, Hamilton ON, Canada

OPEN ACCESS

Edited by:
Simon Patrick Hogan, University of Michigan, United States

Reviewed by:
Hans Oettgen, Boston Children's Hospital and Harvard Medical School, United States
Mark C. Siracusa, Rutgers Biomedical and Health Sciences, United States

***Correspondence:**
Manel Jordana
jordanam@mcmaster.ca

Specialty section:
This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology

Received: *06 September 2020*
Accepted: *10 November 2020*
Published: *10 December 2020*

Citation:
Bruton K, Koenig JFE, Phelps A and Jordana M (2020) Perturbations to Homeostasis in Experimental Models Revealed Innate Pathways Driving Food Allergy. Front. Immunol. 11:603272. doi: 10.3389/fimmu.2020.603272

While type 2 immunity has been conventionally viewed as beneficial against helminths, venoms, and poisons, and harmful in allergy, contemporary research has uncovered its critical role in the maintenance of homeostasis. The initiation of a type 2 immune response involves an intricate crosstalk between structural and immune cells. Structural cells react to physical and chemical tissue perturbations by secreting alarmins, which signal the innate immune system to restore homeostasis. This pathway acts autonomously in the context of sterile injury and in the presence of foreign antigen initiates an adaptive Th2 response that is beneficial in the context of venoms, toxins, and helminths, but not food allergens. The investigation of the triggers and mechanisms underlying food allergic sensitization in humans is elusive because sensitization is a silent process. Therefore, the central construct driving food allergy modeling is based on introducing perturbations of tissue homeostasis along with an allergen which will result in an immunological and clinical phenotype that is consistent with that observed in humans. The collective evidence from multiple models has revealed the pre-eminent role of innate cells and molecules in the elicitation of allergic sensitization. We posit that, with the expanding use of technologies capable of producing formidable datasets, models of food allergy will continue to have an indispensable role to delineate mechanisms and establish causal relationships.

Keywords: food allergy, animal modeling, type 2 immunity, Th2, homeostasis

INTRODUCTION

Our understanding of the underlying pathogenesis of food allergy and its attendant applications has been largely attained through experimental modeling. In fact, modeling, which has been instrumental to scientific progress in general, is an inescapable approach to decipher complex problems such as a disease. Here, we discuss a broad perspective of type 2 immunity, highlighting its role in homeostasis.

Then, we provide a succinct ideology of modeling and examine strategies for food allergy modeling. Lastly, we advance our view on why food allergy modeling will remain an indispensable discovery tool, especially with the continuing development of technologies that facilitate high parameter analyses.

PATHWAYS OF INNATE-DRIVEN TYPE 2 IMMUNITY

Historically, type 2 immunity was conceived as a balancing system for type 1 inflammatory responses and whose primary purpose was to provide defense against helminths (1). Indeed, the symptomatology elicited by type 2 responses, including itching, tearing, intestinal cramps, wheezing, swelling, and, in extreme instances, anaphylaxis, is geared toward containment or expulsion of helminths, poisons, and toxins and are, therefore, beneficial for the host. Antithetically, type 2 immunity promotes harmful responses against food allergens.

Research over the last decade, largely in experimental models, has revolutionized this view ushering in a much richer perspective of both the functions and the engineering of type 2 immunity. With respect to the former, important roles for the cells and molecules of type 2 immunity have been uncovered in a wide range of processes, from tissue repair and metabolism to sensing temperature and chemical imbalances (2). Regarding the engineering, it is clear that the initiation and execution of a type 2 immune response entails an intricate crosstalk between structural and immune cells. Collectively, this research has positioned type 2 immunity as a guardian of homeostasis.

Considerable evidence from multiple contexts has revealed a generic type 2 pathway mediated by innate immune cells and molecules that regulates homeostasis. Structural cells at mucosal sites and the skin, prominently epithelial and endothelial cells, react to tissue perturbations or physiological changes by secreting alarmins, such as IL-33, IL-25, and TSLP (3, 4). These alarmins act on cognate receptors on ILC2s which expand and enact a variety of reparative functions primarily through the secretion of IL-5, IL-13, and amphiregulin (5–7). IL-5 facilitates the accumulation of eosinophils at the tissue site which generally impact homeostasis through their cytokines and granule products (8–12). ILC2s, IL-13, and amphiregulin induce a plethora of developmental and reparative functions. These include but are not limited to promotion of postnatal lung alveolarization (13, 14), stimulating epithelial repair and extracellular matrix production to repair wounds (15–19), regulating muscle cell metabolism and the clearance of necrotic debris during muscular injury and exercise (9, 20, 21), and regulating energy metabolism and the differentiation of beige adipose tissue to increase caloric expenditure and generate heat (22–26). To date, components of this pathway have been implicated in homeostatic maintenance in the skin (18, 19), muscles (9, 20), intestines (27, 28), nervous system (29, 30), liver (8, 31, 32), biliary tract (15), lungs (16), kidney (11), adipose tissue (33), among others. Emergent roles in the initiation and amplification of type 2 immunity are being described for other innate immune cells, such as mast cells and basophils, which produce alarmins and potently secrete other cytokines in some contexts (34, 35). Notably, virtually no adaptive immune involvement has been reported in type 2-mediated homeostasis and, in many cases, occurs in mice without an adaptive immune system.

Adaptive type 2 immunity is also activated in the context of significant tissue perturbations. The distinctive rule of engagement is the presence of foreign antigens in the tissue microenvironment. For example, the life cycle of many helminths involves perforating epithelial barriers and implanting into tissues. Most venoms are proteases which cleave proteins critical for tissue integrity and cause necrotic cell death. Some aeroallergens, like house dust mite, have inherent proteolytic activity (36). It follows that type 2 adaptive immunity may have evolved to respond to foreign antigens that are conspicuously present in the local microenvironment of tissue injury. In these alarmin-rich contexts, epithelial cell-derived cytokines directly act through cognate receptors on dendritic cells to condition the expression of the costimulatory molecule OX40L and downregulate IL-12 expression (37, 38). These dendritic cells also capture local antigen and migrate to secondary lymphoid organs where they engage naïve $CD4^+$ T cells. OX40L is critical to induce IL-4 secretion from $CD4^+$ T cells, which is thought to act in an autocrine manner to upregulate STAT6 and GATA3 resulting in Th2 differentiation (39–41). Some T follicular helper cells acquire a Th2-like phenotype required for the differentiation of IgE-secreting cells (42). Adaptive type 2 immunity is beneficial for the host in the aforementioned contexts; IgE is reported to provide resistance to venoms (43) and Th2 cells and IgE facilitate expeditious clearance of parasites (44–46).

We posit that the type 2 innate and adaptive responses are both instigated by the same triggers, perturbations of homeostasis, differing only by the presence or absence of antigen. When tissue homeostasis is disrupted, alarmins signal the innate immune system to initiate reparative functions to regain homeostasis **Figure 1A**). Simultaneously, some of these alarmins condition nearby dendritic cells, which are actively scanning the microenvironment for foreign antigens. Capture of antigens which colocalize with tissue damage are loaded into MHC II and elicit an adaptive type 2 immune response. If a foreign antigen is encountered that signals through pattern recognition receptors (like a bacteria or virus), type 1 immunity is engaged for pathogen control (**Figure 1B**). When no foreign antigens are encountered, DCs internalize and present only self-antigens which usually will not result in adaptive immunity as self-reactive T cells are eliminated or constrained by central and peripheral tolerance (**Figure 1A**). Type 2 immunity is intuitive for helminths, which cause damage when invading tissues and shed antigenic material that is internalized by DCs, thus mounting Th2 and IgE responses (**Figure 1C**) (45). It follows similarly for toxins and some aeroallergens, which cause damage through proteolytic activity and are small enough to be taken up by DCs (**Figure 1D**). However, it falls short when

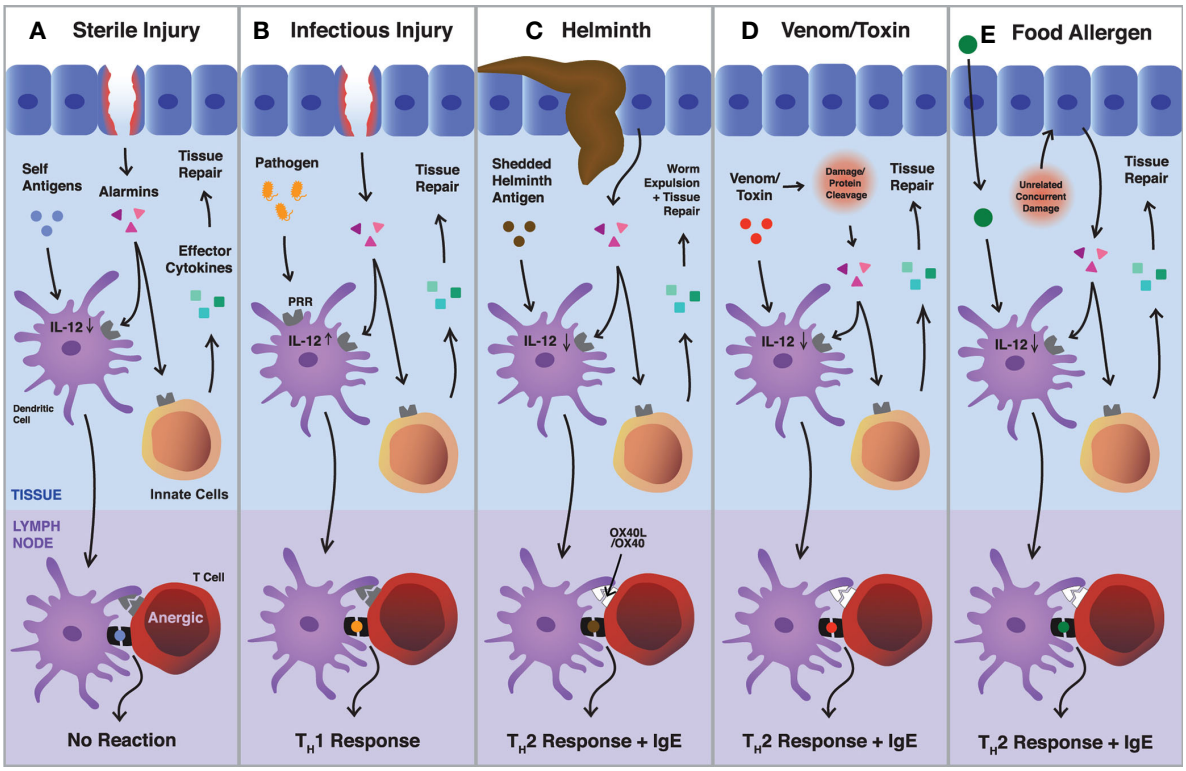


FIGURE 1 | Pathways of Type 2 immune activation *via* tissue perturbations. **(A–D)** Epithelial cells release alarmins (e.g., IL-33, IL-25, and TSLP) in response to tissue damage. Alarmins signal through cognate receptors on innate immune cells, resulting in proliferation and the release of effector cytokines (e.g., IL-5, IL-13, and amphiregulin). These cytokines directly influence tissue repair (e.g., stimulating re-epithelization and extracellular matrix production) or cause the recruitment of other cells (e.g., eosinophils) which aid in tissue repair. Simultaneously, local dendritic cells uptake tissue antigens and respond to alarmin signaling by travelling to the draining lymph nodes and inducing Th2 cell differentiation through OX40-OX40L. **(A)** In the context of sterile injury, only self-antigens are uptaken by dendritic cells and are presented to T cells, which have been limited by central and peripheral tolerance. **(B)** When infectious agents are present, they signal through pattern recognition receptors, upregulating IL-12 in dendritic cells, and initiating Th1 immunity for host defence. **(C, D)** Helminths and venoms, canonical examples of Th2 responses, drive tissue damage by invading tissues or through cleaving proteins, respectively. Antigens that colocalize with damage are presented to T cells and initiate Th2 responses. **(E)** It remains unclear how Th2 immunity is initiated against food allergens. Mouse models require the use of adjuvants (e.g., cholera toxin or tape stripping) to perturb homeostasis, suggesting that food allergic responses are initiated in the presence of damage that is unrelated to the food allergen itself.

rationalizing immune responses against food allergens, which are highly diverse in structure, the vast majority of which are not immunogenic, and, enigmatically, which are essential for survival.

IDEOLOGY OF DISEASE MODELING

Complexity has driven scientists from all branches to develop models, these understood as the functional simplification of an intractable reality. The development of a scientific model is based on the prevailing interpretation of the available information at a given time. Therefore, models are not static but evolve as we enhance our knowledge of a process. A biomedical model is a surrogate for a human being, or a human biological system. Its primary purpose is to understand how the human body works, from genotype to phenotype. Within biomedical models, animal models provide precise genetic and experimental control and are, thus, critical to establish causality. Understanding is not gained by observation but by manipulation, physically, genetically, pharmacologically or immunologically, in ways that would be unreasonable or even unethical to do in a human.

Animal models are critical for investigating disease mechanisms. Diseases are exceptionally intricate, rarely involving a single phenotypic abnormality and often affecting multiple organ systems. A case in point is the multiple phenotypes observed in allergic disease. This presents a conundrum: on the one hand, deciphering this complexity in a human being is an indomitable task; on the other, animal models are a simplification of this reality and can only provide approximations. Consequently, the expectation that findings from a single model may wholly explain a heterogeneous disease is unrealistic. The implication is that multiple models asking multiple questions are necessary to provide a comprehensive view of a disease process, food allergy in this case. Animal models are extensively used as well to interrogate the biological impact of novel therapeutics. These studies along with other experimental approaches are critical to narrow the decision of

which therapeutics should progress to large scale studies in humans. In other words, knowing what not-to-do helps immensely with the decision of what-to-do.

PERSPECTIVE ON FOOD ALLERGY MODELING

Food allergy is typically diagnosed following a clinical reaction in infants or toddlers upon first known exposure (47). However, a prior event must have taken place to trigger allergic sensitization and, ultimately, production of food-specific IgE. As sensitization in humans is a silent process neither its initiation nor its evolution can be interrogated. Moreover, there appears to be no unifying genetic or environmental exposures, highlighting the heterogenous nature of food allergy. Accordingly, a diversity of animal models—primarily mouse models—have provided a tangible, ethical, and indispensable approach to decipher the cellular and molecular mechanisms underlying allergic sensitization.

Unlike some aeroallergens, toxins, and animal poisons, the vast majority of food antigens are not inherently immunogenic. As a consequence, most humans and experimental animals develop oral tolerance, an active state of non-reactivity, upon first exposure to food antigens (48, 49). In fact, the LEAP study demonstrated that over 98% of infants with atopic comorbidities (eczema and/or egg allergy) receiving their first exposure to peanut before 11 months of age develop oral tolerance (50). Similar to a type 2 homeostatic response, the tolerogenic response to food antigens [reviewed in citations (51, 52)] fundamentally involves DCs. However, acquisition of oral tolerance imperatively lacks the key molecules and the majority of cells that trigger Th2 polarization. It follows that a concomitant subversion of homeostasis is required to prime the immune system to mount a Th2-dominant response against food antigens (**Figure 1E**). In mouse modeling, this subversion is generally achieved through the use of adjuvants—substances which aid in eliciting robust immune responses (53). Cholera toxin (CT), staphylococcal enterotoxin B (SEB), and aluminum hydroxide (alum), are three of the most widely used biological or chemical adjuvants that facilitate a Th2-dominant response in mice (54–58). Damaged skin, due to physical disruption of epidermal barriers (i.e., epicutaneous sensitization), drives sensitization to topically applied allergen (59–63). A second approach to modeling food allergies, also a subversion of homeostasis, involves reducing the threshold of reactivity *via* genetic mutations. For example, *Il4ra*^{F709} mice which contain a loss-of-function mutation in the *Il4ra* immunotyrosine inhibitory motif, display a lower sensitization threshold upon food allergen exposure (64, 65). Thus, the use of adjuvanted models has played a key role in overcoming the elusiveness of allergic sensitization in humans.

Basic discoveries that discriminate tolerant (homeostatic) *versus* pathogenic (allergic) responses to foods have been described using models in which mice are sensitized with a single or repeated administration of CT along with a food antigen (56, 66–68). CT is classified as an AB_5 toxin; this class of toxins are produced by many enterotropic bacteria, such as *Escherichia coli* and *Bordetella pertussis* (69). Oral administration of CT enhances $CD103^+$ $CD11c^+$ DC migration to the mesenteric lymph nodes as well as expression of MHC II and costimulatory molecules (e.g., CD86) (70–72). These effects *per se* do not rationalize the elicitation of dominant Th2 priming. However, studies investigating the gene expression profile of mesenteric lymph node DCs in CT-immunized mice showed an upregulation of OX40L (70). Antibody-mediated blockade and genetic knockouts demonstrated a critical role of OX40L to promote Th2 skewing by enabling the initial burst of IL-4 from CD4 T cells (28, 41, 70). Further studies exposed the involvement of the IL-33/ST2 innate axis, where IL-33-signaling facilitates OX40L upregulation (41). Intriguingly, use of an ILC2 depletion strategy revealed a dispensable role of ILC2s in oral sensitization to foods, despite a dominant presence in intestinal tissues and a well-defined role in type 2 homeostatic processes (41).

A relationship between skin atopic abnormalities and sensitization to food allergens has become clear through large-scale longitudinal studies (73, 74). A primary example of this are loss-of-function mutations in the filaggrin gene (*Flg*) that correlate with peanut allergy in humans (75). To elucidate the immunological mechanisms of this relationship, models of epicutaneous sensitization have been employed. Sensitization is typically achieved through tape stripping, which removes the outermost epidermal layer, causing skin inflammation (63). These models have been referred to as "adjuvant-free", which is misleading as tape stripping provides the same function as an adjuvant—induction of an immune response *via* an external input (76–78). Animal models have revealed an essential role of epithelial cells pertaining to barrier function and alarmin production during epicutaneous allergen exposure. In mice, disturbed production of filaggrin, a protein involved in epidermal barrier function, facilitated allergic sensitization to allergen applied topically (79). Damaged epithelial cells are an important source of alarmins such as IL-33, TSLP, and uric acid which, as reviewed earlier, enable DC activation and subsequent CD4 T cell priming (80–81). Tape-strip models have also revealed a role of ILC2s which, in response to IL-25 and IL-33 secretion from tape-stripped skin, proliferate in intestinal tissues to drive IL-4-dependent mast cell expansion (82).

In addition to elucidating the innate immunological events underlying allergic sensitization, animal modeling has enabled discoveries pertaining to allergic reactions. Alarmins involved in sensitization also participate in the effector phase. IL-33 interacts directly with mast cells to potentiate mast cell degranulation (84). Moreover, FcεRI-mediated mast cell activation stimulates production of IL-25, which is hypothesized to further drive the Th2 phenotype (85). Mouse models have elucidated the vasoactive mediators that facilitate anaphylaxis. In humans a correlation between anaphylactic severity and serum platelet activating factor (PAF) levels, but not histamine levels, was reported (86). Use of mouse models determined that blockade of histamine receptors (H1 and H2) was insufficient at preventing anaphylaxis, but that concurrent treatment with antihistamines and a PAF-receptor antagonist could significantly reduce anaphylactic severity (87).

In mouse models, allergen challenges vary greatly in dose and route (intraperitoneal, oral, intradermal, and intravenous) (56, 58, 88, 89). Routes of challenge differ in the clinical phenotype that is induced. For example, oral challenges tend to result in diarrhea and, sometimes, a mild drop in core body temperature. Intraperitoneal challenges, on the other hand, facilitate a much more robust drop in core body temperature and other severe systemic reactions (e.g., seizures). The severity of clinical reactivity may, in part, be dependent on antigen availability and mast cell density at the site of allergen administration (90). As well, IgG has also been shown to mediate anaphylaxis in food allergy mouse models, although an equivalent IgG-mediated food-induced anaphylactic pathway in humans remains contentious (63, 91, 92). In all instances—independent of allergen administration route—modeling allergen challenge reveals downstream functions of allergen-specific immunoglobulins (e.g., the events following allergen cross-linking of mast cell-bound IgE). Thus, the selection of the route of allergen challenge should be guided by the research question, where intraperitoneal and intravenous challenges are best suited to assess systemic anaphylaxis, oral challenge to evaluate gut-local effects (e.g., allergic diarrhea, goblet cell hyperplasia), and intradermal challenge to measure localized vascular permeability. Use of anti-FcγRII/III blocking antibodies may also be employed to further discriminate between IgE- and IgG-mediated allergen reactivity (91).

The use of animal models of food allergy for translational research has been scrutinized due to the necessity of "artificial/experimental sensitization" (i.e., requiring eccentric interventions). The validity of animal models for translational research can, however, be evaluated by three criteria: 1) face validity, 2) predictive validity, and 3) target validity (93, 94). Face validity evaluates the similarity of disease biology and symptomology between humans and the animal model (93). The biological processes of food allergy at large are conserved between mice and humans. For example, the immune response is dominantly Th2 polarized, IL-33 and uric acid are elevated compared to non-allergic controls, and clinical reactivity is IgE-mediated (84, 92, 95). Many of the clinical signs of an allergic reaction are also alike including itching, diarrhea, local inflammation, and systemic shock (76, 96). Predictive validity compares the effectiveness of interventions in humans and in the relevant animal model (93). Due to the small number of interventions tried in human food allergy, evaluation of predictive validity is limited, but oral immunotherapy provides a key example. Oral immunotherapy in both mice and humans can increase the threshold of reactivity (i.e., desensitization) to food allergens, although this desensitized state is, just as it is in the majority of humans, unsustained (68, 97). This limited assessment of predictive validity will be greatly improved come the initiation of clinical trials testing novel biotherapeutics (e.g., anti-IL-4Rα). Lastly, target validity establishes that a particular target (e.g., a cytokine) has the same function in humans and the disease model (93). Many of the molecules and cells involved in human food allergy provide similar actions in animal models. For instance, IL-4 promotes Th2 polarization and IgE class-switch in both mice and human food allergy (95). In contrast, the role of IgG in human food-induced anaphylaxis remains contentious and, therefore, mouse models would provide low target validity for investigation of IgG-targeted interventions. In summary, despite not knowing the "adjuvants" at play in human food allergy, adjuvant-based experimental models have revealed cellular and molecular signatures that exist in humans. Moreover, regardless of experimental model, it is clear that an innate program drives allergic sensitization.

CONCLUDING REMARKS

Over the last few decades, there have been fundamental advances in our understanding of allergic disease, including food allergy. These advances have led to a dramatic re-conceptualization of the functions of type 2 immunity and the critical role of the innate system in programming these functions. We must recognize, however, that the current state of knowledge is vastly incomplete. For example, how gut dysbiosis stresses local tissue environments and impacts primary and secondary immune responses to foods is an emerging area already providing novel insights (98, 99). We surmise that experimental models will continue providing seminal discoveries on the machinery (cells and molecules) and the engineering (operating mechanisms) that underlie allergy. Indeed, the ability to overexpress or repress individual genes in animals, either permanently or on-demand, pervasively or in specific cell types, unlocks unique opportunities to decipher both the pathogenesis and the trajectory of the allergic diathesis. Lastly, experimental modeling remains an unparalleled sieve to guide what should be investigated in humans.

The ever-increasing application of technologies that deliver formidable datasets will help to delineate complex inter- and intracellular pathways, thus enlightening the astounding phenotypic diversity and plasticity of the allergic response. Mass cytometry has dramatically expanded upon flow cytometry in the analysis of protein expression at the single cell level and is drastically reshaping histology allowing for the measurement of very many markers on a single section. Bulk and single cell RNA sequencing is rapidly expanding in read depth, resulting in greater appreciation of the genetic signatures and subpopulations that define disease states. Constant advances in bioinformatics and machine learning continue to unveil new ways of mining these massive datasets for pathways and cell differentiation trajectories. There is no doubt that the next generation of large data analysis is already being conceived and developed beyond the gaze of immunologists. The logic of applying these analyses to human samples is clear. However, applying these technologies to animal research will remain imperative in at least two areas that are not currently possible in humans: 1) To address questions that require manipulation (genotype and exposure), not only observation, to understand how the system reacts and 2) To efficiently deconvolute the temporal and spatial evolution of a given perturbation or process; particularly those that are obscured by the complex exposure

history of humans. Furthermore, emerging discoveries from these datasets in both humans and models will have to be validated through mechanistic studies in experimental models using the long-established immunological toolkit to decipher causal relationships.

AUTHOR CONTRIBUTIONS

KB, JK, AP, and MJ wrote and edited the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by funds from Food Allergy Canada, Walter and Maria Schroeder Foundation, Michael Zych Family, and Canadian Asthma, Allergy, and Immunology Foundation (CAAIF). KB holds a Canada Graduate Scholarship. AP holds The Eva Eugenia Lillian Cope Scholarship.

ACKNOWLEDGMENTS

We kindly thank C. Spadafora for her illustrations.

REFERENCES

1. Mosmann TR, Coffman RL. TH1 and TH2 Cells: Different Patterns of Lymphokine Secretion Lead to Different Functional Properties. *Annu Rev Immunol* (1989) 7:145–73. doi: 10.1146/annurev.iy.07.040189.001045
2. Lloyd CM, Snelgrove RJ. Type 2 immunity: Expanding our view. *Sci Immunol* (2018) 3:1604. doi: 10.1126/sciimmunol.aat1604
3. Molofsky AB, Savage AK, Locksley RM. Interleukin-33 in Tissue Homeostasis, Injury, and Inflammation. *Immunity* (2015) 42:1005–19. doi: 10.1016/j.immuni.2015.06.006
4. Yang D, Han Z, Oppenheim JJ. Alarmins and immunity. *Immunol Rev* (2017) 280:41–56. doi: 10.1111/imr.12577
5. Neill DR, Wong SH, Bellosi A, Flynn RJ, Daly M, Langford TKA, et al. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature* (2010) 464:1367–70. doi: 10.1038/nature08900
6. Moro K, Yamada T, Tanabe M, Takeuchi T, Ikawa T, Kawamoto H, et al. Innate production of TH 2 cytokines by adipose tissue-associated c-Kit+ Sca-1+ lymphoid cells. *Nature* (2010) 463:540–4. doi: 10.1038/nature08636
7. Price AE, Liang HE, Sullivan BM, Reinhardt RL, Eisley CJ, Erle DJ, et al. Systemically dispersed innate IL-13-expressing cells in type 2 immunity. *Proc Natl Acad Sci U S A* (2010) 107:11489–94. doi: 10.1073/pnas.1003988107
8. Goh YPS, Henderson NC, Heredia JE, Eagle AR, Odegaard JI, Lehwald N, et al. Eosinophils secrete IL-4 to facilitate liver regeneration. *Proc Natl Acad Sci U S A* (2013) 110:9914–9. doi: 10.1073/pnas.1304046110
9. Heredia JE, Mukundan L, Chen FM, Mueller AA, Deo RC, Locksley RM, et al. Type 2 innate signals stimulate fibro/adipogenic progenitors to facilitate muscle regeneration. *Cell* (2013) 153:376–88. doi: 10.1016/j.cell.2013.02.053
10. Nussbaum JC, Van Dyken SJ, Von Moltke J, Cheng LE, Mohapatra A, Molofsky AB, et al. Type 2 innate lymphoid cells control eosinophil homeostasis. *Nature* (2013) 502:245–8. doi: 10.1038/nature12526
11. Riedel JH, Becker M, Kopp K, Düster M, Brix SR, Meyer-Schwesinger C, et al. IL-33-Mediated expansion of type 2 innate lymphoid cells protects from progressive glomerulosclerosis. *J Am Soc Nephrol* (2017) 28:2068–80. doi: 10.1681/ASN.2016080877
12. Weller PF, Spencer LA. Functions of tissue-resident eosinophils. *Nat Rev Immunol* (2017) 17:746–60. doi: 10.1038/nri.2017.95
13. Loering S, Cameron GJM, Starkey MR, Hansbro PM. Lung development and emerging roles for type 2 immunity. *J Pathol* (2019) 247:686–96. doi: 10.1002/path.5211
14. de Kleer IM, Kool M, de Bruijn MJW, Willart M, van Moorleghem J, Schuijs MJ, et al. Perinatal Activation of the Interleukin-33 Pathway Promotes Type 2 Immunity in the Developing Lung. *Immunity* (2016) 45:1285–98. doi: 10.1016/j.immuni.2016.10.031
15. Li J, Razumilava N, Gores GJ, Walters S, Mizuochi T, Mourya R, et al. Biliary repair and carcinogenesis are mediated by IL-33-dependent cholangiocyte proliferation. *J Clin Invest* (2014) 124:3241–51. doi: 10.1172/JCI73742
16. Monticelli LA, Sonnenberg GF, Abt MC, Alenghat T, Ziegler CGK, Doering TA, et al. Innate lymphoid cells promote lung-tissue homeostasis after infection with influenza virus. *Nat Immunol* (2011) 12:1045–54. doi: 10.1038/ni.2131
17. Yin H, Li X, Hu S, Liu T, Yuan B, Gu H, et al. IL-33 accelerates cutaneous wound healing involved in upregulation of alternatively activated macrophages. *Mol Immunol* (2013) 56:347–53. doi: 10.1016/j.molimm.2013.05.225
18. Rankin AL, Mumm JB, Murphy E, Turner S, Yu N, McClanahan TK, et al. IL-33 Induces IL-13–Dependent Cutaneous Fibrosis. *J Immunol* (2010) 184:1526–35. doi: 10.4049/jimmunol.0903306
19. Rak GD, Osborne LC, Siracusa MC, Kim BS, Wang K, Bayat A, et al. IL-33-Dependent Group 2 Innate Lymphoid Cells Promote Cutaneous Wound Healing. *J Invest Dermatol* (2016) 136:487–96. doi: 10.1038/JID.2015.406
20. Knudsen NH, Stanya KJ, Hyde AL, Chalom MM, Alexander RK, Liou YH, et al. Interleukin-13 drives metabolic conditioning of muscle to endurance exercise. *Sci (80-)* (2020) 368:eaat3987. doi: 10.1126/science.aat3987
21. Kastenschmidt J. A role for group 2 innate lymphoid cells in muscular dystrophy. *J Immunol* (2019) 202:1350. doi: 10.3390/ijms21041350
22. Hams E, Locksley RM, McKenzie ANJ, Fallon PG. Cutting Edge: IL-25 Elicits Innate Lymphoid Type 2 and Type II NKT Cells That Regulate Obesity in Mice. *J Immunol* (2013) 191:5349–53. doi: 10.4049/jimmunol.1301176
23. Stanya KJ, Jacobi D, Liu S, Bhargava P, Dai L, Gangl MR, et al. Direct control of hepatic glucose production by interleukin-13 in mice. *J Clin Invest* (2013) 123:261–71. doi: 10.1172/JCI64941
24. Brestoff JR, Kim BS, Saenz SA, Stine RR, Monticelli LA, Sonnenberg GF, et al. Group 2 innate lymphoid cells promote beiging of white adipose tissue and limit obesity. *Nature* (2015) 519:242–6. doi: 10.1038/nature14115
25. Lee MW, Odegaard JI, Mukundan L, Qiu Y, Molofsky AB, Nussbaum JC, et al. Activated type 2 innate lymphoid cells regulate beige fat biogenesis. *Cell* (2015) 160:74–87. doi: 10.1016/j.cell.2014.12.011
26. Qiu Y, Nguyen KD, Odegaard JI, Cui X, Tian X, Locksley RM, et al. Eosinophils and type 2 cytokine signaling in macrophages orchestrate development of functional beige fat. *Cell* (2014) 157:1292–308. doi: 10.1016/j.cell.2014.03.066
27. Waddell A, Vallance JE, Moore PD, Hummel AT, Wu D, Shanmukhappa SK, et al. IL-33 Signaling Protects from Murine Oxazolone Colitis by Supporting Intestinal Epithelial Function. *Inflammation Bowel Dis* (2015) 21:2737–46. doi: 10.1097/MIB.0000000000000532
28. Monticelli LA, Osborne LC, Noti M, Tran SV, Zaiss DMW, Artis D. IL-33 promotes an innate immune pathway of intestinal tissue protection dependent on amphiregulin-EGFR interactions. *Proc Natl Acad Sci U S A* (2015) 112:10762–7. doi: 10.1073/pnas.1509070112
29. Gadani SP, Smirnov I, Smith AT, Overall CC, Kipnis J. Characterization of meningeal type 2 innate lymphocytes and their response to CNS injury. *J Exp Med* (2017) 214:285–96. doi: 10.1084/jem.20161982
30. Gadani SP, Walsh JT, Smirnov I, Zheng J, Kipnis J. The Glia-Derived Alarmin IL-33 Orchestrates the Immune Response and Promotes Recovery following CNS Injury. *Neuron* (2015) 85:703–9. doi: 10.1016/j.neuron.2015.01.013
31. Steinmann S, Schoedsack M, Heinrich F, Breda PC, Ochel A, Tiegs G, et al. Hepatic ILC2 activity is regulated by liver inflammation-induced cytokines and effector CD4+ T cells. *Sci Rep* (2020) 10:1–13. doi: 10.1038/s41598-020-57985-w
32. Gieseck RL, Ramalingam TR, Hart KM, Vannella KM, Cantu DA, Lu WY, et al. Interleukin-13 Activates Distinct Cellular Pathways Leading to Ductular Reaction, Steatosis, and Fibrosis. *Immunity* (2016) 45:145–58. doi: 10.1016/j.immuni.2016.06.009
33. Bénézech C, Jackson-Jones LH. ILC2 Orchestration of Local Immune Function in Adipose Tissue. *Front Immunol* (2019) 10:171. doi: 10.3389/fimmu.2019.00171

34. Sokol CL, Medzhitov R. Emerging functions of basophils in protective and allergic immune responses. *Mucosal Immunol* (2010) 3:129–37. doi: 10.1038/mi.2009.137
35. Krystel-Whittemore M, Dileepan KN, Wood JG. Mast cell: A multi-functional master cell. *Front Immunol* (2016) 6:1–12. doi: 10.3389/fimmu.2015.00620
36. Chevigné A, Jacquet A. Emerging roles of the protease allergen Der p 1 in house dust mite–induced airway inflammation. *J Allergy Clin Immunol* (2018) 142:398–400. doi: 10.1016/j.jaci.2018.05.027
37. Hussaarts L, Yazdanbakhsh M, Guigas B. Priming Dendritic Cells for Th2 Polarization: Lessons Learned from Helminths and Implications for Metabolic Disorders. *Front Immunol* (2014) 5:499. doi: 10.3389/fimmu.2014.00499
38. Chu DK, Llop-Guevara A, Walker TD, Flader K, Goncharova S, Boudreau JE, et al. IL-33, but not thymic stromal lymphopoietin or IL-25, is central to mite and peanut allergic sensitization. *J Allergy Clin Immunol* (2013) 131:187–200.e1–8. doi: 10.1016/j.jaci.2012.08.002
39. Liu Z, Liu Q, Hamed H, Anthony RM, Foster A, Finkelman FD, et al. IL-2 and Autocrine IL-4 Drive the In Vivo Development of Antigen-Specific Th2 T Cells Elicited by Nematode Parasites. *J Immunol* (2005) 174:2242–9. doi: 10.4049/jimmunol.174.4.2242
40. Noben-Trauth N, Hu-Li J, Paul WE. IL-4 secreted from individual naive CD4 + T cells acts in an autocrine manner to induce Th2 differentiation. *Eur J Immunol* (2002) 32:1428–33. doi: 10.1002/1521-4141(200205)32:5<1428::AID-IMMU1428>3.0.CO;2-0
41. Chu DK, Mohammed-Ali Z, Jiménez-Saiz R, Walker TD, Goncharova S, Llop-Guevara A, et al. T helper cell IL-4 drives intestinal Th2 priming to oral peanut antigen, under the control of OX40L and independent of innate-like lymphocytes. *Mucosal Immunol* (2014) 7:1395–404. doi: 10.1038/mi.2014.29
42. Gowthaman U, Chen JS, Zhang B, Flynn WF, Lu Y, Song W, et al. Identification of a T follicular helper cell subset that drives anaphylactic IgE. *Sci (80-)* (2019) 365:eaaw6433. doi: 10.1126/science.aaw6433
43. Marichal T, Starkl P, Reber LL, Kalesnikoff J, Oettgen HC, Tsai M, et al. A Beneficial Role for Immunoglobulin E in Host Defense against Honeybee Venom. *Immunity* (2013) 39:963–75. doi: 10.1016/j.immuni.2013.10.005
44. Gurish MF, Bryce PJ, Tao H, Kisselgof AB, Thornton EM, Miller HR, et al. IgE Enhances Parasite Clearance and Regulates Mast Cell Responses in Mice Infected with Trichinella spiralis. *J Immunol* (2004) 172:1139–45. doi: 10.4049/jimmunol.172.2.1139
45. White RR, Artavanis-Tsakonas K. How helminths use excretory secretory fractions to modulate dendritic cells. *Virulence* (2012) 3:668–77. doi: 10.4161/viru.22832
46. King CL, Xianli J, Malhotra I, Liu S, Mahmoud AA, Oettgen HC. Mice with a targeted deletion of the IgE gene have increased worm burdens and reduced granulomatous inflammation following primary infection with Schistosoma mansoni. *J Immunol* (1997) 158:294–300.
47. Savage J, Johns CB. Food allergy: epidemiology and natural history. *Immunol Allergy Clin North Am* (2015) 35:45–59. doi: 10.1016/j.iac.2014.09.004
48. Husby S, Mestecky J, Moldoveanu Z, Holland S, Elson CO. Oral tolerance in humans. T cell but not B cell tolerance after antigen feeding. *J Immunol* (1994) 152:4663–70.
49. Friedman A, Weiner HL. Induction of anergy or active suppression following oral tolerance is determined by antigen dosage. *Proc Natl Acad Sci U S A* (1994) 91:6688–92. doi: 10.1073/pnas.91.14.6688
50. Du Toit G, Roberts G, Sayre PH, Bahnson HT, Radulovic S, Santos AF, et al. Randomized Trial of Peanut Consumption in Infants at Risk for Peanut Allergy. *N Engl J Med* (2015) 372:803–13. doi: 10.1056/NEJMoa1414850
51. Pabst O, Mowat AM. Oral tolerance to food protein. *Mucosal Immunol* (2012) 5:232–9. doi: 10.1038/mi.2012.4
52. Satitsuksanoa P, Jansen K, Głobińska A, van de Veen W, Akdis M. Regulatory Immune Mechanisms in Tolerance to Food Allergy. *Front Immunol* (2018) 9:2939. doi: 10.3389/fimmu.2018.02939
53. Awate S, Babiuk LA, Mutwiri G. Mechanisms of action of adjuvants. *Front Immunol* (2013) 4:114:114. doi: 10.3389/fimmu.2013.00114
54. Snider DP, Marshall JS, Perdue MH, Liang H. Production of IgE antibody and allergic sensitization of intestinal and peripheral tissues after oral immunization with protein Ag and cholera toxin. *J Immunol* (1994) 153:647–57.
55. Marinaro M, Staats HF, Hiroi T, Jackson RJ, Coste M, Boyaka PN, et al. Mucosal adjuvant effect of cholera toxin in mice results from induction of T helper 2 (Th2) cells and IL-4. *J Immunol* (1995) 155:4621–9.
56. Sun J, Arias K, Alvarez D, Fattouh R, Walker T, Goncharova S, et al. Impact of CD40 Ligand, B Cells, and Mast Cells in Peanut-Induced Anaphylactic Responses. *J Immunol* (2007) 179:6696–703. doi: 10.4049/jimmunol.179.10.6696
57. Ganeshan K, Neilsen CV, Hadsaitong A, Schleimer RP, Luo X, Bryce PJ. Impairing oral tolerance promotes allergy and anaphylaxis: A new murine food allergy model. *J Allergy Clin Immunol* (2009) 123:231. doi: 10.1016/J.JACI.2008.10.011
58. Brandt EB, Strait RT, Hershko D, Wang Q, Muntel EE, Scribner TA, et al. Mast cells are required for experimental oral allergen–induced diarrhea. *J Clin Invest* (2003) 112:1666. doi: 10.1172/JCI19785
59. Wang LF, Lin JY, Hsieh KH, Lin RH. Epicutaneous exposure of protein antigen induces a predominant Th2-like response with high IgE production in mice. *J Immunol* (1996) 156:4077–82.
60. Hsieh K-Y, Tsai C-C, Herbert Wu CH, Lin R-H. Epicutaneous Exposure to Protein Antigen and Food Allergy. *Clin Exp Allergy* (2003) 33:1067–75. doi: 10.1046/J.1365-2222.2003.01724.X
61. Tordesillas L, Goswami R, Benedé S, Grishina G, Dunkin D, Järvinen KM, et al. Skin exposure promotes a Th2-dependent sensitization to peanut allergens. *J Clin Invest* (2014) 124:4965. doi: 10.1172/JCI75660
62. Noti M, Kim BS, Siracusa MC, Rak GD, Kubo M, Moghaddam AE, et al. Exposure to food allergens through inflamed skin promotes intestinal food allergy through the thymic stromal lymphopoietin-basophil axis. *J Allergy Clin Immunol* (2014) 133:1390–9, 1399.e1–6. doi: 10.1016/j.jaci.2014.01.021
63. Jiménez-Saiz R, Ellenbogen Y, Koenig J, Gordon ME, Walker TD, Rosace D, et al. IgG1 $^{+}$ B cell immunity predates IgE responses in epicutaneous sensitization to foods. *Allergy* (2018) 74:165–75. doi: 10.1111/all.13481
64. Tachdjian R, Al Khatib S, Schwinglshackl A, Kim HS, Chen A, Blasioli J, et al. In vivo regulation of the allergic response by the IL-4 receptor alpha chain immunoreceptor tyrosine-based inhibitory motif. *J Allergy Clin Immunol* (2010) 125:1128–36. doi: 10.1016/j.jaci.2010.01.054
65. Burton OT, Noval Rivas M, Zhou JS, Logsdon SL, Darling AR, Koleoglou KJ, et al. Immunoglobulin E signal inhibition during allergen ingestion leads to reversal of established food allergy and induction of regulatory T cells. *Immunity* (2014) 41:141–51. doi: 10.1016/J.IMMUNI.2014.05.017
66. Zhang B, Liu E, Gertie JA, Joseph J, Xu L, Pinker EY, et al. Divergent T follicular helper cell requirement for IgA and IgE production to peanut during allergic sensitization. *Sci Immunol* (2020) 5:eaay2754. doi: 10.1126/sciimmunol.aay2754
67. Li XM, Serebrisky D, Lee SY, Huang CK, Bardina L, Schofield BH, et al. A murine model of peanut anaphylaxis: T- and B-cell responses to a major peanut allergen mimic human responses. *J Allergy Clin Immunol* (2000) 106:150–8. doi: 10.1067/mai.2000.107395
68. Leonard SA, Martos G, Wang W, Nowak-Węgrzyn A, Berin MC. Oral immunotherapy induces local protective mechanisms in the gastrointestinal mucosa. *J Allergy Clin Immunol* (2012) 129:1579–87.e1. doi: 10.1016/j.jaci.2012.04.009
69. Beddoe T, Paton AW, Le Nours J, Rossjohn J, Paton JC. Structure, biological functions and applications of the AB5 toxins. *Trends Biochem Sci* (2010) 35:411–8. doi: 10.1016/j.tibs.2010.02.003
70. Blázquez AB, Berin MC, Viney JL. Gastrointestinal dendritic cells promote Th2 skewing via OX40L. *J Immunol* (2008) 180:4441–50. doi: 10.4049/jimmunol.180.7.4441
71. Fahlén-Yrlid L, Gustafsson T, Westlund J, Holmberg A, Strömbeck A, Blomquist M, et al. CD11c(high)dendritic cells are essential for activation of CD4+ T cells and generation of specific antibodies following mucosal immunization. *J Immunol* (2009) 183:5032–41. doi: 10.4049/jimmunol.0803992
72. Anjuère F, Luci C, Lebens M, Rousseau D, Hervouet C, Milon G, et al. In vivo adjuvant-induced mobilization and maturation of gut dendritic cells after oral administration of cholera toxin. *J Immunol* (2004) 173:5103–11. doi: 10.4049/jimmunol.173.8.5103
73. Lack G, Fox D, Northstone K, Golding J. Factors Associated with the Development of Peanut Allergy in Childhood. *N Engl J Med* (2003) 348:977–85. doi: 10.1056/NEJMoa013536
74. Hill DJ, Hosking CS. Food allergy and atopic dermatitis in infancy: an epidemiologic study. *Pediatr Allergy Immunol* (2004) 15:421–7. doi: 10.1111/j.1399-3038.2004.00178.x
75. Brown SJ, Asai Y, Cordell HJ, Campbell LE, Zhao Y, Liao H, et al. Loss-of-function variants in the filaggrin gene are a significant risk factor for peanut allergy. *J Allergy Clin Immunol* (2011) 127:661–7. doi: 10.1016/j.jaci.2011.01.031

76. Berin MC, Mayer L. Immunophysiology of experimental food allergy. *Mucosal Immunol* (2009) 2:24–32. doi: 10.1038/mi.2008.72
77. Kanagaratham C, Sallis BF, Fiebiger E. Experimental Models for Studying Food Allergy. *Cell Mol Gastroenterol Hepatol* (2018) 6:356–69. doi: 10.1016/J.JCMGH.2018.05.010
78. Tordesillas L, Mondoulet L, Blazquez A, Benhamou P, Sampson HA, Berin MC. Epicutaneous Immunotherapy Induces Gastrointestinal LAP + Regulatory T Cells and Prevents Food-Induced Anaphylaxis. *J Allergy Clin Immunol* (2017) 139:189–201. doi: 10.1016/J.JACI.2016.03.057
79. Fallon PG, Sasaki T, Sandilands A, Campbell LE, Saunders SP, Mangan NE, et al. A homozygous frameshift mutation in the murine filaggrin gene facilitates enhanced percutaneous allergen priming. *Nat Genet* (2009) 41:602. doi: 10.1038/NG.358
80. Bruhs A, Proksch E, Schwarz T, Schwarz A. Disruption of the Epidermal Barrier Induces Regulatory T Cells via IL-33 in Mice. *J Invest Dermatol* (2018) 138:570–9. doi: 10.1016/J.JID.2017.09.032
81. Oyoshi MK, Larson RP, Ziegler SF, Geha RS. Mechanical injury polarizes skin dendritic cells to elicit a Th2 response by inducing cutaneous TSLP expression. *J Allergy Clin Immunol* (2010) 126:976. doi: 10.1016/J.JACI.2010.08.041
82. Kong J, Chalcraft K, Mandur TS, Jimenez-Saiz R, Walker TD, Goncharova S, et al. Comprehensive metabolomics identifies the alarmin uric acid as a critical signal for the induction of peanut allergy. *Allergy* (2015) 70:495–505. doi: 10.1111/all.12579
83. Leyva-Castillo J-M, Galand C, Kam C, Burton O, Gurish M, Musser MA, et al. Mechanical Skin Injury Promotes Food Anaphylaxis by Driving Intestinal Mast Cell Expansion. *Immunity* (2019) 50:1262–75.e4. doi: 10.1016/J.IMMUNI.2019.03.023
84. Galand C, Leyva-Castillo JM, Yoon J, Han A, Lee MS, McKenzie ANJ, et al. IL-33 promotes food anaphylaxis in epicutaneously sensitized mice by targeting mast cells. *J Allergy Clin Immunol* (2016) 138:1356–66. doi: 10.1016/j.jaci.2016.03.056
85. Ikeda K, Nakajima H, Suzuki K, Kagami S, Hirose K, Suto A, et al. Mast cells produce interleukin-25 upon Fc epsilon RI-mediated activation. *Blood* (2003) 101:3594–6. doi: 10.1182/blood-2002-09-2817
86. Vadas P, Gold M, Perelman B, Liss GM, Lack G, Blyth T, et al. Platelet-Activating Factor, PAF Acetylhydrolase, and Severe Anaphylaxis. *N Engl J Med* (2008) 358:28–35. doi: 10.1056/NEJMoa070030
87. Arias K, Baig M, Colangelo M, Chu D, Walker T, Goncharova S, et al. Concurrent blockade of platelet-activating factor and histamine prevents life-threatening peanut-induced anaphylactic reactions. *J Allergy Clin Immunol* (2009) 124:307–14, 314.e1–2. doi: 10.1016/j.jaci.2009.03.012
88. He J-S, Subramaniam S, Narang V, Srinivasan K, Saunders SP, Carbajo D, et al. IgG1 memory B cells keep the memory of IgE responses. *Nat Commun* (2017) 8:641. doi: 10.1038/s41467-017-00723-0
89. Oettgen HC, Martin TR, Wynshaw-Boris A, Deng C, Drazen JM, Leder P. Active anaphylaxis in IgE-deficient mice. *Nature* (1994) 370:367–70. doi: 10.1038/370367a0
90. Hogan SP, Wang YH, Strait R, Finkelman FD. Food-induced anaphylaxis: Mast cells as modulators of anaphylactic severity. *Semin Immunopathol* (2012) 34:643–53. doi: 10.1007/s00281-012-0320-1
91. Arias K, Chu DK, Flader K, Botelho F, Walker TD, Arias N, et al. Distinct immune effector pathways contribute to the full expression of peanut-induced anaphylactic reactions in mice. *J Allergy Clin Immunol* (2011) 127:1552–61.e1. doi: 10.1016/j.jaci.2011.03.044
92. Finkelman FD, Simons FER, Frew AJ, Ansotegui IJ, Bochner BS, Golden DBK, et al. Anaphylaxis: lessons from mouse models. *J Allergy Clin Immunol* (2007) 120:506–25. doi: 10.1016/j.jaci.2007.07.033
93. Denayer T, Stöhr T, Van Roy M. Animal models in translational medicine: Validation and prediction. *New Horizons Transl Med* (2014) 2:5–11. doi: 10.1016/J.NHTM.2014.08.001
94. Ferreira GS, Veening-Griffioen DH, Boon WPC, Moors EHM, Gispen-de Wied CC, Schellekens H, et al. A standardised framework to identify optimal animal models for efficacy assessment in drug development. *PloS One* (2019) 14:e0218014. doi: 10.1371/journal.pone.0218014
95. Bruton K, Spill P, Vohra S, Baribeau O, Manzoor S, Gadkar S, et al. Interrupting reactivation of immunological memory reprograms allergy and averts anaphylaxis. *bioRxiv* (2020). doi: 10.1101/2020.03.04.975706. 2020.03.04.975706.
96. Tordesillas L, Berin MC, Sampson HA. Immunology of Food Allergy. *Immunity* (2017) 47:32–50. doi: 10.1016/j.immuni.2017.07.004
97. Chinthrajah RS, Purington N, Andorf S, Long A, O'Laughlin KL, Lyu SC, et al. Sustained outcomes in oral immunotherapy for peanut allergy (POISED study): a large, randomised, double-blind, placebo-controlled, phase 2 study. *Lancet (London England)* (2019) 394:1437–49. doi: 10.1016/S0140-6736(19)31793-3
98. Plunkett CH, Nagler CR. The Influence of the Microbiome on Allergic Sensitization to Food. *J Immunol* (2017) 198:581–9. doi: 10.4049/jimmunol.1601266
99. Iweala OI, Nagler CR. The Microbiome and Food Allergy. *Annu Rev Immunol* (2019) 37:377–403. doi: 10.1146/annurev-immunol-042718-041621

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 11 December 2020
doi: 10.3389/fimmu.2020.603050

IgE and IgG Antibodies as Regulators of Mast Cell and Basophil Functions in Food Allergy

Cynthia Kanagaratham[1,2], *Yasmeen S. El Ansari*[1,3], *Owen L. Lewis*[1] *and Hans C. Oettgen*[1,2]*

[1] Division of Immunology, Department of Pediatrics, Boston Children's Hospital, Boston, MA, United States, [2] Department of Pediatrics, Harvard Medical School, Boston, MA, United States, [3] Institute of Laboratory Medicine, Philipps University Marburg, Marburg, Germany

OPEN ACCESS

Edited by:
Simon Patrick Hogan,
University of Michigan, United States

Reviewed by:
Clinton Mathias,
Western New England University, United States
Marat Khodoun,
University of Cincinnati, United States
Hajime Karasuyama,
Tokyo Medical and Dental University, Japan
Michael D. Kulis,
University of North Carolina at Chapel Hill, United States

***Correspondence:**
Hans C. Oettgen
hans.oettgen@childrens.harvard.edu

Specialty section:
This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology

Received: 04 September 2020
Accepted: 22 October 2020
Published: 11 December 2020

Citation:
Kanagaratham C, El Ansari YS, Lewis OL and Oettgen HC (2020) IgE and IgG Antibodies as Regulators of Mast Cell and Basophil Functions in Food Allergy. Front. Immunol. 11:603050. doi: 10.3389/fimmu.2020.603050

Food allergy is a major health issue, affecting the lives of 8% of U.S. children and their families. There is an urgent need to identify the environmental and endogenous signals that induce and sustain allergic responses to ingested allergens. Acute reactions to foods are triggered by the activation of mast cells and basophils, both of which release inflammatory mediators that lead to a range of clinical manifestations, including gastrointestinal, cutaneous, and respiratory reactions as well as systemic anaphylaxis. Both of these innate effector cell types express the high affinity IgE receptor, FcεRI, on their surface and are armed for adaptive antigen recognition by very-tightly bound IgE antibodies which, when cross-linked by polyvalent allergen, trigger degranulation. These cells also express inhibitory receptors, including the IgG Fc receptor, FcγRIIb, that suppress their IgE-mediated activation. Recent studies have shown that natural resolution of food allergies is associated with increasing food-specific IgG levels. Furthermore, oral immunotherapy, the sequential administration of incrementally increasing doses of food allergen, is accompanied by the strong induction of allergen-specific IgG antibodies in both human subjects and murine models. These can deliver inhibitory signals *via* FcγRIIb that block IgE-induced immediate food reactions. In addition to their role in mediating immediate hypersensitivity reactions, mast cells and basophils serve separate but critical functions as adjuvants for type 2 immunity in food allergy. Mast cells and basophils, activated by IgE, are key sources of IL-4 that tilts the immune balance away from tolerance and towards type 2 immunity by promoting the induction of Th2 cells along with the innate effectors of type 2 immunity, ILC2s, while suppressing the development of regulatory T cells and driving their subversion to a pathogenic pro-Th2 phenotype. This adjuvant effect of mast cells and basophils is suppressed when inhibitory signals are delivered by IgG antibodies signaling *via* FcγRIIb. This review summarizes current understanding of the immunoregulatory effects of mast cells and basophils and how these functions are modulated by IgE and IgG antibodies. Understanding these pathways could provide important insights into innovative strategies for preventing and/or reversing food allergy in patients.

Keywords: mast cells, IgG, IgE, food allergy, Fc receptor, oral immunotherapy, basophil activation test

INTRODUCTION

Global surveillance by the World Allergy Organization, shows that the prevalence of food allergies has been rising over the last decade in both developed and developing countries (1). This increase has been considered as the "second wave" of the allergy epidemic, following the "first wave" that was driven by allergic respiratory illnesses. In the United States alone, food allergies affect approximately 8% of children and 2%–3% of adults (2). The most common food allergens in the US are peanut, cow's milk, hen's egg, tree nut, soy, fish, wheat, and shellfish (3).

Food allergies are hypersensitivity reactions that can be mediated by a wide range of humoral and cellular mechanisms. IgE-mediated food allergy is the most common and will be the focus of this review. It occurs in individuals who produce food-specific IgE antibodies. These subjects are often referred to as "sensitized". These IgE antibodies are bound to the innate granulocytic effector cells of anaphylaxis, mast cells and basophils. Upon interaction with allergen and cell-bound IgE, the granule contents of these cells are released and, along with prostaglandin and leukotriene mediators rapidly produced by the same cells, act on a range of target tissues to trigger immediate physiologic responses (4). In the vasculature, these mediators cause dilation of blood vessels and increased plasma leak which manifest locally in tissues as hives and angioedema (including laryngeal edema) and systemically as hypovolemic shock (4). The mediators additionally cause smooth muscle constriction, leading to bronchospasm, vomiting, and diarrhea, and also bind to neuronal receptors triggering pain and itch (4). When multiple organ systems are involved, the reaction is designated systemic anaphylaxis (3). Acute reactions often resolve within the first few hours; however, some patients experience recurrence of symptoms 8–12 h following the first reaction (biphasic reactions) (5).

The mechanisms of immunological priming leading to IgE production in food allergic subjects are unclear. Some patients have prior histories of ingestion, suggesting immunological sensitization *via* the gut. However, many children experience adverse reactions following their initial ingestion of a food, suggesting alternate routes of immune priming. Emerging evidence suggests that sensitization can occur following cutaneous contact, especially in the setting of a disrupted skin barrier, as occurs in atopic dermatitis (6, 7).

Our understanding of the pathways of immunological sensitization, effector cell activation and regulation of IgE-mediated food allergy has grown rapidly since just over 50 years ago when reagin, the fraction of serum responsible for transferring skin test responsiveness from an allergic individual to a naïve recipient, was identified as IgE. The factors regulating IgE-mediated food allergy have been of great interest with a particular emphasis in the role of regulatory T cells (Tregs) in constraining both the emergence of food allergen-specific T helper cells and the production of allergen-specific IgE. However, in recent years, the ability of mast cells and basophils to exert adjuvant functions in immune sensitization to allergens and of IgG antibodies to block IgE-mediated food allergy has been recognized and the role of the inhibitory IgG receptor, FcγRIIb, in potently inhibiting food allergies has really come into focus. In the first part of this review, we briefly discussed the mechanisms, pathophysiology and key players in the disease. In the second part, we cover the evidence for a regulatory functions of mast cells, basophils, IgE and IgG and how they may be targeted clinically to counter food allergy.

Abbreviations: Mouse names: IgE knockout, *Igh-7*$^{-/-}$; *Kit*$^{W\text{-}sh}$ mice, B6.Cg-*Kit*$^{W\text{-}sh}$/HNihrJaeBsmGlliJ; Mcpt5cre iDTR, B6.Tg(Mcpt5^{cre})*Gt(ROSA)26Sor*$^{tm1(HBEGF)Awai}$.

MECHANISMS, PATHOPHYSIOLOGY, AND TREATMENT OF FOOD ALLERGIES

Food Allergy, a Breakdown of Oral Tolerance

Our ability to maintain systemic unresponsiveness to orally ingested antigens is an active process occurring in gut-associated lymphoid tissues. Food antigens can cross the epithelial barrier following damage to the epithelium, through specialized intraepithelial passages, or *via* sampling by antigen presenting cells (APCs) (8). Oral exposure promotes the development of Foxp3$^+$ Tregs, including RORγt$^+$ Tregs that are induced by microbial signals in a Myd88-dependent manner (9–11). These prevent the development of allergen-specific IgE specialized CD103$^+$ dendritic cells in the gut, *via* a process involving TGF-β and retinoic acid, promote the differentiation of naïve T cells into Tregs (12). A break in tolerance can occur when the cytokine environment in the intestine favors the emergence of effector T helper 2 (Th2) cells and/or the reprogramming of Tregs to a pathogenic phenotype. Cytokines produced by gut epithelial cells, including IL-25, IL-33 and thymic stromal lymphopoietin (TSLP), may be particularly important drivers of this shift away from tolerance. IL-25 expression has been shown to be high in the small intestine in mouse models of food allergy, and overexpression of IL-25 increases Th2 cytokine production by type 2 innate lymphoid cells (ILC2s) and amplifies allergic responses. Conversely, lack of IL-25 is protective (13, 14). TSLP is similarly expressed in gastrointestinal epithelial cells in the setting of food allergy. Khodoun and colleagues demonstrated that monoclonal antibodies against IL-25, IL-33, or TSLP could each individually prevent the development of food allergy induced by oral gavage with egg white and medium chain triglycerides in mice (15). IL-33 produced by intestinal epithelial cells leads to an increase in OX40L expression on CD103$^+$ dendritic cells, which skews the immune profile towards Th2 (16, 17). IL-33 produced by keratinocytes following epicutaneous allergen exposure to damaged skin in mice, as can occur in atopic dermatitis, has been shown to promote activation of ILC2s, Th2 skewing, intestinal mast cell expansion and susceptibility to anaphylaxis (18). By increasing the production of IL-4 by ILC2s, IL-33 can also suppress the development of Tregs (19). Researchers at Stanford have shown that etokimab (anti-IL-33) (NCT02920021) can protect against reactions to oral peanut

challenge, reduce allergen-specific IgE, and decrease the levels of cytokines associated with food allergies such as IL-9 and IL-13 (20).

Some of the pro-sensitizing effects for food allergens are exerted by these same cytokines in the skin. In a mouse model of atopic dermatitis driven by the cutaneous application of ovalbumin (OVA) along with the Vitamin D analogue MC903, Noti, and colleagues observed that TSLP produced by keratinocytes promotes basophil expansion and the induction of a Th2 response (driven by basophil-derived IL-4) with food allergy (21, 22). Muto and colleagues reported a similar TSLP- and basophil-driven food allergy response in mice epicutaneously sensitized in the presence of SDS (23). Leyva-Castillo and colleagues have shown that exposure to food allergens *via* disrupted skin barrier has also been shown to promote mast cell expansion at a distance in the small intestine in a manner dependent on the induction of intestinal ILC2s by keratinocyte-derived IL-33 along with intestinal tuft cell-derived IL-25 (18).

Gastrointestinal Mast Cell Expansion in Food Allergy: IL-4, IL-9, and MMC9

In addition to IL-25 and IL-33, the Th2 cytokines IL-4 and IL-9 have been shown to play an important role in food allergy development. IL-9 is produced both by Th2 cells and by a subset of mast cells, IL-9-producing mucosal mast cells (MMC9). IL-9 promotes mast cell growth and lack of IL-9 prevents the induction of food allergy in mouse models (24, 25). Transgenic overexpression of IL-9 promotes the food allergy response by increasing mast cell number in the small intestine and enhancing gut permeability (24, 25). Th2 cells contribute to the development of MMC9 that provide a positive feedback loop for the expansion of mast cell numbers following oral challenge (26). IL-4, in addition to its role in promoting Th2 responses and driving IgE isotype switching in B cells, is also needed to support intestinal mast cell expansion following food allergen ingestion (27). This cytokine induces the antiapoptotic genes, Bcl-2 and Bcl-X(L), in mast cells in a STAT6-dependent manner (27). Competitive reconstitution studies using wild type mouse mast cells mixed with mast cells lacking the IL-4 receptor α-chain have revealed a strong competitive advantage of IL-4 receptor-bearing cells. Hogan and colleagues have recently demonstrated that IL-4, signaling *via* IL-4Rα induces MMC9 in a BATF-dependent mechanism, nicely linking the observed roles of IL-9 and IL-4 in mast cell homeostasis (28). IL-4 signals can subvert Tregs from their normally suppressive phenotype to one in which they express the Th2 transcription factor GATA-3 and Th2 cytokines, including IL-4, and thereby drive the disease phenotype (29). Investigators at Stanford and elsewhere are conducting trials exploring the potential of dupilumab (anti-IL-4Rα monoclonal antibody that prevents the binding of IL-4 and IL-13 to its receptor) in combination with anti-IgE (NCT03679676) during peanut oral immunotherapy (OIT).

Treatments for Food Allergy

Currently, there are no curative treatments for food allergies. The standard approach is education of individuals and families regarding strategies of allergen avoidance. While this is effective in preventing potentially serious reactions, it can significantly impact the quality of life of the patient and their family members. Avoidance paradoxically deprives these patients of one of the best-known paths to achieving tolerance to an antigen, namely ingestion. Furthermore, broad avoidance diets can result in nutritional deficiencies in multi-sensitized children (30, 31). Some children will eventually experience natural resolution of their allergy. Still, while this tends to occur for some foods, like egg and dairy, it is rare for others, like peanuts and tree nuts. In addition to practicing allergen avoidance, patients diagnosed with food allergies, must be presented with effective treatment plans on how to manage their reactions in case of accidental exposure. Mild reactions to foods, such as itching and hives, can be treated with anti-histamines, such as diphenhydramine and cetirizine. Though anti-histamines can alleviate the symptoms associated with allergy, they do not hinder the progression of the disease or reverse the disease-associated immune profile. Systemic anaphylaxis, the severe, life threatening reaction, presents as difficulty breathing or swallowing, and is treated immediately with epinephrine. Immunotherapy, which involves exposure to increasing doses of the allergen over a period of time, is the only disease-modifying treatment. Immunotherapy can be administered *via* several routes, including sublingual (SLIT), epicutaneous (EPIT) and oral (OIT) (32). Peanut powder (Palforzia®) OIT is the only current FDA-approved treatment for immunotherapy. A major limitation of OIT is that the food unresponsive state that is achieved is transient and maintaining it requires continued regular ingestion of the food (33). Adjunctive anti-cytokine treatments that might enhance safety as well as durability of immunotherapy are actively being explored (20, 34).

KEY PLAYERS INVOLVED IN FOOD ALLERGY: MAST CELLS AND BASOPHILS, IgE, AND IgE RECEPTORS

Mast Cells and Basophils: Effectors of Immediate Hypersensitivity

Mast cells and basophils arise from $CD34^+$ hematopoietic progenitor cells. Mast cells are long-lived tissue resident cells that differentiate locally from bloodborne progenitors (35). They are often found in vascularized sites that are exposed to the external environment and microbiome, such as the mucosa of the gastrointestinal and respiratory tracts (36). Basophils are short-lived cells that mature in the bone marrow, enter the circulation and can either be activated intravascularly or traffic to sites of inflammation to exert their functions (37–39). Because of their anatomic localization in barrier tissues, mast cells are likely to be one of the first cell types to encounter and respond to pathogens, making them important effector cells of the innate immune response. Several groups have identified their protective roles in bacterial infection and they are also critical in the immune response to parasites (36, 40). They express pathogen recognition receptors and can release anti-microbial peptides

upon activation. Mast cell granule proteases play an important role in detoxifying insect, scorpion, and reptile venoms (41–43). Like mast cells, basophils have been demonstrated to play key roles in host defense not only in the setting of Th2 responses, but also in the inflammatory reactions leading to helminth expulsion or tissue encystment and in resistance to ticks (44–48).

In addition to acting as effector cells of innate immunity, mast cells and basophils live at the interface of innate and adaptive immunity. Since they express Fc receptors, for IgE and IgG, they are armed with the adaptive immune capability to recognize specific antigens and participate in recall responses. They also regulate the emergence of adaptive responses. Granule components such as histamine can regulate T cell immunity and the antibody response, and mast cells and basophils are major producers of IL-4 and IL-13 (49–52). These two cytokines promote both Th2 cell differentiation and isotype switching of B cells to produce IgE.

The classic trigger for mast cell and basophil activation is through the IgE-mediated crosslinking the IgE receptor, FcεRI. This activation results in the degranulation of the cells, releasing preformed mediators (such as histamine, neutral proteases, and TNF-α), *de novo* synthesis of pro-inflammatory lipid mediators, and production of growth factors, cytokines, and chemokines (53, 54).

FcεRI and Its Downstream Signaling Pathways

IgE is the least abundant antibody in circulation. Its concentration in plasma is roughly 10^5 times less (100 ng/ml vs 10 mg/ml) than that of IgG. In addition to being present at such low concentrations, IgE has a notably short half-life, less than 1 day, while that of IgG is around 3 weeks (55). However, most of the IgE in the body is found in a cell-bound state, owing to the incredibly high affinity for IgE by FcεRI (K_d $1x10^{-9}$mol/L). IgE effectively remains permanently attached to FcεRI until internalized, which makes the tissue half-life of IgE to likely be on the order of weeks to months (55, 56).

There exist two isoforms of the high affinity IgE receptor, FcεRI. The tetrameric $\alpha\beta\gamma_2$ form is found on mast cells and basophils. The trimeric $\alpha\gamma_2$ form, though less abundant than the tetrameric, is present on eosinophils, platelets, monocytes, and dendritic cells. The alpha chain has the ligand binding site, with two Ig-like domains that bind IgE. The β chain contains four transmembrane spanning regions and it amplifies the signal generated by the γ subunit. The γ chains are dimeric disulfide-linked transmembrane proteins. The β and γ chains contain immunoreceptor tyrosine-based activation motifs (ITAMs). An ITAM is a conserved sequence containing tyrosine separated from a leucine or isoleucine by two amino acids (YxxL/I). The ITAM tyrosine is a substrate for phosphorylation by signaling kinases such as Lyn and Syk. Two ITAMs are often found together separated by 6 to 8 amino acids (YxxL/I x(6-8) YxxL/I).

When multivalent antigens bind to FcεRI-bound IgE, proximal FcεRI aggregate into lipid rafts that are rich in cholesterol, sphingolipids, protein tyrosine kinases (PTKs), and GPI-anchored proteins. Recognition of one antigen molecule by Fab sites on two different IgE molecules bound to neighboring FcεRI, results in receptor crosslinking and transphosphorylation of the ITAMs. Antigen recognition mediates a rapid cascade of signaling events that induce the activation of PTKs of the SRC and TEC families (57–59). This activation leads to the release of preformed, rapidly formed, and newly synthesized mediators from the effector cells (60) (see **Figure 1**).

Tyrosine residues on ITAMs on β and γ chains are phosphorylated by receptor-associated Lyn tyrosine kinase found constitutively associated with the β chain. Phosphorylation of tyrosine residues in the ITAMs provides docking sites for Src homology 2 (SH2) domain-containing kinases, such as Syk PTK to the γ chains and recruits additional Lyn PTK. Syk is phosphorylated by Lyn and activated by conformational changes (61, 62). Phosphorylated Syk subsequently phosphorylates adapter molecules LAT1, LAT2, SLP-76, Grb2, and VAV at tyrosine sites leading to the formation of supramolecular plasma membrane-localized signaling complexes. These signaling events lead to the activation of other signalling pathways such as PI3K, PLC-γ, RAS/ERK, JNK, p38, and AKT. They also lead to the immediate exocytosis of granules and their pre-formed mediators, rapid synthesis of prostaglandins and leukotrienes and eventual transcription of cytokines genes, such as IL-4, IL-6, IL-10, and TNF-α (60).

In addition to driving the allergic effector functions of mast cells and basophils, there is evidence that some IgE antibodies can provide survival signals to mast cells even in the absence of allergen. These have been designated "cytokinergic" IgEs by Kawakami and colleagues (63). In the absence of the survival cytokines necessary for mast cells, such as SCF, cytokinergic IgE antibodies can provide a survival-enhancing effect (64, 65). Furthermore, they have been shown to induce cytokine production, calcium flux, histamine release, and leukotriene synthesis (66–68). Cytokinergic IgE antibodies may act by self-associating or binding to autoantigens in order to aggregate FcεRIs.

Glycosylation of IgE molecules has been shown to correlate with IgE binding to FcεRI and for effective signaling upon receptor cross-linking. IgE antibodies are very heavily glycosylated when compared with other immunoglobulin isotypes. Anthony and colleagues demonstrated the functional significance of IgE glycans. They found that one at N394 was obligatory for FcεRI binding (69). Subsequent comparison of plasma IgE molecules between allergic and non-allergic individuals by mass spectrometry by the same group showed that the "allergic IgE" has more terminal sialylation of its glycans than "non-allergic IgE". The degree of sialylation of IgE does not affect its binding to FcεRI but modulates the signaling downstream of the receptor and activation of the cell (70).

CD23: The Low Affinity Receptor for IgE

IgE can also bind to and exert its effects through its low affinity receptor CD23 (FcεRII). CD23 is broadly distributed with expression on B cells, dendritic cells, eosinophils, gastrointestinal and respiratory epithelial cells, and others (71). It is a type II transmembrane protein (N-terminus intracellular)

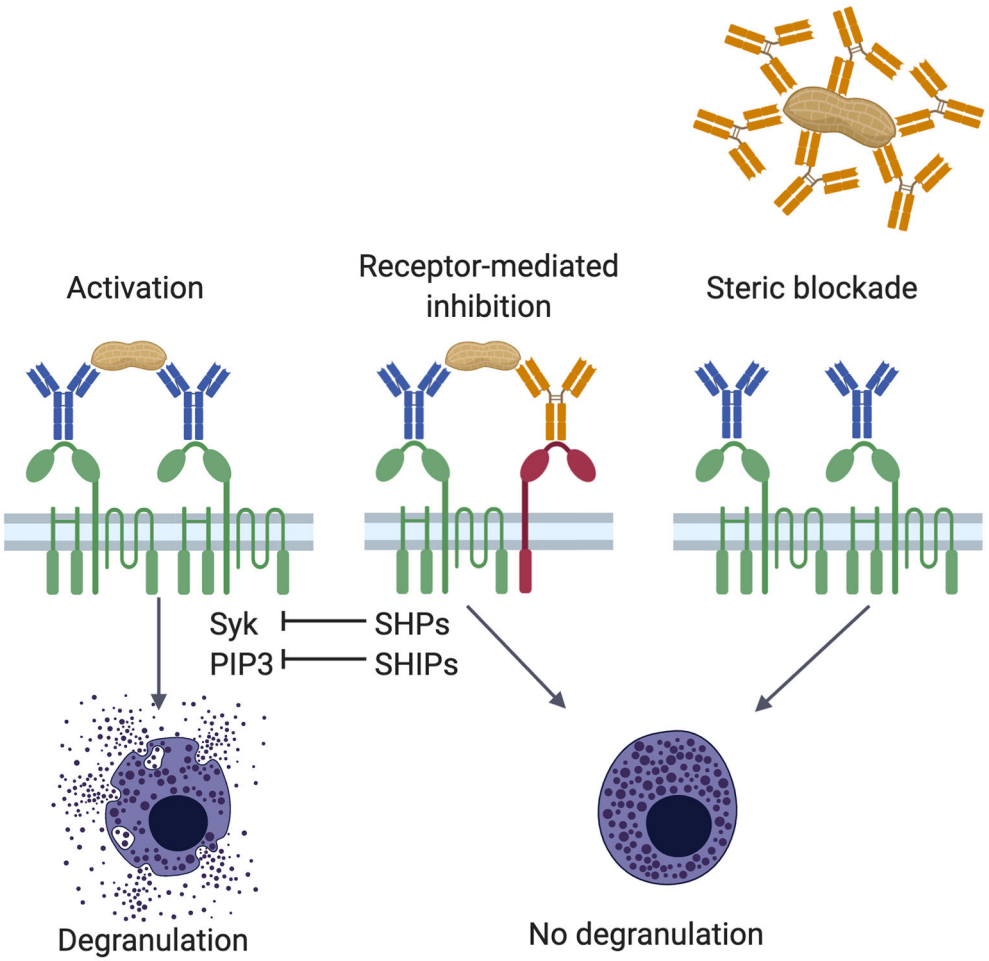


FIGURE 1 | Mechanisms of IgG mediated inhibition. Antigen encounter by neighboring FcεRI (green)-bound IgE (blue) on the surface of mast cells or basophils induces receptor crosslinking, phosphorylation of cytosolic immunoreceptor tyrosine-based *activation* motif (ITAM) sequences in the tetraspanning β- and disulfide-linked γ-chain dimers, and activation of various signaling pathways involving protein tyrosine kinases, such as Syk, and inositol intermediates, including PIP_3 (left panel). These positive signals culminate in the degranulation of the cell. Allergen specific IgG antibodies (orange) counter the effects of IgE in two ways, receptor-mediated inhibition (center panel) and steric blockade (right panel). In receptor-mediated inhibition, when polyvalent allergens are simultaneously engaged by FcεRI-bound IgE and FcγRIIb (red)-bound IgG, crosslinking of the two receptors leads to phosphorylation of FcγRIIb cytosolic immunoreceptor tyrosine-based *inhibition* motifs (ITIMs). These recruit protein tyrosine phosphatases and inositol phosphatases, such as SHPs and SHIPs, respectively. Phosphatases can neutralize phosphoprotein (such as Syk) and phospholipid (such as PIP_3) signaling intermediates induced by FcεRI activation. In steric blockade, IgG antibodies bind the allergen before it reaches receptor-bound IgE. By masking IgE binding epitopes, these blocking IgG antibodies inhibit interaction with IgE and thereby prevent FcεRI-mediated mast cell activation.

assembled as a multimer with α-helical coiled-coil stalks terminating in C-type lectin heads that bind to IgE (see **Figure 2**) (72). CD23 is the only Fc receptor that is not part of the immunoglobulin superfamily. In addition to binding IgE, it also interacts with the B cell surface protein, CD21, which functions as complement receptor 2 (CR2) and is the binding site and entry point for Epstein-Barr virus in B cells (73). Protease-sensitive sites present in the stalks of CD23 can be cleaved by endogenous proteases such as the metalloproteinase, ADAM10, and by protease allergens including the dust mite protease, *Der p 1*. The released oligomeric CD23 heads are called soluble CD23 (sCD23) and retain their ability to bind IgE.

IgG RECEPTORS

In addition to FcεRI, human and mouse mast cells and basophils also express Fcγ receptors (FcγRs) that bind to IgG antibodies. FcγRs belong to the immunoglobulin superfamily and their patterns of expression vary among leukocytes (74–77). The general consensus is that human mast cells and basophils express FcγRIIa and FcγRIIb, while mouse mast cells and basophils express FcγRIIIa and FcγRIIb (78). FcγRIIb is the only inhibitory IgG receptor, and all others are activating. IgG and FcγRs are involved in a number of immune defense mechanisms including toxin neutralization, antibody

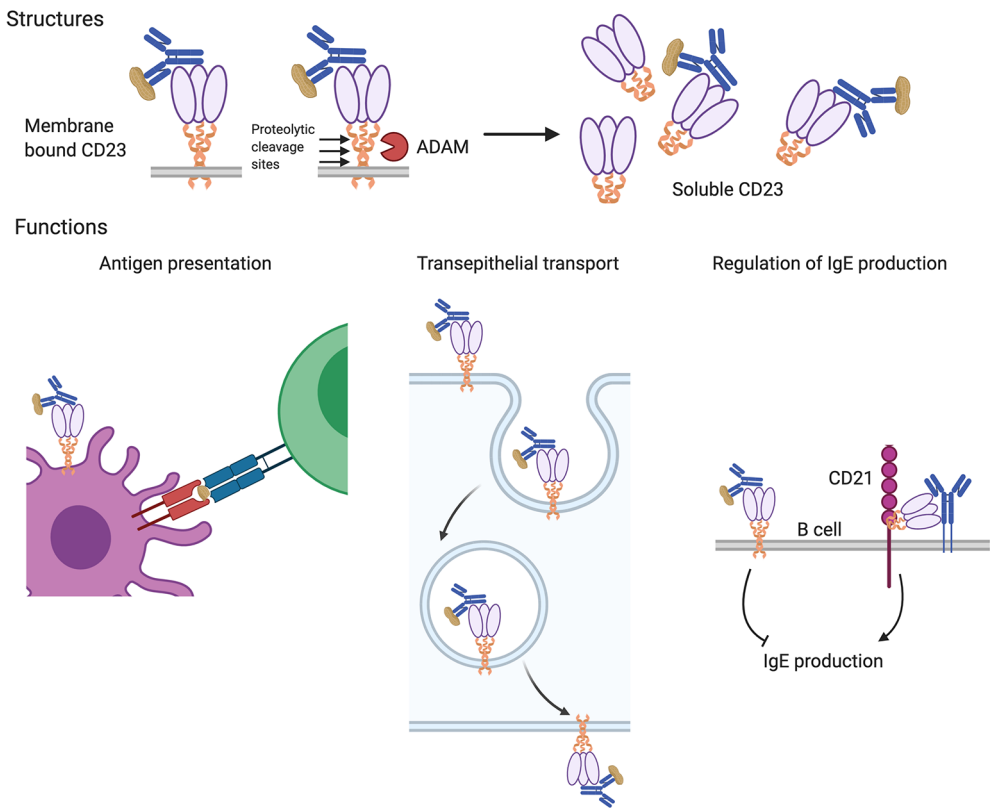


FIGURE 2 | Structures and functions of the low-affinity IgE receptor, CD23. CD23 is expressed as a multimer of subunits consisting of coiled-coil stalks with lectin-family domain heads that bind to IgE (upper panels). Membrane-bound CD23 can be converted to a soluble form that retains IgE-binding ability following cleavage at protease sites by endogenous (ADAM) or allergen (e.g. *Der p 1*) proteases. Various functions have been attributed to CD23 (lower panels). It can facilitate antigen uptake for presentation by B cells and antigen presenting cells to T cells (left panel) and mediate the transport of allergens across polarized epithelium in the gut and airway (center panel). CD23 also regulates IgE production; the transmembrane form on B cells suppresses their production of IgE and the soluble form, *via* interactions with B cell surface CD21 and IgE, enhances IgE production (right panel).

dependent cell-mediated cytotoxicity, phagocytosis, and cytokine production.

Activating IgG Receptors: IgG-Mediated Anaphylaxis

The classical paradigm for allergic reactions centers on antigen aggregation of IgE, bound to FcεRI on mast cells and basophils, leading to their activation. However, even before the discovery of IgE, IgG antibodies had been shown to be able to activate mast cells. In the 1950s, Ovary, Benacerraf and others found that the ability of serum to transfer cutaneous hypersensitivity from one animal to another resided in both a heat-stable gamma-globulin fraction of serum (IgG) and in a heat-labile reagin fraction (79, 80). The relevance of these observations in cutaneous hypersensitivity was confirmed for systemic anaphylaxis more than four decades later when it was reported that active systemic anaphylaxis can be elicited in *i.v.*-challenged mice lacking the Cε exons encoding the IgE heavy chain (76, 81–83). Like FcεRI, crosslinking of activating IgG receptors by immune complexes results in the phosphorylation of ITAMs and a cascade of signaling events (84, 85). While FcεRI-mediated anaphylaxis is histamine dependent, FcγRIIIa-mediated anaphylaxis involves the release of platelet activating factor from macrophages (86). As demonstrated in mice, IgG-mediated anaphylaxis, unlike IgE-mediated anaphylaxis, occurs predominantly following intravascular antigen exposure and has been shown to require much higher doses of the antigen (86). The existence of IgG-mediated anaphylaxis in humans has not been directly proven and remains somewhat controversial. However, the elicitation of reactions with the physiologic features of anaphylaxis after intravascular administration of agents to which no IgE antibody response can be detected suggests the existence of an IgG pathway. The integration of input signals from activating and inhibitory receptors determines the outcome of the local and systemic inflammatory responses.

FcγRIIb: The Inhibitory IgG Receptor

FcγRIIb is the only inhibitory IgG receptor and one of its main function is to turn off signals initiated by activating Fc receptors and the B cell receptor (BCR). The importance of IgG-mediated feedback control of humoral immunity by FcγRIIb has long been appreciated (87). Co-aggregation of FcγRIIb with the BCR

increases the BCR activation threshold and suppresses B cell-mediated antigen presentation. In the absence of FcγRIIb, the amount of antigen needed to activate B cells is increased, and production of antibodies to T cell-dependent antigens is suppressed (88). FcγRIIb signals can inhibit the maturation of dendritic cells as well as their FcγR-mediated antigen presentation and T cell priming (89, 90). In macrophages, FcγRIIb inhibits FcγR-mediated phagocytosis and release of cytokines (91–93). Overall, FcγRIIb is the most widely expressed FcγR and its presence on various cell types is required for maintenance of peripheral tolerance.

Unlike activating receptors, the aggregation of FcγRIIb alone is of no consequence and binding of IgG or immune complexes solely to FcγRIIb does not inhibit the functions of a resting cell. The inhibitory receptor is expressed on the surface of cells in conjunction with activating receptors, and arming the inhibitory function of FcγRIIb requires an initial licensing signal by an activating receptor. When immunoglobulins on activating and inhibitory receptors recognize independent epitopes in cis on a common antigen, the receptors co-aggregate in lipid rafts for crosslinking to occur (94, 95). FcγRIIb lacks the gamma chain and therefore has no ITAMs. Instead it contains an immunoreceptor tyrosine-based inhibitory motif (ITIM) in its cytosolic domain. ITIMs recruit protein tyrosine phosphatases and inositol phosphatases (96).

ITIMs were first identified in FcγRIIb and subsequent sequence alignments revealed their presence in a large number of inhibitory receptors (96, 97). Following ligand binding and aggregation with an activating receptor, tyrosine residues in these ITIMs are phosphorylated (98, 99). These ITIM phosphotyrosines are docking sites for Src homology 2 (SH2) domain-containing protein tyrosine phosphatases (SHPs) and inositol phosphatases (SHIPs). SHIP-1 dephosphorylates phosphatidylinositol (3, 4, 5)-triphosphate (PIP_3) to phosphatidylinositol diphosphate (PIP_2) (see **Figure 1**). This reaction depletes membrane PIP_3 docking sites for PH-domain-containing signaling intermediates, such as BTK, and consumes the substrate in the formation of IP_3, which participates in mast cell activation by inducing calcium release from endoplastic reticulum stores. Mast cells deficient in SHIP-1 degranulate at lower concentrations of IgE and antigen, and they also degranulate much more strongly (100). Therefore, SHIP-1, at baseline, raises the threshold for FcεRI mediated mast cell activation.

It is commonly understood that the inhibitory function of FcγRIIb is mediated by its coaggregation with an activating receptor by the same multivalent antigen. This is described as FcγRIIb-mediated "cis-inhibition". However, Malbec et al. show that FcγRIIb can also mediate inhibition of activating receptors triggered independently of, and not co-aggregated with, FcγRIIb (101). The authors describe this phenomenon as "trans-inhibition". Trans-inhibition can decrease the release of β-hexosaminidase, histamine, LTC-4, MIP1-α, and TNF-α from mast cells and basophils, and decrease anaphylaxis. The $F(ab)_2$ portion of IgG was not capable of mediating this inhibitory effect, suggesting that the phenotype is dependent on receptor-mediated action of IgG.

FcγRIIb in Disease

Due to its known roles in inhibiting the activation of immune cells, FcγRIIb has been considered as a potential regulator in various immunological disorders, especially in autoimmune diseases.

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with autoantibody production affecting many organs including the skin, brain, joints, and kidneys. Abnormal B cell responses to immune complexes containing autoantigens is a feature of SLE. Immune complexes exert their effects *via* activating FcγRs, and impaired FcγRIIb function has been shown to be involved in the disease pathogenesis (102). In rheumatoid arthritis (RA), autoantibodies mediate the destruction of the synovial membrane. Variants in FcγRIIb have been shown to be associated with RA (103). In a collagen-induced arthritis mouse model of RA, lack of FcγRIIb increases the disease score, cartilage destruction, and concentration of collagen specific IgG. In patients with idiopathic thrombocytopenic purpura (ITP), the immune recognition of autoantigens on platelets triggers their destruction. The same variant form of FcγRIIb identified in patients with RA has been shown to be associated with ITP (104). In a mouse model of multiple sclerosis, lack of FcγRIIb is associated with increase in disease score and more activation of myelin-specific T cells. Administration of intravenous immunoglobulin (IVIG) has been successfully used to treat patients with autoimmune diseases during acute flares. IVIG induces the expression of FcγRIIb on blood basophils, monocytes, and eosinophils (105). In contrast, lack of FcγRIIb, or variants of the protein that decrease its function may confer an advantage for fighting infections (106). For example, FcγRIIb deficiency is associated with resistance to streptococcal infections in mice (93, 107). Variants of FcγRIIb associated with SLE ($FCGR2B^{T232}$) have been shown to be more prevalent in areas where malaria is endemic and may confer protection against the infection (108).

REGULATION OF IMMUNE RESPONSES BY IgE AND IgG ANTIBODIES ACTING ON MAST CELLS AND BASOPHILS

The opposing effects of IgE antibodies, triggering activating input *via* FcεRI, and IgG antibodies, sending ITIM-mediated inhibitory signals *via* FcγRIIb, are critical in controlling the acute effector functions of mast cells and basophils. These are the pathways that respectively drive immediate hypersensitivity reactions in food allergy, including anaphylaxis, and suppress acute reactions in subjects who have developed food-specific IgG responses. However, the opposing roles of IgE and IgG in food allergy extend significantly beyond these direct effects on effector cell activation, with immunoregulatory influences ranging from

modulation of IgE receptor density and signaling thresholds in effector cells, to the IL-4-driven priming and maintenance of effective Th2 responses to food allergens and corresponding suppression and/or pathogenic reprogramming of Tregs. These immediate and downstream functions of food allergen-specific IgE and IgG antibodies extend the rationale for IgE blockade by omalizumab during OIT and form the mechanistic basis for the development of food allergen-specific IgG monoclonal antibodies as novel therapeutics for food allergy. Here we provide a detailed review of the immunomodulatory effects of IgE and IgG antibodies as they relate both to innate effector cell functions and to downstream regulation of adaptive immune responses in food allergy.

IgE and Regulation of IgE Receptor Density

IgE and its receptors have symbiotic relationships. It has been known for many years that plasma IgE levels and basophil FcεRI expression are regulated in tandem in atopic patients (109), but until recently it had not been possible to distinguish between correlation and causation. Saini and colleagues showed that the relationship between circulating IgE and basophil FcεRI levels holds up across a spectrum of allergic, infectious, and immunodeficiency disorders associated with dysregulated IgE levels, indicating that atopy, itself, is not the driver and suggesting that IgE antibodies themselves have an effect on FcεRI levels (110). Such a direct effect of IgE on FcεRI expression was supported by studies of a rat basophilic leukemia line in which FcεRI levels increased when IgE antibodies were added to the culture (111). Subsequent mouse genetic analyses showed that this interaction is indeed operative *in vivo* at physiologic IgE levels. $IgE^{-/-}$ mice had greatly reduced FcεRI expression both on circulating basophils and on tissue mast cells, and both cell types rapidly upregulated FcεRI following IgE infusion (112, 113). This phenomenon could be reproduced in culture using freshly isolated primary mast cells, or cultured bone marrow derived mast cells (BMMCs), and has subsequently been confirmed in human mast cells, basophils, and dendritic cells (114–117). IgE-mediated modulation of FcεRI levels, in turn, regulates effector cell activation thresholds. In the presence of low ambient IgE, decreased basophil surface FcεRI is associated with an increased threshold for activation by allergen (115). The presence of IgE stabilizes FcεRI, preventing internalization and degradation. As FcεRI continues to be synthesized within a cell, the presence of IgE favors the capture and accumulation of FcεRI at the cell surface (118). A similar relationship exists for IgE and CD23. Occupancy of CD23 by IgE protects its protease-sensitive sites from cleavage, and CD23 levels are low in animals lacking IgE and increase directly in relation to ambient IgE levels (56).

IgE and Mast Cell Homeostasis

In addition to enhancing FcεRI levels on the surface of mast cells, IgE antibodies promote mast cell survival and proliferation *in vitro* and *in vivo*. The derivation and propagation of mast cells from bone marrow stem cells require the presence of IL-3 and SCF. Without these cytokines, BMMCs undergo apoptotic cell death. However, the addition of IgE both enhances the proliferation of BMMCs in the presence of IL-3 and SCF, and protects them from apoptosis upon withdrawal of IL-3, suggesting an important role for IgE in mast cell expansion in settings of type 2 inflammation, and for survival in growth factor limiting conditions (64, 65). Such a function of promoting mast cell expansion and survival has in fact been observed in mouse models of parasitic worm infections. IgE promotes splenic mast cell expansion and parasite clearance in the course of *Trichinella spiralis* infection (119). Similarly, in an asthma model, sensitization of mice by inhalation of the fungus *Aspergillus fumigatus* drives mast cell expansion in the bronchus, trachea, and spleen and this expansion is dependent on the presence of IgE antibodies (120). Together, these findings reveal that IgE antibodies not only act to trigger mast cell degranulation and regulate FcεRI levels, but also promote mast cell survival and expansion.

Downregulation of IgE Receptor by Omalizumab

Omalizumab is a recombinant humanized IgG antibody that recognizes the Fc portion of IgE. Its affinity for free IgE is greater than that of IgE for its receptor so it can effectively compete for free IgE and prevent its binding to cell surface FcεRI. However, omalizumab cannot effectively disrupt established interactions between IgE and FcεRI. Once free IgE is captured by omalizumab, immune complexes are formed that are eventually cleared from circulation. By reducing the ambient free IgE concentration, omalizumab leads to a downregulation of FcεRI. Allergic patients that have been treated with omalizumab have low density of FcεRI on the surface of basophils, mast cells, and dendritic cells (114–117). Thus, more than the removal of circulating allergen-specific IgE, this secondary effect of omalizumab on FcεRI density is critical in its mechanism of action in the treatment of hypersensitivity reactions.

Mast Cells and Basophils as Producers of Th2 and Pro-Inflammatory Cytokines

The role of IgE and mast cells as effectors of immediate hypersensitivity in food allergy is well characterized. However, in addition to rapidly releasing the vasoactive mediators of anaphylaxis, IgE-activated mast cells serve as an important source of immunomodulatory cytokines. Cytokine production requires the activation of a transcriptional program and is therefore delayed. Thus, several hours after FcεRI is crosslinked, as the symptoms of immediate hypersensitivity abate, IgE-activated mast cells produce a range of chemokines and cytokines important for orchestrating the influx of innate immune cells (eosinophils and basophils) and T cells important in driving type 2 inflammation (121, 122). Indeed, IL-4, the critical inducer of Th2 responses, was first reported to be produced by mast cells, in a study conducted by Marshall Plaut, Bill Paul, and colleagues (49). Mast cells are also prolific inducers of IL-6 and TNF-α, cytokines critical in the activation of APCs and induction of inflammation. Thus, mast cells, present in abundance in the gastrointestinal tract, are prime candidates to be the innate

immune inducers of immunological sensitization and Th2 responses. Similar to mast cells, IgE-activated basophils are potent producers of cytokines, particularly IL-4 and, following adjuvant exposure, TSLP and IL-25 (50–52, 123).

Mast Cells, Basophils, ILC2s, and IgE Antibodies Act as Adjuvants in Mouse Models

Upon IgE-mediated activation *via* FcεRI, mast cells positioned at mucosal surfaces and in the skin as well as basophils recruited to these sites can prime the allergic immune response by influencing both innate and adaptive immune cells. Effective induction of T cell responses to contact sensitizers applied to the skin occurs only when mast cells and IgE are present, and local cutaneous inflammatory responses to superantigens are mast cell dependent (124, 125). Mast cell-derived TNF-α is thought to induce Langerhans migration from the epidermis to draining lymph nodes in contact sensitivity models (126) and mast cell derived histamine may activate Langerhans activation in mice injected intradermally with IgE followed by antigen challenge (127). Although basophils have not been implicated in pathways of immune priming in contact sensitivity, they have, as already noted, been shown to contribute to immunological priming in the skin in the setting of allergic inflammation induced by M903 application or barrier disruption by SDS (21, 23). When basophils are depleted or when basophils lack the ability to produce IL-4, the outcomes of sensitization and challenge are altered, with reduced allergen-induced responses (22, 23). In some models of asthma, mast cells, basophils, and IgE play important roles in orchestrating allergic sensitization and effector responses (128–130). The role of mast cells as endogenous adjuvants is most pronounced in settings where artificial adjuvants are not employed, as shown by Galli and colleagues in an alum-independent mouse model of OVA-driven asthma (128).

The constitutive presence of mast cells in the intestinal mucosa has drawn attention to their potential contribution to immunological priming in the gut in food allergy. An adjuvant role of mast cells in food allergy was demonstrated by Burton and colleagues using a mouse model of peanut allergy (131). They found that two independent strains of mice lacking mast cells, $Kit^{W\text{-}sh}$ and $Mcpt5^{cre}$iDTR, exhibited decreased peanut-specific IgE production and impaired peanut-specific Th2 responses, but maintained relatively robust induction of Tregs. They further established that signaling pathways downstream of FcεRI are needed to drive allergic sensitization. For instance, mast cell lineage-specific deletion of Syk kinase in $Mctp5^{cre}$-$Syk^{fl/fl}$ mice, and pharmacologic inhibition of Syk kinase function, both separately recapitulated the phenotype of suppressed peanut allergy. Furthermore, blockade of IgE with anti-IgE antibodies and genetic removal of IgE ($IgE^{-/-}$ mice) both independently led to impaired Th2 responses to peanut ingestion and still permitted the development and expansion of Tregs. The observation that mast cell-deficient mice reconstituted with wild type, but not IL-4-deficient, mast cells had restored Th2 responses to peanut strongly implicated mast cells as a key source of IL-4 in this system. IL-4 also negatively impacts the numbers and functions of Tregs so that, while promoting Th2 immunity, it also suppresses and potentially subverts the mechanisms that keep it in check. Chatila and colleagues showed that IL-4 can subvert Tregs to a pathogenic phenotype, expressing Th2 transcription factor GATA-3 as well as IL-4, a state which contributes to, rather than suppresses, allergic disease (29). Taken together these findings established the critical roles of gut mast cells as inducers of Th2 immunity, a function that, during recurrent allergen exposures and evolving adaptive immune responses, is amplified by food-specific antibodies, which signal *via* FcεRI.

In contrast to the analyses of basophil contributions to immune sensitization in the skin, the role of basophils in priming immune sensitization to ingested allergens has been less extensively studied. Using basophil depletion (Ba103 anti-CD200R3 mAb) or basophil-deleted Bas-TRECK mice, Kawakami and colleagues demonstrated attenuation of clinical scores, diarrhea incidence and plasma levels of the mast cell protease mMCP-1 following intragastric allergen instillation in mice primed intraperitoneally and then enterally challenged with OVA (132). Total and OVA-specific IgE responses were not different between basophil-deficient and -sufficient mice arguing against adjuvant immune priming effect in this model. Along with mast cells, basophils also contribute to systemic anaphylaxis. In a mouse model of peanut induced anaphylaxis, selective, or inducible ablation of basophils, without affecting the mast cell compartment, has been shown to reduce hypothermia (133).

In addition to mast cells and basophils, ILC2s have been identified as critical innate immune sources of IL-4 and inducers of Type 2 immune responses. It turns out there is a critical interplay between these cell types, revealed in murine models of food allergy. Like Th2 cells, ILC2s express the transcription factor GATA-3 and secrete Th2 cytokines, including IL-5 and IL-13. Unlike Th2 cells, they lack T cell receptor (TCR) and cannot recognize antigen. In response to epithelial-derived cytokines, such as IL-25 and IL-33, they produce large amounts of IL-5, IL-9, and IL-13 (134). Recent findings indicate that in addition to being primed by epithelial cell-derived cytokines, ILC2s can also be activated in a mast cell driven manner, and conversely that effects of ILC2s on mast cells can influence the severity of anaphylaxis in food allergy. In mouse models of food allergy using OVA or peanut, the induction of ILC2s was significantly impaired in mice lacking IgE antibodies and those lacking mast cells (135). Furthermore, in these same murine models, IL-13 produced by ILC2s can regulate the severity of anaphylaxis by increasing sensitivity of target organs to mediators of hypersensitivity reactions (135). Recent work by Leyva-Castillo et al. in studies of food allergy-induced by epicutaneous food exposure revealed that the interaction between mast cells and ILC2s might be bidirectional. They found that intestinal mast cell expansion driven by mechanical skin injury and allergen exposure requires IL-4 and IL-13 derived from ILC2s (18).

Regulation of Allergic Responses by IgE Antibodies in Humans and Mice With Humanized IgE Receptor Expression

The roles of mast cells and IgE in regulating Th2 responses in allergic disease in humans are not as clearly established but there is some evidence for such a connection. Testing the immunomodulatory effects of IgE blockade in OIT with food-allergic subjects offers an opportunity to test this question. In humans, omalizumab has been reported to facilitate more aggressive up-dosing, while also reducing allergic reactions during the course of the treatment (136–138). Stranks et al. hypothesized that IgE blockade might also alter the immunological changes that are induced by OIT. The authors tested this in the PRROTECT cohort of highly peanut-allergic subjects, randomized to receive either standard OIT or OIT in combination with omalizumab (139). While their analysis was somewhat limited by the fact that patients had the option to switch to open-label omalizumab part way through the study, which most did, the analysis revealed that those initially assigned to the omalizumab group, who therefore received the initial peanut dose escalation in the setting of IgE blockade, exhibited a more robust induction of anti-peanut IgG antibodies, one of the key markers of successful OIT (139). This finding suggests that in human food allergy IgE antibodies might have an immunoregulatory effect and that blockade of its receptors or their signaling pathways in mast cells might be an effective strategy for preventing or reversing food allergy. In addition to enhancing tissue-resident mast cell production of IL-4, to prime and consolidate local Th2 and IgE responses, IgE antibodies may further promote adaptive immune responses by enhancing the ability of APCs to prime T cells. Both IgE receptors, FcεRI and CD23, are expressed by APCs and can mediate internalization of allergen complexed with IgE (140).

In humans, the trimeric form of the high affinity IgE receptor, FcεRI, is expressed on APCs. Studies of the skin of patients affected by atopic dermatitis have revealed the presence of several FcεRI$^+$ APCs. These include Langerhans cells and inflammatory dendritic cells (which do not contain Birbeck granules) in the epidermis, and dermal dendritic cells. FcεRI is markedly upregulated on these cell types during allergic flares. However, the lack of FcεRI expression by murine APCs has made it challenging to investigate whether it, like CD23, might promote T cell responses *in vivo*. Mice with humanized expression of FcεRI, using an FcεRI α–chain transgene driven by the CD11c promoter constitutively active in APCs, have proven useful in answering this question. These animals were used to show that allergen-specific IgE, acting *via* FcεRI on APCs, instructed naïve T cells to differentiate into Th2 cells, resulting in augmented allergen-specific Th2 responses *in vivo* (141).

Immunoregulatory Effects of CD23

The ability of CD23 to participate in the priming of T cell responses *in vivo* was first reported in mice as facilitated antigen presentation, a process whereby IgE antibodies generated in response to a previous allergen encounter, amplify Th2 responses upon re-exposure to that allergen in a mechanism mediated by CD23 (see **Figure 2**). Recent studies of facilitated antigen presentation by Heyman and colleagues suggest that IgE: allergen complexes, bound to circulating B cells *via* CD23 enter splenic B cell follicles, where antigen is transferred to resident dendritic cells *via* B cell exosomes generated in a protease (ADAM10) and CD23-dependent process, activating them for efficient antigen presentation (142). Consistent with this model, exogenous IgE does not augment T cell responses in CD23$^{-/-}$ mice but does enhance humoral and cellular immunity following reconstitution with CD23$^+$ B cells. Both the diversity of the IgE repertoire for specific allergens (the range of recognized epitopes) and the avidity of the pooled IgE for antigen affect the efficiency of facilitated antigen presentation (143).

CD23 expressed by B cells appears to play a role in regulating IgE synthesis. Ligation of the receptor by IgE suppresses IgE production and CD23-deficient mice exhibit stronger and longer-lasting IgE responses after immunization (144–146). Conversely CD23 transgenic animals exhibit decreased IgE production (147, 148). In humans, treatment with lumiliximab, a CD23-blocking monoclonal antibody, lowers IgE levels (149). In contrast, soluble fragments of CD23 (sCD23) seem to promote IgE synthesis, perhaps by competing for IgE binding with cell-bound CD23 (see **Figure 2**) (149–151).

THE REGULATORY EFFECTS OF IgG, SIGNALING *VIA* FcγRIIb IN IgE-MEDIATED FOOD ALLERGY

Natural Resolution of Food Allergy Is Associated With IgG Induction

Many children with low to moderate levels of food-specific IgE antibodies can ingest the foods to which they are sensitized without exhibiting any reaction. On the one hand, this observation creates a tremendous challenge for allergy clinicians trying to establish or rule out food allergy using IgE testing. Oral food challenges, in a clinical setting, are often required to establish with certainty whether a child is tolerant or allergic. On the other hand, the inconsistent correlation between food-specific IgE and reactivity provides an important clue regarding the regulatory factors that might block immune responses to foods. There is now abundant evidence that IgG antibodies account both for natural protection from allergic reactions to foods in patients harboring food allergen-specific IgE, and that the induction of IgG responses underlies, at least in part, the protective effects of OIT. An analogous situation has been described for respiratory allergy. In large population-based cohort studies in Australia and the UK, Custovic and colleagues have established that aeroallergen-sensitized children, with aeroallergen-specific IgE antibodies, often have no symptoms. This led to the concept of "benign Th2 immunity" and further analysis of these subjects revealed that those with higher allergen-specific IgG/IgE ratios had fewer symptoms and their sera inhibited the activation of basophils sensitized with

aeroallergen-specific IgE (152, 153). One challenge in diagnosis is that circulating IgE and IgG levels may not reflect the amounts of these antibodies present in the mucosal tissues where allergic reactions are initiated.

Several investigations have revealed that natural resolution of milk allergy in children is associated with increasing IgG levels (154, 155). Similarly, among subjects who test positive for IgE antibodies to peanut, Santos and colleagues found that higher levels of specific IgG4 correlate with tolerance (156). For the most part, analyses of any protective effects of IgG in food allergy have focused on the IgG4 isotype. A protective role for IgG4 had previously been established to be a very strong biomarker of efficacy in subcutaneous allergen immunotherapy (SCIT) (157). The IgG4 findings in SCIT led to a focus on this subclass in food allergy studies and it is the only subclass of IgG for which specific tests have been commercially developed. IgG4 is the least abundant isotype of IgG in human serum, often representing only about 5% of total IgG (158, 159). However, with chronic antigen exposure, IgG4 can increase to account for a larger fraction of total IgG (160). Like IgE, IgG4 is induced during Th2 immune responses under the action of IL-4 and IL-13 on B cells. The concept of a "modified Th2 response," has evolved to describe a scenario where IL-10 is present along with IL-4, and IgG4 class switching and production is promoted over IgE (159, 161–163).

IgG4 is the sole subclass of IgG in that it does not mediate common IgG effector functions such as antibody-dependent cell-mediated cytotoxicity or complement dependent-cytotoxicity. IgG4 antibodies also exhibit a unique ability to undergo Fab arm exchange (FAE). In this process, heavy chains of IgG4 antibodies can separate into half antibodies, each consisting of one heavy chain and one light chain. Half antibodies originating from different parent IgG4s can combine to form bispecific antibodies (164). This re-assortment of Fab regions potentially allows a single IgG4 to recognize two epitopes on an allergen, both increasing overall binding avidity and facilitating crosslinking. Due to its presence in the serum at higher concentration than IgE, its ability to recognize more epitopes and its limited ability to form immune complexes and mediate effector function, it has been proposed that IgG4 might be uniquely suited to function as a blocking antibody, intercepting allergens before they can be engaged by FcεRI-bound IgE on the surface of effector cells.

Induction of High Levels of Specific IgG Following OIT

Perhaps the most compelling evidence for a role of IgG antibodies in regulating food allergen responsiveness has come from OIT studies. In OIT, an allergenic food is administered orally in daily doses that incrementally increase, often up-dosing weekly, over the course of several months. Upon completion of OIT, patients can typically tolerate significant amounts of the allergen without reaction. As this acquired ability to ingest the food is temporary and Tregs which maintain immunological tolerance at the T cell level have not been implicated, this state is referred to as food "unresponsiveness" rather than tolerance (165). Maintenance of this unresponsive status requires ongoing ingestion of the allergenic food. It is striking that at the completion of OIT patients still have very high levels of food allergen-specific IgE antibodies, despite their ability to ingest the allergen without incident. In fact, the IgE titers typically seen after OIT can be unchanged from pre-OIT levels, and are of a magnitude that would strongly predict a significant reaction if obtained in a patient who had not undergone OIT (166). These observations are highly suggestive that OIT induces a suppressive factor, one that inhibits IgE-mediated anaphylaxis.

A clear clue as to the identity of the suppressive factor has been provided by the highly consistent observation that OIT of foods, including milk, egg, and peanut, induces strong food allergen-specific IgG4 responses (167–172). The success of baked-egg challenge has been correlated with these increases in egg-protein IgG4 following OIT (173). In contrast to prior observations with SCIT, the IgG response in OIT encompasses all IgG subclasses, not just IgG4. For instance, patients undergoing OIT for peanut allergy in the PRROTECT trial had several log increases in levels of peanut-specific IgG1, IgG2, IgG3, IgG4, and IgA, as well as the ratio of peanut-specific IgG4/IgE with the greatest increases evident in peanut-specific IgG2 and IgG4 which were increased by two logs (139, 174). The groups of Galli and Nadeau reported that robust IgG responses and elevated IgG4/IgE ratios correlate with sustained unresponsiveness following peanut OIT (175). When modeled in mice with established peanut allergy, OIT also induces a strong IgG response, inclusive of all the murine IgG subclasses (174).

FcγRIIb-Mediated Suppression of IgE-Mediated Mast Cell Activation by Food-Specific IgG Induced During OIT

Mechanisms of OIT and the inhibitory effects of IgG have been studied both in mouse models and in mechanistic investigations in human clinical trials. Since BMMCs can be easily cultured using IL-3 and SCF, and they can be sensitized with food allergen-specific IgE, they provide an excellent tool with which to interrogate the serum of OIT-treated mice for any potential inhibitory activity of induced IgG. Surface expression of the granule marker LAMP-1 (CD107a), which is extruded upon degranulation, is a sensitive marker of mast cell activation. Within minutes of exposure to allergen, mast cells degranulate and an increase in LAMP-1 on the cell surface can be detected. Burton and colleagues reported that sera from mice that underwent OIT for peanut allergy can inhibit the peanut-induced activation of BMMCs sensitized with specific IgE in an IgG-dependent manner (174). In addition to inhibiting IgE-induced degranulation, OIT-induced food allergen-specific IgG antibodies also inhibit the production of IL-4 and IL-13 by activated BMMCs (176). Under these conditions, suppression of IgE activation of IgG is not observed in FcγRIIb$^{-/-}$ BMMCs, indicating a requirement for this receptor in IgG-mediated inhibition. At high doses of IgG, however, inhibition of mast cell activation can be exerted even in cells lacking FcγRIIb, indicating that, in addition to sending negative signals *via* FcγRIIb, IgG can also act as a blocking antibody, sterically

preventing the interaction of allergen with FcεRI-bound IgE (see **Figure 1**) (174). Prior to the identification of the key role of FcγRIIb in mediating the suppressive actions of IgG on mast cells and basophils, this steric blocking effect had commonly been presumed to be the dominant inhibitory mechanism of IgG in allergy, especially following SCIT, which is why such IgG antibodies are still commonly referred to as blocking IgG.

The physiologic relevance of IgG : FcγRIIb-mediated inhibition of IgE-triggered mast cell activation to allergic reactions *in vivo*, particularly to anaphylaxis, has been demonstrated using mouse models. FcγRIIb-deficient mice have provided an excellent genetic tool to analyze this biology. Sensitized FcγRIIb-deficient mice exhibit enhanced anaphylaxis upon allergen challenge (176). Furthermore, inhibition of anaphylaxis by passive transfer of allergen-specific IgG occurs in wild type but not FcγRIIb-deficient mice, indicating that restoration of tolerance *via* IgG requires IgG : FcγRIIb interactions. Although $FcγRIIb^{-/-}$ mice lack the receptor on all cells that would normally express the receptor, reconstitution of the mice with cultured $FcγRIIb^{+/+}$ mast cells from wild type donors restores the protective effects exerted by passive administration of exogenous allergen-specific IgG, confirming the central role of mast cell FcγRIIb in regulating IgE-mediated anaphylaxis *in vivo* (see **Figure 1**) (176).

Inhibition of Basophil Activation by Post-OIT Serum in Human Subjects

The same receptor-mediated inhibition of IgE-induced activation by IgG has also now been clearly demonstrated in patients undergoing OIT. Using an indirect basophil activation test (iBAT), Burton and colleagues analyzed suppressive activity in the serum of peanut-allergic patients who underwent OIT (174). In the iBAT, basophils from a non-allergic donor are used to interrogate the sera of study subjects for both activating and suppressive factors (see **Figure 3**). IgE-containing serum, typically from a peanut allergic patient, is added to these basophils in culture. After the addition of peanut extract, granule extrusion is measured by flow cytometric quantitation of CD63, a granule protein that is similar to the LAMP-1 marker used in the murine system. Using this assay, the group found decreased basophil activation by iBAT following completion of OIT. When both pre- and post-OIT serum from the same individual were incubated with donor basophils, the degranulation was lower than that induced by pre-OIT sera alone, indicating that the suppressive activity was OIT-induced (176). It was determined that this suppression is IgG-mediated and could be blocked by antibodies to FcγRIIb.

Similar observations were subsequently reported by Santos et al. in a separate peanut-allergic cohort. Rather than using basophils from non-allergic donors to query the OIT sera, this group used the human mast cell line, LAD2 (156). They too observed IgG-mediated suppressive activity in LAD2 activation by post-OIT sera, and found that specific depletion of IgG4 reduced the suppression leading them to the conclusion that post-OIT suppression is IgG4-mediated. In reviewing the Santos report, however, it is important to note that while IgG4 removal reduced the degree of suppression exerted by post-OIT sera, the effect was incomplete. IgG4-depleted sera from post-OIT subjects in their cohort exerted >50% suppression of basophil activation compared with 80% in sham-depleted sera, which actually suggests that most of the suppressive activity might in fact be accounted for by non-IgG4 isotypes (156). At this point the argument could be made that suppression of FcεRI signaling by FcγRIIb in food allergy is not uniquely accounted for by IgG4, but is also exerted by other IgG subclasses, all of which are known to have measurable affinity for FcγRIIb. Like OIT, serum from patients that had undergone SLIT can also inhibit basophil reactivity in an IgG dependent manner (177). Future studies with monoclonal IgG antibodies, expressed as IgG isotype swap variants, are needed to clearly delineate the potential contributions of these isotypes to FcγRIIb-mediated basophil suppression.

The discovery that food-specific IgG antibodies account, at least in part, for suppression of food reactions in IgE^{+} food-tolerant subjects and those who have completed OIT has seeded interest in potential therapeutic applications of IgG in food allergy. Although inhibitory food-specific IgG antibodies are still in development by pharmaceutical companies, and there are not yet any direct data regarding the efficacy of IgG in preventing food anaphylaxis, there are preclinical studies that support the approach. Burton and colleagues recently developed a humanized mouse model in which NOD-scid $IL2Rγ^{null}$ mice (NSG,NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ), given human $CD34^{+}$ stem cells, exhibit robust T cell expansion, including $Foxp3^{+}CD25^{+}$ Tregs, $IFNγ^{+}$ Th1 cells and $IL\text{-}4^{+}$ Th2 cells, and B cell engraftment (178). These animals are readily sensitized to ingested peanut, producing specific IgE and exhibiting anaphylaxis with elevated plasma tryptase levels upon challenge (178). If post-OIT serum is administered 24 h prior to allergen challenge, the mice are protected from peanut-induced anaphylaxis (179). This IgG-mediated inhibition can be blocked by injecting mice with anti-FcγRIIb (179). However, blocking FcγRIIb does not fully restore the anaphylaxis phenotype, suggesting that IgG is able to exert some inhibition in a manner independent of FcγRIIb, most likely through steric hinderance (179).

Tissue-Specific Variations in FcγRIIb Expression

The expression of FcgRIIb on human mast cells is still an active area of investigation including the factors that regulate the expression of the receptor. Comparison of the expression of FcγRIIb on mast cells residing in various human tissues has revealed that FcγRIIb is absent from dermal mast cells, but is expressed on those in the gastrointestinal tract (179, 180). This is recapitulated in humanized mice, in which FcγRIIb is detectable by qPCR and flow cytometry in the small intestine and spleen, but not in the skin (179). As would be anticipated by the lack of FcγRIIb, *in vitro* IgG-mediated inhibition of mast cell degranulation does not manifest in human skin mast cells. This low, or completely absent, expression of FcγRIIb on dermal mast cells likely explains why patients who have

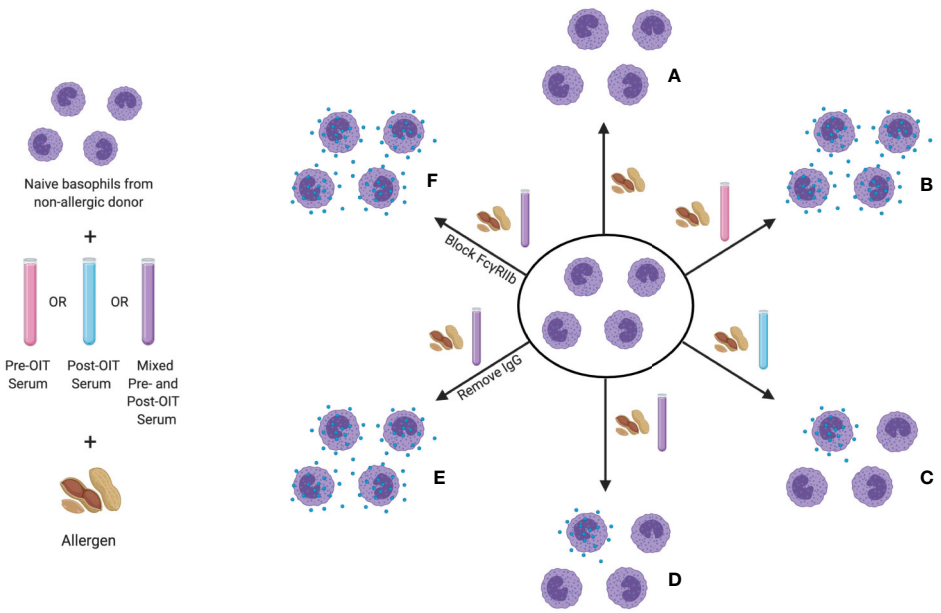


FIGURE 3 | The indirect basophil activation test (iBAT) as a probe for inhibitory food allergen specific IgG. In this assay, basophils from a non-allergic donor are sensitized with IgE from an allergic donor, then incubated with serum to be queried (typically pre-OIT serum, post-OIT serum, or a mix of the two) and then exposed to allergen. Conditions assayed include (proceeding clockwise starting from top center): **(A)** basophil incubation with allergen in the absence of serum (no activation), **(B)** with serum from an allergic donor (full degranulation), **(C)** with post-OIT serum (suppressed activation), **(D)** with a mix of pre- and post-OIT serum (suppressed activation if inhibitory activity is present), **(E)** post-OIT serum with IgG removed (to query the contribution of IgG to suppression) and **(F)** post-OIT serum with antibodies to FcγRIIb (to test whether inhibition is receptor-mediated).

successfully undergone desensitization therapy are able to consume the allergen without symptoms, but they may still exhibit positive skin prick test responses to allergen (181–184).

The relevance of FcγRIIb to physiologic regulation of allergic responses is further suggested by genetic associations. An asthma family cohort study that included 370 atopic, 239 non-atopic, and 169 asthmatic subjects, identified a functional SNP in FCGR2B (187Ile>Thr) that was associated with atopy and IgE production (185). Functional analysis of this variation, that is located in the transmembrane segment of the receptor, showed that 187Ile>Thr FCGR2B is less effective at mediating inhibitory signals than the common allele (186–188).

IgG-Mediated Inhibition of Sensitization to Ingested Antigens

As one might predict, based on their ability to prevent IgE-induced production of Th2 cytokines by mast cells in culture, IgG antibodies that are administered prophylactically prior to initial allergen ingestion can hinder the development of Th2 responses and IgE antibodies. This possibility was assessed in a recent investigation using mouse model of food allergy involving repeated administration of OVA in *Il4raF709* mice, a strain rendered inherently atopic by knock-in of a variant IL-4R α-chain (176). Administration of allergen-specific, but not control, IgG during the sensitization phase markedly suppressed production of OVA-specific Th2 cells, and was permissive for the expansion of Tregs which was not observed in controls (176). Allergen-driven mast cell expansion was also suppressed in the IgG-treated mice. As would be expected in the setting of decreased Th2 immunity, IgE responses were suppressed by more than one log. The combination of low IgE and decreased mast cell numbers rendered the OVA IgG-treated animals completely resistant to anaphylaxis, with no signs of hypothermia, a cardinal physiologic feature of anaphylaxis in mice, or elevated levels of plasma mast cell protease-1 (MMCP-1), a granule protease and marker of mast cell activation analogous to tryptase in humans. Taken together, these findings show that, in addition to blocking phenotypes of IgE-mediated immediate hypersensitivity, like anaphylaxis in the setting of established food allergy, IgG antibodies can block the development of food allergy by blunting the Th2 adjuvant function of mast cells (see **Figure 4**). Analysis of FcγRIIb$^{-/-}$ mice in the same study revealed a key role for the inhibitory receptor in mediating the protective effect of IgG.

In a study of fish allergy, active immune induction of IgG antibodies by vaccinating mice with a hypoallergenic mutant of the fish allergen *Cyp c 1*, has also been shown to protect against allergy (189). These findings suggest that an IgG-based preventive strategy might be beneficial as a prophylactic treatment for children at risk for developing food allergies. The recent findings of Oyoshi and colleagues from studies done in mice, in which IgG:allergen immune complexes passed by food allergen-tolerant mothers to their offspring *via* breast milk, support the induction of Tregs and suppress IgE responses,

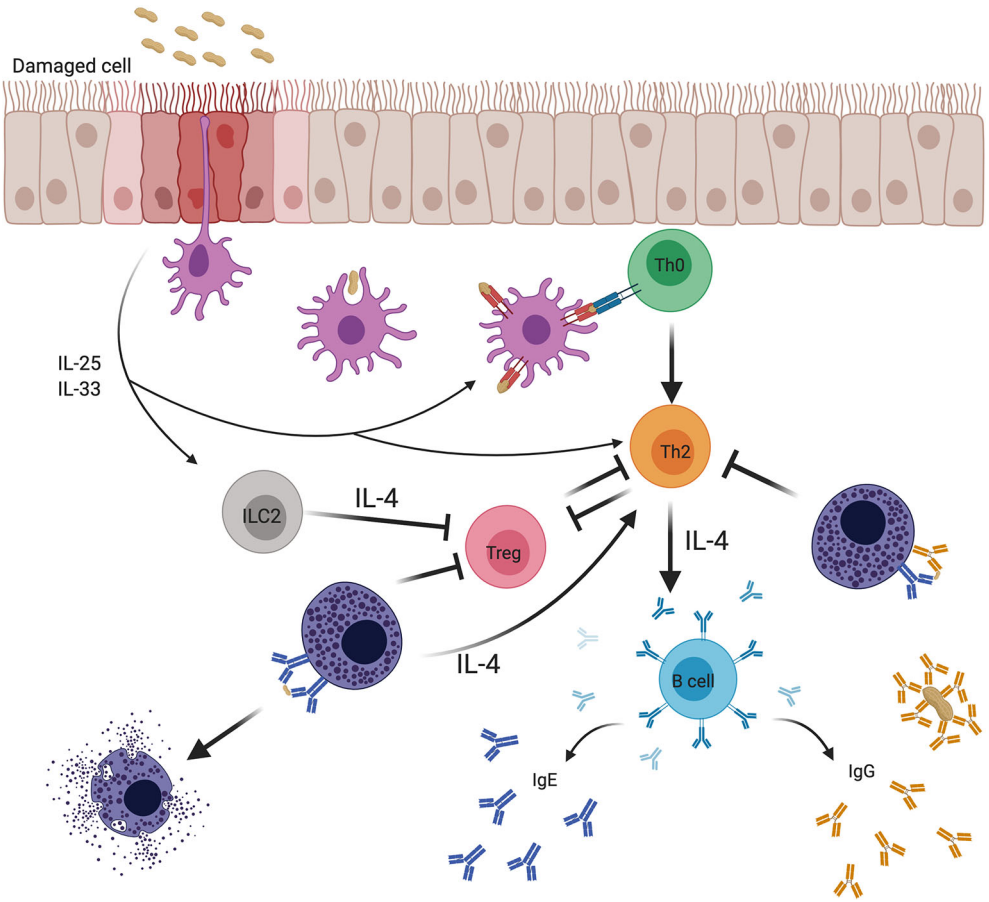


FIGURE 4 | Effects of mast cells on adaptive immune responses to food allergens and the regulation of these effects by IgE and IgG antibodies. Food antigens pass through damaged epithelium, specialized intraepithelial passages (8), or are sampled by antigen presenting cells (APCs). Epithelial cells subjected to stress or microbial signals secrete cytokines such as IL-25 and IL-33 that promote the activity of various cellular mediators involved in the breakdown of tolerance. Mucosal APCs present antigen to naïve T cells that mature into Th2 cells in the context of a Th2-conducive environment. Th2 cells are known to both depend on IL-4 for their differentiation and survival, and to produce IL-4 that drives IgE isotype switching by B cells and mast cell expansion, while inhibiting the production of regulatory T cells (Tregs) and subverting their function. Mast cells sensitized with allergen-specific IgE and type 2 innate lymphoid cells (ILC2s) provide a priming source of IL-4, initiating and sustaining the Th2 environment. In contrast, allergen-specific IgG antibodies induced during natural allergen resolution or during OIT can inhibit mast cell activation *via* signaling through FcγRIIb receptor. Inhibition of mast cell activation by IgG can break the positive feedback loop between mast cells and Th2.

implicate IgG in physiologic maternal transfer of tolerance (190). Furthermore, offspring of mothers who have high levels of allergen-specific IgG in their plasma, cord blood, and breast milk, have lower incidence of allergen sensitization (191). However, the role of mast cells and FcγRIIb in mediating this tolerogenic mechanism have not yet been explored.

Restoration of Tolerance During Adjunctive Therapy With IgG During OIT

OIT can also be modeled in OVA-sensitized *Il4raF709* mice. Mouse models of food allergy using this line have been applied to test the hypothesis that allergen-specific IgG given during the course of OIT might enhance the effectiveness of OIT, by dampening Th2-inducing signals. As expected, OIT, even without adjunctive IgG, resulted in diminished allergen sensitivity, as assessed by anaphylaxis (hypothermia) and MMCP-1 release. However, the protective effects of IgG were dramatically amplified in mice receiving OVA-specific IgG during their OIT with complete abrogation of anaphylaxis and markedly blunted Th2 and IgE responses (176).

IgG Antibodies in Allergic Diarrhea

IgE-mediated gastrointestinal reactions, including diarrhea, are common in IgE-mediated food allergy. The effects of food allergen-specific IgG antibodies in both anaphylaxis and diarrhea were investigated by Kucuk and colleagues in a hybrid model of food allergy involving both active sensitization and passive transfer of IgG (192). Though anaphylaxis and diarrhea are both IgE- and FcεRI-mediated, the mast cell mediators driving these phenotypes appear to be different. Histamine is associated with anaphylactic shock, while platelet activating factor and serotonin are associated with diarrhea (86, 193). To address whether IgG can confer protection against the diarrhea phenotype, Kucuk et al. tested if anti-TNP IgG1 administration

prevents the diarrhea induced by TNP-BSA challenge in sensitized mice. When the investigators tested the phenotypes on the FcγRIIb$^{-/-}$ background, protection against anaphylaxis by IgG1 antibodies was no longer observed while IgG1-mediated inhibition of diarrhea was, surprisingly, retained (192). These findings suggest that while IgG-mediated inhibition of shock is dependent on signaling *via* FcγRIIb as has been confirmed by others, that inhibition of diarrhea may be due to steric hinderance of antigen-IgE binding rather than a receptor-mediated mechanism (see **Figure 1**).

Allergen-Specific IgG and IgE Repertoire Overlap and the Role of IgG$^+$ B Cells as Custodians of IgE Memory

For optimal choreography of allergen-specific interactions between IgE and IgG antibodies in activating or suppressing food allergen responses one might predict that overlap of their repertoires would be advantageous. Recent findings regarding the relationship between IgE and IgG memory suggest that such coordination exists. The process of B cell isotype class switching to IgG$^+$ from IgM$^+$ precursors as well as the evolution of high affinity IgG responses to antigens through affinity maturation, and the creation of memory B cell clones all occur in germinal centers of lymph nodes. It turns out that, in contrast to IgG$^+$ B cells, mouse germinal center IgE$^+$ B cells are susceptible to apoptosis and tend to rapidly transition to a CD138$^+$ plasmablast phenotype. An analysis of sorted human IgE$^+$ B cells from allergic subjects by Croote et al. *via* single cell sequencing revealed that they, like their murine equivalents, almost all have plasmablast transcriptional signatures (194). This unique fate of IgE$^+$ B cells may be related to their very low surface levels of IgE compared with surface IgG or surface IgM on B cells expressing those isotypes (195–198). Both IgE affinity maturation and memory seem to require an intermediate IgG$^+$ stage during which this process occurs followed, sequentially, by a second isotype switch from IgG$^+$ to IgE$^+$. The presence of hybrid switch sequences, Sμ-Sγ-Sε in many IgE$^+$ B cells serves as a footprint of their previous existence as IgG$^+$ clones. The requirement for the intermediate IgG stage in affinity maturation is demonstrated by the lack of high-affinity IgE responses in mice lacking the Cγ locus (199). Deep sequencing of millions of peripheral blood IgH genes in allergic subjects by Boyd and colleagues revealed a phylogenetic lineage progression in which all somatically-mutated IgE sequences were derived from identically-mutated IgG parent clones (200). These findings are consistent with a mechanism in which IgE$^+$ and IgG$^+$ B cells have a shared allergen specific repertoire, with memory residing in the IgG$^+$ population.

Furthermore, T follicular helper (Tfh) cells are required for antibody isotype switching by B cells (201, 202). IL4 producing T follicular helper (Tfh) cells are needed for IgE production and IgE production can be limited by regulatory T follicular (Tfr) cells (203). Eisenbarth and colleagues have recently described a specific subset of IL-4- and IL-13-producing Tfh cells that can drive the production of IgE with high affinity to the antigen (204).

Therapeutic Applications of IgG

Harnessing the evolving understanding of the importance of allergen specific IgG in regulating both immediate hypersensitivity and chronic type 2 responses in allergy, researchers have developed recombinant allergen specific IgG antibodies or molecules that bind FcγRIIb and could be used to treat IgE-mediated allergies. In a recent clinical trial in cat-allergic subjects, Orengo et al. reported that administration of a single high dose of a pair of *Fel d 1*-specific monoclonal IgG4 antibodies in humans prevented symptoms following cat allergen exposure. The dose of IgG4 results in levels in plasma that are in vast excess of the circulating IgE, comparable to the IgG levels induced during successful immunotherapy. In as little as 8 days following injection of *Fel d 1* specific IgG4, subjects challenged with cat allergen had a decrease in total nasal symptom score and an increase in peak inspiratory flow compared to controls. By day 29, their skin prick test reactivity was decreased (205). As animal studies, discussed above, show that allergen-specific IgG exerts immunomodulatory effects, skewing the T cell compartment away from a pro-inflammatory Th2 profile, it would be interesting to see if the protective effects of allergen specific humanized IgG antibodies extend beyond immediate hypersensitivity and whether these monoclonal antibodies might be valuable adjuncts for SCIT in subjects with respiratory allergy (176). Given the strong induction of allergen-specific IgG antibodies in food-allergic subjects undergoing OIT and the clear immunomodulatory effects of these antibodies, we anticipate that food-specific monoclonal antibodies currently in development will also prove beneficial.

A significant limitation of the IgG antibody approach is that any given allergen-specific IgG therapeutic would only target a single allergen. It is unclear if IgG specific for each component allergen would be required for effective therapy. Since the inhibitory signal delivered to the mast cell by IgG against any protein within a food, it is possible that targeting just one component would be sufficient. A variety of alternative approaches have been explored using bispecific antibodies or fusion proteins that bring bridge FcεRI and FcγRIIb (206, 207). Several groups have reported the development of bifunctional FcεRI crosslinkers composed of the Fc portion of IgG1 and the Fc portion of IgE or an allergen (208, 209). However, molecules containing the Fc portion of IgE might be limited in their activity by the availably of free FcεRI sites. Tam et al. designed a bispecific antibody consisting of a Fab' fragment that recognizes human IgE and a Fab' fragment that recognizes FcγRIIb. Incubation of IgE-sensitized cord blood derived mast cells and basophils with the bispecific antibody blocked histamine release following antigen challenge (210). Similarly, Jackman and colleagues described a bispecific antibody in which one arm recognizes FcεRI at a site not blocked by IgE binding, while the other arm binds to FcγRIIb (211).

While these molecules are interesting because of their potential to broadly inhibit allergic responses, they have several limitations. Chronic administration of these compounds is made challenging by their immunogenicity and their short half-life *in vivo*. Furthermore, experience has shown that even a very low clinical risk of IgE receptor cross linking and anaphylaxis with

molecules bearing an FcεRI-binding moiety can pose very significant barriers to their clinical advancement.

DISCUSSION

The quest to understand why some individuals with allergen-specific IgE experience life-threatening reactions, while others have no symptoms at all following ingestion has led to the discovery of allergen-specific IgG as the serum factor that is responsible for conferring protection against allergic reactions. Food allergen-specific IgG levels are higher in individuals that are sensitized but unresponsive to the allergen, in those who have outgrown their food allergies and in subjects who have acquired food unresponsiveness after successful completion of OIT. Furthermore, research in animal models has convincingly demonstrated that the presence of allergen specific IgG during sensitization can inhibit the production of allergen specific IgE, and subsequent promotion of Th2 immunity, while promoting effective induction of Tregs. While not studied in animals, IgE and IgG antibodies may be important to sustain food allergy in a maintenance phase, in addition to induction. Similarly, in these models, an adjunctive therapy with IgG during OIT also impairs Th2 responses and promotes the development of Tregs (176).

The factors driving IgE and IgG responses to ingested antigens are complex. Outside the scope of this review but critically important to shaping immune responses in the gut is the microbiome. Mice with reduced numbers of intestinal microbes or diminished microbial diversity (germ free mice or antibiotic-fed mice) have increased susceptibility to sensitization and food allergy (212, 213). Germ free mice exhibit higher baseline IL-33 expression in their small intestine and also greater IgE levels in their serum (9, 129). Certain species of bacteria, including some in the genus *Clostridia*, have been shown to confer protection against development of food allergies by contributing to the development of peripherally expanded protective RORγ^+ T cells in a MYD88-dependent manner (9, 11, 213).

The specific contribution of IgG4 relative to other IgG isotypes in the inhibition of IgE-mediated effector cell activation *via* FcεRI and in exerting the immunomodulatory effects of IgG requires further study. We believe that investigations in the field have been skewed by the exclusive availability of IgG4 reagents in the ImmunoCAP platform used by most clinical investigators. This has prevented consideration and analysis of the other isotypes. However, as noted in this review, all food-specific antibodies of all four IgG isotypes are induced during OIT and binding to FcγRIIb is clearly not specific to IgG4, begging the question of whether IgG1-3 contribute.

As presented in this review, data correlating allergen-specific IgG with protection against food allergy phenotypes and the role of FcγRIIb in blocking mast cell and basophil activation has been repeatedly shown by many groups. Though the antibody and the receptor can work together to block the activity of effector cells, research has also shown that they are also capable of exerting inhibitory effects independent of one another *via* a number of ways. These observations have been harnessed to engineer antibodies against specific allergen epitopes, and to design small molecules that can aggregate FcγRIIb to FcεRI-bound IgE bound to antigen. As our understanding of the roles of IgG antibodies both in preventing IgE-triggered anaphylaxis and in regulating Th2 immune responses continues to evolve and as approaches for engineering both allergen-specific and broad-spectrum therapeutics are further developed, we are optimistic that effective strategies will emerge for prevention and treatment in the worldwide problem of food allergies.

AUTHOR CONTRIBUTIONS

Under the supervision of HCO, the manuscript was written by CK with YSEA and OLL. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by NIH grant 1R01AI119918 (HO). CK is supported by a postdoctoral fellowship from the Fonds de recherche du Québec—Santé (258617 and 285834). All figures were created with BioRender.com.

REFERENCES

1. Prescott SL, Pawankar R, Allen KJ, Campbell DE, Sinn J, Fiocchi A, et al. A global survey of changing patterns of food allergy burden in children. *World Allergy Organ J* (2013) 6(1):21. doi: 10.1186/1939-4551-6-21
2. Gupta RS, Warren CM, Smith BM, Jiang J, Blumenstock JA, Davis MM, et al. Prevalence and Severity of Food Allergies Among US Adults. *JAMA Netw Open* (2019) 2(1):e185630. doi: 10.1001/jamanetworkopen.2018.5630
3. Boyce JA, Assa'ad A, Burks AW, Jones SM, Sampson HA, Wood RA, et al. Guidelines for the Diagnosis and Management of Food Allergy in the United States: Summary of the NIAID-Sponsored Expert Panel Report. *J Allergy Clin Immunol* (2010) 126(6):1105–18. doi: 10.1016/j.jaci.2010.10.007
4. Jimenez-Rodriguez TW, Garcia-Neuer M, Alenazy LA, Castells M. Anaphylaxis in the 21st century: phenotypes, endotypes, and biomarkers. *J Asthma Allergy* (2018) 11:121–42. doi: 10.2147/JAA.S159411
5. Lee S, Bellolio MF, Hess EP, Erwin P, Murad MH, Campbell RL. Time of Onset and Predictors of Biphasic Anaphylactic Reactions: A Systematic Review and Meta-analysis. *J Allergy Clin Immunol Pract* (2015) 3(3):408–16.e1-2. doi: 10.1016/j.jaip.2014.12.010
6. Tordesillas L, Goswami R, Benede S, Grishina G, Dunkin D, Jarvinen KM, et al. Skin exposure promotes a Th2-dependent sensitization to peanut allergens. *J Clin Invest* (2014) 124(11):4965–75. doi: 10.1172/JCI75660
7. Brough HA, Liu AH, Sicherer S, Makinson K, Douiri A, Brown SJ, et al. Atopic dermatitis increases the effect of exposure to peanut antigen in dust on peanut sensitization and likely peanut allergy. *J Allergy Clin Immunol* (2015) 135(1):164–70. doi: 10.1016/j.jaci.2014.10.007
8. Kulkarni DH, Gustafsson JK, Knoop KA, McDonald KG, Bidani SS, Davis JE, et al. Goblet cell associated antigen passages support the induction and maintenance of oral tolerance. *Mucosal Immunol* (2020) 13(2):271–82. doi: 10.1038/s41385-019-0240-7

9. Ohnmacht C, Park JH, Cording S, Wing JB, Atarashi K, Obata Y, et al. MUCOSAL IMMUNOLOGY. The microbiota regulates type 2 immunity through RORgammat(+) T cells. *Science* (2015) 349(6251):989–93. doi: 10.1126/science.aac4263
10. Sefik E, Geva-Zatorsky N, Oh S, Konnikova L, Zemmour D, McGuire AM, et al. MUCOSAL IMMUNOLOGY. Individual intestinal symbionts induce a distinct population of RORgamma(+) regulatory T cells. *Science* (2015) 349 (6251):993–7. doi: 10.1126/science.aaa9420
11. Abdel-Gadir A, Stephen-Victor E, Gerber GK, Noval Rivas M, Wang S, Harb H, et al. Microbiota therapy acts via a regulatory T cell MyD88/RORgammat pathway to suppress food allergy. *Nat Med* (2019) 25(7):1164–74. doi: 10.1038/s41591-019-0461-z
12. Shan M, Gentile M, Yeiser JR, Walland AC, Bornstein VU, Chen K, et al. Mucus enhances gut homeostasis and oral tolerance by delivering immunoregulatory signals. *Science* (2013) 342(6157):447–53. doi: 10.1126/science.1237910
13. Han H, Thelen TD, Comeau MR, Ziegler SF. Thymic stromal lymphopoietin-mediated epicutaneous inflammation promotes acute diarrhea and anaphylaxis. *J Clin Invest* (2014) 124(12):5442–52. doi: 10.1172/JCI77798
14. Lee JB, Chen CY, Liu B, Mugge L, Angkasekwinai P, Facchinetti V, et al. IL-25 and CD4(+) TH2 cells enhance type 2 innate lymphoid cell-derived IL-13 production, which promotes IgE-mediated experimental food allergy. *J Allergy Clin Immunol* (2016) 137(4):1216–25 e5. doi: 10.1016/j.jaci.2015.09.019
15. Khodoun MV, Tomar S, Tocker JE, Wang YH, Finkelman FD. Prevention of food allergy development and suppression of established food allergy by neutralization of thymic stromal lymphopoietin, IL-25, and IL-33. *J Allergy Clin Immunol* (2018) 141(1):171–9.e1. doi: 10.1016/j.jaci.2017.02.046
16. Blazquez AB, Berin MC. Gastrointestinal dendritic cells promote Th2 skewing via OX40L. *J Immunol* (2008) 180(7):4441–50. doi: 10.4049/jimmunol.180.7.4441
17. Chu DK, Llop-Guevara A, Walker TD, Flader K, Goncharova S, Boudreau JE, et al. IL-33, but not thymic stromal lymphopoietin or IL-25, is central to mite and peanut allergic sensitization. *J Allergy Clin Immunol* (2013) 131 (1):187–200.e1-8. doi: 10.1016/j.jaci.2012.08.002
18. Leyva-Castillo JM, Galand C, Kam C, Burton O, Gurish M, Musser MA, et al. Mechanical Skin Injury Promotes Food Anaphylaxis by Driving Intestinal Mast Cell Expansion. *Immunity* (2019) 50(5):1262–75.e4. doi: 10.1016/j.immuni.2019.03.023
19. Noval Rivas M, Burton OT, Oettgen HC, Chatila T. IL-4 production by group 2 innate lymphoid cells promotes food allergy by blocking regulatory T-cell function. *J Allergy Clin Immunol* (2016) 138(3):801–11.e9. doi: 10.1016/j.jaci.2016.02.030
20. Chinthrajah S, Cao S, Liu C, Lyu SC, Sindher SB, Long A, et al. Phase 2a randomized, placebo-controlled study of anti-IL-33 in peanut allergy. *JCI Insight* (2019) 4(22). doi: 10.1172/jci.insight.131347
21. Noti M, Kim BS, Siracusa MC, Rak GD, Kubo M, Moghaddam AE, et al. Exposure to food allergens through inflamed skin promotes intestinal food allergy through the thymic stromal lymphopoietin-basophil axis. *J Allergy Clin Immunol* (2014) 133(5):1390–9.e1-6. doi: 10.1016/j.jaci.2014.01.021
22. Hussain M, Borcard L, Walsh KP, Pena Rodriguez M, Mueller C, Kim BS, et al. Basophil-derived IL-4 promotes epicutaneous antigen sensitization concomitant with the development of food allergy. *J Allergy Clin Immunol* (2018) 141(1):223–34.e5. doi: 10.1016/j.jaci.2017.02.035
23. Muto T, Fukuoka A, Kabashima K, Ziegler SF, Nakanishi K, Matsushita K, et al. The role of basophils and proallergic cytokines, TSLP and IL-33, in cutaneously sensitized food allergy. *Int Immunol* (2014) 26(10):539–49. doi: 10.1093/intimm/dxu058
24. Forbes EE, Groschwitz K, Abonia JP, Brandt EB, Cohen E, Blanchard C, et al. IL-9- and mast cell-mediated intestinal permeability predisposes to oral antigen hypersensitivity. *J Exp Med* (2008) 205(4):897–913. doi: 10.1084/jem.20071046
25. Osterfeld H, Ahrens R, Strait R, Finkelman FD, Renauld JC, Hogan SP. Differential roles for the IL-9/IL-9 receptor alpha-chain pathway in systemic and oral antigen-induced anaphylaxis. *J Allergy Clin Immunol* (2010) 125 (2):469–76.e2. doi: 10.1016/j.jaci.2009.09.054
26. Chen CY, Lee JB, Liu B, Ohta S, Wang PY, Kartashov AV, et al. Induction of Interleukin-9-Producing Mucosal Mast Cells Promotes Susceptibility to IgE-Mediated Experimental Food Allergy. *Immunity* (2015) 43(4):788–802. doi: 10.1016/j.immuni.2015.08.020
27. Burton OT, Darling AR, Zhou JS, Noval-Rivas M, Jones TG, Gurish MF, et al. Direct effects of IL-4 on mast cells drive their intestinal expansion and increase susceptibility to anaphylaxis in a murine model of food allergy. *Mucosal Immunol* (2013) 6(4):740–50. doi: 10.1038/mi.2012.112
28. Tomar S, Ganesan V, Sharma A, Zeng C, Waggoner L, Smith A, et al. IL-4-BATF signaling directly modulates IL-9 producing mucosal mast cell (MMC9) function in experimental food allergy. *J Allergy Clin Immunol* (2020) S0091-6749(20):31409–3. doi: 10.1016/j.jaci.2020.08.043
29. Noval Rivas M, Burton OT, Wise P, Charbonnier LM, Georgiev P, Oettgen HC, et al. Regulatory T cell reprogramming toward a Th2-cell-like lineage impairs oral tolerance and promotes food allergy. *Immunity* (2015) 42 (3):512–23. doi: 10.1016/j.immuni.2015.02.004
30. Hobbs CB, Skinner AC, Burks AW, Vickery BP. Food allergies affect growth in children. *J Allergy Clin Immunol Pract* (2015) 3(1):133–4.e1. doi: 10.1016/j.jaip.2014.11.004
31. Sova C, Feuling MB, Baumler M, Gleason L, Tam JS, Zafra H, et al. Systematic review of nutrient intake and growth in children with multiple IgE-mediated food allergies. *Nutr Clin Pract* (2013) 28(6):669–75. doi: 10.1177/0884533613505870
32. Albuhairi S, Rachid R. Novel Therapies for Treatment of Food Allergy. *Immunol Allergy Clin North Am* (2020) 40(1):175–86. doi: 10.1016/j.iac.2019.09.007
33. Chinthrajah RS, Cao S, Dunham T, Sampath V, Chandra S, Chen M, et al. Oral immunotherapy for peanut allergy: The pro argument. *World Allergy Organ J* (2020) 13(8):100455. doi: 10.1016/j.waojou.2020.100455
34. Bauer RN, Manohar M, Singh AM, Jay DC, Nadeau KC. The future of biologics: applications for food allergy. *J Allergy Clin Immunol* (2015) 135 (2):312–23. doi: 10.1016/j.jaci.2014.12.1908
35. Galli SJ. The Mast Cell-IgE Paradox: From Homeostasis to Anaphylaxis. *Am J Pathol* (2016) 186(2):212–24. doi: 10.1016/j.ajpath.2015.07.025
36. Valent P, Akin C, Hartmann K, Nilsson G, Reiter A, Hermine O, et al. Mast cells as a unique hematopoietic lineage and cell system: From Paul Ehrlich's visions to precision medicine concepts. *Theranostics* (2020) 10(23):10743–68. doi: 10.7150/thno.46719
37. Voehringer D. Protective and pathological roles of mast cells and basophils. *Nat Rev Immunol* (2013) 13(5):362–75. doi: 10.1038/nri3427
38. Karasuyama H, Miyake K, Yoshikawa S, Yamanishi Y. Multifaceted roles of basophils in health and disease. *J Allergy Clin Immunol* (2018) 142(2):370–80. doi: 10.1016/j.jaci.2017.10.042
39. Voehringer D. Recent advances in understanding basophil functions in vivo. *F1000Res* (2017) 6:1464. doi: 10.12688/f1000research.11697.1
40. Mukai K, Tsai M, Starkl P, Marichal T, Galli SJ. IgE and mast cells in host defense against parasites and venoms. *Semin Immunopathol* (2016) 38 (5):581–603. doi: 10.1007/s00281-016-0565-1
41. Metz M, Piliponsky AM, Chen CC, Lammel V, Abrink M, Pejler G, et al. Mast cells can enhance resistance to snake and honeybee venoms. *Science* (2006) 313(5786):526–30. doi: 10.1126/science.1128877
42. Marichal T, Starkl P, Reber LL, Kalesnikoff J, Oettgen HC, Tsai M, et al. A beneficial role for immunoglobulin E in host defense against honeybee venom. *Immunity* (2013) 39(5):963–75. doi: 10.1016/j.immuni.2013.10.005
43. Starkl P, Marichal T, Gaudenzio N, Reber LL, Sibilano R, Tsai M, et al. IgE antibodies, FcepsilonRIalpha, and IgE-mediated local anaphylaxis can limit snake venom toxicity. *J Allergy Clin Immunol* (2016) 137(1):246–57.e11. doi: 10.1016/j.jaci.2015.08.005
44. Schwartz C, Turqueti-Neves A, Hartmann S, Yu P, Nimmerjahn F, Voehringer D. Basophil-mediated protection against gastrointestinal helminths requires IgE-induced cytokine secretion. *Proc Natl Acad Sci U S A* (2014) 111(48):E5169–77. doi: 10.1073/pnas.1412663111
45. Siracusa MC, Saenz SA, Hill DA, Kim BS, Headley MB, Doering TA, et al. TSLP promotes interleukin-3-independent basophil haematopoiesis and type 2 inflammation. *Nature* (2011) 477(7363):229–33. doi: 10.1038/nature10329
46. Giacomin PR, Siracusa MC, Walsh KP, Grencis RK, Kubo M, Comeau MR, et al. Thymic stromal lymphopoietin-dependent basophils promote Th2

cytokine responses following intestinal helminth infection. *J Immunol* (2012) 189(9):4371–8. doi: 10.4049/jimmunol.1200691
47. Wada T, Ishiwata K, Koseki H, Ishikura T, Ugajin T, Ohnuma N, et al. Selective ablation of basophils in mice reveals their nonredundant role in acquired immunity against ticks. *J Clin Invest* (2010) 120(8):2867–75. doi: 10.1172/JCI42680
48. Anyan WK, Seki T, Kumagai T, Obata-Ninomiya K, Furushima-Shimogawara R, Kwansa-Bentum B, et al. Basophil depletion downregulates Schistosoma mansoni egg-induced granuloma formation. *Parasitol Int* (2013) 62(6):508–13. doi: 10.1016/j.parint.2013.07.003
49. Plaut M, Pierce JH, Watson CJ, Hanley-Hyde J, Nordan RP, Paul WE. Mast cell lines produce lymphokines in response to cross-linkage of Fc epsilon RI or to calcium ionophores. *Nature* (1989) 339(6219):64–7. doi: 10.1038/339064a0
50. Seder RA, Paul WE, Dvorak AM, Sharkis SJ, Kagey-Sobotka A, Niv Y, et al. Mouse splenic and bone marrow cell populations that express high-affinity Fc epsilon receptors and produce interleukin 4 are highly enriched in basophils. *Proc Natl Acad Sci U S A* (1991) 88(7):2835–9. doi: 10.1073/pnas.88.7.2835
51. Gibbs BF, Haas H, Falcone FH, Albrecht C, Vollrath IB, Noll T, et al. Purified human peripheral blood basophils release interleukin-13 and preformed interleukin-4 following immunological activation. *Eur J Immunol* (1996) 26 (10):2493–8. doi: 10.1002/eji.1830261033
52. MacGlashan D Jr., White JM, Huang SK, Ono SJ, Schroeder JT, Lichtenstein LM. Secretion of IL-4 from human basophils. The relationship between IL-4 mRNA and protein in resting and stimulated basophils. *J Immunol* (1994) 152(6):3006–16.
53. Grimbaldeston MA, Metz M, Yu M, Tsai M, Galli SJ. Effector and potential immunoregulatory roles of mast cells in IgE-associated acquired immune responses. *Curr Opin Immunol* (2006) 18(6):751–60. doi: 10.1016/j.coi.2006.09.011
54. Metcalfe DD, Baram D, Mekori YA. Mast cells. *Physiol Rev* (1997) 77 (4):1033–79. doi: 10.1152/physrev.1997.77.4.1033
55. Oettgen HC. Fifty years later: Emerging functions of IgE antibodies in host defense, immune regulation, and allergic diseases. *J Allergy Clin Immunol* (2016) 137(6):1631–45. doi: 10.1016/j.jaci.2016.04.009
56. Kisselgof AB, Oettgen HC. The expression of murine B cell CD23, in vivo, is regulated by its ligand, IgE. *Int Immunol* (1998) 10(9):1377–84. doi: 10.1093/intimm/10.9.1377
57. Kawakami Y, Yao L, Miura T, Tsukada S, Witte ON, Kawakami T. Tyrosine phosphorylation and activation of Bruton tyrosine kinase upon Fc epsilon RI cross-linking. *Mol Cell Biol* (1994) 14(8):5108–13. doi: 10.1128/MCB.14.8.5108
58. Hutchcroft JE, Geahlen RL, Deanin GG, Oliver JM. Fc epsilon RI-mediated tyrosine phosphorylation and activation of the 72-kDa protein-tyrosine kinase, PTK72, in RBL-2H3 rat tumor mast cells. *Proc Natl Acad Sci U S A* (1992) 89(19):9107–11. doi: 10.1073/pnas.89.19.9107
59. Eiseman E, Bolen JB. Engagement of the high-affinity IgE receptor activates src protein-related tyrosine kinases. *Nature* (1992) 355(6355):78–80. doi: 10.1038/355078a0
60. Gilfillan AM, Rivera J. The tyrosine kinase network regulating mast cell activation. *Immunol Rev* (2009) 228(1):149–69. doi: 10.1111/j.1600-065X.2008.00742.x
61. El-Hillal O, Kurosaki T, Yamamura H, Kinet JP, Scharenberg AM. syk kinase activation by a src kinase-initiated activation loop phosphorylation chain reaction. *Proc Natl Acad Sci U S A* (1997) 94(5):1919–24. doi: 10.1073/pnas.94.5.1919
62. Kimura T, Sakamoto H, Appella E, Siraganian RP. Conformational changes induced in the protein tyrosine kinase p72syk by tyrosine phosphorylation or by binding of phosphorylated immunoreceptor tyrosine-based activation motif peptides. *Mol Cell Biol* (1996) 16(4):1471–8. doi: 10.1128/MCB.16.4.1471
63. Kawakami T, Kitaura J. Mast cell survival and activation by IgE in the absence of antigen: a consideration of the biologic mechanisms and relevance. *J Immunol* (2005) 175(7):4167–73. doi: 10.4049/jimmunol.175.7.4167
64. Asai K, Kitaura J, Kawakami Y, Yamagata N, Tsai M, Carbone DP, et al. Regulation of mast cell survival by IgE. *Immunity* (2001) 14(6):791–800. doi: 10.1016/S1074-7613(01)00157-1
65. Kalesnikoff J, Huber M, Lam V, Damen JE, Zhang J, Siraganian RP, et al. Monomeric IgE stimulates signaling pathways in mast cells that lead to cytokine production and cell survival. *Immunity* (2001) 14(6):801–11. doi: 10.1016/S1074-7613(01)00159-5
66. Kitaura J, Song J, Tsai M, Asai K, Maeda-Yamamoto M, Mocsai A, et al. Evidence that IgE molecules mediate a spectrum of effects on mast cell survival and activation via aggregation of the FcepsilonRI. *Proc Natl Acad Sci U S A* (2003) 100(22):12911–6. doi: 10.1073/pnas.1735525100
67. Tanaka S, Takasu Y, Mikura S, Satoh N, Ichikawa A. Antigen-independent induction of histamine synthesis by immunoglobulin E in mouse bone marrow-derived mast cells. *J Exp Med* (2002) 196(2):229–35. doi: 10.1084/jem.20012037
68. Pandey V, Mihara S, Fensome-Green A, Bolsover S, Cockcroft S. Monomeric IgE stimulates NFAT translocation into the nucleus, a rise in cytosol Ca2+, degranulation, and membrane ruffling in the cultured rat basophilic leukemia-2H3 mast cell line. *J Immunol* (2004) 172(7):4048–58. doi: 10.4049/jimmunol.172.7.4048
69. Shade KT, Platzer B, Washburn N, Mani V, Bartsch YC, Conroy M, et al. A single glycan on IgE is indispensable for initiation of anaphylaxis. *J Exp Med* (2015) 212(4):457–67. doi: 10.1084/jem.20142182
70. Shade KC, Conroy ME, Washburn N, Kitaoka M, Huynh DJ, Laprise E, et al. Sialylation of immunoglobulin E is a determinant of allergic pathogenicity. *Nature* (2020) 582(7811):265–70. doi: 10.1038/s41586-020-2311-z
71. Lee WT, Conrad DH. Murine B cell hybridomas bearing ligand-inducible Fc receptors for IgE. *J Immunol* (1986) 136(12):4573–80.
72. Dierks SE, Bartlett WC, Edmeades RL, Gould HJ, Rao M, Conrad DH. The oligomeric nature of the murine Fc epsilon RII/CD23. Implications for function. *J Immunol* (1993) 150(6):2372–82.
73. Aubry JP, Pochon S, Gauchat JF, Nueda MA, Holers VM, Graber P, et al. CD23 interacts with a new functional extracytoplasmic domain involving N-linked oligosaccharides on CD21. *J Immunol* (1994) 152(12):5806–13.
74. Nimmerjahn F, Bruhns P, Horiuchi K, Ravetch JV. FcgammaRIV: a novel FcR with distinct IgG subclass specificity. *Immunity* (2005) 23(1):41–51. doi: 10.1016/j.immuni.2005.05.010
75. Bruhns P, Jonsson F. Mouse and human FcR effector functions. *Immunol Rev* (2015) 268(1):25–51. doi: 10.1111/imr.12350
76. Nimmerjahn F, Ravetch JV. Fcgamma receptors as regulators of immune responses. *Nat Rev Immunol* (2008) 8(1):34–47. doi: 10.1038/nri2206
77. Nimmerjahn F, Ravetch JV. Fcgamma receptors: old friends and new family members. *Immunity* (2006) 24(1):19–28. doi: 10.1016/j.immuni.2005.11.010
78. Daeron M. Innate myeloid cells under the control of adaptive immunity: the example of mast cells and basophils. *Curr Opin Immunol* (2016) 38:101–8. doi: 10.1016/j.coi.2015.12.004
79. Ovary Z. Quantitative studies in passive cutaneous anaphylaxis of the guinea pig. *Int Arch Allergy Appl Immunol* (1952) 3(2):162–74. doi: 10.1159/000227958
80. Nussenzweig RS, Merryman C, Benacerraf B. Electrophoretic Separation and Properties of Mouse Antihapten Antibodies Involved in Passive Cutaneous Anaphylaxis and Passive Hemolysis. *J Exp Med* (1964) 120:315–28. doi: 10.1084/jem.120.2.315
81. Oettgen HC, Martin TR, Wynshaw-Boris A, Deng C, Drazen JM, Leder P. Active anaphylaxis in IgE-deficient mice. *Nature* (1994) 370(6488):367–70. doi: 10.1038/370367a0
82. Perez LG, Costa MR, Todd CA, Haynes BF, Montefiori DC. Utilization of immunoglobulin G Fc receptors by human immunodeficiency virus type 1: a specific role for antibodies against the membrane-proximal external region of gp41. *J Virol* (2009) 83(15):7397–410. doi: 10.1128/JVI.00656-09
83. Verma A, Ngundi MM, Meade BD, De Pascalis R, Elkins KL, Burns DL. Analysis of the Fc gamma receptor-dependent component of neutralization measured by anthrax toxin neutralization assays. *Clin Vaccine Immunol* (2009) 16(10):1405–12. doi: 10.1128/CVI.00194-09
84. Reth M. Antigen receptor tail clue. *Nature* (1989) 338(6214):383–4. doi: 10.1038/338383b0
85. Okayama Y, Tkaczyk C, Metcalfe DD, Gilfillan AM. Comparison of Fc epsilon RI- and Fc gamma RI-mediated degranulation and TNF-alpha synthesis in human mast cells: selective utilization of phosphatidylinositol-3-kinase for Fc gamma RI-induced degranulation. *Eur J Immunol* (2003) 33 (5):1450–9. doi: 10.1002/eji.200323563

86. Strait RT, Morris SC, Yang M, Qu XW, Finkelman FD. Pathways of anaphylaxis in the mouse. *J Allergy Clin Immunol* (2002) 109(4):658–68. doi: 10.1067/mai.2002.123302
87. Nicholas R, Sinclair SC. Regulation of the immune response. I. Reduction in ability of specific antibody to inhibit long-lasting IgG immunological priming after removal of the Fc fragment. *J Exp Med* (1969) 129(6):1183–201. doi: 10.1084/jem.129.6.1183
88. Takai T, Ono M, Hikida M, Ohmori H, Ravetch JV. Augmented humoral and anaphylactic responses in Fc gamma RII-deficient mice. *Nature* (1996) 379(6563):346–9. doi: 10.1038/379346a0
89. Kalergis AM, Ravetch JV. Inducing tumor immunity through the selective engagement of activating Fcgamma receptors on dendritic cells. *J Exp Med* (2002) 195(12):1653–9. doi: 10.1084/jem.20020338
90. Li F, Smith P, Ravetch JV. Inhibitory Fcgamma receptor is required for the maintenance of tolerance through distinct mechanisms. *J Immunol* (2014) 192(7):3021–8. doi: 10.4049/jimmunol.1302934
91. Clynes R, Maizes JS, Guinamard R, Ono M, Takai T, Ravetch JV. Modulation of immune complex-induced inflammation in vivo by the coordinate expression of activation and inhibitory Fc receptors. *J Exp Med* (1999) 189(1):179–85. doi: 10.1084/jem.189.1.179
92. Yuasa T, Kubo S, Yoshino T, Ujike A, Matsumura K, Ono M, et al. Deletion of fcgamma receptor IIB renders H-2(b) mice susceptible to collagen-induced arthritis. *J Exp Med* (1999) 189(1):187–94. doi: 10.1084/jem.189.1.187
93. Clatworthy MR, Smith KG. FcgammaRIIb balances efficient pathogen clearance and the cytokine-mediated consequences of sepsis. *J Exp Med* (2004) 199(5):717–23. doi: 10.1084/jem.20032197
94. Katsumata O, Hara-Yokoyama M, Sautes-Fridman C, Nagatsuka Y, Katada T, Hirabayashi Y, et al. Association of FcgammaRII with low-density detergent-resistant membranes is important for cross-linking-dependent initiation of the tyrosine phosphorylation pathway and superoxide generation. *J Immunol* (2001) 167(10):5814–23. doi: 10.4049/jimmunol.167.10.5814
95. Kwiatkowska K, Sobota A. The clustered Fcgamma receptor II is recruited to Lyn-containing membrane domains and undergoes phosphorylation in a cholesterol-dependent manner. *Eur J Immunol* (2001) 31(4):989–98. doi: 10.1002/1521-4141(200104)31:4<989::AID-IMMU989>3.0.CO;2-V
96. Daeron M, Latour S, Malbec O, Espinosa E, Pina P, Pasmans S, et al. The same tyrosine-based inhibition motif, in the intracytoplasmic domain of Fc gamma RIIB, regulates negatively BCR-, TCR-, and FcR-dependent cell activation. *Immunity* (1995) 3(5):635–46. doi: 10.1016/1074-7613(95)90134-5
97. Long EO. Regulation of immune responses through inhibitory receptors. *Annu Rev Immunol* (1999) 17:875–904. doi: 10.1146/annurev.immunol.17.1.875
98. D'Ambrosio D, Hippen KL, Minskoff SA, Mellman I, Pani G, Siminovitch KA, et al. Recruitment and activation of PTP1C in negative regulation of antigen receptor signaling by Fc gamma RIIB1. *Science* (1995) 268 (5208):293–7. doi: 10.1126/science.7716523
99. Malbec O, Fong DC, Turner M, Tybulewicz VL, Cambier JC, Fridman WH, et al. Fc epsilon receptor I-associated lyn-dependent phosphorylation of Fc gamma receptor IIB during negative regulation of mast cell activation. *J Immunol* (1998) 160(4):1647–58.
100. Huber M, Helgason CD, Damen JE, Liu L, Humphries RK, Krystal G. The src homology 2-containing inositol phosphatase (SHIP) is the gatekeeper of mast cell degranulation. *Proc Natl Acad Sci U S A* (1998) 95(19):11330–5. doi: 10.1073/pnas.95.19.11330
101. Malbec O, Cassard L, Albanesi M, Jonsson F, Mancardi D, Chicanne G, et al. Trans-inhibition of activation and proliferation signals by Fc receptors in mast cells and basophils. *Sci Signal* (2016) 9(459):ra126. doi: 10.1126/scisignal.aag1401
102. Bolland S, Ravetch JV. Spontaneous autoimmune disease in Fc(gamma) RIIB-deficient mice results from strain-specific epistasis. *Immunity* (2000) 13 (2):277–85. doi: 10.1016/S1074-7613(00)00027-3
103. Radstake TR, Franke B, Wenink MH, Nabbe KC, Coenen MJ, Welsing P, et al. The functional variant of the inhibitory Fcgamma receptor IIb (CD32B) is associated with the rate of radiologic joint damage and dendritic cell function in rheumatoid arthritis. *Arthritis Rheum* (2006) 54(12):3828–37. doi: 10.1002/art.22275
104. Bruin M, Bierings M, Uiterwaal C, Revesz T, Bode L, Wiesman ME, et al. Platelet count, previous infection and FCGR2B genotype predict development of chronic disease in newly diagnosed idiopathic thrombocytopenia in childhood: results of a prospective study. *Br J Haematol* (2004) 127(5):561–7. doi: 10.1111/j.1365-2141.2004.05235.x
105. Petry J, Rahmoller J, Duhring L, Lilienthal GM, Lehrian S, Buhre JS, et al. Enriched blood IgG sialylation attenuates IgG-mediated and IgG-controlled-IgE-mediated allergic reactions. *J Allergy Clin Immunol* (2020 S0091-6749 (20):30887–3. doi: 10.1016/j.jaci.2020.05.056
106. Smith KG, Clatworthy MR. FcgammaRIIB in autoimmunity and infection: evolutionary and therapeutic implications. *Nat Rev Immunol* (2010) 10 (5):328–43. doi: 10.1038/nri2762
107. Gjertsson I, Kleinau S, Tarkowski A. The impact of Fcgamma receptors on Staphylococcus aureus infection. *Microb Pathog* (2002) 33(4):145–52. doi: 10.1006/mpat.2002.0524
108. Willcocks LC, Carr EJ, Niederer HA, Rayner TF, Williams TN, Yang W, et al. A defunctioning polymorphism in FCGR2B is associated with protection against malaria but susceptibility to systemic lupus erythematosus. *Proc Natl Acad Sci U S A* (2010) 107(17):7881–5. doi: 10.1073/pnas.0915133107
109. Malveaux FJ, Conroy MC, Adkinson NF Jr., Lichtenstein LM. IgE receptors on human basophils. Relationship to serum IgE concentration. *J Clin Invest* (1978) 62(1):176–81. doi: 10.1172/JCI109103
110. Saini SS, Klion AD, Holland SM, Hamilton RG, Bochner BS, Macglashan DW Jr. The relationship between serum IgE and surface levels of FcepsilonR on human leukocytes in various diseases: correlation of expression with FcepsilonRI on basophils but not on monocytes or eosinophils. *J Allergy Clin Immunol* (2000) 106(3):514–20. doi: 10.1067/mai.2000.108431
111. Furuichi K, Rivera J, Isersky C. The receptor for immunoglobulin E on rat basophilic leukemia cells: effect of ligand binding on receptor expression. *Proc Natl Acad Sci U S A* (1985) 82(5):1522–5. doi: 10.1073/pnas.82.5.1522
112. Lantz CS, Yamaguchi M, Oettgen HC, Katona IM, Miyajima I, Kinet JP, et al. IgE regulates mouse basophil Fc epsilon RI expression in vivo. *J Immunol* (1997) 158(6):2517–21.
113. Yamaguchi M, Lantz CS, Oettgen HC, Katona IM, Fleming T, Miyajima I, et al. IgE enhances mouse mast cell Fc(epsilon)RI expression in vitro and in vivo: evidence for a novel amplification mechanism in IgE-dependent reactions. *J Exp Med* (1997) 185(4):663–72. doi: 10.1084/jem.185.4.663
114. Beck LA, Marcotte GV, MacGlashan D, Togias A, Saini S. Omalizumab-induced reductions in mast cell Fce psilon RI expression and function. *J Allergy Clin Immunol* (2004) 114(3):527–30. doi: 10.1016/j.jaci.2004.06.032
115. MacGlashan DWJr., Bochner BS, Adelman DC, Jardieu PM, Togias A, McKenzie-White J, et al. Down-regulation of Fc(epsilon)RI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. *J Immunol* (1997) 158(3):1438–45.
116. Prussin C, Griffith DT, Boesel KM, Lin H, Foster B, Casale TB. Omalizumab treatment downregulates dendritic cell FcepsilonRI expression. *J Allergy Clin Immunol* (2003) 112(6):1147–54. doi: 10.1016/j.jaci.2003.10.003
117. Saini SS, MacGlashan DW Jr., Sterbinsky SA, Togias A, Adelman DC, Lichtenstein LM, et al. Down-regulation of human basophil IgE and FC epsilon RI alpha surface densities and mediator release by anti-IgE-infusions is reversible in vitro and in vivo. *J Immunol* (1999) 162(9):5624–30.
118. Borkowski TA, Jouvin MH, Lin SY, Kinet JP. Minimal requirements for IgE-mediated regulation of surface Fc epsilon RI. *J Immunol* (2001) 167(3):1290–6. doi: 10.4049/jimmunol.167.3.1290
119. Gurish MF, Bryce PJ, Tao H, Kisselgof AB, Thornton EM, Miller HR, et al. IgE enhances parasite clearance and regulates mast cell responses in mice infected with Trichinella spiralis. *J Immunol* (2004) 172(2):1139–45. doi: 10.4049/jimmunol.172.2.1139
120. Mathias CB, Freyschmidt EJ, Caplan B, Jones T, Poddighe D, Xing W, et al. IgE influences the number and function of mature mast cells, but not progenitor recruitment in allergic pulmonary inflammation. *J Immunol* (2009) 182(4):2416–24. doi: 10.4049/jimmunol.0801569
121. Burd PR, Thompson WC, Max EE, Mills FC. Activated mast cells produce interleukin 13. *J Exp Med* (1995) 181(4):1373–80. doi: 10.1084/jem.181.4.1373
122. Ikeda K, Nakajima H, Suzuki K, Kagami S, Hirose K, Suto A, et al. Mast cells produce interleukin-25 upon Fc epsilon RI-mediated activation. *Blood* (2003) 101(9):3594–6. doi: 10.1182/blood-2002-09-2817
123. Huang FJ, Ma YL, Tang RY, Gong WC, Li J, Chen CX, et al. Interleukin-4- and NACHT, LRR and PYD domains-containing protein 3-independent

mechanisms of alum enhanced T helper type 2 responses on basophils. *Immunology* (2016) 149(2):238–51. doi: 10.1111/imm.12636
124. Bryce PJ, Miller ML, Miyajima I, Tsai M, Galli SJ, Oettgen HC. Immune sensitization in the skin is enhanced by antigen-independent effects of IgE. *Immunity* (2004) 20(4):381–92. doi: 10.1016/S1074-7613(04)00080-9
125. Ando T, Matsumoto K, Namiranian S, Yamashita H, Glatthorn H, Kimura M, et al. Mast cells are required for full expression of allergen/SEB-induced skin inflammation. *J Invest Dermatol* (2013) 133(12):2695–705. doi: 10.1038/jid.2013.250
126. Suto H, Nakae S, Kakurai M, Sedgwick JD, Tsai M, Galli SJ. Mast cell-associated TNF promotes dendritic cell migration. *J Immunol* (2006) 176 (7):4102–12. doi: 10.4049/jimmunol.176.7.4102
127. Jawdat DM, Albert EJ, Rowden G, Haidl ID, Marshall JS. IgE-mediated mast cell activation induces Langerhans cell migration in vivo. *J Immunol* (2004) 173(8):5275–82. doi: 10.4049/jimmunol.173.8.5275
128. Yu M, Tsai M, Tam SY, Jones C, Zehnder J, Galli SJ. Mast cells can promote the development of multiple features of chronic asthma in mice. *J Clin Invest* (2006) 116(6):1633–41. doi: 10.1172/JCI25702
129. Hill DA, Siracusa MC, Abt MC, Kim BS, Kobuley D, Kubo M, et al. Commensal bacteria-derived signals regulate basophil hematopoiesis and allergic inflammation. *Nat Med* (2012) 18(4):538–46. doi: 10.1038/nm.2657
130. Motomura Y, Morita H, Moro K, Nakae S, Artis D, Endo TA, et al. Basophil-derived interleukin-4 controls the function of natural helper cells, a member of ILC2s, in lung inflammation. *Immunity* (2014) 40(5):758–71. doi: 10.1016/j.immuni.2014.04.013
131. Burton OT, Noval Rivas M, Zhou JS, Logsdon SL, Darling AR, Koleoglou KJ, et al. Immunoglobulin E signal inhibition during allergen ingestion leads to reversal of established food allergy and induction of regulatory T cells. *Immunity* (2014) 41(1):141–51. doi: 10.1016/j.immuni.2014.05.017
132. Kashiwakura JI, Ando T, Karasuyama H, Kubo M, Matsumoto K, Matsuda T, et al. The basophil-IL-4-mast cell axis is required for food allergy. *Allergy* (2019) 74(10):1992–6. doi: 10.1111/all.13834
133. Reber LL, Marichal T, Mukai K, Kita Y, Tokuoka SM, Roers A, et al. Selective ablation of mast cells or basophils reduces peanut-induced anaphylaxis in mice. *J Allergy Clin Immunol* (2013) 132(4):881–8.e1-11. doi: 10.1016/j.jaci.2013.06.008
134. Lei A, He Y, Yang Q, Li X, Li R. Role of myeloid cells in the regulation of group 2 innate lymphoid cell-mediated allergic inflammation. *Immunology* (2020) 161(1):18–24. doi: 10.1111/imm.13232
135. Burton OT, Medina Tamayo J, Stranks AJ, Miller S, Koleoglou KJ, Weinberg EO, et al. IgE promotes type 2 innate lymphoid cells in murine food allergy. *Clin Exp Allergy* (2018) 48(3):288–96. doi: 10.1111/cea.13075
136. Nadeau KC, Schneider LC, Hoyte L, Borras I, Umetsu DT. Rapid oral desensitization in combination with omalizumab therapy in patients with cow's milk allergy. *J Allergy Clin Immunol* (2011) 127(6):1622–4. doi: 10.1016/j.jaci.2011.04.009
137. MacGinnitie AJ, Rachid R, Gragg H, Little SV, Lakin P, Cianferoni A, et al. Omalizumab facilitates rapid oral desensitization for peanut allergy. *J Allergy Clin Immunol* (2017) 139(3):873–81.e8. doi: 10.1016/j.jaci.2016.08.010
138. Schneider LC, Rachid R, LeBovidge J, Blood E, Mittal M, Umetsu DT. A pilot study of omalizumab to facilitate rapid oral desensitization in high-risk peanut-allergic patients. *J Allergy Clin Immunol* (2013) 132(6):1368–74. doi: 10.1016/j.jaci.2013.09.046
139. Stranks AJ, Minnicozzi SC, Miller SJ, Burton OT, Logsdon SL, Spergel JM, et al. Immunoglobulin E blockade during food allergen ingestion enhances the induction of inhibitory immunoglobulin G antibodies. *Ann Allergy Asthma Immunol* (2019) 122(2):213–5. doi: 10.1016/j.anai.2018.10.024
140. Rosenwasser LJ. Mechanisms of IgE Inflammation. *Curr Allergy Asthma Rep* (2011) 11(2):178–83. doi: 10.1007/s11882-011-0179-6
141. Sallmann E, Reininger B, Brandt S, Duschek N, Hoflehner E, Garner-Spitzer E, et al. High-affinity IgE receptors on dendritic cells exacerbate Th2-dependent inflammation. *J Immunol* (2011) 187(1):164–71. doi: 10.4049/jimmunol.1003392
142. Ding Z, Dahlin JS, Xu H, Heyman B. IgE-mediated enhancement of CD4(+) T cell responses requires antigen presentation by CD8alpha(-) conventional dendritic cells. *Sci Rep* (2016) 6:28290. doi: 10.1038/srep28290
143. Holm J, Willumsen N, Wurtzen PA, Christensen LH, Lund K. Facilitated antigen presentation and its inhibition by blocking IgG antibodies depends on IgE repertoire complexity. *J Allergy Clin Immunol* (2011) 127(4):1029–37. doi: 10.1016/j.jaci.2011.01.062
144. Cernadas M, De Sanctis GT, Krinzman SJ, Mark DA, Donovan CE, Listman JA, et al. CD23 and allergic pulmonary inflammation: potential role as an inhibitor. *Am J Respir Cell Mol Biol* (1999) 20(1):1–8. doi: 10.1165/ajrcmb.20.1.3299
145. Haczku A, Takeda K, Hamelmann E, Loader J, Joetham A, Redai I, et al. CD23 exhibits negative regulatory effects on allergic sensitization and airway hyperresponsiveness. *Am J Respir Crit Care Med* (2000) 161(3 Pt 1):952–60. doi: 10.1164/ajrccm.161.3.9905046
146. Yu P, Kosco-Vilbois M, Richards M, Kohler G, Lamers MC. Negative feedback regulation of IgE synthesis by murine CD23. *Nature* (1994) 369 (6483):753–6. doi: 10.1038/369753a0
147. Payet ME, Woodward EC, Conrad DH. Humoral response suppression observed with CD23 transgenics. *J Immunol* (1999) 163(1):217–23.
148. Payet-Jamroz M, Helm SL, Wu J, Kilmon M, Fakher M, Basalp A, et al. Suppression of IgE responses in CD23-transgenic animals is due to expression of CD23 on nonlymphoid cells. *J Immunol* (2001) 166(8):4863–9. doi: 10.4049/jimmunol.166.8.4863
149. Rosenwasser LJ, Busse WW, Lizambri RG, Olejnik TA, Totoritis MC. Allergic asthma and an anti-CD23 mAb (IDEC-152): results of a phase I, single-dose, dose-escalating clinical trial. *J Allergy Clin Immunol* (2003) 112 (3):563–70. doi: 10.1016/S0091-6749(03)01861-X
150. Saxon A, Kurbe-Leamer M, Behle K, Max EE, Zhang K. Inhibition of human IgE production via Fc epsilon R-II stimulation results from a decrease in the mRNA for secreted but not membrane epsilon H chains. *J Immunol* (1991) 147(11):4000–6.
151. Rosenwasser LJ, Meng J. Anti-CD23. *Clin Rev Allergy Immunol* (2005) 29 (1):61–72. doi: 10.1385/CRIAI:29:1:061
152. Holt PG, Strickland D, Bosco A, Belgrave D, Hales B, Simpson A, et al. Distinguishing benign from pathologic TH2 immunity in atopic children. *J Allergy Clin Immunol* (2016) 137(2):379–87. doi: 10.1016/j.jaci.2015.08.044
153. Custovic A, Soderstrom L, Ahlstedt S, Sly PD, Simpson A, Holt PG. Allergen-specific IgG antibody levels modify the relationship between allergen-specific IgE and wheezing in childhood. *J Allergy Clin Immunol* (2011) 127(6):1480–5. doi: 10.1016/j.jaci.2011.03.014
154. Savilahti EM, Rantanen V, Lin JS, Karinen S, Saarinen KM, Goldis M, et al. Early recovery from cow's milk allergy is associated with decreasing IgE and increasing IgG4 binding to cow's milk epitopes. *J Allergy Clin Immunol* (2010) 125(6):1315–21.e9. doi: 10.1016/j.jaci.2010.03.025
155. Ruiter B, Knol EF, van Neerven RJ, Garssen J, Bruijnzeel-Koomen CA, Knulst AC, et al. Maintenance of tolerance to cow's milk in atopic individuals is characterized by high levels of specific immunoglobulin G4. *Clin Exp Allergy* (2007) 37(7):1103–10. doi: 10.1111/j.1365-2222.2007.02749.x
156. Santos AF, James LK, Bahnson HT, Shamji MH, Couto-Francisco NC, Islam S, et al. IgG4 inhibits peanut-induced basophil and mast cell activation in peanut-tolerant children sensitized to peanut major allergens. *J Allergy Clin Immunol* (2015) 135(5):1249–56. doi: 10.1016/j.jaci.2015.01.012
157. Wisniewski J, Agrawal R, Woodfolk JA. Mechanisms of tolerance induction in allergic disease: integrating current and emerging concepts. *Clin Exp Allergy* (2013) 43(2):164–76. doi: 10.1111/cea.12016
158. Aalberse RC, Stapel SO, Schuurman J, Rispens T. Immunoglobulin G4: an odd antibody. *Clin Exp Allergy* (2009) 39(4):469–77. doi: 10.1111/j.1365-2222.2009.03207.x
159. Davies AM, Sutton BJ. Human IgG4: a structural perspective. *Immunol Rev* (2015) 268(1):139–59. doi: 10.1111/imr.12349
160. Aalberse RC, van der Gaag R, van Leeuwen J. Serologic aspects of IgG4 antibodies. I. Prolonged immunization results in an IgG4-restricted response. *J Immunol* (1983) 130(2):722–6.
161. Francis JN, James LK, Paraskevopoulos G, Wong C, Calderon MA, Durham SR, et al. Grass pollen immunotherapy: IL-10 induction and suppression of late responses precedes IgG4 inhibitory antibody activity. *J Allergy Clin Immunol* (2008) 121(5):1120–5.e2. doi: 10.1016/j.jaci.2008.01.072
162. Jeannin P, Lecoanet S, Delneste Y, Gauchat JF, Bonnefoy JY. IgE versus IgG4 production can be differentially regulated by IL-10. *J Immunol* (1998) 160 (7):3555–61.
163. Satoguina JS, Weyand E, Larbi J, Hoerauf A. T regulatory-1 cells induce IgG4 production by B cells: role of IL-10. *J Immunol* (2005) 174(8):4718–26. doi: 10.4049/jimmunol.174.8.4718
164. Aalberse RC, Schuurman J. IgG4 breaking the rules. *Immunology* (2002) 105 (1):9–19. doi: 10.1046/j.0019-2805.2001.01341.x

165. Frischmeyer-Guerrerio PA, Masilamani M, Gu W, Brittain E, Wood R, Kim J, et al. Mechanistic correlates of clinical responses to omalizumab in the setting of oral immunotherapy for milk allergy. *J Allergy Clin Immunol* (2017) 140(4):1043–53.e8. doi: 10.1016/j.jaci.2017.03.028
166. Santos AF, James LK, Kwok M, McKendry RT, Anagnostou K, Clark AT, et al. Peanut oral immunotherapy induces blocking antibodies but does not change the functional characteristics of peanut-specific IgE. *J Allergy Clin Immunol* (2020) 145(1):440–3.e5. doi: 10.1016/j.jaci.2019.09.005
167. Skripak JM, Nash SD, Rowley H, Brereton NH, Oh S, Hamilton RG, et al. A randomized, double-blind, placebo-controlled study of milk oral immunotherapy for cow's milk allergy. *J Allergy Clin Immunol* (2008) 122 (6):1154–60. doi: 10.1016/j.jaci.2008.09.030
168. Jones SM, Pons L, Roberts JL, Scurlock AM, Perry TT, Kulis M, et al. Clinical efficacy and immune regulation with peanut oral immunotherapy. *J Allergy Clin Immunol* (2009) 124(2):292–300.e1-97. doi: 10.1016/j.jaci.2009.05.022
169. Vickery BP, Pons L, Kulis M, Steele P, Jones SM, Burks AW. Individualized IgE-based dosing of egg oral immunotherapy and the development of tolerance. *Ann Allergy Asthma Immunol* (2010) 105(6):444–50. doi: 10.1016/j.anai.2010.09.030
170. Kim EH, Bird JA, Kulis M, Laubach S, Pons L, Shreffler W, et al. Sublingual immunotherapy for peanut allergy: clinical and immunologic evidence of desensitization. *J Allergy Clin Immunol* (2011) 127(3):640–6.e1. doi: 10.1016/j.jaci.2010.12.1083
171. Bedoret D, Singh AK, Shaw V, Hoyte EG, Hamilton R, DeKruyff RH, et al. Changes in antigen-specific T-cell number and function during oral desensitization in cow's milk allergy enabled with omalizumab. *Mucosal Immunol* (2012) 5(3):267–76. doi: 10.1038/mi.2012.5
172. Vickery BP, Lin J, Kulis M, Fu Z, Steele PH, Jones SM, et al. Peanut oral immunotherapy modifies IgE and IgG4 responses to major peanut allergens. *J Allergy Clin Immunol* (2013) 131(1):128–34.e1-3. doi: 10.1016/j.jaci.2012.10.048
173. Caubet JC, Bencharitiwong R, Moshier E, Godbold JH, Sampson HA, Nowak-Wegrzyn A. Significance of ovomucoid- and ovalbumin-specific IgE/IgG(4) ratios in egg allergy. *J Allergy Clin Immunol* (2012) 129 (3):739–47. doi: 10.1016/j.jaci.2011.11.053
174. Burton OT, Logsdon SL, Zhou JS, Medina-Tamayo J, Abdel-Gadir A, Noval Rivas M, et al. Oral immunotherapy induces IgG antibodies that act through FcgammaRIIb to suppress IgE-mediated hypersensitivity. *J Allergy Clin Immunol* (2014) 134(6):1310–7.e6. doi: 10.1016/j.jaci.2014.05.042
175. Tsai M, Mukai K, Chinthrajah RS, Nadeau KC, Galli SJ. Sustained successful peanut oral immunotherapy associated with low basophil activation and peanut-specific IgE. *J Allergy Clin Immunol* (2020) 145(3):885–96.e6. doi: 10.1016/j.jaci.2019.10.038
176. Burton OT, Tamayo JM, Stranks AJ, Koleoglou KJ, Oettgen HC. Allergen-specific IgG antibody signaling through FcgammaRIIb promotes food tolerance. *J Allergy Clin Immunol* (2018) 141(1):189–201.e3. doi: 10.1016/j.jaci.2017.03.045
177. Orgel K, Burk C, Smeekens J, Suber J, Hardy L, Guo R, et al. Blocking antibodies induced by peanut oral and sublingual immunotherapy suppress basophil activation and are associated with sustained unresponsiveness. *Clin Exp Allergy* (2019) 49(4):461–70. doi: 10.1111/cea.13305
178. Burton OT, Stranks AJ, Tamayo JM, Koleoglou KJ, Schwartz LB, Oettgen HC. A humanized mouse model of anaphylactic peanut allergy. *J Allergy Clin Immunol* (2017) 139(1):314–22.e9. doi: 10.1016/j.jaci.2016.04.034
179. Burton OT, Epp A, Fanny ME, Miller SJ, Stranks AJ, Teague JE, et al. Tissue-Specific Expression of the Low-Affinity IgG Receptor, FcgammaRIIb, on Human Mast Cells. *Front Immunol* (2018) 9:1244. doi: 10.3389/fimmu.2018.01244
180. Zhao W, Kepley CL, Morel PA, Okumoto LM, Fukuoka Y, Schwartz LB. Fc gamma RIIa, not Fc gamma RIIb, is constitutively and functionally expressed on skin-derived human mast cells. *J Immunol* (2006) 177(1):694–701. doi: 10.4049/jimmunol.177.1.694
181. Burks AW, Jones SM, Wood RA, Fleischer DM, Sicherer SH, Lindblad RW, et al. Oral immunotherapy for treatment of egg allergy in children. *N Engl J Med* (2012) 367(3):233–43. doi: 10.1056/NEJMoa1200435
182. Begin P, Winterroth LC, Dominguez T, Wilson SP, Bacal L, Mehrotra A, et al. Safety and feasibility of oral immunotherapy to multiple allergens for food allergy. *Allergy Asthma Clin Immunol* (2014) 10(1):1. doi: 10.1186/1710-1492-10-1
183. Syed A, Garcia MA, Lyu SC, Bucayu R, Kohli A, Ishida S, et al. Peanut oral immunotherapy results in increased antigen-induced regulatory T-cell function and hypomethylation of forkhead box protein 3 (FOXP3). *J Allergy Clin Immunol* (2014) 133(2):500–10. doi: 10.1016/j.jaci.2013.12.1037
184. Vickery BP, Scurlock AM, Kulis M, Steele PH, Kamilaris J, Berglund JP, et al. Sustained unresponsiveness to peanut in subjects who have completed peanut oral immunotherapy. *J Allergy Clin Immunol* (2014) 133(2):468–75. doi: 10.1016/j.jaci.2013.11.007
185. Wu J, Lin R, Huang J, Guan W, Oetting WS, Sriramarao P, et al. Functional Fcgamma receptor polymorphisms are associated with human allergy. *PLoS One* (2014) 9(2):e89196. doi: 10.1371/journal.pone.0089196
186. Li X, Wu J, Carter RH, Edberg JC, Su K, Cooper GS, et al. A novel polymorphism in the Fcgamma receptor IIB (CD32B) transmembrane region alters receptor signaling. *Arthritis Rheum* (2003) 48(11):3242–52. doi: 10.1002/art.11313
187. Kono H, Kyogoku C, Suzuki T, Tsuchiya N, Honda H, Yamamoto K, et al. FcgammaRIIB Ile232Thr transmembrane polymorphism associated with human systemic lupus erythematosus decreases affinity to lipid rafts and attenuates inhibitory effects on B cell receptor signaling. *Hum Mol Genet* (2005) 14(19):2881–92. doi: 10.1093/hmg/ddi320
188. Floto RA, Clatworthy MR, Heilbronn KR, Rosner DR, MacAry PA, Rankin A, et al. Loss of function of a lupus-associated FcgammaRIIb polymorphism through exclusion from lipid rafts. *Nat Med* (2005) 11(10):1056–8. doi: 10.1038/nm1288
189. Freidl R, Gstoettner A, Baranyi U, Swoboda I, Stolz F, Focke-Tejkl M, et al. Blocking antibodies induced by immunization with a hypoallergenic parvalbumin mutant reduce allergic symptoms in a mouse model of fish allergy. *J Allergy Clin Immunol* (2017) 139(6):1897–905.e1. doi: 10.1016/j.jaci.2016.10.018
190. Ohsaki A, Venturelli N, Buccigrosso TM, Osganian SK, Lee J, Blumberg RS, et al. Maternal IgG immune complexes induce food allergen-specific tolerance in offspring. *J Exp Med* (2018) 215(1):91–113. doi: 10.1084/jem.20171163
191. Lupinek C, Hochwallner H, Johansson C, Mie A, Rigler E, Scheynius A, et al. Maternal allergen-specific IgG might protect the child against allergic sensitization. *J Allergy Clin Immunol* (2019) 144(2):536–48. doi: 10.1016/j.jaci.2018.11.051
192. Kucuk ZY, Strait R, Khodoun MV, Mahler A, Hogan S, Finkelman FD. Induction and suppression of allergic diarrhea and systemic anaphylaxis in a murine model of food allergy. *J Allergy Clin Immunol* (2012) 129(5):1343–8. doi: 10.1016/j.jaci.2012.03.004
193. Brandt EB, Strait RT, Hershko D, Wang Q, Muntel EE, Scribner TA, et al. Mast cells are required for experimental oral allergen-induced diarrhea. *J Clin Invest* (2003) 112(11):1666–77. doi: 10.1172/JCI19785
194. Croote D, Darmanis S, Nadeau KC, Quake SR. High-affinity allergen-specific human antibodies cloned from single IgE B cell transcriptomes. *Science* (2018) 362(6420):1306–9. doi: 10.1126/science.aau2599
195. Yang Z, Sullivan BM, Allen CD. Fluorescent in vivo detection reveals that IgE (+) B cells are restrained by an intrinsic cell fate predisposition. *Immunity* (2012) 36(5):857–72. doi: 10.1016/j.immuni.2012.02.009
196. Laffleur B, Duchez S, Tarte K, Denis-Lagache N, Peron S, Carrion C, et al. Self-Restrained B Cells Arise following Membrane IgE Expression. *Cell Rep* (2015) 10(6):900–9. doi: 10.1016/j.celrep.2015.01.023
197. Haniuda K, Fukao S, Kodama T, Hasegawa H, Kitamura D. Autonomous membrane IgE signaling prevents IgE-memory formation. *Nat Immunol* (2016) 17(9):1109–17. doi: 10.1038/ni.3508
198. Tong P, Granato A, Zuo T, Chaudhary N, Zuiani A, Han SS, et al. IgH isotype-specific B cell receptor expression influences B cell fate. *Proc Natl Acad Sci U S A* (2017) 114(40):E8411–E20. doi: 10.1073/pnas.1704962114
199. Xiong H, Dolpady J, Wabl M, Curotto de Lafaille MA, Lafaille JJ. Sequential class switching is required for the generation of high affinity IgE antibodies. *J Exp Med* (2012) 209(2):353–64. doi: 10.1084/jem.20111941
200. Looney TJ, Lee JY, Roskin KM, Hoh RA, King J, Glanville J, et al. Human B-cell isotype switching origins of IgE. *J Allergy Clin Immunol* (2016) 137 (2):579–86.e7. doi: 10.1016/j.jaci.2015.07.014
201. Kobayashi T, Iijima K, Dent AL, Kita H. Follicular helper T cells mediate IgE antibody response to airborne allergens. *J Allergy Clin Immunol* (2017) 139 (1):300–13.e7. doi: 10.1016/j.jaci.2016.04.021

202. Meli AP, Fontes G, Leung Soo C, King IL. T Follicular Helper Cell-Derived IL-4 Is Required for IgE Production during Intestinal Helminth Infection. *J Immunol* (2017) 199(1):244–52. doi: 10.4049/jimmunol.1700141
203. Clement RL, Daccache J, Mohammed MT, Diallo A, Blazar BR, Kuchroo VK, et al. Follicular regulatory T cells control humoral and allergic immunity by restraining early B cell responses. *Nat Immunol* (2019) 20(10):1360–71. doi: 10.1038/s41590-019-0472-4
204. Gowthaman U, Chen JS, Zhang B, Flynn WF, Lu Y, Song W, et al. Identification of a T follicular helper cell subset that drives anaphylactic IgE. *Science* (2019) 365(6456). doi: 10.1126/science.aaw6433
205. Orengo JM, Radin AR, Kamat V, Badithe A, Ben LH, Bennett BL, et al. Treating cat allergy with monoclonal IgG antibodies that bind allergen and prevent IgE engagement. *Nat Commun* (2018) 9(1):1421. doi: 10.1038/s41467-018-03636-8
206. Bargou R, Leo E, Zugmaier G, Klinger M, Goebeler M, Knop S, et al. Tumor regression in cancer patients by very low doses of a T cell-engaging antibody. *Science* (2008) 321(5891):974–7. doi: 10.1126/science.1158545
207. van Spriel AB, van Ojik HH, van De Winkel JG. Immunotherapeutic perspective for bispecific antibodies. *Immunol Today* (2000) 21(8):391–7. doi: 10.1016/S0167-5699(00)01659-5
208. Zhu D, Kepley CL, Zhang M, Zhang K, Saxon A. A novel human immunoglobulin Fc gamma Fc epsilon bifunctional fusion protein inhibits Fc epsilon RI-mediated degranulation. *Nat Med* (2002) 8(5):518–21. doi: 10.1038/nm0502-518
209. Zhu D, Kepley CL, Zhang K, Terada T, Yamada T, Saxon A. A chimeric human-cat fusion protein blocks cat-induced allergy. *Nat Med* (2005) 11 (4):446–9. doi: 10.1038/nm1219
210. Tam SW, Demissie S, Thomas D, Daeron M. A bispecific antibody against human IgE and human FcgammaRII that inhibits antigen-induced histamine release by human mast cells and basophils. *Allergy* (2004) 59 (7):772–80. doi: 10.1111/j.1398-9995.2004.00332.x
211. Jackman J, Chen Y, Huang A, Moffat B, Scheer JM, Leong SR, et al. Development of a two-part strategy to identify a therapeutic human bispecific antibody that inhibits IgE receptor signaling. *J Biol Chem* (2010) 285(27):20850–9. doi: 10.1074/jbc.M110.113910
212. Cahenzli J, Koller Y, Wyss M, Geuking MB, McCoy KD. Intestinal microbial diversity during early-life colonization shapes long-term IgE levels. *Cell Host Microbe* (2013) 14(5):559–70. doi: 10.1016/j.chom.2013.10.004
213. Stefka AT, Feehley T, Tripathi P, Qiu J, McCoy K, Mazmanian SK, et al. Commensal bacteria protect against food allergen sensitization. *Proc Natl Acad Sci U S A* (2014) 111(36):13145–50. doi: 10.1073/pnas.1412008111

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 14 December 2020
doi: 10.3389/fimmu.2020.602660

Oral Immunotherapy and Basophil and Mast Cell Reactivity in Food Allergy

*Anuya Paranjape[1,2], Mindy Tsai[1,2], Kaori Mukai[1], Ramona A. Hoh[1], Shilpa A. Joshi[1], R. Sharon Chinthrajah[2,3], Kari C. Nadeau[2,3], Scott D. Boyd[1,2] and Stephen J. Galli[1,2,4]**

[1] Department of Pathology, Stanford University School of Medicine, Stanford, CA, United States, [2] Sean N. Parker Center for Allergy and Asthma Research, Stanford University School of Medicine, Stanford, CA, United States, [3] Division of Pulmonary, Allergy and Critical Care Medicine and Division of Allergy, Immunology and Rheumatology, Stanford University, Stanford University School of Medicine, Stanford, CA, United States, [4] Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA, United States

OPEN ACCESS

Edited by:
Ana Olivera,
National Institutes of Health (NIH), United States

Reviewed by:
Edward Knol,
University Medical Center Utrecht, Netherlands
Michael D. Kulis,
University of North Carolina at Chapel Hill, United States

***Correspondence:**
Stephen J. Galli
sgalli@stanford.edu

Specialty section:
This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology

Received: 04 September 2020
Accepted: 10 November 2020
Published: 14 December 2020

Citation:
Paranjape A, Tsai M, Mukai K, Hoh RA, Joshi SA, Chinthrajah RS, Nadeau KC, Boyd SD and Galli SJ (2020) Oral Immunotherapy and Basophil and Mast Cell Reactivity in Food Allergy. Front. Immunol. 11:602660. doi: 10.3389/fimmu.2020.602660

Basophil activation tests (BATs) can closely monitor, *in vitro*, a patient's propensity to develop type I hypersensitivity reactions. Because of their high specificity and sensitivity, BATs have become promising diagnostic tools, especially in cases with equivocal clinical histories, skin prick test results, and/or levels of specific IgE to allergen extracts. BATs also are useful as tools for monitoring the effects of treatment, since oral immunotherapy (OIT) studies report a diminution in patients' basophil responsiveness over the course of OIT. This review will discuss the BAT findings obtained before, during, and after OIT for food allergy. We will mainly focus on the association of basophil responsiveness, and alterations in basophil surface markers, with clinical outcomes and other clinical features, such as blood levels of specific IgG and IgE antibodies. The detailed analysis of these correlations will ultimately facilitate the use of BATs, along with other blood biomarkers, to differentiate short-term desensitization versus sustained unresponsiveness and to improve treatment protocols. Given the critical anatomic location of mast cells adjacent to the many IgE^+ plasma cells found in the gastrointestinal tissues of allergic individuals, we will also discuss the role of gastrointestinal mast cells in manifestations of food allergies.

Keywords: food oral immunotherapy, basophil activation tests, mast cells, skin prick tests, IgE, IgG, B cells

INTRODUCTION

Human and mouse studies have shown that mast cells and basophils are the primary immune effector cells in IgE-mediated food allergy (1–4). Most commonly, food allergy manifests as a form of immediate hypersensitivity, in which engagement of IgE bound to FcεRI on mast cells and basophils by specific food allergens leads to the release of pre-formed and newly synthesized mediators that elicit a range of pathological responses in several target tissues. Such responses range

from hives, itching, mild gastrointestinal discomfort, and diarrhea to intense systemic reactions which, in some cases, result in rapidly fatal anaphylaxis (5, 6).

Although FcεRI are highly expressed on both mast cells and basophils, these effectors are distinct cell populations that are regulated by different transcription factors, express distinct cell surface receptors, reside in anatomically distinct locations (7–10) and exhibit different activation thresholds to IgE-dependent stimulation, including that mediated by food allergens (11). In mouse models of food allergy, TSLP-elicited basophil expansion appears to be pivotal for cutaneous sensitization with food allergens (12–14) whereas IL-9-producing mucosal mast cells appear to be critical for intestinal mastocytosis after intragastric allergen exposure (3). In humans, studies of cat and peanut allergies have indicated that anti-IgE treatments might exhibit different response kinetics in skin mast cells and blood basophils (15, 16). It therefore should be kept in mind that basophils and mast cells may have complementary but distinct roles in the context of mouse or human food allergy.

This review will focus on basophil activation tests (BATs), which directly assess basophil reactivity, and skin prick tests (SPTs), which assess skin mast cell reactivity, obtained during food oral immunotherapy (OIT). We will discuss these in the context of clinical outcomes and will consider the use of these tools in monitoring OIT treatments.

BASOPHIL ACTIVATION TESTS (BATS)

BATs are *ex vivo* flow cytometry-based assays for measuring basophil activation. In 1991, Knol et al. noted increased expression of CD63 on the plasma membrane of purified basophils following their activation with anti-IgE or fMLP and also found the close correlation of increased basophil expression of CD63 with histamine release (17). The development of flow cytometry-based techniques to gate on human blood basophils made the assessment of CD63 possible in whole blood without any purification step (18, 19). Subsequent studies established the reliability of another activation maker, CD203c (20, 21), but differences in the activation kinetics of CD203c vs CD63 pointed towards differences in their mechanisms of up-regulation (22). While CD63 and CD203c remain the most popular markers, many other activation markers, such as CD107a, CD13, CD164, CD69, CD11b, and diamine oxidase (23, 24), have been used for assessment of basophil activation (25–27). The pros and cons of various basophil gating strategies and activation markers have been discussed in detail elsewhere (27–29). Because of their specificity and sensitivity, BATs are being evaluated for the diagnosis of food, drug, and venom allergies, and for monitoring the effects of immunotherapy or the natural resolution of allergies (27–30).

However, BAT studies have differed in anticoagulants used for blood collection, temperature and duration of blood storage before the test, and the presence or absence of IL-3 priming (21, 31, 32). Mukai et al. compared CD63 and CD203c expression at baseline and post activation among four conditions of storage (at room temperature for 4 or 24 h and at 4°C for 4 or 24 h) using blood collected in either EDTA or heparin (33). Activation-induced CD63 upregulation cannot be noted in blood samples collected in EDTA, emphasizing the need for extracellular physiological calcium/magnesium for CD63 upregulation (34). However, blood collected in heparin yielded similar outputs in CD63 and CD203c upregulation 4 or 24 h post blood draw, if stored at 4°C (33).

Another major difference in BAT protocols is the use of whole blood or enriched preparations of basophils (35). Whole blood preparations not only better mirror the physiological or pathological *in vivo* conditions [e.g., the presence of soluble factors and blocking antibodies (26, 32, 36, 37)], but also allow insight into resting levels of activation marker expression (26). However, since CD63 is a non-exclusive marker for basophils, there have been concerns that platelets binding to basophils might falsely increase "basophil CD63 expression" in whole blood assays (38, 39). Importantly, using flow cytometry and an immunohistochemical staining analysis with the platelet-specific marker CD41, Mukai et al. found that the appearance of $CD63^{hi}$ basophils is primarily due to basophil-derived CD63 (33).

An alternate approach to conventional BATs is the use of fluorescent avidin. Positively-charged avidin binds to negatively-charged granule constituents that are exteriorized on the cell surface post activation (39). While this method is relatively new, it holds the promise of offering a more sensitive and specific method for quantifying basophil activation in whole blood (40).

BASOPHIL RESPONSIVENESS AND CLINICAL PHENOTYPE

Two studies have analyzed basophil function among milk allergic subjects exhibiting different clinical phenotypes: allergic, heated milk tolerant, and outgrown. Basophil reactivity [quantified as % $CD63^{hi}$ cells (41), or expressed as %$CD63^{+}$ cells (42)], tested over a range of crude milk protein concentrations, was significantly lower among subjects tolerant to heated forms of milk than in those reacting to it (40). However, basophil reactivity was significantly higher in the heated milk-tolerant patients than in subjects who had outgrown milk allergy or were non-allergic. Notably, among heated milk-tolerant subjects, those with regular ingestion of heated milk exhibited less basophil reactivity, especially at lower milk protein concentrations (41). While basophil reactivity to anti-IgE stimulation was also lower in this heated milk-tolerant group, fMLP stimulation showed no differences.

Notably, Rubio et al. (43) also analyzed the value of BATs (assessed by upregulation of CD63) in distinguishing between children exhibiting persistent allergies to cow's milk and those who had developed tolerance naturally. They developed a decisional algorithm incorporating a combination of BAT results, together with specific IgE levels and SPTs, which successfully distinguished (at the 94% level) between children who had developed tolerance naturally (these had low BAT

results, as well as low specific IgE and SPT results) vs those who exhibited persistent allergies to cow's milk. However, the most important of the three measurements used in their algorithm was the BAT result.

Taken together, these studies indicate that assessment of allergen-specific basophil responses might be a useful tool for monitoring acquisition of allergen unresponsiveness during food allergy immunotherapies.

MEASUREMENTS OF BASOPHIL RESPONSIVENESS DURING ORAL IMMUNOTHERAPY

Results of BAT assays are commonly presented as mean fluorescence intensity (MFI) of activation markers or percentage of cells that are $CD63^+$ or $CD63^{hi}$. Basophil reactivity (also known as maximal response or CDmax) and sensitivity (i.e., effective dose at 50% of the maximal activation, ED_{50} or CD_{sens}) differ from patient to patient, and some studies recommend testing basophil responses over a range of allergen concentrations and expressing the results as area under the curve (AUC) of the dose response curve (44). When interpreting BAT data obtained from OIT studies it is important to keep in mind the particular representation used, as that might affect the interpretation of the data. The experimental settings that have been used to perform a BAT assay are listed in **Table 1**. The food OIT studies shown in **Table 2** list the BAT data obtained at various times during OIT treatment, and subsequent sections will discuss the clinical outcomes of some of these studies (45, 49, 51–53, 55) or the immune parameters (41, 46, 49, 51) measured. Although this review focuses on food OIT, we should note that a decrease in basophil responsiveness for food allergens has also been documented during studies of sublingual immunotherapy (SLIT) (49, 50, 57–59) and epicutaneous immunotherapy (EPIT) (60).

BASOPHIL RESPONSES AND CLINICAL OUTCOMES

In a SLIT/OIT study of 30 milk allergic patients showing favorable clinical outcomes, no reduction was observed during OIT in allergen or anti-IgE stimulated basophil histamine release, or in constitutive basophil Syk expression (50). However, spontaneous basophil histamine release was decreased in the SLIT/OIT group, beginning 20 weeks from initiating treatment. When subjects were divided based on whether or not they developed sustained unresponsiveness (passing a food challenge 6 weeks post avoidance), an increase in constitutive expression of CD63 and CD203c during the build-up phase was observed among those not developing sustained unresponsiveness. For none of the parameters, including spontaneous histamine release, did baseline values predict development of sustained unresponsiveness (50). However, this study used basophil-enriched mononuclear cells (BECs) obtained after double Percoll density centrifugation, not whole blood preparations, raising the possibility that the enrichment process might have affected the results by disrupting binding of IgG or other serum inhibitory factors (36, 37). The few studies using whole blood basophil preparations suggest that basophil responsiveness can help to predict the threshold and severity of allergic reactions during oral food challenge (43, 61, 62). In contrast, in a study of enriched basophil suspensions, Gorelik et al. (49) found no significant correlation between basophil activation (CD63 expression) and specific number or severity of allergic reactions to oral food challenges during a peanut OIT/SLIT crossover trial. They also found a negative correlation between achievement of sustained unresponsiveness (passing a food challenge at 4 to 6 weeks post avoidance) and peanut-induced histamine release, CD63 induction and IL-4 production analyzed at baseline. Histamine release and CD63 were measured with BECs after double Percoll density centrifugation and basophil intracellular IL-4 was measured in whole blood preparations. These studies suggest that processing of basophils before *ex vivo* activation can significantly affect BAT results.

Another important finding by Gorelik et al. (49) is that the significant negative correlation between basophil activation markers and development of sustained unresponsiveness was evident only at the lowest concentration of allergen extract used for *in vitro* basophil activation. This information may help in interpreting OIT results that found no significant correlations between basophil activation markers and development of sustained unresponsiveness, despite favorable clinical outcomes (50, 52). In Syed at al. (52), a single dose of peanut was used to measure activation-induced upregulation of CD203c MFI in whole blood basophils of 23 OIT subjects. Although CD203c MFI was significantly decreased in participants undergoing OIT compared to controls, there were no significant differences among those who developed unresponsiveness (no detectable

TABLE 1 | Experimental settings that have been used to perform BAT assays.

Starting material	Whole blood (45–48) or basophil-enriched mononuclear cells (BECs) obtained after double Percoll density centrifugation (49, 50)
Anticoagulant used to collect blood	Heparin (33, 47, 51) or EDTA (49, 50, 52, 53)
Storage time and temperature	Up to 24 h at 4°C (33, 51) or within 4 h at room temperature (21, 32)
Experimental conditions	Negative Control - media with (45–47, 54) or without (51) IL-3 Single (45, 50, 52) or multiple (46–49, 51, 54, 55) concentrations of the allergen Positive Control - anti-IgE and/or fMLP
Activation conditions	30 min (45–48, 51, 52, 54) or 15 min (53) at 37°C
Frequently used activation markers	CD63 (45–48, 50, 54), CD203c (45, 46, 50, 52)

TABLE 2 | BAT data obtained at various time points before, during, and after OIT in food allergy subjects.

Study size	Food extract concentration	Time points for BAT analysis	Basophil activation reported as	Major findings with respect to time into the treatment	Reference
29	Peanut (10, 1, 0.1 µg/ml)	Baseline, <4 months of OIT, 4–6 months, >6 months	%CD63hi	At a peanut concentration of 10 µg/ml, basophil activation was significantly reduced within first 4 months, and continued to decline beyond 6 months.	Jones et al. (47)
10	Egg (egg white, ovalbumin, ovomucoid) (500, 50, 5 ng/ml)	Baseline, 1 month post build-up	%CD63+, CD63 MFI	Significant decrease in CD63 expression in all patients.	Vila et al. (56)
28	Peanut (10-10^-5 µg/ml). Egg white (1-10^-3 µg/ml).	Baseline, day 21–156, day 157–423	%CD63high, CD203c (MFI)	Significant suppression of peanut induced CD63 upregulation over time only in the peanut OIT group, no change in the placebo group. Significant reduction of CD63 upregulation at the highest egg concentration tested, the trend was evident at lower egg concentrations only in peanut OIT group. $	Thyagarajan et al. (46)
49	Peanut (1–1,000 ng/ml)	Baseline, at the time of desensitization OFC, and at the time of sustained unresponsiveness OFC	%CD63+	Basophil responsiveness did not increase during the 4-week avoidance period between desensitization and sustained unresponsiveness.	Kulis et al. (54)
99	Peanut (0.001–100 µg/ml)	Baseline, post desensitization	AUC for %CD63+, CD63 (MFI)	No significant within-patient differences identified after treatment.	Anagnostou et al. (48)
21	Peanut (0.1, 1, 10 ng/ml)	Baseline, end of blinded escalation phase, 6 months into the maintenance phase, 12 months into maintenance phase, 6 months into continued/add on therapy, 4–6 weeks off treatment	Histamine release (% of total), CD63 (% of total basophils), CD203 (MFI), Intracellular IL-4	For subjects receiving OIT, peanut-induced histamine release and CD63 significantly suppressed at the end of dose escalation and at 6 months into maintenance, but did reverse towards the end of maintenance phase. Peanut-induced IL-4 expression significantly reduced from the end of dose escalation through maintenance compared to baseline, but increased 4–6 weeks after the subjects were taken off the therapy. #	Gorelik et al. (49)
55	Egg (0.1, 0.01 ug/ml)	Baseline, 10 months into the trial, 22 months (end of desensitization), after avoidance (24 months)	%CD63+	Basophil reactivity showed significant reduction post baseline in children receiving egg OIT compared to those receiving placebo. #	Burks et al. (55)
15	Unheated milk (100 ng/ml), heated milk (100 ng/ml)	Baseline, 12 months into the OIT	Percentage of CD63 or CD203c expression above baseline levels	#	Goldberg et al. (45)
23	Peanut (1 µg/ml)	At baseline, 3, 6, 9, 12, 18, 24, 27, and 30 months	CD203c (MFI)	Peanut induced CD203c expression in participants undergoing OIT decreased significantly at 3 months and kept reducing until 9 months compared to that in control subjects.	Syed et al. (52)
30	Peanut, Ara h 1, 2, and 6	Baseline, 3 months into active OIT, post maintenance phase (12 months), post avoidance (13 months)	AUC (%CD63hi), sensitivity (measured by using the dose that induced 50% of the maximum response)	#	Patil et al. (53)
30	Milk (10 µg/ml)	Baseline, end of build-up phase, end of maintenance, post avoidance	CD63 MFI, CD203c MFI	Spontaneous histamine release significantly reduced by week 6 in SLIT/OIT arm and remained reduced throughout (86 weeks) the study. No change in milk-induced histamine release. No change in constitutive CD63, Syk expression.	Keet et al. (50)
120	Peanut (0.1, 1, 10, 100, and 1,000 ng/ml)	Baseline, week 12, 52,104, and 117 of OIT	AUC (%CD63high), CD203c (MFI)	Peanut and anti-IgE induced AUC (%CD63hi) significantly reduced in OIT arm as early as 12 weeks and remained suppressed throughout the maintenance phase (week 104). Basophil responses were significantly lower in both arms of OIT (avoidance and low maintenance dose)	Tsai and Mukai et al. (51)

(Continued)

TABLE 2 | Continued

Study size	Food extract concentration	Time points for BAT analysis	Basophil activation reported as	Major findings with respect to time into the treatment	Reference
				at the primary endpoint (week 117), but were not significantly different vs each other. $ #	

$Results further discussed in Basophil Responses and Serum Immunoglobulins.
#Results further discussed in Basophil Responses and Clinical Outcomes.

clinical reaction 3 months post withdrawal of therapy) versus those who did not. Therefore, it is important to test a wide range of allergen concentrations in BATs.

The form of allergen used for *in vitro* basophil activation can also influence the results. In a peanut OIT study (53), SU (sustained unresponsiveness) and TD (transient desensitization) were evaluated in 22 participants 4 weeks post avoidance, and *in vitro* basophil responses induced by whole peanut extract or by various peanut allergen proteins were analyzed in basophils in whole blood. After desensitization by 12 months of OIT, followed by 4 weeks of peanut avoidance, basophil sensitivity (i.e., ED50, defined as the dose inducing 50% of the maximum response) to Ara h 2 significantly decreased in the SU group but not in the TD group. However, basophil sensitivity to whole peanut showed no significant difference between the groups. Moreover, when basophil reactivity was quantified as AUC, reactivity to Ara h 2 or to whole peanut was suppressed equally by the end of desensitization in both the SU and TD groups, but only the TD group rebounded post avoidance. Trends for activation with Ara h 6 were similar to those for Ara h 2, in terms of both sensitivity and reactivity (AUC).

A study of egg OIT involving 55 subjects (15 placebo, 40 OIT) tested basophil responses in whole blood and clinical desensitization at 10 and 22 months (55). Basophil responses (expressed as $\%CD63^{+}$ cells) at 10 months of OIT were significantly lower among desensitized vs non-desensitized subjects. However, no significant differences in basophil responses were observed between subjects who did or did not develop sustained unresponsiveness at 24 months (2 months after withdrawal of OIT). Notably, this study did not comment on basophil reactivity at baseline.

Different results were reported in a study of baked milk OIT in 15 milk-allergic subjects (49). Successful completion of the trial was defined as reaching the primary outcome dose of baked milk without adverse reactions at 1 year of treatment. Those succeeding in this trial exhibited a significantly lower mean difference between heated (180°C for 30 min) milk-induced and unheated milk-induced basophil CD203c expression (tested in whole blood at the beginning of the trial) than those who could not complete the trial (45). The group that successfully completed the trial also exhibited a trend toward lower values of heated (hypoallergenic form) milk-driven minus unheated (hyper-allergenic form) milk-driven basophil CD63 expression. This study suggested that patients whose basophils reacted less to the hypoallergenic vs more allergenic forms of this antigen at baseline became desensitized to the hypoallergenic form by the end of the trial.

Finally, our study of peanut OIT (51) divided 120 participants (25 placebo, 95 OIT) into groups depending on whether or not they developed sustained unresponsiveness (passing the oral food challenge either 13 weeks post avoidance or after 13 weeks of a low maintenance dose consumption, i.e. week 117 of OIT). A retrospective analysis of the groups with or without sustained unresponsiveness revealed that peanut-specific basophil responses were significantly lower among the group that developed sustained unresponsiveness at all time-points (weeks 12, 52, 104, 117) tested during OIT, and also at baseline (**Figure 1**).

Notably, we (51) also grouped peanut OIT participants according to baseline basophil responsiveness to peanut (calculated as AUC for $\%CD63^{high}$ cells) into LR (low responders), IR (intermediate responders) and HR (high responders) (**Figures 2A, B**). We found that LRs tolerated 2–3 times more peanut protein at the time of enrollment, pointing towards a relationship between basophil responsiveness and severity of allergic reactions during food challenges. Furthermore, while a larger fraction of the LR group (91%) developed sustained unresponsiveness at the primary endpoint (**Figure 2C**), those subjects from the IR and HR groups who showed substantial reduction (80–90%) of their peanut-induced basophil responses during the trial also achieved sustained unresponsiveness. This analysis thus revealed two groups of subjects that achieved sustained unresponsiveness post OIT - allergic patients with mild antigen-specific basophil responsiveness at the beginning of the trial and patients who undergo significant reduction of allergen specific basophil responses due to OIT.

BASOPHIL RESPONSES AMONG "RELEASER" AND "NON-RELEASER" BASOPHILS

Several studies have shown that blood basophils from some individuals fail to degranulate upon stimulation through the FcεRI (63–72). These basophils have been widely called "non-releasers" (64–71) or sometimes "anergic" (72), depending on the study. During a year-long study by Kepley et al. (68), there were three "non-atopic, non-releasers", defined as patients whose basophils failed to release histamine in response to anti-IgE

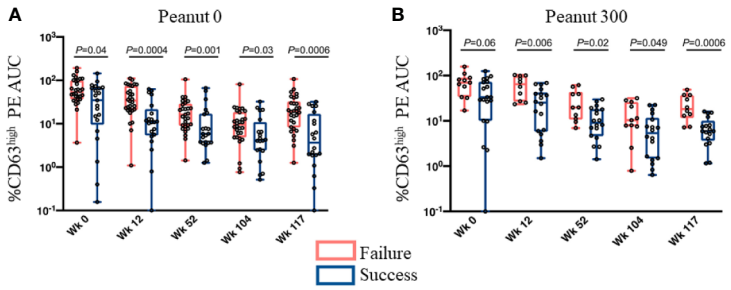


FIGURE 1 | Basophil responsiveness in OIT treatment outcome groups (i.e., Success vs Failure) is significantly different at all times tested. Basophil responsiveness to peanut extract (%CD63^{high} PE AUC) evaluated at multiple time points during OIT. Subjects were divided into two groups based on whether they did (Success) or did not (Failure) develop sustained unresponsiveness assessed by an oral food challenge at week 117. **(A)** Peanut 0 – the treatment arm in which subjects completely avoided peanut consumption after the end of desensitization phase (Week 104). **(B)** Peanut 300 – the treatment arm that maintained subjects at a low dose (i.e., 300 mg/day) of peanut consumption, from week 104 onwards. Whiskers represent the range (minimum to maximum values of AUC), boxes extend from 25th to 75th percentiles. The lines in the middle of the boxes are medians. Individual values are shown as circles. P values were determined by Mann-Whitney test. These are from Figures 4A, B of Tsai and Mukai et al. (51).

antibody (calculated using basophil-enriched cell populations isolated by Percoll gradient centrifugation yielding 25–60% basophil purity). However, the basophils from one of these subjects converted into "releasers". As "releasers", these basophils had detectable Syk protein expression (analyzed in western blots performed with >99% pure basophils obtained by sequential positive and negative selection and flow sorting) that was undetectable in basophils obtained during the "non-releaser" phase in the same patient.

A study by Puan et al. (72) divided individuals according to the functional state of their whole blood basophils. HDM^{R} (house dust mite responders) had CD63^{+} basophils after HDM stimulation (using an empirically determined threshold of at least 38% CD63^{+} cells). HDM^{NR} (HDM non-responders) were defined as giving no response to HDM allergens but at least 38% CD63^{+} basophils after anti-FcεRI stimulation. They defined "anergic" basophils as those that responded neither to HDM allergens nor to anti-FcεRI stimulation. For 38 individuals, the functional state of their basophils was defined at two time points separated by a period of approximately 2 years. While 26 of these 38 individuals remained in the same functional state, 13 underwent transitions between one of the reactive states (HDM^{R} or HDM^{NR}) and the anergic state. Moreover, such transitions happened in both directions. Conversion between releaser and non-releaser phenotype was also noted by Youseff et al. (71), in a four year study. This study categorized individuals as non-releasers if their basophils, obtained as basophil-enriched cell populations (1–55% basophil purity) by Percoll gradient centrifugation of anti-coagulated blood, released less than 12.7% histamine, a cut-off decided by applying statistical methods to the entire data set. Youseff et al. (71) found that 8 of 8 asthmatic non-releasers (13% of the asthmatic subjects enrolled in the study) and 16 of 23 control non-releasers (28% of control subjects enrolled), converted to releaser status at least once over the course of the study.

Overall, each of these studies suggests that basophils from individual donors may be able to cycle in and out of responsiveness over time. Interestingly, Yuoseff et al. (71) observed that the presence of non-releaser basophils does not

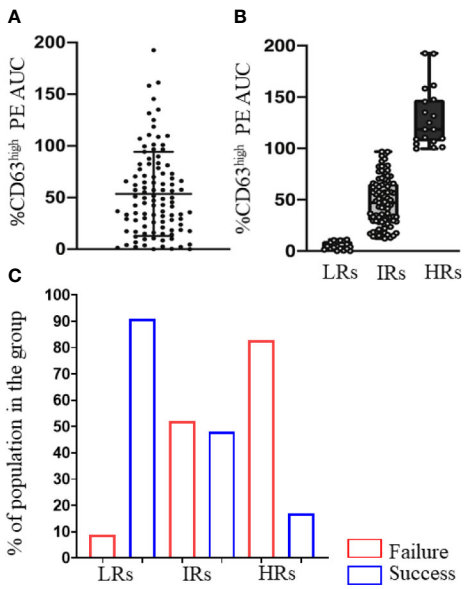


FIGURE 2 | Low basophil activation at week 0 is associated with sustained unresponsiveness after OIT. **(A)** Peanut-induced %CD63^{high} basophils (% CD63^{high} PE AUC) in the 120 participants at baseline. Scatter dot plot with ± SD. **(B)** Peanut-induced %CD63^{high} basophils of LRs (PE AUC < 12.09), IRs (PE AUC >12.09 and <97.37) and HRs (PE AUC >97.37). Individual values are shown as circles. **(C)** Percentage of the subjects in LR, IR, HR groups that showed sustained unresponsiveness (Success) or not (Failure) at week 117, assessed by an oral food challenge. These are from Figures 6A, B and Table 1 of Tsai and Mukai et al. (51).

rule out the diagnosis of asthma. By contrast, Puan et al. (72) suggested that anergic individuals are less likely to develop atopy (assessed by HDM SPTs) and symptoms of allergic rhinitis than those who responded to HDM (i.e., HDM^R). It therefore will be interesting to investigate further, in different diseases, the potential relationships between fluctuations in the functional state of basophils during OIT, or other clinical interventions, and overall clinical outcomes.

BASOPHIL RESPONSES AND SERUM IMMUNOGLOBULINS

A study comparing basophil responses among clinical phenotypes characterized as allergic, heat-denatured milk tolerant and outgrown (41), noted a strong correlation between basophil responses (quantified as $\%CD63^{hi}$ cells) and specific IgE levels among all groups. Since then, many OIT studies have recorded longitudinal changes in the levels of serum antibodies during OIT, but very few comment on correlations between such changes and basophil responses.

A peanut OIT study involving 28 subjects recorded basophil responses in whole blood at various times (day 0, days 21–156 and day 157–423) (46). Compared to baseline, peanut OIT resulted in significantly lower peanut-induced basophil responses (% $CD63^{high}$) for all 6 concentrations of peanut used for *in vitro* activation. This coincided with significantly increased peanut-specific IgG4 levels. Interestingly, in peanut-egg dual allergic subjects (9 among the 28 enrolled), basophil responses to egg were also decreased. However, a significant reduction in basophil responses was only detected with the highest of four tested concentrations of egg extract. There were no changes in egg-specific IgG4, which might be the reason for this mild effect.

In another peanut OIT study involving 21 subjects (49), baseline levels of basophil IL-4 expression (quantified using basophils in whole blood) in response to all three doses of peanut used for *in vitro* activation was positively correlated with peanut-specific serum IgE levels. However, when peanut-induced basophil CD63 and histamine release were evaluated using BECs obtained after double Percoll density centrifugation, no correlation with any serum antibody levels was detected. These results thus may have been influenced by studying basophils in whole blood vs post enrichment.

We studied peanut OIT in 120 participants, testing basophils in whole blood (51). We analyzed the relationship between basophil responses and serum antibody levels at baseline (week 0) and at the primary endpoint (week 117). At both time points, peanut-induced basophil responses (calculated as AUC for % $CD63^{high}$ cells) showed weakly significant positive correlations with levels of serum IgE against peanut and peanut-components (e.g., Ara h 1, Ara h 2 and Ara h 3) and with the specific IgE/total IgE ratio, and a negative correlation with the specific IgG4/specific IgE ratio. We noted no significant correlations of basophil activation with levels of specific IgG4. A similar conclusion was supported when we classified our participants, according to basophil responsiveness at baseline, into LR (low responders), IR (intermediate responders) and HR (high responders) (51). Thus, at the time of enrollment, the LR group differed from the IR or HR subjects not only in having lower peanut-specific and component-specific IgE levels and a smaller ratio of specific IgE/total IgE, but also in having a higher ratio of specific IgG4/specific IgE.

These observations suggest that basophil responses to allergens reflect the coordinated actions of both activating and inhibitory immunoglobulins—and that the proportion of such immunoglobulins is more critical than their absolute levels. However, the proportion of immunoglobulins does not take into account their relative avidity, their affinity, or the epitopes they recognize. These also are important factors that must be considered, in addition to ratios of activating/inhibitory immunoglobulins, when interpreting basophil responses.

TISSUE MAST CELLS AND ORAL IMUNOTHERAPY

Compared to blood basophils, it has been difficult to evaluate mast cell populations that may participate in food allergy. However, food allergy studies in mice have described the critical role played by mucosal mast cells, both in acquisition of susceptibility towards food allergens and in their contribution to the severity of the allergic reactions (3, 4). Duodenal biopsies from food allergic patients have also showed enhanced expression of mast cell-associated transcripts compared to control subjects (3).

Nevertheless, many challenges have hindered the detailed study of human mucosal mast cells at the site of the disease (i.e., GI tissues). Examples of such problems include the sparse distribution of gut mast cells, making it difficult to obtain sufficient numbers for many studies. Indeed, isolation of these cells for *in vitro* analysis requires enzymatic and mechanical tissue dispersion, processes that likely change the intrinsic activities of the cells. Moreover, *in vitro* studies may not recapitulate mast cell actions *in situ*, as these cells' responses to microenvironmental cues can change their phenotype and function (73, 74). Due to these limitations, it has become common to evaluate instead the responses of skin mast cells, which can be conveniently studied *in situ* (5).

Indeed, whenever IgE-mediated food allergy is suspected, SPTs are commonly recommended to identify the causative allergen, along with measurements of serum levels of allergen-specific IgE. SPTs provoke allergen-mediated mast cell degranulation in the skin, leading to measurable wheal-and-flare reactions. For some common food allergens, wheal size thresholds have helped to confirm sensitization and to indicate a high probability of food allergy (75, 76). However, although SPT diameters differ between allergic and non-allergic subjects, they might not distinguish subjects that have naturally outgrown allergy from those who are still allergic (41, 42). It also should be remembered that skin mast cells live for months or longer and take weeks to change responsiveness to antigens recognized by

cell-bound IgE, while mature blood basophils live only for days (7, 10). Such considerations suggest that losses in skin responses to allergens might occur substantially slower than the development of clinical unresponsiveness, which may be more reflective of changes in basophils or, perhaps, gastrointestinal mast cells.

The few studies of food OIT (47, 50, 52, 55, 77) and SLIT (50, 57–59, 78) containing longitudinal analyses of SPT diameters note their reduction, either during or toward the end of the study. In analyzing peanut OIT in 29 participants, Jones et al. found SPT diameters began to significantly decrease beginning at 6 months into the trial (47). This study did not discuss the correlations between the SPT results and the clinical outcomes. However, just 3 months into the OIT, peanut-specific IgG and IgG4 increased significantly, followed by significant suppression of basophil responses (measured as %$CD63^{bright}$ for individual peanut concentrations) 4 months into the trial (47). In another study of peanut OIT involving 23 participants (52), basophil responses and SPT results followed a similar trend, but with different kinetics of reduction: peanut-induced CD203c MFI was significantly reduced in OIT subjects vs controls at 3 months into therapy, whereas SPT diameters significantly diminished at 12 months. Nevertheless, both basophil responses (assessed with a single dose of food allergen) and SPT results did not significantly differ among subjects developing sustained unresponsiveness (at 3 months post therapy withdrawal) vs those who did not.

Another study monitoring the response to Omalizumab in 14 peanut-allergic subjects noticed a significant reduction (more than 80% from baseline) in peanut-induced histamine release AUC in 5 subjects within 8 weeks of initiating therapy (16). These five did not have reduced SPT responses this early in the treatment. In the other nine subjects, peanut-induced basophil histamine release AUC was unchanged by week 8 but 10-fold more peanut allergen was required to induce the maximal histamine release. Although this shift in basophil sensitivity was smaller than the treatment-induced improvement (approximately 50 fold) in the dose of allergen needed to induce a clinical response, there was a temporal association between basophil dose response and clinical response as early as week 8 of treatment. However, SPT responses did diminish by the end of treatment (week 24).

Overall, studies of OIT indicate that progressive desensitization evolves much faster than the changes in skin mast cell reactivity. Another probable explanation reflects the mechanism of OIT-induced desensitization. Data from milk- or egg-allergic subjects showed that food-specific IgG levels not only exhibit an inverse correlation with the reaction severity but also increase in parallel with natural resolution of the allergy (79, 80). Both OIT and early food introduction strategies elicit food-specific IgG antibodies (47, 57, 81–83). These then can act through inhibitory Fcγ receptors (i.e., FcγRIIb) to inhibit IgE-FcεRI mediated hypersensitivity (4). Studies of both mouse (84–87) and human (88) mast cells and basophils (36, 37) have provided evidence for this counter-regulatory mechanism. This also may explain why some patients with food-specific IgE can safely ingest food with no reaction and why the presence of allergen-specific IgE is needed but not sufficient to induce a clinical reaction (89). Notably, human skin mast cells do not ordinarily express the inhibitory receptor FcγRIIb (**Figure 3A**) (90, 91). It therefore seems very likely that this counter-regulatory mechanism is ineffective in skin mast cells.

Burton et al. have demonstrated the expression of FcγRIIb on human mast cells throughout the gastrointestinal tract, including the tongue, esophagus, small and large intestine, and (weakly) in the stomach (90). Using a humanized mouse model, they also tested the physiologic role of the IgG-FcγRIIb pathway in suppression of IgE-triggered systemic anaphylaxis, evidence that the receptor was functional (90). These observations highlight the heterogeneity among tissue mast cells (**Figure 3B**). They also provide an explanation for the

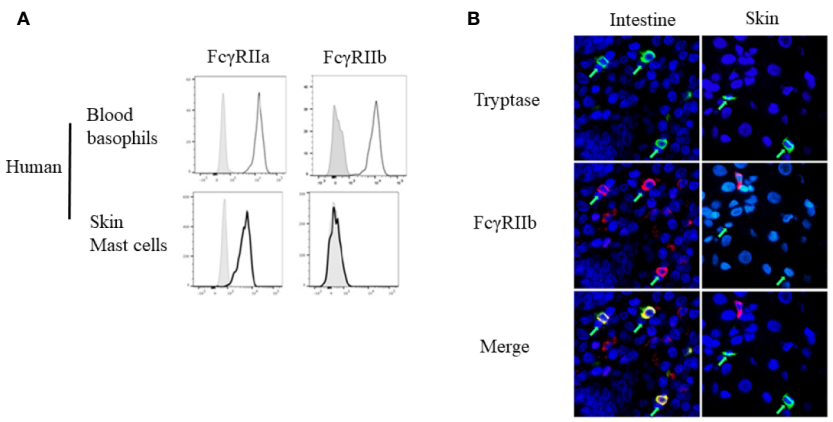


FIGURE 3 | Differential expression of Fcγ receptor (FcγR)IIb by basophils and different mast cell populations. **(A)** FcγRIIa and FcγRIIb expression by human peripheral blood basophils and human Skin mast cells, assessed by flow cytometry. **(B)** FCγRIIb expression by human intestinal or skin mast cells, assessed by immunofluorescence staining for mast cell tryptase (green) and FcγRIIb (red) of human tissue sections. Mast cells are indicated by green arrows. These are from Figure 1A, and Figure 3 of Burton et al. (90) with permission.

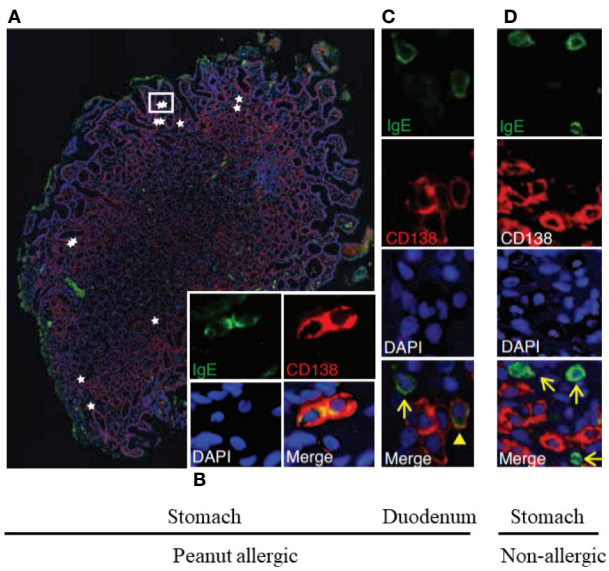


FIGURE 4 | Stomach and duodenum are reservoirs of IgE$^+$ B lineage cells in peanut allergic people. Immunofluorescence of stomach **(A, B)** and duodenal **(C)** biopsies from peanut allergic donors or from non-allergic stomach **(D)**. IgE (green), plasma cell marker CD138 (red), and nuclei (DAPI; blue) **(A)**. IgE$^+$CD138$^+$plasma cells (stars) localized singly and in clusters between gastric glands; a white rectangle outlines two IgE$^+$CD138$^+$plasma cells, for which single-channel staining is shown in **(B)**. **(C)** IgE$^+$CD138$^+$ plasma cell (arrowhead) and IgE$^+$CD138$^-$ putative mast cell (arrow). **(D)** Representative image from a non-allergic donor. IgE$^+$CD138$^-$ putative mast cells were observed (arrows), but IgE$^+$CD138$^+$ plasma cells were absent. These are from Figures 1E–H of Hoh and Joshi et al. (92).

relative contribution of FcγRIIb in suppressing hypersensitivity in mice vs humans.

A study by Hoh and Joshi, et al. provides yet another perspective into the events taking place in the GI tract of allergic subjects (92). By performing high throughput DNA sequencing on biopsies of esophagus, stomach, and duodenum from peanut-allergic patients and controls, they found that peanut-allergic patients harbored large numbers of somatically-mutated, clonally-expanded, allergen-specific IgE$^+$ B lineage cells, including cells with a plasma cell phenotype, in their GI tissues (**Figure 4**). Furthermore, the co-occurrence of IgE-expressing and non-IgE-expressing clonally-related B lineage cells in the same biopsy samples indicated local isotype switching. Common convergent heavy chain sequences shared between allergic donors suggested that common immunoglobulin genetic rearrangements contributed to the pathogenesis of the disease.

Such IgE$^+$ plasma cells present throughout the GI tract of allergic subjects could disproportionately contribute to the FcεRI bound IgE on mast cells present at the same locations (**Figure 4**). Regional differences in local IgE$^+$ plasma cell clones might lead to differences in local mast cell IgE loading, contributing to the clinical heterogeneity of patient symptoms and thresholds post allergen exposure. Stomach and duodenal IgE$^+$ clone counts in allergic patients correlated with peanut allergen-specific IgE levels in serum, where they will affect responses of circulating blood basophils. Indeed, basophils may be the first responders to food antigens that gain access to the blood. However, it will be interesting to determine whether the effects of OIT on gut mucosal mast cells may actually precede those influencing mast cells present in the skin. Specifically, it is possible that reductions in the reactivity of basophils and gut mast cells during OIT will exhibit more similar kinetics than the later reduction observed in the responses of skin mast cells.

CONCLUSIONS

Basophil responses measured *ex vivo* before, during and after immunotherapy can help to differentiate between transient desensitization and sustained unresponsiveness. Moreover, considerations such as study size, evaluation of basophil activation in whole blood vs in enriched preparations, the range of antigen dosages used, and which basophil responses are assessed (e.g., %CD63$^+$ vs AUC of %CD63$^+$ vs CD203c MFI, etc.) may critically influence the information gained from a study. For testing relatively large numbers of specimens that may originate at significant distances from the test site, we currently favor the approach described in **Table 3**. However, clearly, more investigations are required to establish which type of BAT measurements, and which BAT thresholds, can identify those allergic subjects who can benefit most from OIT. Finally, an important role for mucosal mast cells in food allergy has been

TABLE 3 | Approach for testing relatively large number of BAT specimens that originate at significant distances from the testing site.

Starting material	Whole blood
Anticoagulant used	Heparin
Storage time and temperature	Up to 4 h at room temperature or up to 24 h at 4°C
Experimental conditions	Negative control (RPMI) 5 Concentrations of allergen - e.g., 0.1, 1, 10, 100, 1,000 ng/ml of Peanut extract Positive controls - anti-IgE (1 μg/ml) and IL-3 (2 ng/ml) (tested separately)
Activation conditions	30 min at 37°C
Gating strategy	CD123+HLA-DR-
Activation markers evaluated	CD63 (%CD63high), CD203c (ΔCD203c MFI)

If BATs are to be performed on a large number of samples obtained at a significant distance from the testing site, we favor immediately bringing the blood to 4°C and then keeping the blood at that temperature for ~24 h before testing (33,51). This keeps the storage conditions uniform for all of the samples being evaluated.

suggested by work in both humans and mice. For example, our high throughput DNA sequencing study revealed that the gastrointestinal tract of food allergic patients is a reservoir of IgE^+ B lineage cells. This finding emphasizes the need to study, in particular, those gastrointestinal mast cells that are near such IgE-producing plasma cells.

AUTHOR CONTRIBUTIONS

AP wrote most of the text, with help from SG. All authors participated in the editing, and oversight of the content, of the text. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Reber LL, Marichal T, Mukai K, Kita Y, Tokuoka SM, Roers A, et al. Selective ablation of mast cells or basophils reduces peanut-induced anaphylaxis in mice. *J Allergy Clin Immunol* (2013) 132:881–8. doi: 10.1016/j.jaci.2013.06.008
2. Dua S, Dowey J, Foley L, Islam S, King Y, Ewan P, et al. Diagnostic Value of Tryptase in Food Allergic Reactions: A Prospective Study of 160 Adult Peanut Challenges. *J Allergy Clin Immunol Pract* (2018) 6:1692–8. doi: 10.1016/j.jaip.2018.01.006
3. Chen C-Y, Lee J-B, Liu B, Ohta S, Wang P-Y, Kartashov AV, et al. Induction of Interleukin-9-Producing Mucosal Mast Cells Promotes Susceptibility to IgE-Mediated Experimental Food Allergy. *Immunity* (2015) 43:788–802. doi: 10.1016/j.immuni.2015.08.020
4. Burton OT, Noval Rivas M, Zhou JS, Logsdon SL, Darling AR, Koleoglou KJ, et al. Immunoglobulin E Signal Inhibition during Allergen Ingestion Leads to Reversal of Established Food Allergy and Induction of Regulatory T Cells. *Immunity* (2014) 41:141–51. doi: 10.1016/j.immuni.2014.05.017
5. Yu W, Freeland DMH, Nadeau KC. Food allergy: immune mechanisms, diagnosis and immunotherapy. *Nat Rev Immunol* (2016) 16:751–65. doi: 10.1038/nri.2016.111
6. Renz H, Allen KJ, Sicherer SH, Sampson HA, Lack G, Beyer K, et al. Food allergy. *Nat Rev Dis Primers* (2018) 4:17098. doi: 10.1038/nrdp.2017.98
7. Karasuyama H, Miyake K, Yoshikawa S, Yamanishi Y. Multifaceted roles of basophils in health and disease. *J Allergy Clin Immunol* (2018) 142:370–80. doi: 10.1016/j.jaci.2017.10.042
8. Korošec P, Gibbs BF, Rijavec M, Custovic A, Turner PJ. Important and specific role for basophils in acute allergic reactions. *Clin Exp Allergy* (2018) 48:502–12. doi: 10.1111/cea.13117
9. Varricchi G, Raap U, Rivellese F, Marone G, Gibbs BF. Human mast cells and basophils-How are they similar how are they different? *Immunol Rev* (2018) 282:8–34. doi: 10.1111/imr.12627
10. Schroeder JT, MacGlashan DW, Lichtenstein LM. Human basophils: mediator release and cytokine production. *Adv Immunol* (2001) 77:93–122. doi: 10.1016/S0065-2776(01)77015-0
11. Nolte H, Schiøtz PO, Kruse A, Skov PS. Comparison of intestinal mast cell and basophil histamine release in children with food allergic reactions. *Allergy* (1989) 44:554–65. doi: 10.1111/j.1398-9995.1989.tb04200.x
12. Noti M, Kim BS, Siracusa MC, Rak GD, Kubo M, Moghaddam AE, et al. Exposure to food allergens through inflamed skin promotes intestinal food allergy through the thymic stromal lymphopoietin–basophil axis. *J Allergy Clin Immunol* (2014) 133:1390–9. doi: 10.1016/j.jaci.2014.01.021
13. Muto T, Fukuoka A, Kabashima K, Ziegler SF, Nakanishi K, Matsushita K, et al. The role of basophils and proallergic cytokines, TSLP and IL-33, in cutaneously sensitized food allergy. *Int Immunol* (2014) 26:539–49. doi: 10.1093/intimm/dxu058
14. Hussain M, Borcard L, Walsh KP, Pena Rodriguez M, Mueller C, Kim BS, et al. Basophil-derived IL-4 promotes epicutaneous antigen sensitization concomitant with the development of food allergy. *J Allergy Clin Immunol* (2018) 141:223–34. doi: 10.1016/j.jaci.2017.02.035
15. Eckman JA, Sterba PM, Kelly D, Alexander V, Liu MC, Bochner BS, et al. Effects of omalizumab on basophil and mast cell responses using an intranasal cat allergen challenge. *J Allergy Clin Immunol* (2010) 125:889–95.e7. doi: 10.1016/j.jaci.2009.09.012
16. Savage JH, Courneya J-P, Sterba PM, MacGlashan DW, Saini SS, Wood RA. Kinetics of mast cell, basophil, and oral food challenge responses in omalizumab-treated adults with peanut allergy. *J Allergy Clin Immunol* (2012) 130:1123–9.e2. doi: 10.1016/j.jaci.2012.05.039
17. Knol EF, Mul FPJ, Jansen H, Calafat J, Roos D. Monitoring human basophil activation via CD63 monoclonal antibody 435. *J Allergy Clin Immunol* (1991) 88:328–38. doi: 10.1016/0091-6749(91)90094-5
18. Gane P, Pecquet C, Lambin P, Abuaf N, Leynadier F, Rouger P. Flow cytometric evaluation of human basophils. *Cytometry* (1993) 14:344–8. doi: 10.1002/cyto.990140316
19. Monneret G, Gutowski MC, Bienvenu J. Detection of allergen-induced basophil activation by expression of CD63 antigen using a tricolour flow cytometric method. *Clin Exp Immunol* (1999) 115:393–6. doi: 10.1046/j.1365-2249.1999.00841.x
20. Platz IJ, Binder M, Marxer A, Lischka G, Valent P, Bühring H-J. Hymenoptera-Venom-Induced Upregulation of the Basophil Activation Marker Ecto-Nucleotide Pyrophosphatase/Phosphodiesterase 3 in Sensitized Individuals. *Int Arch Allergy Immunol* (2001) 126:335–42. doi: 10.1159/000049531
21. Ocmant A, Peignois Y, Mulier S, Hanssens L, Michils A, Schandené L. Flow cytometry for basophil activation markers: The measurement of CD203c up-regulation is as reliable as CD63 expression in the diagnosis of cat allergy. *J Immunol Methods* (2007) 320:40–8. doi: 10.1016/j.jim.2006.12.002
22. Hennersdorf F, Florian S, Jakob A, Baumgärtner K, Sonneck K, Nordheim A, et al. Identification of CD13, CD107a, and CD164 as novel basophil-activation markers and dissection of two response patterns in time kinetics of IgE-dependent upregulation. *Cell Res* (2005) 15:325–35. doi: 10.1038/sj.cr.7290301
23. Ebo DG, Bridts CH, Mertens CH, Hagendorens MM, Stevens WJ, De Clerck LS. Analyzing histamine release by flow cytometry (HistaFlow): A novel instrument to study the degranulation patterns of basophils. *J Immunol Methods* (2012) 375:30–8. doi: 10.1016/j.jim.2011.09.003
24. Nullens S, Sabato V, Faber M, Leysen J, Bridts CH, De Clerck LS, et al. Basophilic histamine content and release during venom immunotherapy: Insights by flow cytometry. *Cytometry* (2013) 84B:173–8. doi: 10.1002/cyto.b.21084
25. Kleine-Tebbe J, Erdmann S, Knol EF, MacGlashan DWJr, Poulsen LK, Gibbs BF. Diagnostic Tests Based on Human Basophils: Potentials, Pitfalls and Perspectives. *Int Arch Allergy Immunol* (2006) 141:79–90. doi: 10.1159/000094495
26. MacGlashan DW. Basophil activation testing. *J Allergy Clin Immunol* (2013) 132:777–87. doi: 10.1016/j.jaci.2013.06.038
27. Santos AF, Shreffler WG. Road map for the clinical application of the basophil activation test in food allergy. *Clin Exp Allergy* (2017) 47:1115–24. doi: 10.1111/cea.12964
28. McGowan EC, Saini S. Update on the Performance and Application of Basophil Activation Tests. *Curr Allergy Asthma Rep* (2013) 13:101–9. doi: 10.1007/s11882-012-0324-x
29. Hoffmann HJ, Knol EF, Ferrer M, Mayorga L, Sabato V, Santos AF, et al. Pros and Cons of Clinical Basophil Testing (BAT). *Curr Allergy Asthma Rep* (2016) 16:56. doi: 10.1007/s11882-016-0633-6
30. Hemmings O, Kwok M, McKendry R, Santos AF. Basophil Activation Test: Old and New Applications in Allergy. *Curr Allergy Asthma Rep* (2018) 18:77. doi: 10.1007/s11882-018-0831-5
31. Chirumbolo S. The use of IL-3 in basophil activation tests is the real pitfall. *Cytometry* (2011) 80B:137–8. doi: 10.1002/cyto.b.20570
32. Ebo DG, Bridts CH, Hagendorens MM, Aerts NE, De Clerck LS, Stevens WJ. Basophil activation test by flow cytometry: Present and future applications in allergology. *Cytometry* (2008) 74B:201–10. doi: 10.1002/cyto.b.20419
33. Mukai K, Gaudenzio N, Gupta S, Vivanco N, Bendall SC, Maecker HT, et al. Assessing basophil activation by using flow cytometry and mass cytometry in blood stored 24 hours before analysis. *J Allergy Clin Immunol* (2017) 139:889–99.e11. doi: 10.1016/j.jaci.2016.04.060

34. MacGlashan D. Expression of CD203c and CD63 in human basophils: relationship to differential regulation of piecemeal and anaphylactic degranulation processes: Basophil activation markers and mode of degranulation. *Clin Exp Allergy* (2010) 40:1365–77. doi: 10.1111/j.1365-2222.2010.03572.x
35. Gibbs BF, Papenfuss K, Falcone FH. A rapid two-step procedure for the purification of human peripheral blood basophils to near homogeneity. *Clin Exp Allergy* (2008) 38:480–5. doi: 10.1111/j.1365-2222.2007.02919.x
36. Cassard L, Jönsson F, Arnaud S, Daëron M. Fcγ Receptors Inhibit Mouse and Human Basophil Activation. *J Immunol* (2012) 189:2995–3006. doi: 10.4049/jimmunol.1200968
37. Cady CT, Powell MS, Harbeck RJ, Giclas PC, Murphy JR, Katial RK, et al. IgG antibodies produced during subcutaneous allergen immunotherapy mediate inhibition of basophil activation via a mechanism involving both FcγRIIA and FcγRIIB. *Immunol Lett* (2010) 130:57–65. doi: 10.1016/j.imlet.2009.12.001
38. Azorsa D, Hyman J, Hildreth J. CD63/Pltgp40: a platelet activation antigen identical to the stage- specific, melanoma-associated antigen ME491. *Blood* (1991) 78:280–4. doi: 10.1182/blood.V78.2.280.bloodjournal782280
39. de Bruijne-Admiraal LG, Modderman PW. P-Selectin Mediates Ca2+-DependentAdhesion of Activated Platelets to Many Different Types of Leukocytes: Detection by Flow Cytometry. *Blood* (1992) 80(1):134–42. doi: 10.1182/blood.V80.1.134.bloodjournal801134
40. Mukai K, Chinthrajah RS, Nadeau KC, Tsai M, Gaudenzio N, Galli SJ. A new fluorescent-avidin–based method for quantifying basophil activation in whole blood. *J Allergy Clin Immunol* (2017) 140:1202–6. doi: 10.1016/j.jaci.2017.03.052
41. Wanich N, Nowak-Wegrzyn A, Sampson HA, Shreffler WG. Allergen-specific basophil suppression associated with clinical tolerance in patients with milk allergy. *J Allergy Clin Immunol* (2009) 123:789–94. doi: 10.1016/j.jaci.2008.12.1128
42. Ford LS, Bloom KA, Nowak-Węgrzyn AH, Shreffler WG, Masilamani M, Sampson HA. Basophil reactivity, wheal size, and immunoglobulin levels distinguish degrees of cow's milk tolerance. *J Allergy Clin Immunol* (2013) 131:180–6.e3. doi: 10.1016/j.jaci.2012.06.003
43. Rubio A, Vivinus-Nébot M, Bourrier T, Saggio B, Albertini M, Bernard A. Benefit of the basophil activation test in deciding when to reintroduce cow's milk in allergic children: Basophil activation test in cow's milk allergy. *Allergy* (2011) 66:92–100. doi: 10.1111/j.1398-9995.2010.02432.x
44. Patil SU, Shreffler WG. Immunology in the Clinic Review Series; focus on allergies: basophils as biomarkers for assessing immune modulation: Basophil modulation in immunotherapy. *Clin Exp Immunol* (2012) 167:59–66. doi: 10.1111/j.1365-2249.2011.04503.x
45. Goldberg MR, Nachshon L, Appel MY, Elizur A, Levy MB, Eisenberg E, et al. Efficacy of baked milk oral immunotherapy in baked milk–reactive allergic patients. *J Allergy Clin Immunol* (2015) 136:1601–6. doi: 10.1016/j.jaci.2015.05.040
46. Thyagarajan A, Jones SM, Calatroni A, Pons L, Kulis M, Woo CS, et al. Evidence of pathway-specific basophil anergy induced by peanut oral immunotherapy in peanut-allergic children. *Clin Exp Allergy* (2012) 42:1197–205. doi: 10.1111/j.1365-2222.2012.04028.x
47. Jones SM, Pons L, Roberts JL, Scurlock AM, Perry TT, Kulis M, et al. Clinical efficacy and immune regulation with peanut oral immunotherapy. *J Allergy Clin Immunol* (2009) 124:292–300.e97. doi: 10.1016/j.jaci.2009.05.022
48. Anagnostou K, Islam S, King Y, Foley L, Pasea L, Bond S, et al. Assessing the efficacy of oral immunotherapy for the desensitisation of peanut allergy in children (STOP II): a phase 2 randomised controlled trial. *Lancet* (2014) 383:1297–304. doi: 10.1016/S0140-6736(13)62301-6
49. Gorelik M, Narisety SD, Guerrerio AL, Chichester KL, Keet CA, Bieneman AP, et al. Suppression of the immunologic response to peanut during immunotherapy is often transient. *J Allergy Clin Immunol* (2015) 135:1283–92. doi: 10.1016/j.jaci.2014.11.010
50. Keet CA, Frischmeyer-Guerrerio PA, Thyagarajan A, Schroeder JT, Hamilton RG, Boden S, et al. The safety and efficacy of sublingual and oral immunotherapy for milk allergy. *J Allergy Clin Immunol* (2012) 129:448–55.e5. doi: 10.1016/j.jaci.2011.10.023
51. Tsai M, Mukai K, Chinthrajah RS, Nadeau KC, Galli SJ. Sustained successful peanut oral immunotherapy associated with low basophil activation and peanut-specific IgE. *J Allergy Clin Immunol* (2020) 145:885–96.e6. doi: 10.1016/j.jaci.2019.10.038
52. Syed A, Garcia MA, Lyu S-C, Bucayu R, Kohli A, Ishida S, et al. Peanut oral immunotherapy results in increased antigen-induced regulatory T-cell function and hypomethylation of forkhead box protein 3 (FOXP3). *J Allergy Clin Immunol* (2014) 133:500–10.e11. doi: 10.1016/j.jaci.2013.12.1037
53. Patil SU, Steinbrecher J, Calatroni A, Smith N, Ma A, Ruiter B, et al. Early decrease in basophil sensitivity to Ara h 2 precedes sustained unresponsiveness after peanut oral immunotherapy. *J Allergy Clin Immunol* (2019) 144:1310–9.e4. doi: 10.1016/j.jaci.2019.07.028
54. Kulis M, Yue X, Guo R, Zhang H, Orgel K, Ye P, et al. High- and low-dose oral immunotherapy similarly suppress pro-allergic cytokines and basophil activation in young children. *Clin Exp Allergy* (2019) 49:180–9. doi: 10.1111/cea.13256
55. Burks AW, Jones SM, Wood RA, Fleischer DM, Sicherer SH, Lindblad RW, et al. Oral Immunotherapy for Treatment of Egg Allergy in Children. *N Engl J Med* (2012) 367:233–43. doi: 10.1056/NEJMoa1200435
56. Vila L, Moreno A, Gamboa PM, Martínez-Aranguren R, Sanz ML. Decrease in antigen-specific CD63 basophil expression is associated with the development of tolerance to egg by SOTI in children. *Pediatr Allergy Immunol* (2013) 24:463–8. doi: 10.1111/pai.12070
57. Kim EH, Bird JA, Kulis M, Laubach S, Pons L, Shreffler W, et al. Sublingual immunotherapy for peanut allergy: Clinical and immunologic evidence of desensitization. *J Allergy Clin Immunol* (2011) 127:640–46.e1. doi: 10.1016/j.jaci.2010.12.1083
58. Kim EH, Yang L, Ye P, Guo R, Li Q, Kulis MD, et al. Long-term sublingual immunotherapy for peanut allergy in children: Clinical and immunologic evidence of desensitization. *J Allergy Clin Immunol* (2019) 144:1320–6.e1. doi: 10.1016/j.jaci.2019.07.030
59. Fleischer DM, Burks AW, Vickery BP, Scurlock AM, Wood RA, Jones SM, et al. Sublingual immunotherapy for peanut allergy: A randomized, double-blind, placebo-controlled multicenter trial. *J Allergy Clin Immunol* (2013) 131:119–27.e7. doi: 10.1016/j.jaci.2012.11.011
60. Jones SM, Sicherer SH, Burks AW, Leung DYM, Lindblad RW, Dawson P, et al. Epicutaneous immunotherapy for the treatment of peanut allergy in children and young adults. *J Allergy Clin Immunol* (2017) 139:1242–52.e9. doi: 10.1016/j.jaci.2016.08.017
61. Santos AF, Du Toit G, Douiri A, Radulovic S, Stephens A, Turcanu V, et al. Distinct parameters of the basophil activation test reflect the severity and threshold of allergic reactions to peanut. *J Allergy Clin Immunol* (2015) 135:179–86. doi: 10.1016/j.jaci.2014.09.001
62. Song Y, Wang J, Leung N, Wang LX, Lisann L, Sicherer SH, et al. Correlations between basophil activation, allergen-specific IgE with outcome and severity of oral food challenges. *Ann Allergy Asthma Immunol* (2015) 114:319–26. doi: 10.1016/j.anai.2015.01.006
63. Ishizaka T, Soto CS, Ishizaka K. Mechanisms of Passive Sensitization: III. Number of IgE Molecules and Their Receptor Sites on Human Basophil Granulocytes. *J Immunol* (1973) 111(2):500–11.
64. Bochner BS, MacGlashan DW, Marcotte GV. IgE-dependent regulation of human basophil adherence to vascular endothelium. *J Immunol* (1989) 142 (9):3180–6.
65. Nguyen K-L, Gillis S, MacGlashan DW. A comparative study of releasing and nonreleasing human basophils: Nonreleasing basophils lack an early component of the signal transduction pathway that follows IgE cross-linking. *J Allergy Clin Immunol* (1990) 85:1020–9. doi: 10.1016/0091-6749(90)90046-7
66. Knol EF, Mul FPJ, Kuijpers TW, Verhoeven AJ, Roos D. Intracellular events in anti-lgE nonreleasing human basophils. *J Allergy Clin Immunol* (1992) 90 (1):92–103. doi: 10.1016/s0091-6749(06)80015-1
67. Yamaguchi M, Hirai K, Ohta K, Suzuki K, Kitani S, Takaishi T, et al. Nonreleasing basophils convert to releasing basophils by culturing with IL-3. *J Allergy Clin Immunol* (1996) 97:1279–87. doi: 10.1016/S0091-6749(96)70196-3
68. Kepley CL, Youssef L, Andrews RP, Wilson BS, Oliver JM. Syk deficiency in nonreleaser basophils. *J Allergy Clin Immunol* (1999) 104:279–84. doi: 10.1016/S0091-6749(99)70367-2
69. Lavens-Phillips SE, MacGlashan DW. The Tyrosine Kinases p53/56lyn and p72syk Are Differentially Expressed at the Protein Level but Not at the Messenger RNA Level in Nonreleasing Human Basophils. *Am J Respir Cell Mol Biol* (2000) 23:566–71. doi: 10.1165/ajrcmb.23.4.4123

70. Suzukawa M, Hirai K, Iikura M, Nagase H, Komiya A, Yoshimura-Uchiyama C, et al. IgE- and FcεRI-mediated migration of human basophils. *Int Immunol* (2005) 17:1249–55. doi: 10.1093/intimm/dxh301
71. Youssef LA, Schuyler M, Gilmartin L, Pickett G, Bard JDJ, Tarleton CA, et al. Histamine Release from the Basophils of Control and Asthmatic Subjects and a Comparison of Gene Expression between "Releaser" and "Nonreleaser" Basophils. *J Immunol* (2007) 178:4584–94. doi: 10.4049/jimmunol.178.7.4584
72. Puan KJ, Andiappan AK, Lee B, Kumar D, Lai TS, Yeo G, et al. Systematic characterization of basophil anergy. *Allergy* (2017) 72:373–84. doi: 10.1111/all.12952
73. Galli SJ, Kalesnikoff J, Grimbaldeston MA, Piliponsky AM, Williams CMM, Tsai M. Mast cells as "tunable" effector and immunoregulatory cells: Recent Advances. *Annu Rev Immunol* (2005) 23:749–86. doi: 10.1146/annurev.immunol.21.120601.141025
74. Frossi B, Mion F, Sibilano R, Danelli L, Pucillo CEM. Is it time for a new classification of mast cells? What do we know about mast cell heterogeneity? *Immunol Rev* (2018) 282:35–46. doi: 10.1111/imr.12636
75. Chinthrajah RS, Tupa D, Prince BT, Block WM, Rosa JS, Singh AM, et al. Diagnosis of Food Allergy. *Pediatr Clin North Am* (2015) 62:1393–408. doi: 10.1016/j.pcl.2015.07.009
76. Boyce JA, Assa'ad A, Burks AW, Jones SM, Sampson HA, Wood RA, et al. Guidelines for the Diagnosis and Management of Food Allergy in the United States: Report of the NIAID-Sponsored Expert Panel. *J Allergy Clin Immunol* (2010) 126:S1–58. doi: 10.1016/j.jaci.2010.10.007
77. MacGinnitie AJ, Rachid R, Gragg H, Little SV, Lakin P, Cianferoni A, et al. Omalizumab facilitates rapid oral desensitization for peanut allergy. *J Allergy Clin Immunol* (2017) 139:873–81.e8. doi: 10.1016/j.jaci.2016.08.010
78. Fernández-Rivas M, Garrido Fernández S, Nadal JA, Alonso Díaz de Durana MD, García BE, González-Mancebo E, et al. Randomized double-blind, placebo-controlled trial of sublingual immunotherapy with a Pru p 3 quantified peach extract. *Allergy* (2009) 64:876–83. doi: 10.1111/j.1398-9995.2008.01921.x
79. Savilahti EM, Rantanen V, Lin JS, Karinen S, Saarinen KM, Goldis M, et al. Early recovery from cow's milk allergy is associated with decreasing IgE and increasing IgG4 binding to cow's milk epitopes. *J Allergy Clin Immunol* (2010) 125:1315–21.e9. doi: 10.1016/j.jaci.2010.03.025
80. Caubet JC, Bencharitiwong R, Moshier E, Godbold JH, Sampson HA, Nowak-Węgrzyn A. Significance of ovomucoid- and ovalbumin-specific IgE/IgG4 ratios in egg allergy. *J Allergy Clin Immunol* (2012) 129:739–47. doi: 10.1016/j.jaci.2011.11.053
81. Vickery BP, Pons L, Kulis M, Steele P, Jones SM, Burks AW. Individualized IgE-based dosing of egg oral immunotherapy and the development of tolerance. *Ann Allergy Asthma Immunol* (2010) 105:444–50. doi: 10.1016/j.anai.2010.09.030
82. Du Toit G, Roberts G, Sayre PH, Bahnson HT, Radulovic S, Santos AF, et al. Randomized Trial of Peanut Consumption in Infants at Risk for Peanut Allergy. *N Engl J Med* (2015) 372:803–13. doi: 10.1056/NEJMoa1414850
83. Bedoret D, Singh AK, Shaw V, Hoyte EG, Hamilton R, DeKruyff RH, et al. Changes in antigen-specific T-cell number and function during oral desensitization in cow's milk allergy enabled with omalizumab. *Mucosal Immunol* (2012) 5:267–76. doi: 10.1038/mi.2012.5
84. Takai T, Onot M, Hikida M, Ohmori H, Ravetcht JV. Augmented humoral and anaphylactic responses in FcγRll-deficient mice. *Nature* (1996) 379:346–9. doi: 10.1038/379346a0
85. Zha L, Leoratti FMS, He L, Mohsen MO, Cragg M, Storni F, et al. An unexpected protective role of low-affinity allergen-specific IgG through the inhibitory receptor FcγRIIb. *J Allergy Clin Immunol* (2018) 142:1529–36.e6. doi: 10.1016/j.jaci.2017.09.054
86. Daëron M, Malbec O, Latour S, Arock M, Fridman WH. Regulation of high-affinity IgE receptor-mediated mast cell activation by murine low-affinity IgG receptors. *J Clin Invest* (1995) 95:577–85. doi: 10.1172/JCI117701
87. Shreffler WG. IgG-Blocking Antibodies Inhibit IgE-Mediated Anaphylaxis in Vivo Through Both Antigen Interception and FcγRIIb Cross-linking. *Pediatrics* (2007) 120:S152.2–S153. doi: 10.1542/peds.2007-0846HHHH
88. Kepley CL, Taghavi S, Mackay G, Zhu D, Morel PA, Zhang K, et al. Co-aggregation of FcγRII with FcεRI on Human Mast Cells Inhibits Antigen-induced Secretion and Involves SHIP-Grb2-Dok Complexes. *J Biol Chem* (2004) 279:35139–49. doi: 10.1074/jbc.M404318200
89. Sampson H, Albergo R. Comparison of results of skin tests, RAST, and double-blind, placebo-controlled food challenges in children with atopic dermatitis. *J Allergy Clin Immunol* (1984) 74:26–33. doi: 10.1016/0091-6749(84)90083-6
90. Burton OT, Epp A, Fanny ME, Miller SJ, Stranks AJ, Teague JE, et al. Tissue-Specific Expression of the Low-Affinity IgG Receptor, FcγRIIb, on Human Mast Cells. *Front Immunol* (2018) 9:1244. doi: 10.3389/fimmu.2018.01244
91. Zhao W, Kepley CL, Morel PA, Okumoto LM, Fukuoka Y, Schwartz LB. FcγRIIa, Not FcγRIIb, Is Constitutively and Functionally Expressed on Skin-Derived Human Mast Cells. *J Immunol* (2006) 177:694–701. doi: 10.4049/jimmunol.177.1.694
92. Hoh RA, Joshi SA, Lee J-Y, Martin BA, Varma S, Kwok S, et al. Origins and clonal convergence of gastrointestinal IgE $^{+}$ B cells in human peanut allergy. *Sci Immunol* (2020) 5:eaay4209. doi: 10.1126/sciimmunol.aay4209

Conflict of Interest: SG reports grants from NIH. RC reports grants from NIAID, CoFAR, Aimmune, DBV Technologies, Astellas, Regeneron, is an Advisory member for Alladapt, Genentech, Novartis, and receives personal fees from Before Brands outside the submitted work. KN reports grants from NIAID, NHLBI, NIEHS, Food Allergy Research & Education (FARE); other funding from World Allergy Organization (WAO), Cour Pharma, Before Brands, Alladapt, Latitude, IgGenix, Immune Tolerance Network (ITN), and NIH clinical research centers outside the submitted work. In addition, KN has the following patents pending: Inhibition of Allergic Reaction to Peanut Allergen using an IL-33 Inhibitor; Special Oral Formula for Decreasing Food Allergy Risk and Treatment for Food Allergy; Basophil Activation Based Diagnostic Allergy Test; Granulocyte-based methods for detecting and monitoring immune system disorders; Methods and Assays for Detecting and Quantifying Pure Subpopulations of White Blood Cells in Immune System Disorders; Mixed Allergen Compositions and Methods for Using the Same; Microfluidic Device and Diagnostic Methods for Allergy Testing Based on Detection of Basophil Activation. SB has consulted for Regeneron, Sanofi and Novartis on topics unrelated to this study, owns shares in AbCellera and CareDx, and has patents awarded or submitted related to immunoglobulin gene and protein analysis.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer EK declared a past co-authorship with several of the authors RSC and KN to the handling editor.

frontiers in Immunology

MINI REVIEW
published: 17 December 2020
doi: 10.3389/fimmu.2020.614402

Targeting the FcεRI Pathway as a Potential Strategy to Prevent Food-Induced Anaphylaxis

Melanie C. Dispenza[1]*, *Bruce S. Bochner*[2] *and Donald W. MacGlashan Jr*[1]

[1] Department of Medicine, Division of Allergy and Clinical Immunology, Johns Hopkins University School of Medicine, Baltimore, MD, United States, [2] Department of Medicine, Division of Allergy and Immunology, Northwestern University Feinberg School of Medicine, Chicago, IL, United States

Despite attempts to halt it, the prevalence of food allergy is increasing, and there is an unmet need for strategies to prevent morbidity and mortality from food-induced allergic reactions. There are no known medications that can prevent anaphylaxis, but several novel therapies show promise for the prevention of food-induced anaphylaxis through targeting of the high-affinity IgE receptor (FcεRI) pathway. This pathway includes multiple candidate targets, including tyrosine kinases and the receptor itself. Small molecule inhibitors of essential kinases have rapid onset of action and transient efficacy, which may be beneficial for short-term use for immunotherapy buildup or desensitizations. Short courses of FDA-approved inhibitors of Bruton's tyrosine kinase can eliminate IgE-mediated basophil activation and reduce food skin test size in allergic adults, and prevent IgE-mediated anaphylaxis in humanized mice. In contrast, biologics may provide longer-lasting protection, albeit with slower onset. Omalizumab is an anti-IgE antibody that sequesters IgE, thereby reducing FcεRI expression on mast cells and basophils. As a monotherapy, it can increase the clinical threshold dose of food allergen, and when used as an adjunct for food immunotherapy, it decreases severe reactions during buildup phase. Finally, lirentelimab, an anti-Siglec-8 antibody currently in clinical trials, can prevent IgE-mediated anaphylaxis in mice through mast cell inhibition. This review discusses these and other emerging therapies as potential strategies for preventing food-induced anaphylaxis. In contrast to other food allergy treatments which largely focus on individual allergens, blockade of the FcεRI pathway has the advantage of preventing clinical reactivity from any food.

Keywords: anaphylaxis, Bruton's tyrosine kinase, Siglec, omalizumab, IgE, FcεRI signaling, food allergy

OPEN ACCESS

Edited by:
Ana Olivera,
National Institutes of Health (NIH), United States

Reviewed by:
Hans Oettgen,
Boston Children's Hospital and Harvard Medical School, United States
Stephen J. Galli,
Stanford University, United States

***Correspondence:**
Melanie C. Dispenza
mdispen1@jhmi.edu

Specialty section:
This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology

Received: *06 October 2020*
Accepted: *16 November 2020*
Published: *17 December 2020*

Citation:
Dispenza MC, Bochner BS and MacGlashan DW Jr (2020) Targeting the FcεRI Pathway as a Potential Strategy to Prevent Food-Induced Anaphylaxis. Front. Immunol. 11:614402. doi: 10.3389/fimmu.2020.614402

INTRODUCTION

Approximately 15 million people in the United States have food allergy and are at risk for anaphylaxis, a potentially life-threatening systemic allergic reaction (1). There is no cure for food allergy, and no known therapies are capable of preventing anaphylaxis, so food allergy is primarily managed by avoiding triggering foods. Unfortunately, accidental exposures still occur. In contrast, there are scenarios in which patients are intentionally exposed to known allergens such as during

food oral immunotherapy (OIT). Despite its efficacy in inducing desensitization to protect against food-induced anaphylaxis, many patients stop OIT prior to reaching maintenance due to frequent and/or severe adverse reactions from the OIT doses themselves (2, 3). Therefore, there is an unmet need for novel strategies of preventing food-induced anaphylaxis from both accidental exposures and during therapeutic procedures such as OIT buildup.

Food allergy is mediated by food-specific IgE found in circulation and bound to the high affinity receptor FcεRI on the surface of mast cells and basophils. When food protein binds to its specific IgE and cross-links the receptor, downstream activation of various kinases induces degranulation, leukotriene and prostaglandin production, and *de novo* cytokine production, which collectively cause clinical symptoms (4). Because all IgE-mediated reactions involve signaling through FcεRI, targeting it or its pathway components would be an ideal strategy for preventing food-induced reactions. This review discusses components of the IgE pathway as potential therapeutic targets for preventing food-induced anaphylaxis (**Table 1**).

IgE AND FcεRI

IgE binding to FcεRI stabilizes the receptor's expression on the surface of mast cells and basophils; thus, FcεRI expression is regulated by the level of total serum IgE (5, 6). Because the binding affinity of IgE to FcεRI is extremely high (< 1 nmol/L), disruption of this interaction has proven difficult therapeutically. Strategies which focus on reducing the levels of total IgE or disrupting its binding to FcεRI show promise for the prevention of IgE-mediated reactions to foods. The first demonstration that anti-IgE therapy could raise food threshold doses during oral challenge came from a trial with the anti-IgE monoclonal antibody (mAb) talizumab (developed by Tanox) in peanut-allergic subjects (7). Talizumab never progressed further in clinical trials, but other anti-IgE therapies show promise for this indication.

Omalizumab

Omalizumab (Xolair®; manufacturers Genentech and Novartis) is a humanized antibody that binds to the C3 domain of IgE, thereby sequestering free IgE away from FcεRI (8). The resulting downregulation of surface FcεRI expression on mast cells and basophils renders them less sensitive to allergen-mediated activation. Already FDA-approved for asthma and chronic spontaneous urticaria (CSU), omalizumab has also demonstrated modest effect as a monotherapy for food allergy. Omalizumab treatment for 6–8 weeks increased patients' threshold dose of peanut protein from a median of 80 to 6,500 mg in one open-label study in allergic adults (9), and another trial showed an 81-fold increase in peanut threshold dose in allergic subjects after 24 weeks of omalizumab compared to a 4.1-fold increase in the placebo-treated group (10). In food-allergic children receiving omalizumab for their severe asthma, omalizumab increased threshold doses for milk, egg, wheat, and hazelnut from an average of 1,013 to 8,727 mg of food protein after 16 weeks of treatment (11). Unfortunately, not all subjects in these trials displayed a significant increase in their food threshold dose on omalizumab. More research is needed to determine its efficacy in preventing reactivity from food exposures and to identify which patients would benefit most from omalizumab monotherapy.

Omalizumab has also been recently studied as an adjuvant therapy for food OIT. Early open-label studies suggested that 9 to 12 weeks of omalizumab therapy could facilitate rapid oral desensitization to cow's milk and peanut in high-risk patients (12, 13). Subsequently, Wood et al. demonstrated in a double-blind, placebo-controlled (DBPC) trial that milk-allergic subjects treated with omalizumab experienced fewer adverse reactions during OIT build-up (with 2.1% of doses provoking reactions) compared to those treated with placebo (16.1% of doses) (14). None of the reactions experienced during omalizumab treatment required medical treatment, compared to 3.8% of reactions in subjects on placebo. However, omalizumab had no effect on subjects' ability to pass an exit oral food challenge, nor did it affect rates of sustained unresponsiveness to milk 4 months after cessation of OIT. Another DBPC trial using omalizumab during multi-food OIT showed a significant reduction in the median per-participant percentage of OIT doses causing adverse reactions in those receiving omalizumab (27%) compared to placebo (68%), especially for gastrointestinal and respiratory symptoms (15). Additional trials are ongoing to evaluate omalizumab's ability to facilitate multi-food OIT (NCT03881696).

Ligelizumab

Ligelizumab (Novartis) is another humanized anti-IgE mAb which binds to free IgE with higher affinity than omalizumab. Ligelizumab effectively prevents passive systemic anaphylaxis (PSA) in human FcεRIα transgenic mice (16). Interestingly, though ligelizumab also binds to the C3 domain of IgE, it can bind to CD23-bound IgE on B cells as well, unlike omalizumab (16). Though the mechanism is unclear, this property allows ligelizumab to prevent new IgE production. Ligelizumab has demonstrated superior and more durable suppression of total IgE levels, skin prick test responses to allergens, and basophil surface FcεRI expression in humans compared to omalizumab (17). This may make it more effective than omalizumab in preventing IgE-mediated reactions in patients who have especially high serum IgE levels. It has not yet been tested in food allergy, though clinical trials have shown efficacy in CSU (NCT02477332).

Lumiliximab

The low-affinity IgE receptor, FcεRII or CD23, appears to regulate IgE homeostasis (18). Anti-CD23 treatment of B cells has been shown to reduce IgE production (19). In a phase I trial, a single dose of lumiliximab (Biogen Idec Inc.), an anti-CD23 antibody, significantly reduced circulating serum IgE in asthma patients (20).

TABLE 1 | Investigational therapies for food-induced anaphylaxis that target IgE or its pathway components.

Specific agents by target	Mechanism of action	Advantages	Disadvantages
IgE and FcεRI			
Omalizumab* (anti-IgE mAb)	Prevents IgE binding to FcεRI	Most well-studied potential therapy; favorable safety profile for chronic use; may facilitate food oral immunotherapy	Slow onset of action; incomplete prevention of clinical reactivity; most effective at lower serum IgE levels
Ligelizumab (anti-IgE mAb)	Prevents IgE binding to FcεRI; may reduce B cell production of IgE	Higher affinity for IgE than omalizumab; favorable safety profile; may be more effective than omalizumab at high serum IgE levels	Moderately slow onset of action
Lumiliximab (anti-CD23)	Reduces B cell production of IgE	Long duration of total IgE reduction	Moderately slow onset of action
Quilizumab (anti-IgE mAb)	Depletes IgE-producing B cells	Long duration of total IgE reduction	Moderately slow onset of action
MEDI4212 (anti-IgE mAb)	Prevents IgE binding to FcεRI; depletes IgE-producing B cells	May be more effective than omalizumab at high serum IgE levels	Moderately slow onset of action; shorter duration of action may mean more frequent dosing than omalizumab
E2_79 (anti-IgE darpin)	Prevents IgE binding to FcεRI and dissociates existing complexes	Relatively rapid onset of action for dissociating IgE-FcεRI complexes	No clinical data available
Kinases			
Ibrutinib, acalabrutinib (BTK inhibitors)	Inhibition of FcεRI signaling	Complete inhibition of FcεRI signaling; rapid onset of action; oral dosing; next-generation inhibitors have favorable safety profile; shown to prevent systemic anaphylaxis in humanized mice	Side-effects may prevent chronic use
Fostamatinib** (Syk inhibitor)	Inhibition of FcεRI signaling	Rapid onset of action; oral dosing	Less favorable safety profile compared to other kinase inhibitors
GSK2646264 (Syk inhibitor)	Inhibition of FcεRI signaling	Rapid onset of action	Currently in topical formulation only with unknown efficacy with oral dosing
WZ3146 (Lyn and Fyn inhibitor)	Inhibition of FcεRI signaling	Rapid onset of action	No clinical data available; inhibitors may have increased toxicity due to broad enzyme expression
SHP and SHIP-1			
Anti-CD32b/FcεRI mAb or fusion proteins	Inhibition of FcεRI signaling		No clinical data available; risk of inducing anaphylaxis
Allergen-specific IgG	Inhibition of FcεRI signaling; competitive binding of allergen away from IgE		Allergen-dependent mechanism
Anti-CD300a mAb	Inhibition of FcεRI signaling		No clinical data available; only partial reduction of degranulation *in vitro*
IE1 (anti-CD300a/IgE bispecific Ab)			
AQX-1125	Inhibition of FcεRI signaling	Oral dosing	Only partial reduction of degranulation *in vitro*
Siglecs			
Lirentelimab (AK002; anti-Siglec-8 mAb)	Inhibition of FcεRI signaling	Target receptor has limited expression; favorable safety profile; shown to prevent systemic anaphylaxis in humanized mice; also depletes eosinophils	
Dual targeting liposomes (antigen and anti-Siglec-3/CD33)	Inhibition of FcεRI signaling	Shown to prevent systemic anaphylaxis in mice	No clinical data available; allergen-dependent mechanism
Anti-Siglec-6 mAb	Inhibition of FcεRI signaling	Target receptor is exclusive to mast cells	No clinical data available; only partial reduction of degranulation *in vitro*
Anti-Siglec-7 mAb	Inhibition of FcεRI signaling		No clinical data available; less effective in preventing basophil activation *in vitro*

**FDA-approved for asthma and CSU.*

***FDA-approved for chronic refractory immune thrombocytopenic purpura.*

All agents act in an allergen-independent mechanism unless otherwise noted.

Quilizumab

Quilizumab (Genentech) is a humanized afucosylated anti-IgE antibody in development that binds to M1', a domain specific to membrane-bound IgE in B cells. In this way, it reduces circulating IgE *via* apoptotic depletion of IgE-producing B cells, but does not bind to circulating IgE or IgE already bound to FcεRI. In early clinical trials, a single dose of quilizumab reduced serum IgE levels in patients with allergic rhinitis or

asthma for approximately 6 months (21). Unfortunately, despite reducing IgE levels, it failed to improve symptoms in a trial for antihistamine-refractory CSU (22). The future of quilizumab is unknown, but it may have a potential application in treating food allergy given its ability to prevent further IgE production.

MEDI4212

MEDI4212 is a high-affinity antibody for IgE which can both bind to circulating IgE as well as membrane-bound IgE, thus depleting IgE-producing B cells through antibody-dependent cell-mediated cytotoxicity (23). Because of its dual action in reducing IgE, it may be more effective than omalizumab in patients with very high IgE levels. Its phase I trial demonstrated superior reduction of total IgE levels in atopic subjects after a single MEDI4212 dose compared to subjects treated with a dose of omalizumab (24).

Anti-IgE Darpins

Eggel et al. created a non-immunoglobulin ankyrin repeat protein (DARPin) inhibitor of IgE, E2_79, which not only blocks IgE from binding to FcεRI, but is also able to rapidly dissociate preformed IgE-FcεRI complexes (25). Intravenous infusion of E2_79 just 6 hours prior to antigen challenge was shown to prevent passive cutaneous anaphylaxis (PCA) in human FcεRIα transgenic mice (26). These data suggest that E2_79 could rapidly dissociate food-specific IgE from allergic cells in patients *in vivo*, though it has not yet been tested in clinical trials.

KINASES

Numerous kinases are involved in FcεRI pathway signaling, including spleen tyrosine kinase (Syk), Bruton's tyrosine kinase (BTK), Lyn, Fyn, phospholipase Cγ (PLCγ), PI3 kinase (PI3K), and others (4). All of these enzymes are potential targets for inhibition of IgE-mediated activation of mast cells and basophils. Only recently, with the use of next-generation kinase inhibitors that are more specific for their target enzymes, have we begun to elucidate precisely which kinases are necessary to inhibit in order to prevent IgE-mediated reactions.

BTK

BTK is largely expressed in leukocytes including mast cells, basophils, B cells, neutrophils, monocytes, and NK cells and is thought to be essential for IgE-dependent activation of human cells (27–29). As an essential enzyme for B cell receptor signaling, it has been pharmacologically targeted for the treatment of B cell malignancies, and the relatively recent FDA-approval of selective BTK inhibitors has created the opportunity to repurpose these medications for the prevention of IgE-mediated anaphylaxis. Dispenza et al. showed that just two clinically-relevant oral doses of acalabrutinib, a second-generation irreversible BTK inhibitor, completely prevented moderate-severity PSA in humanized NSG-SGM3 mice which have mature human mast cells and basophils (27). Even more remarkably, it significantly protected against mortality during fatal anaphylaxis in this mouse model. Data *in vivo* in humans is still preliminary, but ibrutinib has been shown to suppress IgE-mediated ex vivo basophil activation and skin prick testing to both aeroallergens and foods in allergic subjects (30–32). Clinical trials using BTK inhibitors to prevent clinically reactivity to foods in food allergic adults are currently ongoing in the authors' laboratory.

Syk

In addition to mast cells and basophils, Syk is expressed in numerous organ systems. Multiple studies have demonstrated Syk inhibition as a potential strategy for preventing anaphylaxis. The active metabolite of fostamatinib (Tavalisse®; Rigel Pharmaceuticals), which was approved in 2018 for chronic refractory immune thrombocytopenic purpura, prevented *ex vivo* basophil activation after a single dose in humans (33), as well as anaphylaxis to peanut in a mouse model of peanut allergy (34). GSK2646264 is a Syk inhibitor in a cream formulation in clinical trials for CSU (NCT02424799) which has been shown to attenuate IgE-mediated histamine release from human mast cells, though it is unclear if this compound would be safe and effective as an oral medication for the prevention of anaphylaxis (35). The Syk inhibitor NVP-QAB205 demonstrated excellent activity in preventing human mast cell and basophil activation (36, 37), but it did not progress to clinical trials due to potential toxicities like several other Syk inhibitors (38–41). Like BTK inhibitors, clinical trials are needed to demonstrate safety and efficacy for using Syk inhibitors to prevent food-induced anaphylaxis.

Lyn and Fyn

Lyn and Fyn are kinases upstream of Syk in the FcεRI pathway and may each play both positive and negative regulatory roles on IgE-mediated activation of human cells. Evidence that their inhibition can prevent IgE-mediated reactions largely arises from studies using non-specific compounds. The EGFR inhibitor WZ3146 effectively blocked mast cell and basophil activation *in vitro* through off-target antagonist activity on Lyn and Fyn (42). AZD7762, and inhibitor of Chk1 with Lyn/Fyn activity, and had similar efficacy in preventing LAD2 mast cell activation (43), but cardiac toxicity in Phase I trials prevented further development. Ultimately, Lyn and Fyn may be useful targets for preventing food allergy reactions if they can be specifically targeted, but even specific inhibitors may have an unfavorable toxicities given the broad expression profile of these kinases.

SHP AND SHIP-1

Upon activation, various mast cell and basophil inhibitory receptors with immunoreceptor tyrosine inhibitory motifs (ITIMs) recruit phosphotyrosine phosphatases (SHP-1 and SHP-2) and inositol phosphatases (SHIP-1), which then provide direct inhibitory feedback on the FcεRI pathway.

CD32b

Expressed on basophils and some tissue mast cells as well as other cells, the low-affinity IgG receptor FcγRIIb (CD32b) is a potential target for the prevention of allergen-mediated anaphylaxis (44). Co-aggregation of CD32b with FcεRI has been shown to inhibit IgE-mediated mast cell and basophil activation (45). Cross-linking these receptors can be achieved in an allergen-independent manner with bispecific antibodies to FcεRI and CD32b or Fcε-Fcγ fusion proteins to prevent allergic reactivity (46–48), though these strategies have yet to move forward to clinical trials. In contrast, specific IgG antibodies can induce allergen-specific IgE-FcεRI-CD32b cross-linking in the presence of allergen, as well as competitively block allergen binding to its specific IgE (49). Clinical trials using cat-specific IgG cocktails for treatment of respiratory allergies are ongoing (NCT03838731) (50), but no trials are currently investigating this approach in food allergy.

CD300a

CD300a is an inhibitory receptor expressed on numerous human immune cells including mast cells and basophils. Cross-linking of CD300a recruits SHP-1 and SHIP-1 to elicit strong inhibitory signals on the FcεRI pathway (51). Monoclonal antibodies to CD300a partially prevented IgE-mediated CD63 upregulation in human basophils (52, 53), and IE1, a bispecific antibody recognizing IgE and CD300a, was shown to inhibit FcεRI signaling and IgE-mediated degranulation in human mast cells in a dose-dependent manner (54).

AQX-1125

As a SHIP-1 activator, AQX-1125 reduced IgE-mediated mast cell degranulation *in vitro* and showed efficacy in murine models of allergic asthma (55). It is currently in clinical trials for atopic dermatitis (NCT02324972).

SIGLECS

Sialic acid-binding immunoglobulin-type lectins (Siglecs) are transmembrane receptors found on the surface of immune cells, so-called because they bind to sialic acid-containing ligands. Most Siglecs have ITIMs or ITIM-like motifs, which upon ligand binding, enable inhibitory signaling to counteract the actions of tyrosine kinases in the FcεRI pathway (56, 57). Their differing ligand specificity and relatively restricted expression profiles make Siglecs good candidate targets for the suppression IgE-mediated activation of mast cells and/or basophils.

Siglec-8

Siglec-8 is the best studied and most promising siglec target for the treatment of allergic diseases, with expression limited to human mast cells, basophils, and eosinophils (58, 59). *In vitro*, pre-incubation of human CD34-derived mast cells with anti-Siglec-8 mAb markedly shifted the anti-FcεRI antibody-induced secretion dose response curve for histamine and PGD2 secretion (60). Siglec-8's ITIM domain was found to be essential for this function, suggesting that its mechanism of action may involve phosphatase recruitment as discussed above. Furthermore, pretreatment with the humanized non-fucosylated IgG1 anti-Siglec-8 antibody lirentelimab (Allakos, Inc.) completely prevented human IgE-mediated PSA in NSG-SGM3 BLT humanized mice (61), suggesting that it may be a useful therapy for preventing anaphylaxis in humans. Lirentelimab has shown efficacy in other mast cell-driven diseases in early clinical trials, including CSU (NCT03436797), atopic keratoconjunctivitis (NCT03379311), and indolent systemic mastocytosis (NCT02808793). The most advanced efforts with lirentelimab involve a phase III study in eosinophilic gastritis and duodenitis (NCT04322604), a disorder characterized by increased numbers of tissue eosinophils and mast cells, based on positive phase II results (62).

Siglec-3/CD33

CD33 (also known as Siglec-3) is expressed on most human myeloid cells, including mast cells and basophils. Duan et al. showed that dual targeting of CD33 and specific IgE on mast cells with liposomes expressing CD33L and a synthetic antigen (TNP) could prevent IgE-mediated anaphylaxis (63). These liposomes prevented TNP-induced degranulation in human mast cells and prevented clinical response during PSA in transgenic mice expressing human CD33. Intriguingly, the liposomes' inhibitory effects were sustained for at least 2–3 days after infusion, potentially due to endocytosis of the TNP-IgE-FcεRI complex in mast cells and/or liposomes' interference of TNP binding to its specific IgE. One limitation of this approach is that these liposomes act in an antigen-specific manner, which may necessitate the use of several different types of liposomes to treat patients with multiple food allergies.

Siglecs-6 and 7

Siglec-7 is expressed on human mast cells, monocytes, eosinophils, NK cells, and, to a lesser extent, basophils (64). Pre-incubation of CD34-derived mast cells with the combination of an activating anti-Siglec-7 antibody, an anti-IgE antibody, and a cross-linking anti-mouse IgG $F(ab')_2$ completely prevented degranulation and partially prevented PGD2 release and GM-CSF production (64). This inhibition was dependent on Siglec-7 directly crosslinking with FcεRI, which has not always been found to be the case for other Siglecs that inhibit FcεRI-mediated signaling such as Siglec-6 or -8 (60, 65). Interestingly, Siglec-7 engagement on human basophils only partially reduces IgE-mediated degranulation, a discrepancy thought to be due to the relatively low expression of Siglec-7 on basophils. Siglec-6 is a geographically unique target, given that its expression is primarily limited to mast cells (66), but its engagement on mast cells has shown only modest (approximately 30%) reduction in IgE-mediated degranulation (65). Further studies are needed to determine the efficacy of targeting either Siglec-6 or -7 for the prevention of IgE-mediated anaphylaxis.

DISCUSSION AND CONCLUSIONS

Despite attempts at its prevention, the prevalence of IgE-mediated food allergy is increasing. Therapies targeting IgE and the FcεRI pathway may fill the need for preventing food-induced anaphylaxis. One major benefit to this approach is that it is not allergen specific, unlike food OIT. Additionally, FcεRI pathway inhibition would prevent the release of all mast cell and basophil mediators, unlike most current allergy treatments (such as antihistamines and leukotriene receptor antagonists) which only counteract the effects of a few of many mediators that participate in causing allergic reactions.

Both short-term and long-term protection strategies to prevent anaphylaxis are needed to improve the quality of life of food allergic patients. Short-term therapies which can facilitate food OIT build-up to prevent adverse reactions would allow more patients to reach maintenance dose. Alternatively, short-term treatments which could protect against accidental exposures during isolated high risk situations (e.g. a family vacation abroad or birthday parties) would reduce morbidity and mortality and help alleviate anxiety for those suffering from food allergies. Perhaps more importantly, therapies are needed which could be used chronically to reliably prevent reactions from accidental food exposures.

Many questions remain regarding the utility and safety of the above therapies, most of which are still experimental or in clinical development. Each therapy's risk-benefit ratio and mechanism of action will determine its specific indication (**Table 1**). BTK inhibitors have a rapid onset of action and are highly effective at preventing IgE-mediated anaphylaxis, making them good candidates for short-term episodic use to prevent reactivity to foods, but they may not be suitable for long-term use based on the safety profile of the currently FDA-approved BTK inhibitors. Omalizumab has a favorable safety profile when used chronically, but it has slower onset of efficacy, and its ability to reliably prevent anaphylaxis (especially fatal anaphylaxis) is unknown. More studies are needed to determine the safety and efficacy of targeting the FcεRI pathway as a protective measure against food-induced anaphylaxis.

AUTHOR CONTRIBUTIONS

MD conceived of the concept and wrote the manuscript. BB and DM contributed sections and provided feedback on the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was funded in part by a K23 mentored career award from NIH to MD (grant AI143965), a U19 grant from NIH to BB (AI136443), the Ludwig Family Foundation, and the Johns Hopkins School of Medicine Clinician Scientist Career Development Award.

REFERENCES

1. Shaker MS, Wallace DV, Golden DBK, Oppenheimer J, Bernstein JA, Campbell RL, et al. Anaphylaxis-a 2020 practice parameter update, systematic review, and Grading of Recommendations, Assessment, Development and Evaluation (GRADE) analysis. *J Allergy Clin Immunol* (2020) 145(4):1082–123. doi: 10.1016/j.jaci.2020.01.017
2. Wood RA. Oral Immunotherapy for Food Allergy. *J Invest Allergol Clin Immunol* (2017) 27(3):151–9. doi: 10.18176/jiaci.0143
3. Vazquez-Cortes S, Jaqueti P, Arasi S, Machinena A, Alvaro-Lozano M, Fernandez-Rivas M. Safety of Food Oral Immunotherapy: What We Know, and What We Need to Learn. *Immunol Allergy Clinics North America* (2020) 40(1):111–33. doi: 10.1016/j.iac.2019.09.013
4. Peavy RD, Metcalfe DD. Understanding the mechanisms of anaphylaxis. *Curr Opin Allergy Clin Immunol* (2008) 8(4):310–5. doi: 10.1097/ACI.0b013e3283036a90
5. Hsu C. and MacGlashan D, Jr. IgE antibody up-regulates high affinity IgE binding on murine bone marrow-derived mast cells. *Immunol Lett* (1996) 52 (2-3):129–34. doi: 10.1016/0165-2478(96)02599-0
6. Lantz CS, Yamaguchi M, Oettgen HC, Katona IM, Miyajima I, Kinet JP, et al. IgE regulates mouse basophil Fc epsilon RI expression in vivo. *J Immunol* (1997) 158(6):2517–21.
7. Leung DY, Sampson HA, Yunginger JW, Burks AW Jr., Schneider LC, Wortel CH, et al. Effect of anti-IgE therapy in patients with peanut allergy. *N Engl J Med* (2003) 348(11):986–93. doi: 10.1056/NEJMoa022613
8. Liu J, Lester P, Builder S, Shire SJ. Characterization of complex formation by humanized anti-IgE monoclonal antibody and monoclonal human IgE. *Biochemistry* (1995) 34(33):10474–82. doi: 10.1021/bi00033a020
9. Savage JH, Courneya JP, Sterba PM, Macglashan DW, Saini SS, Wood RA. Kinetics of mast cell, basophil, and oral food challenge responses in omalizumab-treated adults with peanut allergy. *J Allergy Clin Immunol* (2012) 130(5):1123–9. doi: 10.1016/j.jaci.2012.05.039
10. Sampson HA, Leung DY, Burks AW, Lack G, Bahna SL, Jones SM, et al. randomized, doubleblind, parallelgroup, placebocontrolled oral food challenge trial of Xolair (omalizumab) in peanut allergy. *J Allergy Clin Immunol* (2011) 127(5):1309–10 e1. doi: 10.1016/j.jaci.2011.01.051
11. Fiocchi A, Artesani MC, Riccardi C, Mennini M, Pecora V, Fierro V, et al. Impact of Omalizumab on Food Allergy in Patients Treated for Asthma: A Real-Life Study. *J Allergy Clin Immunol In Pract* (2019) 7(6):1901–9 e5. doi: 10.1016/j.jaip.2019.01.023
12. Nadeau KC, Schneider LC, Hoyte L, Borras I, Umetsu DT. Rapid oral desensitization in combination with omalizumab therapy in patients with cow's milk allergy. *J Allergy Clin Immunol* (2011) 127(6):1622–4. doi: 10.1016/j.jaci.2011.04.009
13. Schneider LC, Rachid R, LeBovidge J, Blood E, Mittal M, Umetsu DT. A pilot study of omalizumab to facilitate rapid oral desensitization in high-risk peanut-allergic patients. *J Allergy Clin Immunol* (2013) 132(6):1368–74. doi: 10.1016/j.jaci.2013.09.046
14. Wood RA, Kim JS, Lindblad R, Nadeau K, Henning AK, Dawson P, et al. A randomized, double-blind, placebo-controlled study of omalizumab combined with oral immunotherapy for the treatment of cow's milk allergy. *J Allergy Clin Immunol* (2016) 137(4):1103–10 e11. doi: 10.1016/j.jaci.2015.10.005
15. Andorf S, Purington N, Block WM, Long AJ, Tupa D, Brittain E, et al. Anti-IgE treatment with oral immunotherapy in multifood allergic participants: a double-blind, randomised, controlled trial. *Lancet Gastroenterol Hepatol* (2018) 3(2):85–94. doi: 10.1016/S2468-1253(17)30392-8
16. Gasser P, Tarchevskaya SS, Guntern P, Brigger D, Ruppli R, Zbaren N, et al. The mechanistic and functional profile of the therapeutic anti-IgE antibody ligelizumab differs from omalizumab. *Nat Commun* (2020) 11(1):165. doi: 10.1038/s41467-019-13815-w

17. Arm JP, Bottoli I, Skerjanec A, Floch D, Groenewegen A, Maahs S, et al. Pharmacokinetics, pharmacodynamics and safety of QGE031 (ligelizumab), a novel high-affinity anti-IgE antibody, in atopic subjects. *Clin Exp Allergy* (2014) 44(11):1371–85. doi: 10.1111/cea.12400
18. Cooper AM, Hobson PS, Jutton MR, Kao MW, Drung B, Schmidt B, et al. Soluble CD23 controls IgE synthesis and homeostasis in human B cells. *J Immunol* (2012) 188(7):3199–207. doi: 10.4049/jimmunol.1102689
19. Nakamura T, Kloetzer WS, Brams P, Hariharan K, Chamat S, Cao X, et al. In vitro IgE inhibition in B cells by anti-CD23 monoclonal antibodies is functionally dependent on the immunoglobulin Fc domain. *Int J Immunopharmacol* (2000) 22 (2):131–41. doi: 10.1016/S0192-0561(99)00068-5
20. Rosenwasser LJ, Busse WW, Lizambri RG, Olejnik TA, Totoritis MC. Allergic asthma and an anti-CD23 mAb (IDEC-152): results of a phase I, single-dose, dose-escalating clinical trial. *J Allergy Clin Immunol* (2003) 112(3):563–70. doi: 10.1016/S0091-6749(03)01861-X
21. Gauvreau GM, Harris JM, Boulet LP, Scheerens H, Fitzgerald JM, Putnam WS, et al. Targeting membrane-expressed IgE B cell receptor with an antibody to the M1 prime epitope reduces IgE production. *Sci Transl Med* (2014) 6 (243):243ra85. doi: 10.1126/scitranslmed.3008961
22. Harris JM, Cabanski CR, Scheerens H, Samineni D, Bradley MS, Cochran C, et al. A randomized trial of quilizumab in adults with refractory chronic spontaneous urticaria. *J Allergy Clin Immunol* (2016) 138(6):1730–2. doi: 10.1016/j.jaci.2016.06.023
23. Nyborg AC, Zacco A, Ettinger R, Jack Borrok M, Zhu J, Martin T, et al. Development of an antibody that neutralizes soluble IgE and eliminates IgE expressing B cells. *Cell Mol Immunol* (2016) 13(3):391–400. doi: 10.1038/cmi.2015.19
24. Sheldon E, Schwickart M, Li J, Kim K, Crouch S, Parveen S, et al. Pharmacokinetics, Pharmacodynamics, and Safety of MEDI4212, an Anti-IgE Monoclonal Antibody, in Subjects with Atopy: A Phase I Study. *Adv Ther* (2016) 33(2):225–51. doi: 10.1007/s12325-016-0287-8
25. Kim B, Eggel A, Tarchevskaya SS, Vogel M, Prinz H, Jardetzky TS. Accelerated disassembly of IgE-receptor complexes by a disruptive macromolecular inhibitor. *Nature* (2012) 491(7425):613–7. doi: 10.1038/nature11546
26. Eggel A, Baravalle G, Hobi G, Kim B, Buschor P, Forrer P, et al. Accelerated dissociation of IgE-FcepsilonRI complexes by disruptive inhibitors actively desensitizes allergic effector cells. *J Allergy Clin Immunol* (2014) 133(6):1709–19 e8. doi: 10.1016/j.jaci.2014.02.005
27. Dispenza MC, Krier-Burris RA, Chhiba KD, Undem BJ, Robida PA, Bochner BS. Bruton's tyrosine kinase inhibition effectively protects against human IgE-mediated anaphylaxis. *J Clin Invest* (2020) 130(9):4759–70. doi: 10.1172/JCI138448
28. Smiljkovic D, Blatt K, Stefanzl G, Dorofeeva Y, Skrabs C, Focke-Tejkl M, et al. BTK inhibition is a potent approach to block IgE-mediated histamine release in human basophils. *Allergy* (2017) 72(11):1666–76. doi: 10.1111/all.13166
29. MacGlashan D Jr., Honigberg LA, Smith A, Buggy J, Schroeder JT. Inhibition of IgE-mediated secretion from human basophils with a highly selective Bruton's tyrosine kinase, Btk, inhibitor. *Int Immunopharmacol* (2011) 11 (4):475–9. doi: 10.1016/j.intimp.2010.12.018
30. Regan JA, Cao Y, Dispenza MC, Ma S, Gordon LI, Petrich AM, et al. Ibrutinib, a Bruton's tyrosine kinase inhibitor used for treatment of lymphoproliferative disorders, eliminates both aeroallergen skin test and basophil activation test reactivity. *J Allergy Clin Immunol* (2017) 140(3):875–9.e1. doi: 10.1016/j.jaci.2017.03.013
31. Advani RH, Buggy JJ, Sharman JP, Smith SM, Boyd TE, Grant B, et al. Bruton tyrosine kinase inhibitor ibrutinib (PCI-32765) has significant activity in patients with relapsed/refractory B-cell malignancies. *J Clin Oncol* (2013) 31 (1):88–94. doi: 10.1200/JCO.2012.42.7906
32. Dispenza MC, Pongracic JA, Singh AM, Bochner BS. Short-term ibrutinib therapy suppresses skin test responses and eliminates IgE-mediated basophil activation in adults with peanut or tree nut allergy. *J Allergy Clin Immunol* (2018) 141(5):1914–6. doi: 10.1016/j.jaci.2017.12.987
33. Braselmann S, Taylor V, Zhao H, Wang S, Sylvain C, Baluom M, et al. R406, an orally available spleen tyrosine kinase inhibitor blocks fc receptor signaling and reduces immune complex-mediated inflammation. *J Pharmacol Exp Ther* (2006) 319(3):998–1008. doi: 10.1124/jpet.106.109058
34. Burton OT, Noval Rivas M, Zhou JS, Logsdon SL, Darling AR, Koleoglou KJ, et al. Immunoglobulin E signal inhibition during allergen ingestion leads to reversal of established food allergy and induction of regulatory T cells. *Immunity* (2014) 41(1):141–51. doi: 10.1016/j.immuni.2014.05.017
35. Ramirez Molina C, Falkencrone S, Skov PS, Hooper-Greenhill E, Barker M, Dickson MC. GSK2646264, a spleen tyrosine kinase inhibitor, attenuates the release of histamine in ex vivo human skin. *Br J Pharmacol* (2019) 176 (8):1135–42. doi: 10.1111/bph.14610
36. MacGlashan D Jr., Undem BJ. Inducing an anergic state in mast cells and basophils without secretion. *J Allergy Clin Immunol* (2008) 121(6):1500–6, 6 e1-4. doi: 10.1016/j.jaci.2008.04.019
37. Patou J, Holtappels G, Affleck K, van Cauwenberge P, Bachert C. Syk-kinase inhibition prevents mast cell activation in nasal polyps. *Rhinology* (2011) 49 (1):100–6. doi: 10.4193/Rhino09.147
38. Nam ST, Park YH, Kim HW, Kim HS, Lee D, Lee MB, et al. Suppression of IgE-mediated mast cell activation and mouse anaphylaxis via inhibition of Syk activation by 8-formyl-7-hydroxy-4-methylcoumarin, 4mu8C. *Toxicol Appl Pharmacol* (2017) 332:25–31. doi: 10.1016/j.taap.2017.07.015
39. Li X, Kwon O, Kim DY, Taketomi Y, Murakami M, Chang HW. NecroX-5 suppresses IgE/Ag-stimulated anaphylaxis and mast cell activation by regulating the SHP-1-Syk signaling module. *Allergy* (2016) 71(2):198–209. doi: 10.1111/all.12786
40. Kato T, Iwasaki H, Kobayashi H, Miyagawa N, Matsuo A, Hata T, et al. JTE-852, a novel spleen tyrosine kinase inhibitor, blocks mediator secretion from mast cells with immunoglobulin E crosslinking. *Eur J Pharmacol* (2017) 801:1–8. doi: 10.1016/j.ejphar.2017.02.048
41. Deng Y, Jin F, Li X, Park SJ, Chang JH, Kim DY, et al. Sauchinone suppresses FcepsilonRI-mediated mast cell signaling and anaphylaxis through regulation of LKB1/AMPK axis and SHP-1-Syk signaling module. *Int Immunopharmacol* (2019) 74:105702. doi: 10.1016/j.intimp.2019.105702
42. Park YH, Kim DK, Kim HS, Lee D, Lee MB, Min KY, et al. WZ3146 inhibits mast cell Lyn and Fyn to reduce IgE-mediated allergic responses in vitro and in vivo. *Toxicol Appl Pharmacol* (2019) 383:114763. doi: 10.1016/j.taap.2019.114763
43. Park YH, Kim DK, Kim HW, Kim HS, Lee D, Lee MB, et al. Repositioning of anti-cancer drug candidate, AZD7762, to an anti-allergic drug suppressing IgE-mediated mast cells and allergic responses via the inhibition of Lyn and Fyn. *Biochem Pharmacol* (2018) 154:270–7. doi: 10.1016/j.bcp.2018.05.012
44. Gomez G. Current Strategies to Inhibit High Affinity FcepsilonRI-Mediated Signaling for the Treatment of Allergic Disease. *Front Immunol* (2019) 10:175. doi: 10.3389/fimmu.2019.00175
45. Daeron M, Malbec O, Latour S, Arock M, Fridman WH. Regulation of high-affinity IgE receptor-mediated mast cell activation by murine low-affinity IgG receptors. *J Clin Invest* (1995) 95(2):577–85. doi: 10.1172/JCI117701
46. Tam SW, Demissie S, Thomas D, Daeron M. A bispecific antibody against human IgE and human FcgammaRII that inhibits antigen-induced histamine release by human mast cells and basophils. *Allergy* (2004) 59(7):772–80. doi: 10.1111/j.1398-9995.2004.00332.x
47. Zhu D, Kepley CL, Zhang M, Zhang K, Saxon A. A novel human immunoglobulin Fc gamma Fc epsilon bifunctional fusion protein inhibits Fc epsilon RI-mediated degranulation. *Nat Med* (2002) 8(5):518–21. doi: 10.1038/nm0502-518
48. Allen LC, Kepley CL, Saxon A, Zhang K. Modifications to an Fcgamma-Fcvarepsilon fusion protein alter its effectiveness in the inhibition of FcvarepsilonRI-mediated functions. *J Allergy Clin Immunol* (2007) 120 (2):462–8. doi: 10.1016/j.jaci.2007.04.019
49. Strait RT, Morris SC, Finkelman FD. IgG-blocking antibodies inhibit IgE-mediated anaphylaxis in vivo through both antigen interception and Fc gamma RIIb cross-linking. *J Clin Invest* (2006) 116(3):833–41. doi: 10.1172/JCI25575
50. Orengo JM, Radin AR, Kamat V, Badithe A, Ben LH, Bennett BL, et al. Treating cat allergy with monoclonal IgG antibodies that bind allergen and prevent IgE engagement. *Nat Commun* (2018) 9(1):1421. doi: 10.1038/s41467-018-03636-8
51. Stenton GR, Mackenzie LF, Tam P, Cross JL, Harwig C, Raymond J, et al. Characterization of AQX-1125, a small-molecule SHIP1 activator: Part 2. Efficacy studies in allergic and pulmonary inflammation models in vivo. *Br J Pharmacol* (2013) 168(6):1519–29. doi: 10.1111/bph.12038
52. Gibbs BF, Sabato V, Bridts CH, Ebo DG, Ben-Zimra M, Levi-Schaffer F. Expressions and inhibitory functions of CD300a receptors on purified human basophils. *Exp Dermatol* (2012) 21(11):884–6. doi: 10.1111/exd.12018
53. Sabato V, Verweij MM, Bridts CH, Levi-Schaffer F, Gibbs BF, De Clerck LS, et al. CD300a is expressed on human basophils and seems to inhibit IgE/

FcepsilonRI-dependent anaphylactic degranulation. *Cytometry B Clin Cytom* (2012) 82(3):132–8. doi: 10.1002/cyto.b.21003
54. Bachelet I, Munitz A, Levi-Schaffer F. Abrogation of allergic reactions by a bispecific antibody fragment linking IgE to CD300a. *J Allergy Clin Immunol* (2006) 117(6):1314–20. doi: 10.1016/j.jaci.2006.04.031
55. Stenton GR, Mackenzie LF, Tam P, Cross JL, Harwig C, Raymond J, et al. Characterization of AQX-1125, a small-molecule SHIP1 activator: Part 1. Effects on inflammatory cell activation and chemotaxis in vitro and pharmacokinetic characterization in vivo. *Br J Pharmacol* (2013) 168 (6):1506–18. doi: 10.1111/bph.12039
56. Bornhofft KF, Goldammer T, Rebl A, Galuska SP. Siglecs: A journey through the evolution of sialic acid-binding immunoglobulin-type lectins. *Dev Comp Immunol* (2018) 86:219–31. doi: 10.1016/j.dci.2018.05.008
57. Karra L, Levi-Schaffer F. *Down-Regulation of Mast Cell Responses through ITIM Containing Inhibitory Receptors*. Boston, MA: Springer (2011). doi: 10.1007/978-1-4419-9533-9_9
58. Kikly KK, Bochner BS, Freeman SD, Tan KB, Gallagher KT, D'Alessio KJ, et al. Identification of SAF-2, a novel siglec expressed on eosinophils, mast cells, and basophils. *J Allergy Clin Immunol* (2000) 105(6 Pt 1):1093–100. doi: 10.1067/mai.2000.107127
59. Floyd H, Ni J, Cornish AL, Zeng Z, Liu D, Carter KC, et al. Siglec-8. A novel eosinophil-specific member of the immunoglobulin superfamily. *J Biol Chem* (2000) 275(2):861–6. doi: 10.1074/jbc.275.2.861
60. Yokoi H, Choi OH, Hubbard W, Lee HS, Canning BJ, Lee HH, et al. Inhibition of FcepsilonRI-dependent mediator release and calcium flux from human mast cells by sialic acid-binding immunoglobulin-like lectin 8 engagement. *J Allergy Clin Immunol* (2008) 121(2):499–505.e1. doi: 10.1016/j.jaci.2007.10.004
61. Youngblood BA, Brock EC, Leung J, Falahati R, Bryce PJ, Bright J, et al. AK002, a Humanized Sialic Acid-Binding Immunoglobulin-Like Lectin-8 Antibody that Induces Antibody-Dependent Cell-Mediated Cytotoxicity against Human Eosinophils and Inhibits Mast Cell-Mediated Anaphylaxis in Mice. *Int Arch Allergy Immunol* (2019) 180(2):91–102. doi: 10.1159/000501637
62. Dellon ES, Peterson KA, Murray JA, Falk GW, Gonsalves N, Chehade M, et al. Anti-Siglec-8 Antibody for Eosinophilic Gastritis and Duodenitis. *N Engl J Med* (2020) 383(17):1624–34. doi: 10.1056/NEJMoa2012047
63. Duan S, Koziol-White CJ, Jester WF Jr., Nycholat CM, Macauley MS, Panettieri RA Jr., et al. CD33 recruitment inhibits IgE-mediated anaphylaxis and desensitizes mast cells to allergen. *J Clin Invest* (2019) 129(3):1387–401. doi: 10.1172/JCI125456
64. Mizrahi S, Gibbs BF, Karra L, Ben-Zimra M, Levi-Schaffer F. Siglec-7 is an inhibitory receptor on human mast cells and basophils. *J Allergy Clin Immunol* (2014) 134(1):230–3. doi: 10.1016/j.jaci.2014.03.031
65. Yu Y, Blokhuis BRJ, Diks MAP, Keshavarzian A, Garssen J, Redegeld FA. Functional Inhibitory Siglec-6 Is Upregulated in Human Colorectal Cancer-Associated Mast Cells. *Front Immunol* (2018) 9:2138. doi: 10.3389/fimmu.2018.02138
66. Plum T, Wang X, Rettel M, Krijgsveld J, Feyerabend TB, Rodewald HR. Human Mast Cell Proteome Reveals Unique Lineage, Putative Functions, and Structural Basis for Cell Ablation. *Immunity* (2020) 52(2):404–16 e5. doi: 10.1016/j.immuni.2020.01.012

Conflict of Interest: MD has received research funding from Acerta Pharma/AstraZeneca in addition to consultant fees from AlphaSights, BVF Partners, and DAVA Oncology. BB has also received research funding from Acerta Pharma/AstraZeneca, as well as consultant fees from Regeneron, Sanofi, and GlaxoSmithkline. Finally, BB receives remuneration for serving on the scientific advisory board of Allakos, Inc. and owns stock in Allakos. He is a co-inventor on existing Siglec-8–related patents and thus may be entitled to a share of royalties received by Johns Hopkins University during development and potential sales of such products. BB is also a co-founder of Allakos; the terms of this arrangement are being managed by Johns Hopkins University and Northwestern University in accordance with their conflict of interest policies. DM consults for Boehringer-Ingelheim.

frontiers in Immunology

ORIGINAL RESEARCH
published: 22 December 2020
doi: 10.3389/fimmu.2020.606837

Protein Disulfide Isomerases Regulate IgE-Mediated Mast Cell Responses and Their Inhibition Confers Protective Effects During Food Allergy

*Dylan Krajewski[1], Stephanie H. Polukort[1], Justine Gelzinis[1], Jeffrey Rovatti[1], Edwin Kaczenski[1], Christine Galinski[1], Megan Pantos[1], Nickul N. Shah[2,3], Sallie S. Schneider[2,3], Daniel R. Kennedy[1] and Clinton B. Mathias[1,3]**

[1] Department of Pharmaceutical and Administrative Sciences, College of Pharmacy and Health Sciences, Western New England University, Springfield, MA, United States, [2] Pioneer Valley Life Sciences Institute, Baystate Medical Center, Springfield, MA, United States, [3] Department of Veterinary and Animal Sciences, University of Massachusetts at Amherst, Amherst, MA, United States

OPEN ACCESS

Edited by:
Ana Olivera, National Institutes of Health (NIH), United States

Reviewed by:
Hariharan Subramanian, Michigan State University, United States
Peter Monk, The University of Sheffield, United Kingdom
Satoshi Tanaka, Kyoto Pharmaceutical University, Japan

***Correspondence:**
Clinton B. Mathias
clinton.mathias@wne.edu

Specialty section:
This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology

Received: *15 September 2020*
Accepted: *17 November 2020*
Published: *22 December 2020*

Citation:
Krajewski D, Polukort SH, Gelzinis J, Rovatti J, Kaczenski E, Galinski C, Pantos M, Shah NN, Schneider SS, Kennedy DR and Mathias CB (2020) Protein Disulfide Isomerases Regulate IgE-Mediated Mast Cell Responses and Their Inhibition Confers Protective Effects During Food Allergy. Front. Immunol. 11:606837. doi: 10.3389/fimmu.2020.606837

The thiol isomerase, protein disulfide isomerase (PDI), plays important intracellular roles during protein folding, maintaining cellular function and viability. Recent studies suggest novel roles for extracellular cell surface PDI in enhancing cellular activation and promoting their function. Moreover, a number of food-derived substances have been shown to regulate cellular PDI activity and alter disease progression. We hypothesized that PDI may have similar roles during mast cell-mediated allergic responses and examined its effects on IgE-induced mast cell activity during cell culture and food allergy. Mast cells were activated *via* IgE and antigen and the effects of PDI inhibition on mast cell activation were assessed. The effects of PDI blockade *in vivo* were examined by treating mice with the irreversible PDI inhibitor, PACMA-31, in an ovalbumin-induced model of food allergy. The role of dietary PDI modulators was investigated using various dietary compounds including curcumin and quercetin-3-rutinoside (rutin). PDI expression was observed on resting mast cell surfaces, intracellularly, and in the intestines of allergic mice. Furthermore, enhanced secretion of extracellular PDI was observed on mast cell membranes during IgE and antigen activation. Insulin turbidimetric assays demonstrated that curcumin is a potent PDI inhibitor and pre-treatment of mast cells with curcumin or established PDI inhibitors such as bacitracin, rutin or PACMA-31, resulted in the suppression of IgE-mediated activation and the secretion of various cytokines. This was accompanied by decreased mast cell proliferation, FcεRI expression, and mast cell degranulation. Similarly, treatment of allergic BALB/c mice with PACMA-31 attenuated the development of food allergy resulting in decreased allergic diarrhea, mast cell activation, and fewer intestinal mast cells. The production of T_H2-specific cytokines was also suppressed. Our

observations suggest that PDI catalytic activity is essential in the regulation of mast cell activation, and that its blockade may benefit patients with allergic inflammation.

Keywords: protein disulfide isomerase, mast cells, food allergy, PDI, propynoic acid carbamoyl methyl amide

INTRODUCTION

IgE-mediated mast cell activation is a critical component in the induction of allergic responses to food-derived antigens (1–3). The cross-linking of food-specific antigens by IgE-bearing mast cells in the intestinal tract induces a stepwise cascade of activation-induced events, resulting first in the release of various pre-formed mediators from mast cell granules, followed by the synthesis of several *de novo* substances including various cytokines and lipid mediators. These events are tightly orchestrated involving several phosphorylative reactions that culminate in the activation of transcription factors which regulate gene expression.

We have previously shown that food-derived components such as curcumin can attenuate the development of mast cell responses during food allergy (4, 5). Curcumin, a natural product found in the spice turmeric has well-known pharmacological properties, including anti-allergic (4, 6, 7), anti-inflammatory (8), and anti-cancer activities (9, 10). Despite the interest in curcumin and its analogues as potential therapeutics (9), there is no consensus on the molecular mechanisms by which it exerts pharmacological action. While some studies have demonstrated curcumin acts upon various transcription factors to regulate the expression of enzymes and cytokines (4, 11), other studies suggest that curcumin exerts these effects by modulating the redox status of the target cell (12).

We hypothesized that dietary components such as curcumin may modulate the mast cell response during food allergy by inhibiting the direct activation of circulating proteins and enzymes and explored likely targets. One common mechanism of protein activation is through an allosteric disulfide bond, where a disulfide will rearrange to alter the intra- or intermolecular structure of the protein to activate or inactivate it (13). The rearrangement of these disulfide bonds is often accomplished through a thiol reductase enzyme such as protein disulfide isomerase (PDI).

Thiol isomerases such as PDI catalyze the breakage, formation and rearrangement of disulfide bonds, regulating protein folding within the endoplasmic reticulum (ER) (14, 15). However more recent studies have determined extracellular roles for PDI including the activation of thrombus formation (16), entry of HIV into lymphocytes (17), and the survival and progression of various cancers (18).

In this study, we verify the PDI inhibitory activity of curcumin and explore the role that PDI plays in the development of mast cell-mediated responses during food allergy using known PDI inhibitors. To date, with the exception of a postulation that PDI catalyzes the formation of IgG4 under conditions of chronic antigen exposure (19), there has been no known role of the enzyme in the development of mast cell-dependent allergic responses. Here, we show for the first time that mast cells express extracellular PDI on their surface and that blockade of PDI in mast cells suppresses their function and attenuates the development of mast cell responses during food allergy. Pretreatment of bone marrow-derived mast cells (BMMCs) with PDI inhibitors including dietary PDI modulators suppressed their activation and degranulation, resulting in the decreased expression and secretion of various mast cell-derived cytokines. Furthermore, treatment of wild-type mice with PACMA-31 (an orally active irreversible PDI inhibitor) in a model of ovalbumin (OVA)-induced food allergy resulted in a significant attenuation in the development of food allergy symptoms including decreases in allergic diarrhea, mast cell activation and allergen-specific IgE. These data demonstrate that PDI plays vital roles during mast cell-mediated responses by regulating mast cell activation and cytokine production. Furthermore, dietary components can modulate mast cell activation during allergic responses by regulating PDI activity, suggesting that blocking PDI function may prove to be of therapeutic benefit in allergic patients.

Abbreviations: OVA, ovalbumin; WT, wild-type; BMMCs, bone marrow-derived mast cells; PACMA-31, propynoic acid carbamoyl methyl amide; Rutin, quercetin-3-rutinoside.

MATERIALS AND METHODS

Animals

BALB/c mice were purchased from Taconic Farms and Envigo. All mice were bred in our facility and all animal research was approved by the IACUC of Western New England University.

Insulin Turbidity Assay

PDI activity was measured *via* PDI-catalyzed reduction of insulin in the insulin turbidity assay as previously described by us and others (20–22). Briefly, the reaction mixture consisted of 100 mM potassium phosphate (pH 7.4), 0.75 mM DTT, 2 mM EDTA, 35 μg/ul of bovine insulin, and 0.8 μg purified human PDI in a total volume of 30 μl in a 384-well plate. The progress of the reaction was monitored for 90 min at 37°C. Curcumin, PACMA-31 or control buffer was added prior to the addition of enzyme at the concentrations indicated. PDI activity in the presence of compound was determined by the following formula: PDI activity (%) = $(OD[_{compound + PDI + DTT}] - OD[_{DTT}])/(OD[_{PDI + DTT}] - OD[_{DTT}]) \times 100\%$. Enzyme inhibition was determined by the following formula: enzyme inhibition = $(1 - [OD_{max\{compound + enzyme\}}/OD_{max\{buffer\ control + enzyme\}}])$.

BMMC Culture

BMMCs were generated from naïve BALB/c mice and cultured with 10 ng/ml of rIL-3 (Shenandoah) and rSCF (Shenandoah)

for >4 weeks as we have previously described (23). Harvested BMMC were positive for c-Kit and FcεRI.

BMMC Activation and Pre-Treatment With PDI Inhibitors

1×10^6 BMMCs/ml were cultured in triplicates with 10 ng/ml IL-3 and SCF. Cells were activated by pre-sensitizing with 1 μg/ml DNP-IgE (clone SPE7, Sigma) or vehicle (medium), followed by treatment with 200 ng/ml DNP-BSA (5, 24). Increasing concentrations of curcumin (Sigma), bacitracin (Sigma), quercetin-3-rutinoside hydrate or rutin (Sigma), and propynoic acid carbamoyl methyl amide (PACMA)-31 (Sigma) or vehicle (DMSO) were added to various experimental groups for different time periods (including 30 min and 24 h) prior to challenge with DNP-BSA. Expression of cytokine genes was assessed by RT-PCR between 30 min to 1 h following activation. Assessment of secreted cytokines was performed 6 to 24 h later.

Measurement of ER Stress

ER stress was induced by adding brefeldin A (1 μg/ml) to unactivated BMMCs or cells activated with IgE and antigen as described above. The effects of PDI inhibition were examined by treating with PDI inhibitors such as curcumin or PACMA-31. Six hours after activation with IgE/Ag, cells were stained for surface antigens such as c-Kit or FcεRI with fluorescently-labeled mAbs. Intracellular cytokine staining was then performed using a kit from Biolegend (San Diego, CA). Cells were fixed, then permeabilized and stained for various cytokines using mAbs. Cytokine-producing cells were enumerated by flow cytometry.

Quantitative PCR Analysis and ELISAs

Quantitative RT-PCR was performed as previously described using Taqman probes (5, 24). Expression of cytokine genes (IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, IL-33, TNF-α, IFN-γ) and PDI (P4HB, PDIA3) was calculated relative to GAPDH transcripts. ELISAs for mMCP-1 (Affymetrix), IL-4, IL-5, IL-6, TNF-α, and IFN-γ (Biolegend), IL-13 (R&D Systems), and OVA-IgE were performed according to manufacturers' protocols as previously described (5, 24).

β-Hexosaminidase (β-Hex) Assay

BMMCs were activated with DNP-IgE and DNP-BSA in the presence or absence of various PDI inhibitors. β-hex activity in cell culture supernatants was assessed as previously described by us (5, 24). Percent cellular content was calculated according to the following formula: (amount released into supernatant)/(amount in supernatant + amount in lysate) × 100.

BMMC Proliferation

BMMCs were cultured with rIL-3 and rSCF as described above. Some groups of cells were treated with various concentrations of bacitracin, rutin or PACMA-31. Cells were counted daily for 4 to 5 days, and live cells were enumerated on the basis of trypan blue exclusion.

Flow Cytometry

BMMCs and MC/9 cells (a murine mast cell line) were incubated with mAbs against mouse c-Kit (Biolegend), FcεRI (Biolegend), PDI (Life Technologies), and isotype controls for PDI (IgG2a/Life Technologies). Expression of cell surface PDI was assessed at various times in cells after activation with IgE and antigen and in unactivated controls. Intracellular PDI expression was assessed 30 min later in fixed and permeabilized cells. Flow cytometric analysis was performed using an Accuri C6 flow cytometer and Flow jo software.

Western Blot

BMMCs and MC/9 cells were activated with DNP-IgE and DNP-BSA and total protein extracts were harvested after 4 h. Western blot was performed as previously described (4). PDI detection was performed using a rabbit anti-PDI mAb (1:1000; Cell Signaling).

Food Allergy Regimen

To induce food allergy, BALB/c mice were *i.p.* immunized with 50 μg chicken egg OVA in 1 mg alum twice as previously described (5, 24). Mice were challenged *i.g.* with 50 mg OVA on 6 alternating days. Control animals were *i.p.* sensitized but not challenged with OVA. One hour after the sixth challenge, mice were sacrificed and assessed as previously described (5, 24, 25). To assess the effects of PACMA-31 exposure in allergic mice, some groups of mice (both controls and OVA-challenged animals) were gavaged with 10 mg/kg (300 μg) of PACMA-31 suspended in 250 μl 1% carboxy methyl cellulose (CMC) as previously described (4). Treatment with PACMA-31 was initiated one day prior to challenge with OVA and continued daily until sacrifice. Mice were sacrificed 1 h after the sixth challenge with OVA and food allergy parameters were assessed as previously described (4, 5, 24). The development of intestinal anaphylaxis was assessed as described below. Blood was collected for evaluation of antibodies and mMCP-1 in serum. Jejunum was collected for histological assessment of mast cells and evaluation of cytokine gene expression by RT-PCR as described above. Spleens were collected for evaluation of systemic cytokine production by T cells.

Measurement of Intestinal Anaphylaxis

Intestinal anaphylaxis was assessed in challenged mice by scoring the percentage of animals exhibiting allergic diarrhea for one h after OVA challenge (4, 25).

Histological Analysis and Enumeration of Mast Cells

Intestinal mast cells were enumerated as previously described by us (26). Tissue sections were stained with chloroacetate esterase (CAE) and mast cells were counted in complete cross-sections of jejunum.

Spleen Stimulation

Spleen cells were cultured with medium, 200 μg/ml OVA or anti-CD3 and anti-CD28 and cytokines were enumerated in supernatants as previously described (5, 24).

Statistical Analysis

Data are expressed as mean ± SEM, unless stated otherwise. Statistical significance comparing different sets of mice (between

2 groups) was determined by the unpaired Student's t-test, whenever applicable. In experiments comparing multiple experimental groups or time points, one or two-way analysis of variance was performed followed by the Dunnett test for multiple comparisons.

RESULTS

Curcumin Inhibits Protein Disulfide Isomerase Activity and Suppresses IgE-Mediated Mast Cell Activation

We have previously demonstrated that food-derived substances such as curcumin can modulate mast cell responses during food allergy by suppressing their activation and pro-allergic effects (4). The proper folding and assembly of proteins catalyzed by PDI as well as their dimerization is a critical component of cellular function, suggesting that the modulation of mast cell responses during allergic inflammation may depend on cell-specific regulation of PDI activity. Furthermore, a number of recent studies suggest novel roles for dietary PDI inhibitors in regulating cellular activation and altering disease progression (27, 28). This includes polyphenolic flavonoids such as the quercetin-3-glycosides, which have been shown to inhibit PDI both *in vitro* and in mice (20). We therefore wondered whether the effects of curcumin on mast cells may also be mediated *via* inhibition of PDI catalytic activity. Using a modified version of the insulin turbidimetric assay that measures PDI catalytic activity (20–22), we therefore explored the potential of curcumin to also inhibit PDI activity (**Figure 1A**). Curcumin inhibited PDI activity in a dose-dependent manner, suggesting that its ability to inhibit mast cell activation *in vivo* may be dependent on regulation of PDI activity (**Figure 1A**). PDI catalytic activity is highly induced during conditions of ER stress and upregulation of the unfolded protein response (UPR). To therefore further assess whether the inhibitory effects of curcumin on mast cells maybe mediated *via* PDI inhibition, we examined its role in IgE-activated mast cells under conditions of ER stress. One way of inducing ER stress in cells is to block protein transport using the chemical, brefeldin A (29). Brefeldin A inhibits transport of proteins from the ER to the Golgi and induces retrograde protein transport from the Golgi apparatus to the ER. This results in the accumulation of unfolded proteins in the ER. Examination of TNF-α cytokine production by BMMCs after IgE-induced activation using intracellular cytokine staining revealed a significant decrease in the generation of TNF-α-producing cells in the presence of curcumin (**Figure 1B**). Furthermore, pretreatment of BMMCs with curcumin prior to activation with IgE and antigen resulted in a significant decrease in the production of the cytokines IL-4, IL-6, IL-13, and TNF-α (**Figures 1C–F**). Collectively, these data demonstrate that curcumin inhibits PDI activity and its effects on mast cells may be mediated *via* PDI inhibition, warranting further assessment of the role of PDI during mast cell-mediated responses.

Bacitracin Pre-Treatment Modulates Cytokine Gene Expression and Secretion in IgE-Activated Mast Cells

The ubiquitous expression of PDI and its importance in protein folding presents a major challenge to examination of its activity using gene knockdown strategies. Knockdown of PDI is lethal in yeast and mammalian cell lines (30) and to date, no viable strains of PDI knockout mice exist. As such, the functions of PDI both *in vitro* and *in vivo* have often been studied using various small molecule inhibitors of PDI as well as PDI-blocking antibodies. To further investigate the role of PDI during mast cell activation and function, we therefore assessed the effects of pre-treatment with various PDI inhibitors on mast cell cytokine production after activation with IgE and antigen. We explored the effects of three well-established PDI inhibitors to verify they all had the same effect and corroborate that the process was PDI-dependent. The following stepwise approach was utilized: assessment of mast cell function using classic PDI inhibitors such as bacitracin, examination of the effects of clinically validated PDI inhibitors such as rutin on mast cells, and confirmation of PDI activity in mast cells using selective PDI inhibitors such as PACMA-31.

The effects of PDI in cell studies have historically been studied using the topical peptide antibiotic, bacitracin (27, 31). Bacitracin is a well-studied, non-selective inhibitor of PDI and blocks PDI function in the high micromolar range (IC_{50} of 70μM in insulin reductase assays) inhibiting disulfide bond formation (32). Bacitracin is also non cell-permeable (27). Therefore, its effects on PDI catalytic activity maybe mediated *via* inhibition of cell surface PDI. To examine the effects of bacitracin pre-treatment on IgE-mediated activation in mast cells, BMMCs were pre-treated with increasing concentrations of the drug overnight prior to activation *via* IgE and antigen. The dose-dependent effects of bacitracin pre-treatment on cytokine were then assessed. As expected, IgE-mediated activation of BMMCs resulted in the enhanced secretion of IL-4, IL-6, IL-13, and TNF-α into cell culture supernatants (**Figures 2A–D**). In contrast, pre-treatment with various doses of bacitracin, resulted in a dose-dependent inhibition of cytokine secretion (**Figures 2A–D**), suggesting that bacitracin suppresses mast cell-mediated cytokine production by inhibiting the activity of PDI during IgE-induced activation. Similar effects were also observed on the induction of cytokine gene transcription (**Supplementary Figures 1A–C**). These data therefore suggest that inhibition of PDI activity can suppress the production of cytokines by IgE-activated mast cells. Furthermore, they also strongly implicate a role for extracellular PDI activity, as bacitracin is a non-cell membrane permeable PDI inhibitor.

Rutin Pre-Treatment Modulates Cytokine Secretion in IgE-Activated Mast Cells

While bacitracin is widely used in research as a PDI antagonist, its clinical use *in vivo* is hampered by its low membrane permeability and adverse side effects such as nephrotoxicity (27). To therefore further confirm that PDI plays an important role in mast cell activation, we utilized a second well-established

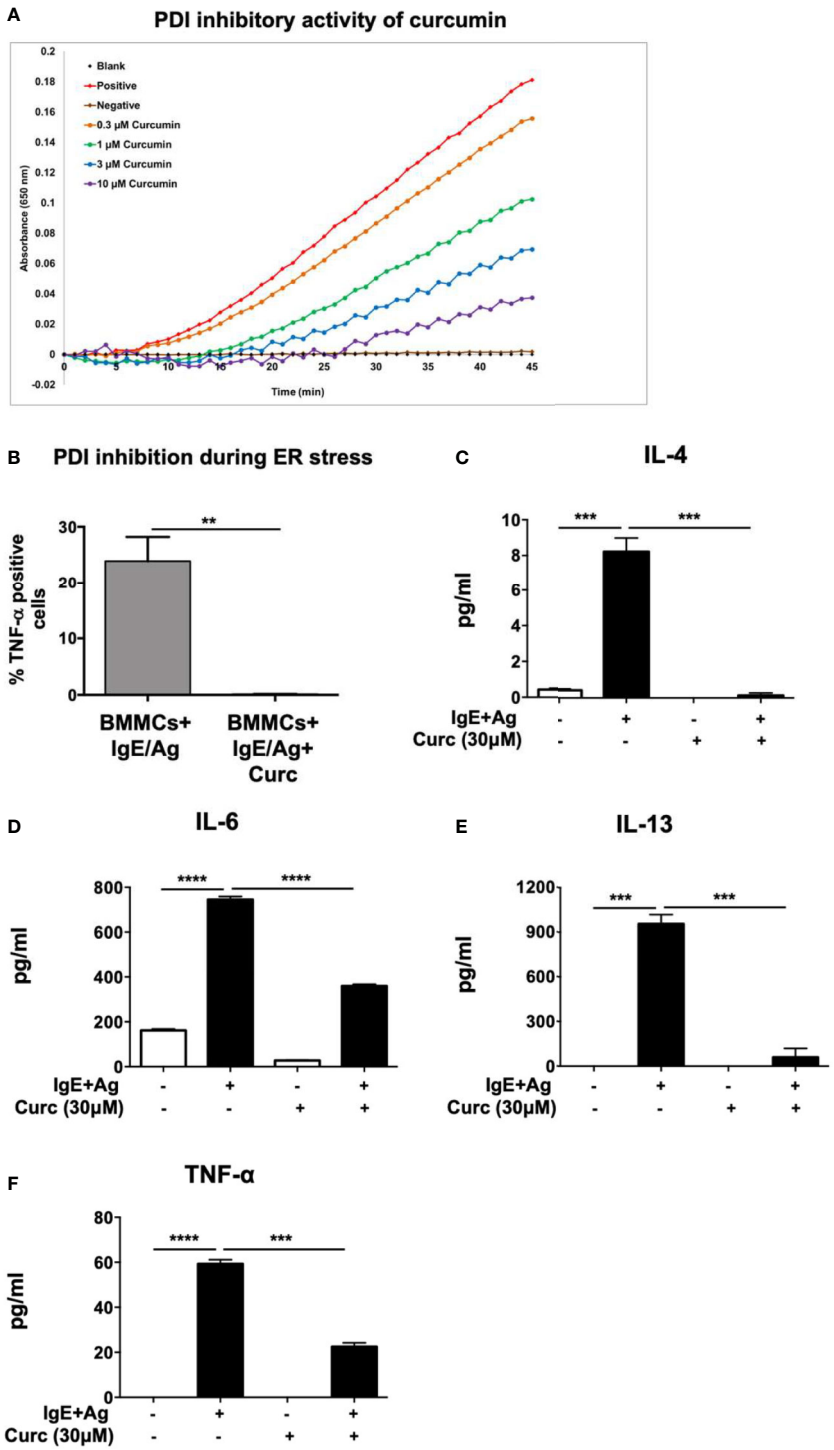


FIGURE 1 | Curcumin inhibits PDI catalytic activity and suppresses IgE-mediated mast cell-derived cytokine production. **(A)** PDI activity was measured using the insulin-based turbidimetric assay in the absence (*red*) or presence of 0.3 μM (orange), 1 μM (green), 3 μM (blue), or 10 μM (purple) curcumin. The brown line represents baseline with DTT in the absence of PDI. **(B)** BMMCs were pre-treated with 30μM curcumin overnight and activated with DNP-IgE (1μg/ml) and DNP-BSA (200 ng/ml) in the presence or absence of Brefeldin A (1 μg/ml). Six h later, TNF-α-positive cells were enumerated using intracellular staining. **(C–F)** BMMCs were pre-treated with 30 μM curcumin and activated *via* DNP-IgE and antigen. Supernatants were collected 12 h later and were evaluated for the presence of IL-4, IL-6, IL-13, and TNF-α by ELISA. Data are representative of three or more independent experiments. ** $p < 0.0051$; ***$p < 0.0005$; ****$p < 0.0001$ (students t-test).

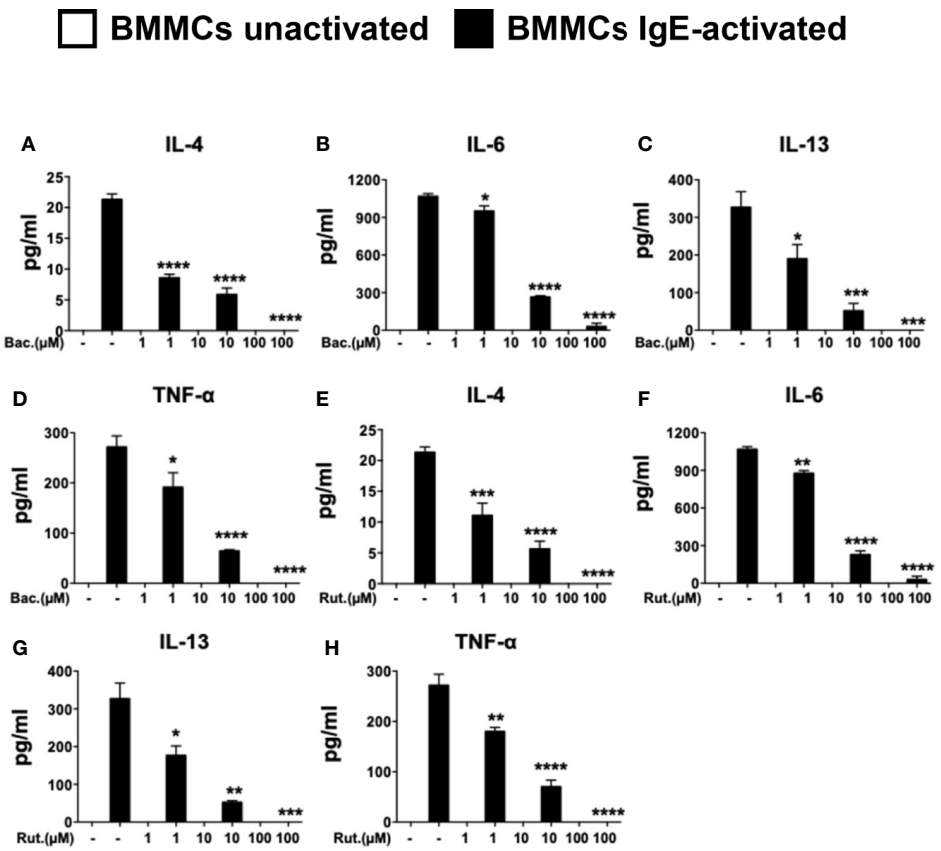


FIGURE 2 | Pre-treatment with PDI inhibitors suppresses the secretion of cytokines in IgE-activated BMMCs. BMMCs were treated with increasing doses of **(A–D)** bacitracin (Bac) or **(E–H)** rutin (Rut) overnight and activated *via* IgE and antigen stimulation as in **Figure 1**. Supernatants were collected 12 h after activation and ELISAs were performed for IL-4, IL-6, IL-13, and TNF-α. Data are representative of two or more independent experiments. Statistical signifance between multiple groups was performed using one-way ANOVA with $p < 0.0001$. Significance for means of groups treated with Bac or Rut compared to mean of untreated cells is shown above bars representing the groups respectively. *$p < 0.03$; **$p < 0.002$; ***$p < 0.0002$; ****$p < 0.0001$ (Dunnet's post-hoc test).

inhibitor of PDI, rutin (IC_{50} 6 μM in insulin reductase assays (27)), in human studies (28, 33, 34). The polyphenolic flavonoid, quercitin-3-rutinoside (rutin) was recently shown to be a potent small molecule inhibitor of PDI (20). Quercetin and its derivatives such as rutin are ubiquitously present in many fruits and vegetables (27), suggesting that they may have the potential to modulate mast cell function in a manner similar to that observed with curcumin (4). To therefore further investigate the importance of PDI during mast cell activation and confirm the effects observed above with bacitracin, we also cultured BMMCs with increasing concentrations of rutin, and assessed the production of mast cell-derived cytokines. As observed above, pre-treatment with increasing concentrations of rutin, also suppressed cytokine gene expression (**Supplementary Figures 1D–F**) and the production of mast cell-derived cytokines in a dose-dependent manner (**Figures 2E–H**), suggesting that mast cell activity during immune responses *in vivo* may be modulated by the fine-tuning of cell-specific PDI activity.

Pre-Treatment With Bacitracin or Rutin Suppresses Release of Pre-Formed Mediators and Influences Long-Term Cell Survival

Our observations above demonstrating a dose-dependent suppression of cytokine expression and secretion by PDI inhibitors are consistent with well-described roles of PDIs as molecular chaperones during the intracellular generation of various proteins. To further determine whether PDI is also involved during mast cell degranulation and the release of pre-formed mediators, we examined cell culture supernatants immediately after activation with DNP-BSA, and assessed the secretion of the enzyme β-hexosaminidase (β-hex) as previously described (24). As observed in **Figure 3A**, IgE-mediated activation induced the secretion of β-hex into cell culture supernatants 15 min later compared with unactivated controls (**Figure 3A**). In contrast, pre-treatment with increasing concentrations of either bacitracin or rutin, resulted in decreased secretion of this enzyme in activated cells (**Figure 3A**). These data suggest that inhibition of PDI can

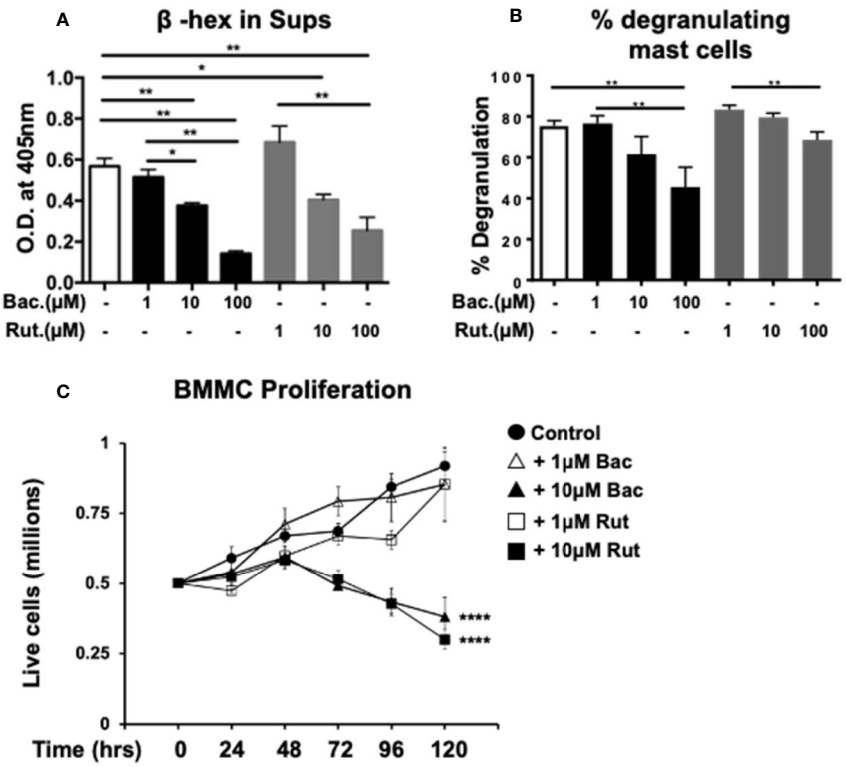


FIGURE 3 | Pre-treatment with PDI inhibitors suppresses mast cell degranulation and cell proliferation. **(A, B)** BMMCs were cultured with varying concentrations of bacitracin or rutin as described above. Mast cell degranulation was assessed by pre-sensitizing BMMCs with DNP-IgE followed by challenge with DNP-BSA. Supernatants and cell lysates were collected and the β-hex assay was performed. **(A)** O.D. values correlating to β-hex release in supernatants and **(B)** percent degranulation (percent cellular content) of BMMCs is shown. **(C)** The effects of treatment with PDI inhibitors on BMMC proliferation and survival were assessed. BMMCs were cultured in triplicates with rIL-3 and rSCF for 5 days in the presence of varying concentrations of bacitracin or rutin and vehicle (DMSO). Cells were counted daily. Numbers of live cells are shown. Data are representative of 2 independent experiments. *$p < 0.05$; **$p < 0.005$ (students t-test). ****$p < 0.0001$ using two-way ANOVA between untreated controls and 10μM-treated groups.

suppress the release of pre-formed mediators from mast cell granules after IgE-mediated activation. Furthermore, the total cellular β-hex content was also decreased in cells treated with PDI inhibitors (data not shown), suggesting that PDI inhibition may also block β-hex production. However, a much smaller effect on the extent of intrinsic mast cell degranulation was observed (**Figure 3B**).

To further investigate the effects of PDI inhibition on mast cells, we also examined the effects of bacitracin and rutin exposure on BMMC proliferation and survival as previously described (24). BMMCs were cultured with IL-3 and SCF, along with various concentrations of bacitracin, rutin, or vehicle. Their proliferation and/or survival was followed for 5 days and live cells were enumerated using trypan blue exclusion. As observed in **Figure 3C** and data not shown, co-culture with bacitracin or rutin for 5 days, resulted in an overall dose-dependent decrease in BMMC proliferation and survival, suggesting that PDI activity may regulate mast cell homeostasis and proliferation possibly by modulating intracellular protein folding during cell growth. This is consistent with the known roles of PDI as an important housekeeping protein necessary for cell survival (30). However, treatment with both PDI inhibitors had no effects on cell viability and proliferation for at least up to 48 h (for the 10μM doses) or longer (for the 1μM doses) after treatment. At these time points, percentages of live cells were comparable between groups and no changes in percentages of dead cells, approximately 10%, were observed. This confirms that the effects of the PDI inhibitors on IgE-mediated mast cell activation described above are not due to cell death but reduction of catalytic activity consistent with reported observations in other (27).

Suppression of Mast Cell Activation and Function by the PDI-Selective Inhibitor, PACMA-31

Lastly, to verify that PDI plays an important role in these processes, we used a PDI selective compound, shown to have potent anti-PDI activity in animal studies. Recently, a class of propynoic acid carbomyl methyl amides (PACMAs) was shown to have broad-spectrum activity against various cancer cell lines (18, 35). Of these, the small molecule compound, PACMA-31, was shown to have irreversible activity against PDI (IC_{50} of 10μM in animal studies (35)), forming a covalent bond with

active site cysteines, and exhibited *in vivo* activity with oral bioavailability in a mouse xenograft model of ovarian cancer (35). Furthermore, PACMA-31 has a greater selectivity for PDI over other thiol isomerases compared to bacitracin. We therefore investigated whether PACMA-31 would also inhibit PDI in our confirmatory assays and assessed its effects on mast cell activation and function in our model. As observed in **Figure 4A**, PACMA-31 is a potent inhibitor of PDI in cell-free enzymatic assays and inhibits the insulin reduction catalytic activity of PDI in a dose-dependent manner. A comparison of the PDI inhibitory activity of PACMA-31 with curcumin is shown in **Supplementary Figure 2**. To further investigate whether PACMA-31 has similar dose-dependent effects on functional studies in mast cells, we treated BMMCs with various concentrations (10μM and 30μM) of PACMA-31. As observed in the case of bacitracin and rutin above, pre-treatment of BMMCs with PACMA-31 overnight (**Figures 4B–E**) resulted in a significant suppression of mast cell activation as evidenced by reduced mast cell cytokine production (**Figures 4B–D**) and decreased overall mast cell survival (**Figure 4E**).

Enhanced Secretion of PDI in Activated Mast Cells

Our data above demonstrates a significant reduction in mast cell functional activity and activation-induced events such as cytokine production when exposed to PDI inhibitors, suggesting that PDI enzymatic activity is induced in mast cells after activation has occurred. This is consistent with the known effects of PDI during cellular activation in various cells. Interestingly, several recent studies have also identified novel functional roles for *extracellular* secreted PDI in various cells (16, 36–40). PDI bound to integrins on the surfaces of these cells has been shown to exert extracellular effects and modulate cellular function. As such, we wondered whether extracellular PDI is also secreted by mast cells during IgE-activation and whether cell-surface PDI may likely be a target of our PDI inhibitors.

To further explore the role of PDI and evaluate its activity during mast cell activation, we assessed the expression of PDI in resting and IgE-activated BMMCs by quantitative PCR. PDI is the gene originally identified as the β-subunit of prolyl-4-hydroxylase (P4HB) that catalyzes the formation of 4-hydroxyproline in collagen (reviewed in (41)). We therefore assessed the expression of the P4HB gene in resting and activated mast cells. As seen in **Figure 5A**, basal expression of the P4HB gene may be observed in resting BMMCs consistent with its role as an intracellular chaperone. However, no further increase in gene expression was observed in IgE-activated BMMCs a few h after activation, suggesting that the intracellular transcriptional levels of PDI remain stable after activation has occurred (**Figure 5A**). This is not surprising as it is the increased enzymatic activity of PDI and not its cellular expression levels that contribute to changes in cellular function.

The secretion and extracellular activity of PDI in endothelial cells and platelets is well-established (16, 40, 42, 43), but its extracellular role in mast cells is unknown. To determine whether PDI can be detected on the surface of mast cells, we assessed the secretion of PDI using flow cytometry. As seen in **Figures 5B, C**, extracellular PDI can be detected on both resting and activated mast cells, with increased expression being observed a few min after activation. Quantification of the median fluorescence intensity of PDI expression revealed a significant increase in the presence of this molecule on the surfaces of cells activated with IgE and antigen (**Figure 5C**). These data therefore suggest that extracellular PDI is present on the cell membranes of resting mast cells, where it has the potential to modulate cellular function *via* its enzymatic activity. The secretion of extracellular PDI is further enhanced soon after IgE activation has occurred. Next, to examine the levels of intracellular PDI protein in mast cells, we evaluated its presence in unactivated and IgE-activated mast cells six h after IgE-activation had occurred. As observed in **Figure 5A** above, intracellular PDI was detected both constitutively and in activated mast cells by flow cytometry (**Figure 5D**). A small but demonstrable increase was observed in activated cells, although the overall levels appeared to be similar (**Figure 5D**), consistent with observations of intracellular PDI levels in other cell types (16, 36, 38–40). This was also confirmed by Western blot analysis (**Figure 5E**). These data therefore demonstrate that PDI is secreted on the surfaces of mast cells, where they may be a likely target of naturally occurring food-derived PDI inhibitors.

PDI Inhibition During Mast Cell Activation Is Sufficient to Suppress IgE-Induced Cytokine Production

The data above suggest that PDI can be secreted by mast cells after activation, with the potential to modulate downstream events such as the transcription of cytokine genes. To further assess the role of PDI inhibition on mast cell activation, we examined the effects of PACMA-31 pre-treatment on the expression of the high affinity receptor for IgE, FcεRI, on resting and activated BMMCs. As observed in **Figure 6A**, resting BMMCs express high levels of unbound FcεRI. In contrast, pre-incubation with PACMA-31 significantly decreased the expression of the receptor in BMMCs. In activated mast cells, the overall intensity of unbound FcεRI expression was decreased as would be expected as a consequence of saturation by IgE molecules. However, this was further reduced in PACMA-31-exposed and IgE-activated BMMCs.

It is possible that in the experiments above, the effects of PACMA-31 were mediated *via* inhibition of constitutively present cell surface PDI. To further investigate the effects of PDI inhibition during mast cell activation and to determine whether activation-associated PDI activity can modulate downstream cellular events, BMMCs were incubated with PACMA-31 starting 30 min prior to activation with DNP-BSA. Six h post-challenge with antigen, the levels of cytokines in supernatants were assessed. As observed in **Figures 6B–D**, the levels of TNF-α, IL-6, and IL-13 were suppressed in the PACMA-31–treated samples compared to untreated controls, suggesting that inhibition of activation-induced PDI activity may be sufficient to alter mast cell responses *in vivo*. Furthermore,

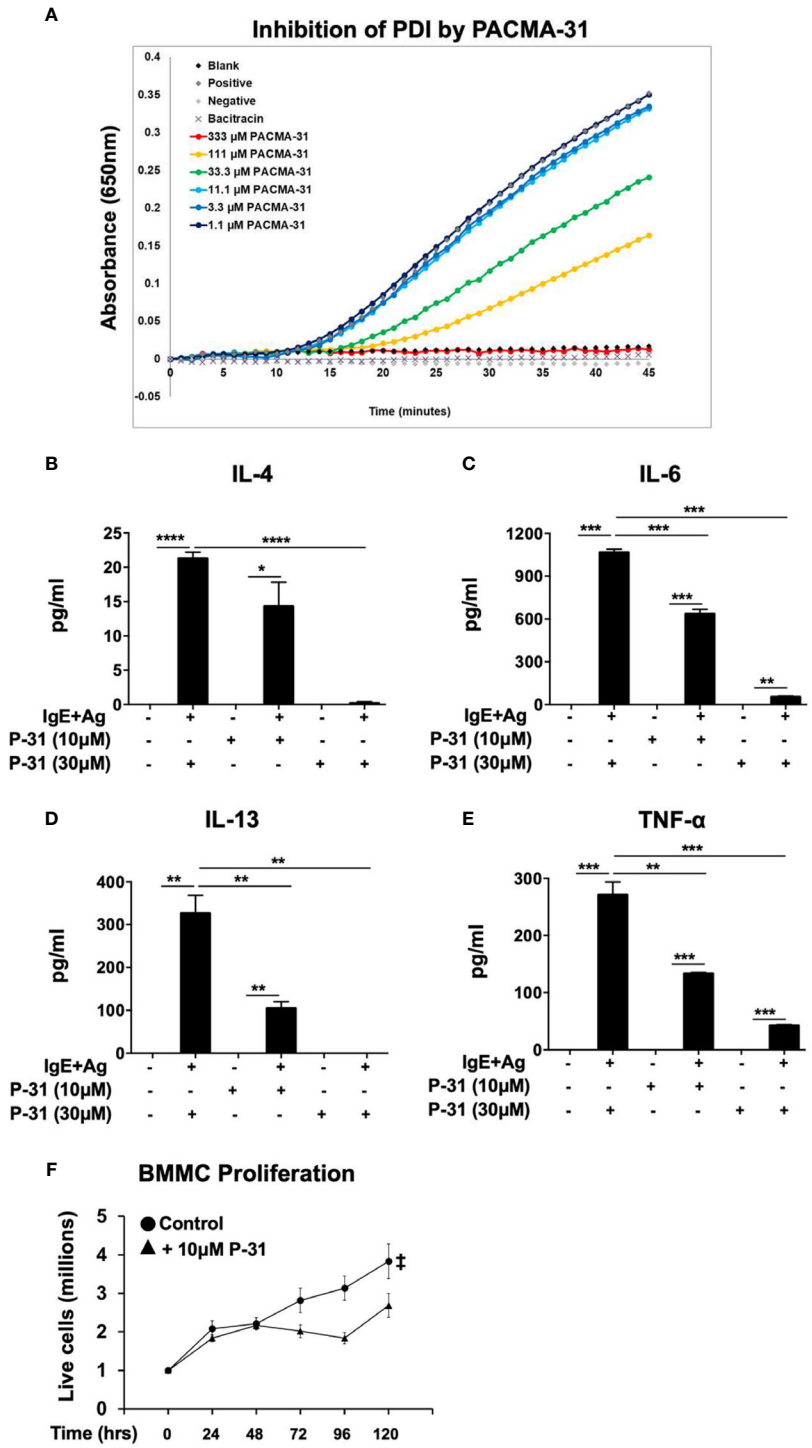


FIGURE 4 | PACMA-31 inhibits PDI and pre-treatment with PACMA-31 suppresses BMMC proliferation and cytokine secretion in IgE-activated mast cells. **(A)** PDI catalytic activity was measured using the insulin-based turbidimetric assay in the absence or presence of various concentrations of PACMA-31. **(B–E)** BMMCs were treated with 10µM or 30µM PACMA-31 (P-31) overnight and activated via IgE and antigen stimulation. Supernatants were collected 12 h after activation and ELISAs were performed for respective cytokines. **(F)** BMMCs were cultured with rIL-3 and rSCF for 5 days and the effects of PACMA-31 treatment on mast cell proliferation were assessed. Numbers of live cells at different time points are shown. Data are representative of 3 or more independent experiments. *$p < 0.05$; **$p < 0.005$; ***$p < 0.001$; ****$p < 0.0001$ (student's t-test). ‡$p < 0.0001$ by two-way ANOVA between untreated cells and P-31–treated groups.

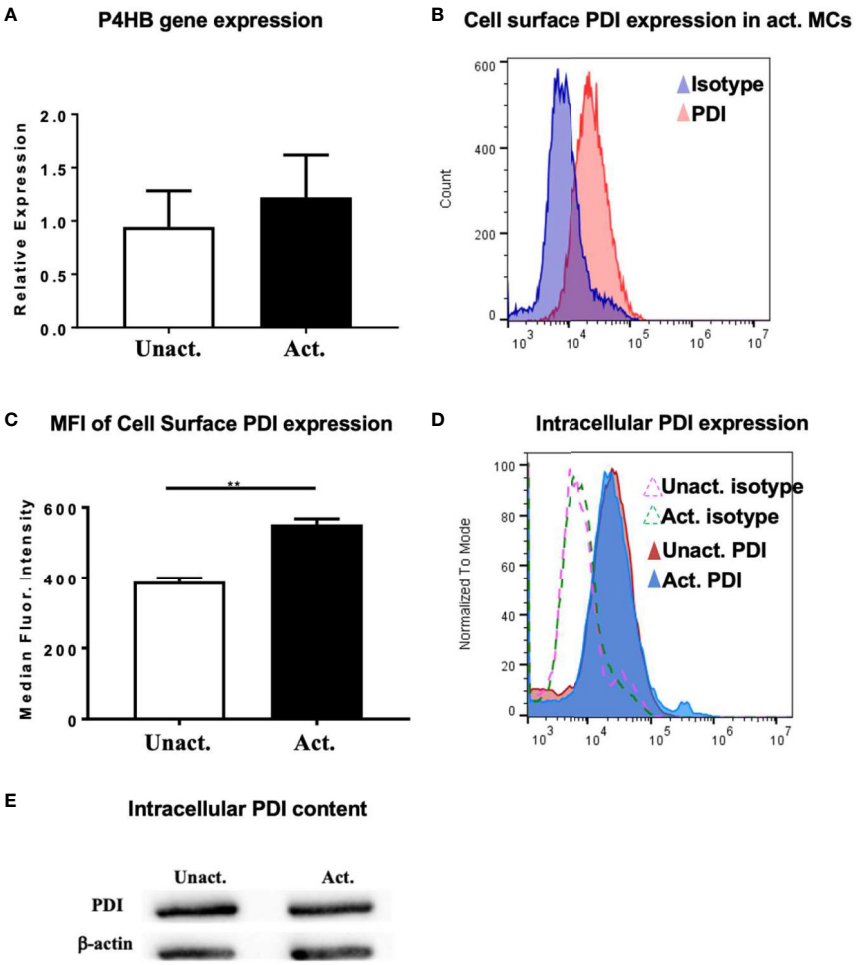


FIGURE 5 | Upregulation of PDI expression in mast cells after activation with IgE and antigen. **(A)** BMMCs were activated with DNP-IgE and antigen and cells were collected for RT-PCR analysis after 4 h. Expression of P4HB is shown. **(B–D)** In other experiments, activated BMMCs or MC/9 cells and controls were examined for the expression of PDI family members by flow cytometry. **(B)** Histogram overlay depicting increase in PDI expression in activated BMMCs compared to isotype control is shown. **(C)** Plot depicting calculated median fluorescence intensity of PDI expression is shown. **(D)** Intracellular expression of PDI in fixed and permeabilized cells is shown. Isotype controls are represented as dashed lines. **(E)** Western blot for PDI in MC/9 cells. Data are representative of 2 independent experiments. **$p < 0.01$ (student's t-test).

inhibition of PDI was sufficient to attenuate the suppression of both pre-formed mediators such as TNF-α as well as *de novo* synthesized cytokines such as IL-13. To further confirm the effects of inhibition of cell surface PDI, BMMCs were treated with an anti-PDI mAb (Abcam), followed by activation *via* IgE and antigen (**Figure 6E**). As expected, extracellular PDI blockade also resulted in suppression of mast cell-derived IL-13 as seen in **Figure 6E**. Lastly, to determine whether PDI blockade by PACMA-31 during ER stress can inhibit the production of mast cell-derived cytokines, BMMCs were cultured with brefeldin A just before activating with DNP-BSA as described in **Figure 1**. Pre-treatment of cells with PACMA-31 significantly attenuated the capacity of BMMCs to produce IL-13 and TNF-α as shown in **Figures 6F to H**. Furthermore, the median fluorescence intensity of both cytokines was also decreased in PACMA-31-treated cells (**Figures 6G–I**). These data therefore strongly suggest that IgE-mediated mast cell activation can be regulated by the catalytic activity of mast cell surface PDI.

PDI Inhibition Suppresses Mast Cell Responses in a Mouse Model of Food Allergy

Our data above demonstrate a significant role for PDI during mast cell activation and function in cell culture. To ascertain whether blocking PDI activity *in vivo* will have similar effects on mast cell responses, we assessed the effects of PACMA-31 treatment in a mast cell-mediated model of intestinal food anaphylaxis. Briefly, mice were sensitized and challenged with the egg allergen OVA as previously described (24) (**Figure 7A**). Beginning one day prior to challenge with OVA, some groups of mice were orally gavaged with PACMA-31 suspended in 1% carboxy methyl cellulose as previously described (4) (**Figure 7A**).

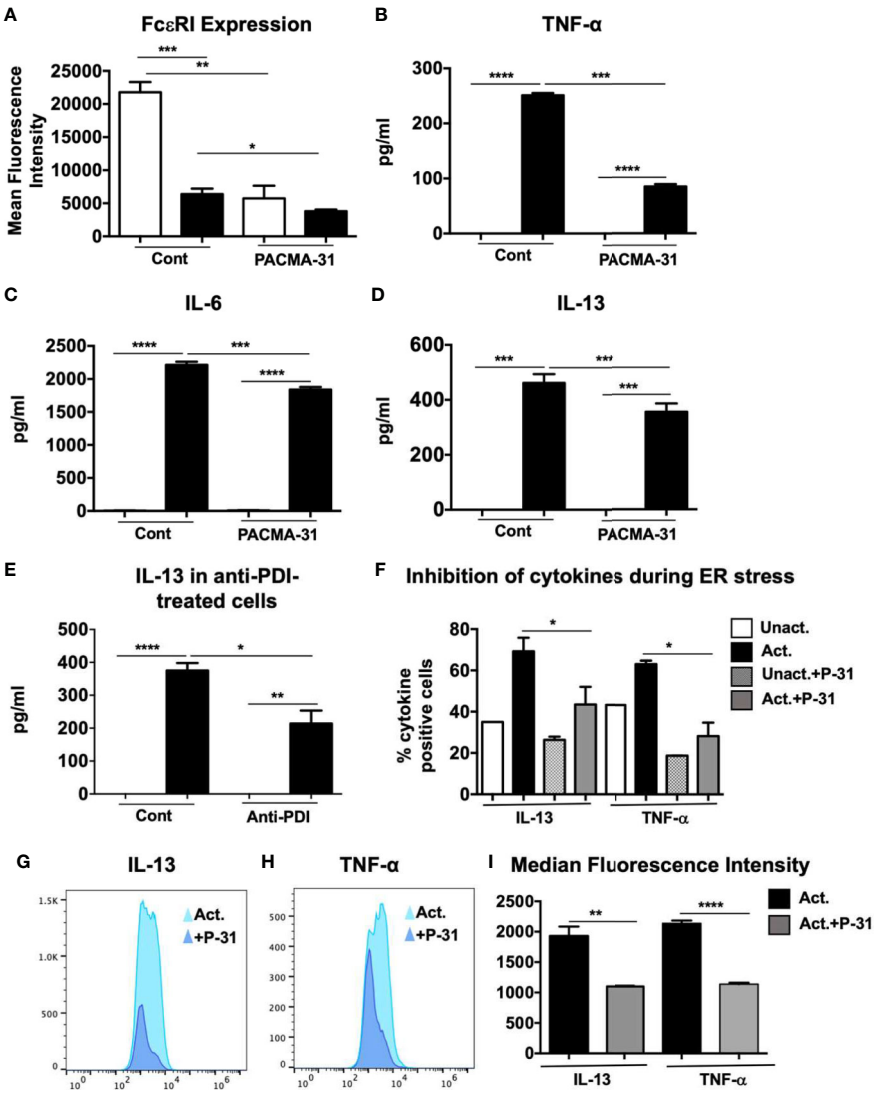


FIGURE 6 | Evaluation of FcεRI expression, cell surface PDI inhibition and ER stress in PACMA-31–treated and IgE-activated BMMCs. **(A)** BMMCs were cultured with PACMA-31 overnight and the expression of FcεRI was assessed in unactivated and activated cells. Mean fluorescence intensity is shown. **(B–D)** BMMCs were treated with 30 μM PACMA-31 for 30 min and activated *via* IgE and antigen stimulation. Supernatants were collected 6 h after activation and ELISAs were performed for respective cytokines. **(E)** BMMCs were pre-treated with anti-PDI mAb for 6 h and activated *via* IgE and Ag. IL-13 secretion into cell supernatants was assessed by ELISA. **(F–I)** BMMCs were pre-treated with PACMA-31 overnight and activated *via* IgE and antigen. ER stress was induced by adding Brefeldin A during activation. Intracellular staining was performed 6 h later. **(F)** Numbers of cytokine producing cells **(G–H)** representative histogram overlays and **(I)** Median Fluorescence intensity for cytokines is shown. Data are representative of 2 independent experiments. *p < 0.05; **p < 0.005; ***p < 0.001; ****p < 0.0001 (student's t-test).

As observed in **Figure 7B**, OVA-sensitized and challenged mice developed profuse diarrhea in comparison with OVA-sensitized controls. This correlated with an increase in OVA-specific IgE production as enumerated in the serum (**Figure 7C**). In contrast, PACMA-31–gavaged animals did not develop diarrhea and exhibited decreased serum OVA-IgE levels (**Figures 7B, C**).

Furthermore, enumeration of mast cells in the small intestine revealed a significant upregulation of chloroacetate esterase-positive mast cells in the jejunum of OVA-sensitized and challenged mice (**Figure 7D** and **Supplementary Figure 3**). Far fewer mast cells were observed in the jejunae of PACMA-31–treated animals (**Figure 7D** and **Supplementary Figure 3**). Similarly, the production of murine mast cell protease-1 (mMCP-1) was enhanced in OVA-challenged allergic mice compared to unchallenged controls (**Figure 7E**). In contrast, serum levels of this enzyme were decreased in PACMA-31–treated mice, suggesting decreased mast cell activation in these animals (**Figure 7E**). Furthermore, the expression of various T_H2-type cytokines including IL-4, IL-5, IL-13, IL-9, and IL-10 was significantly reduced in the OVA-challenged PACMA-31-

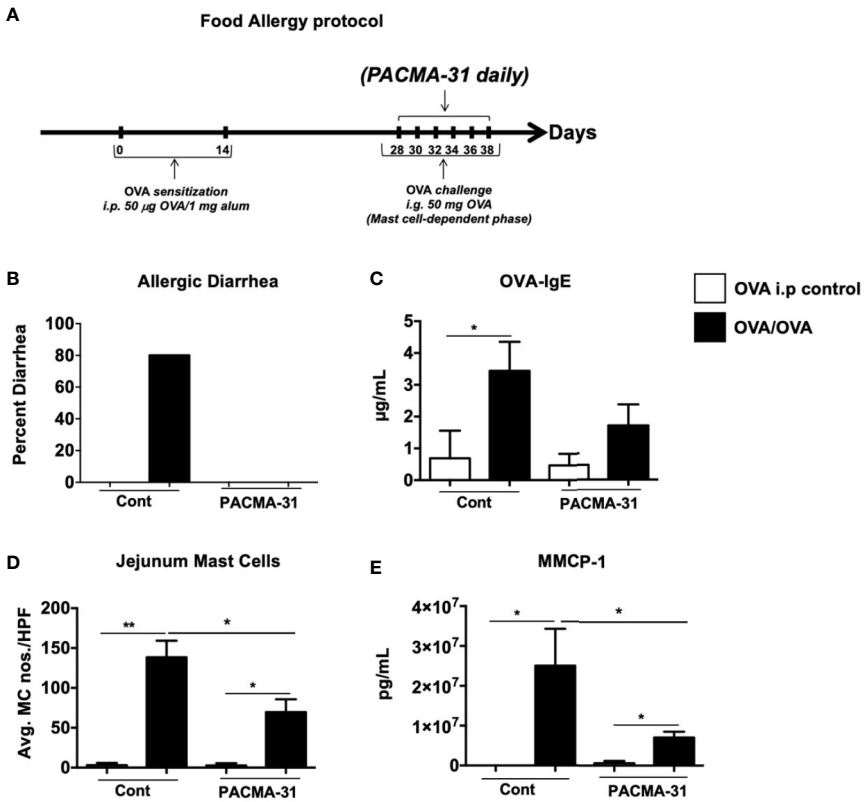


FIGURE 7 | PACMA-31 treatment suppresses the development of food allergy in OVA-sensitized and challenged mice. **(A)** BALB/c mice were sensitized and challenged with OVA as shown. Some groups of animals were also gavaged with 300 µg PACMA-31 suspended in 1% CMC. **(B)** Percent of diarrhea-positive animals. **(C)** Serum OVA-IgE levels **(D)** Numbers of CAE$^+$ jejunal mast cells. **(E)** Serum mMCP-1 levels are shown. n=7 mice/group. Data are representative of 2 independent experiments. *p < 0.05; **p < 0.01 (student's t-test).

treated group compared to the OVA-challenged controls (**Figures 8A–E**).

The data above suggest that PDI activity *in vivo* may be enhanced during allergic responses and its inhibition can modulate mast cell function during food allergy. To determine whether this correlated with increased expression of PDI family members in the intestines of allergic mice, we assessed the levels of P4HB in the jejunae of experimental animals. As expected, expression of these genes was observed in the intestines of both controls and allergic animals and unchanged, consistent with enzymatic inhibition as opposed to decreased expression (**Figure 8F**).

Lastly, the effects of PDI inhibition on systemic T_H2 cytokine production were assessed by stimulating spleen cells with OVA and examining the production of cytokines. As expected, the production of IL-4, IL-5, and IL-13 (**Figures 9A–C**) was enhanced in OVA-stimulated splenic cultures compared to unstimulated controls. In contrast, no enhancement of cytokine production was seen in similarly stimulated cells from PACMA-31–gavaged animals. Also, no differences in IFN-γ production were observed across all groups examined (**Figure 9D**). A similar pattern was observed in cells polyclonally stimulated with T cell agonists (**Supplementary Figure 4**). These data therefore suggest that PDI blockade can suppress T_H2-specific cytokine production during allergic inflammation.

DISCUSSION

In this study, we report for the first time an important role for PDI in the modulation of mast cell homeostasis and mast cell-mediated allergic responses. Collectively, our data demonstrate that mast cells express PDI both constitutively and during IgE-mediated activation. The presence of PDI was also detected in the intestines of enterally-challenged allergic mice. Blockade of PDI activity at both the cellular and physiological levels resulted in a profound suppression of mast cell-mediated responses including decreased mast cell proliferative capacity, reduced secretion of mast cell cytokines, and protection from the development of food allergy.

PDI, a multifunctional ER thiol isomerase, is a 55-kDa protein that is the prototype of the PDI family of proteins. The PDI family comprises of 21 members (44). PDI family members play a critical role in the regulation of protein folding and assembly, both during physiological homeostasis as well as in conditions of cellular stress (15). Increased generation of

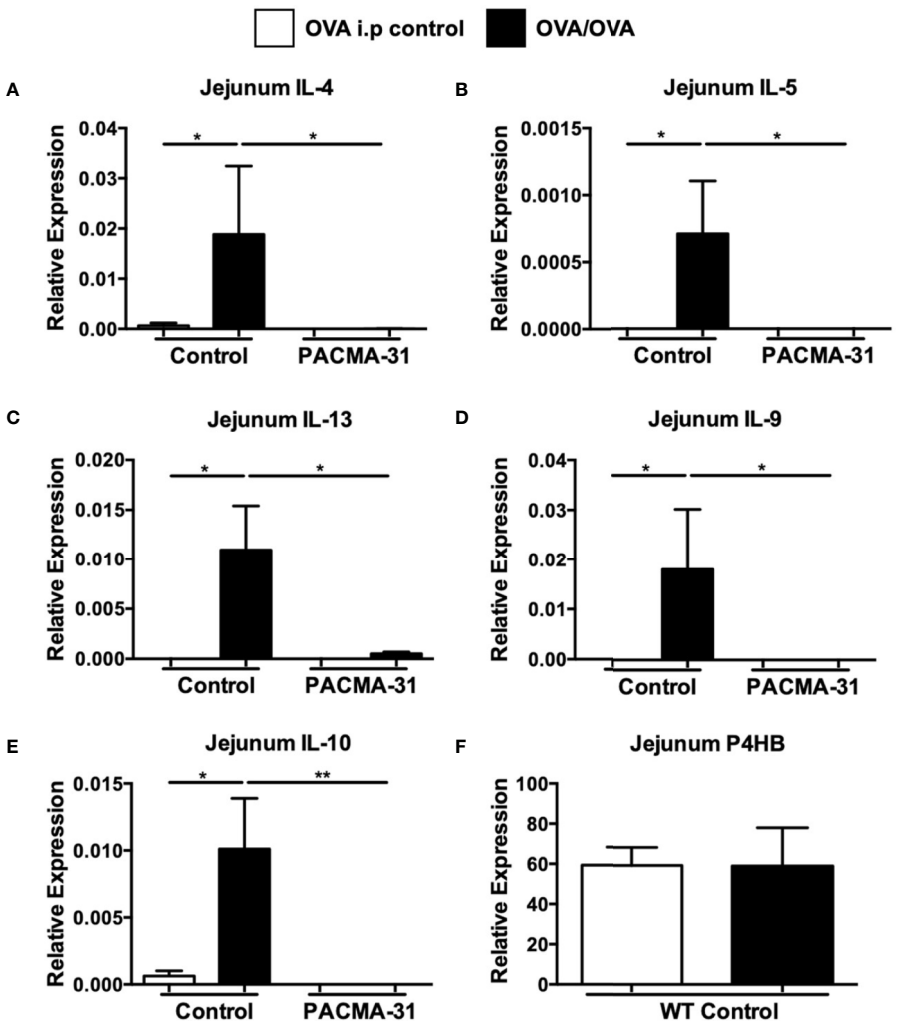


FIGURE 8 | Expression of intestinal PDI in allergic mice and suppression of jejunal Th2 cytokine expression by PACMA-31. BALB/c mice were sensitized and challenged with OVA. Some groups of animals were also gavaged with 300 μg PACMA-31 suspended in 1% CMC. The expression of mRNA for various cytokines **(A–E)**, and **(F)** P4HB was assessed using established Taqman probes and RT-PCR. n=7 mice/group. Data are representative of 2 independent experiments. *$p < 0.05$; **$p < 0.01$ (student's t-test).

unfolded or misfolded proteins during ER stress activates the UPR, resulting in reduced protein synthesis, an increase in the ER curvature, and the activation of PDI and other chaperones (45, 46).

The development of inflammation is thought to activate the UPR, resulting in the enhancement of protein synthesis and folding, and consequently increased PDI activity (47). Complex allergens in particular, such as food-derived proteins, have the potential to induce ER stress and upregulate the function of PDI family members (48). Despite this, the effects of PDI on immune cells has been poorly studied, and very little is known regarding its functions during allergic inflammation. Interestingly, a recent study demonstrated a critical role for a PDI family member, ERp57 in airway allergic responses (49). The expression of ERp57 was increased in lung epithelial cells in both allergen-challenged patients and in mice. Deletion of ERp57 in house dust-mite-challenged mice resulted in decreased airway inflammation and hyperreactivity, accompanied by decreased disulfide bridges in eotaxin, epidermal growth factor, and periostin in the lungs of allergic animals. These data suggest that modulation of PDI activity *in vivo* can influence the outcome of allergic sensitization and challenge.

We hypothesized that food-derived substances may have the potential to modulate mast cell responses during food allergy by inhibiting the activity of PDI. We have previously shown that widely consumed dietary components such as curcumin can suppress mast cell activation and inhibit the development of food allergy in mice (4). The suppression of mast cells by curcumin in this model was dependent on the inhibition of NF-κB activation (4). Similarly, Lee et al. also described anti-allergic effects of curcumin on mast cells (7). They found that the inhibitory effects of curcumin on cultured mast cells were mediated *via* inhibition

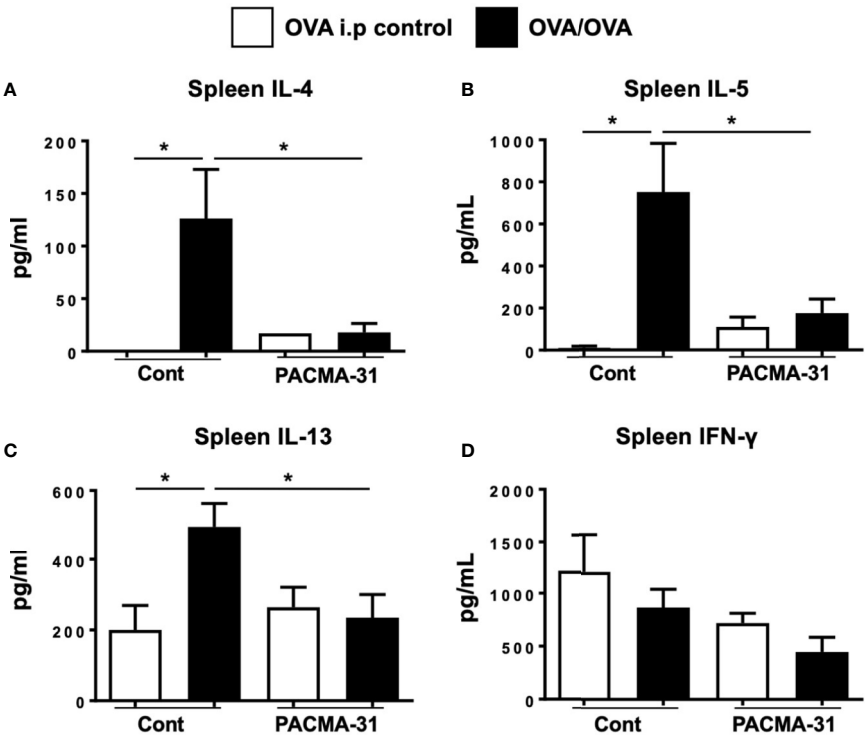


FIGURE 9 | Impaired T_H2 cytokine production by spleen cells from OVA-challenged, PACMA-31–treated mice. BALB/c mice were sensitized and challenged with OVA. Some groups of animals were also gavaged with 300 µg PACMA-31 suspended in 1% CMC. Spleen cells were stimulated with OVA for 72 h. Levels of the cytokines **(A)** IL-4, **(B)** IL-5, **(C)** IL-13, and **(D)** IFN-γ were enumerated in the supernatants by ELISA. n=4 mice/group. Data are representative of 2 independent experiments. *p < 0.05 (student's t-test).

of Syk kinase activity. In this study, we report the PDI inhibitory activity of curcumin, suggesting another potential mechanism for its anti-allergic effects. Since PDI is involved in the formation of disulfide bonds which is critical for the folding of many proteins including transcription factors, it is likely that the previously described suppression of proteins by curcumin and other dietary substances occurs *via* inhibition of PDI catalytic activity. In this context, in addition to curcumin, we demonstrate that another well-known dietary PDI modulator, rutin, also inhibits mast cell activation in our model. This is consistent with reported observations where quercetin and its derivatives, have been demonstrated to have potent anti-PDI activity *in vivo* in various clinical trials (27, 28). In this context, our data suggest that anti-inflammatory compounds such as curcumin and the flavonoids, which are ubiquitous in various types of diets including fruits, vegetables, wines and teas, also have the potential to alter PDI activity in human mast cells and suppress the development of allergic inflammation (27, 28, 50). While we did not specifically examine human mast cells or PDI expression in these cells in this study, it will be important to determine whether PDI has similar effects on IgE-mediated activation in human cells. In this context, interestingly, quercetin and other polyphenols have been shown to modulate human mast cell activity in other studies (51, 52).

Our data suggests that allergen-induced mast cell activation may be a likely target of PDI-dependent modulation by dietary substances, thereby mitigating the overall magnitude of the allergic response. Dietary PDI modulators may possibly attenuate the activity of extracellular or cell surface PDI on mast cells and other immune cells, thereby suppressing their overall activation, and decreasing the production of proinflammatory mediators. This was evidenced by treatment with various PDI inhibitors, which suppressed the IgE-mediated activation of mast cells and inhibited their degranulation and cytokine secretion. Furthermore, treatment with PACMA-31, an orally bioactive, irreversible inhibitor of PDI suppressed mast cell responses both in cell culture and during the development of food allergy, suggesting that therapeutic targeting of PDI in allergic patients may prove to be of benefit. Interestingly, PDI inhibition also attenuated the long-term survival of BMMCs, suggesting that modulation of PDI activity *in vivo* can influence the homeostasis of mast cells. Nonetheless, the effects of PDI inhibition on mast cell degranulation and cytokine secretion were unrelated to its effects on cell viability, as no significant effects on cellular viability were observed within the first 48 h, *i.e.* the percentages of live and dead cells between untreated and treated groups were comparable at this time point (**Figures 3C** and **4F**).

In this report, we demonstrate for the first time the presence of PDI molecules on the surface of mast cells. Here, their enhanced extracellular activity (which can be blocked by PDI inhibitors) during mast cell activation may modulate downstream cellular events, contributing to changes in molecular and cellular function. In this context, cell-surface specific PDI has been found to be important for the functions of hepatocytes, endothelial cells, and platelets (27, 53). Furthermore, PDI has also been shown to be secreted into cell culture supernatants, which we did not ascertain in these studies (reviewed in (15)). It is thought that in these locations, PDI assists with redox protein folding, intramolecular thiol-disulfide exchanges and isomerization activities, as a result of highly specific interactions with various substrates. Future studies aimed at dissecting the contributions of both extracellular and intracellular PDI to mast cell activation will shed further light on the mechanisms by which PDI enhances mast cell responses. In particular, the effects of PDI catalytic activity on the formation of disulfide bonds in various proteins produced by mast cells or that activate them will be important to characterize. In this context, recent studies have elucidated novel roles for the disulfide dimer histamine-releasing factor (HRF) in mast cell-mediated allergic responses (54, 55). HRF forms dimers which can cross-link with IgE on basophils and mast cells and induce the secretion of enhanced levels of histamine, IL-4 and IL-13. Both increased levels of HRF-reactive IgE as well as increased numbers of HRF dimers were found in a recently described study of mast cell-mediated food allergy (54, 55). Similarly, patients with food allergy also had increased levels of serum HRF-reactive IgE and blockade of HRF function in the mouse model as well as oral immunotherapy in patients inhibited the allergic response (54, 55). In light of our data above, it is possible that the generation of HRF dimers *in vivo* may depend on PDI activity, resulting in the enhanced secretion of these proteins during allergic responses and the amplification of mast cell-mediated reactions.

The effects of PDI may also extend beyond its known isomerization activities. For example, recent studies demonstrate that PDI can increase the levels of reactive oxygen species (56) thus directly inducing oxidative stress and apoptosis, as well as activate transcription factors, such as NF- κB and AP-1 (57). In this context, we have previously demonstrated that the protective effects of curcumin on mast cells are mediated *via* inhibition of NF-κB activation (4), whereas another study demonstrated that flavonoids such as rutin can inhibit reactive oxygen species in mast cells (51). As such, further analysis of the effects of PDI inhibition on these and other parameters in mast cells will help elucidate the roles of PDI in these areas.

Lastly, this study demonstrates that PDI blockade has a profound effect on mast cell homeostasis (intestinal mast cell numbers), activation (mMCP-1 levels), and mast cell-mediated effects such as allergic diarrhea in the IgE and mast cell-dependent model of OVA-induced food allergy. However, although we have not examined other immune cell types in this study, it is likely that the activity of PDI family members is also altered in other cells during allergic responses. Particularly, our data in **Figure 9** demonstrate that OVA-specific splenic responses are also suppressed in the food allergy model, thereby implicating a role for PDI in the modulation of allergen-specific T cells. Future studies aimed at examining the effects of PDI on various immune cells during food allergy are therefore necessary to clarify the differential effects of PDI modulation on immune cells and their contribution to the development of food allergy.

Taken together, our data suggest that PDI and its related family members may play vital roles in the regulation of mast cell activation during the allergic response, and that *in vivo* blockade of their activity may prove to be of therapeutic benefit in patients with mast cell-mediated disorders.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by The Institutional Animal Care and Use Committee at Western New England University.

AUTHOR CONTRIBUTIONS

CBM and DRK conceived and directed the project. DK, SP, JG, JR, EK, CG, MP, NS, DRK, and CBM performed experiments and analyzed data. DK, SP, JG, DRK, SSS and CBM prepared figures for the manuscript. CBM, DK and DRK wrote the paper. All authors contributed to the article and approved the submitted version.

FUNDING

This project was supported by funds from the National Institutes of Health grants: NIAID R15AI107668 (CM) and NCI R21CA231000 (DRK).

ACKNOWLEDGMENTS

We thank Dr. Vikas Anathy, University of Vermont Medical Center, for critical reading of the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2020.606837/full#supplementary-material

SUPPLEMENTARY FIGURE 1 | *Pre-treatment with bacitracin and rutin suppresses IgE-induced BMMC cytokine gene expression.* BMMCs were treated with increasing doses of bacitracin (Bac) **(A–C)** or rutin (rut) **(D–F)** and activated *via* IgE and antigen stimulation. Cells were pooled and collected 1 h after activation and RNA and cDNA were prepared using established protocols. qRT-PCR was performed using Taqman probes. Expression of genes was calculated relative to that of GAPDH.

SUPPLEMENTARY FIGURE 2 | *Comparison of the percent PDI inhibitory activity of curcumin and PACMA-31.* The PDI inhibitory activity of curcumin and PACMA-31 were evaluated using the insulin turbidimetric assay. Bar graphs representing inhibition at the 40 min time point for various doses of the inhibitors are shown. Significance at each dose was calculated against the PDI added and untreated control group using one-way ANOVA (p< p=<0.0001).

SUPPLEMENTARY FIGURE 3 | *Histology of small intestine from OVA-sensitized and challenged and PACMA-31–treated mice.* Food allergy was induced in mice by sensitizing and challenging with OVA as in **Figure 7A**. Some groups were treated with PACMA-31 orally. Representative histological images depicting CAE-positive mast cells is shown for the **(A)** WT control **(B)** WT OVA **(C)** P-31 control and **(D, E)** two P-31 OVA groups. Mast cells are shown by an arrow.

SUPPLEMENTARY FIGURE 4 | *Impaired polyclonal cytokine production by spleen cells from OVA-challenged, PACMA-31–treated mice.* BALB/c mice were sensitized and challenged with OVA to induce food allergy. Some groups of animals were also gavaged with 300 µg PACMA-31 suspended in 1% CMC. Upon sacrifice, spleen cells were stimulated with anti-CD3 and anti-CD28 for 72 h. Levels of the cytokines **(A)** IL-4 **(B)** IL-5 **(C)** IL-13, and **(D)** IFN-γ were enumerated in the supernatants by ELISA. n = 4–7 mice/group. Data are representative of 2 independent experiments. *=p<0.05; **=p<0.01; ***=p<0.0001 (student's t-test).

REFERENCES

1. Sampson HA, O'Mahony L, Burks AW, Plaut M, Lack G, Akdis CA. Mechanisms of food allergy. *J Allergy Clin Immunol* (2018) 141(1):11–9. doi: 10.1016/j.jaci.2017.11.005
2. Oettgen HC, Burton OT. IgE receptor signaling in food allergy pathogenesis. *Curr Opin Immunol* (2015) 36:109–14. doi: 10.1016/j.coi.2015.07.007
3. Mathias CB, McAleer JP, Szollosi DE. Inflammatory Diseases of the Gastrointestinal Tract and Pharmacological Treatments. In: CB Mathias, JP McAleer, DE Szollosi, editors. *Pharmacology of Immunotherapeutic Drugs*. Cham: Springer International Publishing (2020). p. 175–205. doi: 10.1007/978-3-030-19922-7_6
4. Kinney SR, Carlson L, Ser-Dolansky J, Thompson C, Shah S, Gambrah A, et al. Curcumin Ingestion Inhibits Mastocytosis and Suppresses Intestinal Anaphylaxis in a Murine Model of Food Allergy. *PLoS One* (2015) 10(7): e0132467. doi: 10.1371/journal.pone.0132467
5. Krajewski D, Kaczenski E, Rovatti J, Polukort S, Thompson C, Dollard C, et al. Epigenetic Regulation via Altered Histone Acetylation Results in Suppression of Mast Cell Function and Mast Cell-Mediated Food Allergic Responses. *Front Immunol* (2018) 9:2414. doi: 10.3389/fimmu.2018.02414
6. Kurup VP, Barrios CS. Immunomodulatory effects of curcumin in allergy. *Mol Nutr Food Res* (2008) 52(9):1031–9. doi: 10.1002/mnfr.200700293
7. Lee JH, Kim JW, Ko NY, Mun SH, Her E, Kim BK, et al. Curcumin, a constituent of curry, suppresses IgE-mediated allergic response and mast cell activation at the level of Syk. *J Allergy Clin Immunol* (2008) 121(5):1225–31. doi: 10.1016/j.jaci.2007.12.1160
8. Jagetia GC, Aggarwal BB. "Spicing up" of the immune system by curcumin. *J Clin Immunol* (2007) 27(1):19–35. doi: 10.1007/s10875-006-9066-7
9. Agrawal DK, Mishra PK. Curcumin and its analogues: potential anticancer agents. *Med Res Rev* (2010) 30(5):818–60. doi: 10.1002/med.20188
10. Shishodia S, Chaturvedi MM, Aggarwal BB. Role of curcumin in cancer therapy. *Curr Probl Cancer* (2007) 31(4):243–305. doi: 10.1016/j.currproblcancer.2007.04.001
11. Goel A, Kunnumakkara AB, Aggarwal BB. Curcumin as "Curecumin": from kitchen to clinic. *Biochem Pharmacol* (2008) 75(4):787–809. doi: 10.1016/j.bcp.2007.08.016
12. Sandur SK, Ichikawa H, Pandey MK, Kunnumakkara AB, Sung B, Sethi G, et al. Role of pro-oxidants and antioxidants in the anti-inflammatory and apoptotic effects of curcumin (diferuloylmethane). *Free Radical Biol Med* (2007) 43(4):568–80. doi: 10.1016/j.freeradbiomed.2007.05.009
13. Schmidt B, Ho L, Hogg PJ. Allosteric disulfide bonds. *Biochemistry* (2006) 45 (24):7429–33. doi: 10.1021/bi0603064
14. Givol D, Goldberger RF, Anfinsen CB. Oxidation and Disulfide Interchange in the Reactivation of Reduced Ribonuclease. *J Biol Chem* (1964) 239:PC3114–16.
15. Parakh S, Atkin JD. Novel roles for protein disulphide isomerase in disease states: a double edged sword? *Front Cell Dev Biol* (2015) 3:30. doi: 10.3389/fcell.2015.00030
16. Cho J, Kennedy DR, Lin L, Huang M, Merrill-Skoloff G, Furie BC, et al. Protein disulfide isomerase capture during thrombus formation in vivo depends on the presence of beta3 integrins. *Blood* (2012) 120(3):647–55. doi: 10.1182/blood-2011-08-372532
17. Ryser HJ, Levy EM, Mandel R, DiSciullo GJ. Inhibition of human immunodeficiency virus infection by agents that interfere with thiol-disulfide interchange upon virus-receptor interaction. *Proc Natl Acad Sci USA* (1994) 91(10):4559–63. doi: 10.1073/pnas.91.10.4559
18. Xu S, Sankar S, Neamati N. Protein disulfide isomerase: a promising target for cancer therapy. *Drug Discov Today* (2014) 19(3):222–40. doi: 10.1016/j.drudis.2013.10.017
19. Aalberse RC, Schuurman J. IgG4 breaking the rules. *Immunology* (2002) 105 (1):9–19. doi: 10.1046/j.0019-2805.2001.01341.x
20. Jasuja R, Passam FH, Kennedy DR, Kim SH, van Hessem L, Lin L, et al. Protein disulfide isomerase inhibitors constitute a new class of antithrombotic agents. *J Clin Invest* (2012) 122(6):2104–13. doi: 10.1172/JCI61228
21. Khodier C VL, Nag PP, Pu J, Wurst J, Pilyugina T, Dockendorff C, et al. *Identification of ML359 as a Small Molecule Inhibitor of Protein Disulfide Isomerase. Probe Reports from the NIH Molecular Libraries Program [Internet]*. Bethesda (MD): National Center for Biotechnology Information (US). (2013 Apr 12 [Updated 2014 Sep 18]).
22. Smith AM, Chan J, Oksenberg D, Urfer R, Wexler DS, Ow A, et al. A high-throughput turbidometric assay for screening inhibitors of protein disulfide isomerase activity. *J Biomol Screen* (2004) 9(7):614–20. doi: 10.1177/1087057104265292
23. Mathias CB, Freyschmidt EJ, Caplan B, Jones T, Poddighe D, Xing W, et al. IgE influences the number and function of mature mast cells, but not progenitor recruitment in allergic pulmonary inflammation. *J Immunol* (2009) 182(4):2416–24. doi: 10.4049/jimmunol.0801569
24. Polukort SH, Rovatti J, Carlson L, Thompson C, Ser-Dolansky J, Kinney SR, et al. IL-10 Enhances IgE-Mediated Mast Cell Responses and Is Essential for the Development of Experimental Food Allergy in IL-10-Deficient Mice. *J Immunol* (2016) 196(12):4865–76. doi: 10.4049/jimmunol.1600066
25. Brandt EB, Strait RT, Hershko D, Wang Q, Muntel EE, Scribner TA, et al. Mast cells are required for experimental oral allergen-induced diarrhea. *J Clin Invest* (2003) 112(11):1666–77. doi: 10.1172/JCI19785
26. Mathias CB, Hobson SA, Garcia-Lloret M, Lawson G, Poddighe D, Freyschmidt EJ, et al. IgE-mediated systemic anaphylaxis and impaired tolerance to food antigens in mice with enhanced IL-4 receptor signaling. *J Allergy Clin Immunol* (2011) 127(3):795–805.e1-6. doi: 10.1016/j.jaci.2010.11.009
27. Flaumenhaft R, Furie B, Zwicker JI. Therapeutic implications of protein disulfide isomerase inhibition in thrombotic disease. *Arterioscler Thromb Vasc Biol* (2015) 35(1):16–23. doi: 10.1161/ATVBAHA.114.303410
28. Zwicker JI, Schlechter BL, Stopa JD, Liebman HA, Aggarwal A, Puligandla M, et al. Targeting protein disulfide isomerase with the flavonoid isoquercetin to improve hypercoagulability in advanced cancer. *JCI Insight* (2019) 4(4):1–12. doi: 10.1172/jci.insight.125851
29. Oslowski CM, Urano F. Measuring ER stress and the unfolded protein response using mammalian tissue culture system. *Methods Enzymol* (2011) 490:71–92. doi: 10.1016/B978-0-12-385114-7.00004-0

30. Appenzeller-Herzog C, Ellgaard L. The human PDI family: versatility packed into a single fold. *Biochim Biophys Acta* (2008) 1783(4):535–48. doi: 10.1016/j.bbamcr.2007.11.010
31. Mandel R, Ryser HJ, Ghani F, Wu M, Peak D. Inhibition of a reductive function of the plasma membrane by bacitracin and antibodies against protein disulfide-isomerase. *Proc Natl Acad Sci USA* (1993) 90(9):4112–6. doi: 10.1073/pnas.90.9.4112
32. Dickerhof N, Kleffmann T, Jack R, McCormick S. Bacitracin inhibits the reductive activity of protein disulfide isomerase by disulfide bond formation with free cysteines in the substrate-binding domain. *FEBS J* (2011) 278 (12):2034–43. doi: 10.1111/j.1742-4658.2011.08119.x
33. Guyette J, Cherubin P, Serrano A, Taylor M, Abedin F, O'Donnell M, et al. Quercetin-3-Rutinoside Blocks the Disassembly of Cholera Toxin by Protein Disulfide Isomerase. *Toxins (Basel)* (2019) 11(8). doi: 10.3390/toxins11080458
34. Lin L, Gopal S, Sharda A, Passam F, Bowley SR, Stopa J, et al. Quercetin-3-rutinoside Inhibits Protein Disulfide Isomerase by Binding to Its b'x Domain. *J Biol Chem* (2015) 290(39):23543–52. doi: 10.1074/jbc.M115.666180
35. Xu S, Butkevich AN, Yamada R, Zhou Y, Debnath B, Duncan R, et al. Discovery of an orally active small-molecule irreversible inhibitor of protein disulfide isomerase for ovarian cancer treatment. *Proc Natl Acad Sci USA* (2012) 109(40):16348–53. doi: 10.1073/pnas.1205226109
36. Delom F, Mallet B, Carayon P, Lejeune PJ. Role of extracellular molecular chaperones in the folding of oxidized proteins. Refolding of colloidal thyroglobulin by protein disulfide isomerase and immunoglobulin heavy chain-binding protein. *J Biol Chem* (2001) 276(24):21337–42. doi: 10.1074/jbc.M101086200
37. Essex DW, Chen K, Swiatkowska M. Localization of protein disulfide isomerase to the external surface of the platelet plasma membrane. *Blood* (1995) 86(6):2168–73. doi: 10.1182/blood.V86.6.2168.bloodjournal8662168
38. Essex DW, Li M. Protein disulphide isomerase mediates platelet aggregation and secretion. *Br J Haematol* (1999) 104(3):448–54. doi: 10.1046/j.1365-2141.1999.01197.x
39. Ros M, Nguyen AT, Chia J, Le Tran S, Le Guezennec X, McDowall R, et al. ER-resident oxidoreductases are glycosylated and trafficked to the cell surface to promote matrix degradation by tumour cells. *Nat Cell Biol* (2020) 22:1371–81. doi: 10.1038/s41556-020-00590-w
40. Wan SW, Lin CF, Lu YT, Lei HY, Anderson R, Lin YS. Endothelial cell surface expression of protein disulfide isomerase activates beta1 and beta3 integrins and facilitates dengue virus infection. *J Cell Biochem* (2012) 113(5):1681–91. doi: 10.1002/jcb.24037
41. Myllyharju J. Prolyl 4-hydroxylases, the key enzymes of collagen biosynthesis. *Matrix Biol* (2003) 22(1):15–24. doi: 10.1016/s0945-053x(03)00006-4
42. Chen K, Detwiler TC, Essex DW. Characterization of protein disulphide isomerase released from activated platelets. *Br J Haematol* (1995) 90(2):425–31. doi: 10.1111/j.1365-2141.1995.tb05169.x
43. Thon JN, Peters CG, Machlus KR, Aslam R, Rowley J, Macleod H, et al. T granules in human platelets function in TLR9 organization and signaling. *J Cell Biol* (2012) 198(4):561–74. doi: 10.1083/jcb.201111136
44. Kozlov G, Maattanen P, Thomas DY, Gehring K. A structural overview of the PDI family of proteins. *FEBS J* (2010) 277(19):3924–36. doi: 10.1111/j.1742-4658.2010.07793.x
45. Klappa P, Hawkins HC, Freedman RB. Interactions between protein disulphide isomerase and peptides. *Eur J Biochem* (1997) 248(1):37–42. doi: 10.1111/j.1432-1033.1997.t01-1-00037.x
46. Molinari M, Galli C, Piccaluga V, Pieren M, Paganetti P. Sequential assistance of molecular chaperones and transient formation of covalent complexes during protein degradation from the ER. *J Cell Biol* (2002) 158(2):247–57. doi: 10.1083/jcb.200204122
47. Zhang K, Kaufman RJ. From endoplasmic-reticulum stress to the inflammatory response. *Nature* (2008) 454(7203):455–62. doi: 10.1038/nature07203
48. Hoffman SM, Tully JE, Nolin JD, Lahue KG, Goldman DH, Daphtary N, et al. Endoplasmic reticulum stress mediates house dust mite-induced airway epithelial apoptosis and fibrosis. *Respir Res* (2013) 14(1):141. doi: 10.1186/1465-9921-14-141
49. Hoffman SM, Chapman DG, Lahue KG, Cahoon JM, Rattu GK, Daphtary N, et al. Protein disulfide isomerase-endoplasmic reticulum resident protein 57 regulates allergen-induced airways inflammation, fibrosis, and hyperresponsiveness. *J Allergy Clin Immunol* (2016) 137(3):822–32.e7. doi: 10.1016/j.jaci.2015.08.018
50. Galinski CN, Zwicker JI, Kennedy DR. Revisiting the mechanistic basis of the French Paradox: Red wine inhibits the activity of protein disulfide isomerase in vitro. *Thromb Res* (2016) 137:169–73. doi: 10.1016/j.thromres.2015.11.003
51. Chen S, Gong J, Liu F, Mohammed U. Naturally occurring polyphenolic antioxidants modulate IgE-mediated mast cell activation. *Immunology* (2000) 100(4):471–80. doi: 10.1046/j.1365-2567.2000.00045.x
52. Shaik Y, Caraffa A, Ronconi G, Lessiani G, Conti P. Impact of polyphenols on mast cells with special emphasis on the effect of quercetin and luteolin. *Cent Eur J Immunol* (2018) 43(4):476–81. doi: 10.5114/ceji.2018.81347
53. Turano C, Coppari S, Altieri F, Ferraro A. Proteins of the PDI family: unpredicted non-ER locations and functions. *J Cell Physiol* (2002) 193 (2):154–63. doi: 10.1002/jcp.10172
54. Ando T, Kashiwakura JI, Itoh-Nagato N, Yamashita H, Baba M, Kawakami Y, et al. Histamine-releasing factor enhances food allergy. *J Clin Invest* (2017) 127(12):4541–53. doi: 10.1172/JCI96525
55. Kashiwakura JI, Ando T, Kawakami T. Role of Histamine-releasing Factor in Allergic Inflammatory Reactions. *Yakugaku Zasshi* (2017) 137(5):517–21. doi: 10.1248/yakushi.16-00239-3
56. Fernandes DC, Manoel AH, Wosniak JJr., Laurindo FR. Protein disulfide isomerase overexpression in vascular smooth muscle cells induces spontaneous preemptive NADPH oxidase activation and Nox1 mRNA expression: effects of nitrosothiol exposure. *Arch Biochem Biophys* (2009) 484(2):197–204. doi: 10.1016/j.abb.2009.01.022
57. Clive DR, Greene JJ. Cooperation of protein disulfide isomerase and redox environment in the regulation of NF-kappaB and AP1 binding to DNA. *Cell Biochem Funct* (1996) 14(1):49–55. doi: 10.1002/cbf.638

Conflict of Interest: CBM and DRK are authors on a patent application submitted by Western New England University regarding the role of thiol isomerases in mast cell activation.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 08 January 2021
doi: 10.3389/fimmu.2020.616020

Antigen-Presenting Cells in Food Tolerance and Allergy

Elise G. Liu[1,2,3†], *Xiangyun Yin*[1,2†], *Anush Swaminathan*[2] *and Stephanie C. Eisenbarth*[1,2,3*]

[1] Department of Laboratory Medicine, Yale University School of Medicine, New Haven, CT, United States, [2] Department of Immunobiology, Yale University School of Medicine, New Haven, CT, United States, [3] Section of Rheumatology, Allergy & Immunology, Yale University School of Medicine, New Haven, CT, United States

Food allergy now affects 6%–8% of children in the Western world; despite this, we understand little about why certain people become sensitized to food allergens. The dominant form of food allergy is mediated by food-specific immunoglobulin E (IgE) antibodies, which can cause a variety of symptoms, including life-threatening anaphylaxis. A central step in this immune response to food antigens that differentiates tolerance from allergy is the initial priming of T cells by antigen-presenting cells (APCs), primarily different types of dendritic cells (DCs). DCs, along with monocyte and macrophage populations, dictate oral tolerance versus allergy by shaping the T cell and subsequent B cell antibody response. A growing body of literature has shed light on the conditions under which antigen presentation occurs and how different types of T cell responses are induced by different APCs. We will review APC subsets in the gut and discuss mechanisms of APC-induced oral tolerance versus allergy to food identified using mouse models and patient samples.

Keywords: food allergy, dendritic cells, oral tolerance, monocytes, gut, mesenteric lymph node, Peyer's patches, macrophages

OPEN ACCESS

Edited by:
Pamela Guerrerio, National Institutes of Health (NIH), United States

Reviewed by:
Even Fossum, Oslo University Hospital, Norway
Suryasarathi Dasgupta, Takeda (California), United States

***Correspondence:**
Stephanie C. Eisenbarth
stephanie.eisenbarth@yale.edu

†These authors have contributed equally to this work

Specialty section:
This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology

Received: *10 October 2020*
Accepted: *20 November 2020*
Published: *08 January 2021*

Citation:
Liu EG, Yin X, Swaminathan A and Eisenbarth SC (2021) Antigen-Presenting Cells in Food Tolerance and Allergy. Front. Immunol. 11:616020. doi: 10.3389/fimmu.2020.616020

INTRODUCTION

Food allergy is a growing epidemic in the developed world, with 6%–8% of children and about 2% of the general population affected in the United States (1–4). A small group of foods including peanut, tree nuts, egg, milk, soy, wheat, fish, shellfish, and sesame cause over 90% of food allergies in the United States. For sufferers of food allergy, consuming the target allergen can lead to various body-wide symptoms including hives, swelling, gastrointestinal distress, cardiovascular, and respiratory compromise, and in rare instances, fatal anaphylaxis (5). The standard of care for food allergy treatment is to avoid consuming the allergenic food and to carry emergency medications in case of accidental ingestion (6). Despite advances made in food allergy treatment with oral immunotherapy, a cure is still elusive. Food allergy greatly affects quality of life, so more treatment options are direly needed (7). To identify therapeutic targets and advance research, it is crucial to understand the mechanisms underlying food allergy.

Food allergy is a type 2 immune reaction to dietary antigens that can manifest in several ways depending on the pathophysiological endotype (8); some forms of food allergy are dominated by the type 2 cellular response, whereas others primarily present with symptoms of the humoral type 2 response. This review will cover what is known about the regulation of the cellular and humoral

immune reactions to food antigens by the dominant antigen-presenting cells of the immune system, dendritic cells (DCs).

The cellular immune response of type 2 immunity is coordinated by Th2 $CD4^+$ T cells, which produce IL-4, -5, and -13 cytokines as well as chemokines and other chemical mediators; a subset of these T cells also make IL-9. The ensuing cellular response includes recruitment and activation of eosinophils, group 2 innate lymphoid cells, (ILC2s) and basophils, as well as changes to the epithelial barrier (9). ILC2s amplify the Th2 response within the gut by producing IL-5 and -13 and quickly react to the production of alarmin cytokines such as IL-25 from the gut epithelium (10). Th2 cells also induce a population of mucosal mast cells that produce IL-4, IL-9, and IL-13 (11), which expands the intestinal mast cell population while suppressing regulatory T (Treg) cell generation, enhancing susceptibility of anaphylaxis to food allergens (12, 13). Tregs are responsible for oral tolerance, the induction of non-responsiveness to gut antigens including food. Many such Tregs are induced within the gut [peripheral Tregs (pTreg)], and we will cover what is known about this important step in avoiding allergic sensitization to food.

The humoral immune response of type 2 immunity is epitomized by IgE, which is driven by two closely related populations of $CD4^+$ T follicular helper (Tfh) cells, IL-4-producing Tfh2 and Il-4 and -13 producing Tfh13 cells (14). In contrast to allergic airway inflammation, mast cells are essential for the allergic IgE-mediated form of food allergy (15, 16). Cross-linking of high-affinity IgE on mature mast cell membranes induces release of the chemical mediators of anaphylaxis, the "weep and sweep" response; this eliminates the target of the IgE antibodies but can be life-threatening. There is also ample data that a positive feedback loop ensues from IgE-mediated mast cell activation, resulting in enhanced cellular type 2 immunity to food allergens (13). Other innate immune cells have also been implicated in contributing to anaphylactic responses in both human and mouse studies including basophils, platelets, macrophages, and neutrophils (17).

Mounting both of these adaptive immune responses begins by activating the correct type of antigen-presenting cell (APC). This requires innate immune activation, since in the absence of activating signals, APCs should induce antigen-specific T cell tolerance. Tolerance is the primary response of the gut immune system to food antigens. Antigen can be acquired by APCs in the gut lamina propria (LP) through multiple access points, including goblet cell-associated passages (18, 19), microfold (M) cell sampling in Peyer's patches (PPs), and gut lumen sampling $CX3CR1^+$ macrophages that pass off antigen to migratory DCs (20). These DCs migrate in a CCR7-dependent manner to provide either activating or tolerizing signals to naïve lymphocytes within gut-associated lymphoid tissues (GALT) (21). GALT are located throughout the intestine and include PPs, mesenteric lymph nodes (MLNs) and isolated lymphoid follicles (ILFs). These are unique cellular niches for induction of tolerance but are also sites for T cell priming and B cell activation. It is important to note that many theories on sensitization to food allergens implicate the skin rather than the gut as the relevant site based on clinical and experimental data (22). Therefore, we will also cover what is known about the APC response in the skin to food allergens.

APC POPULATIONS IN THE GUT

APCs encompass DCs, monocytes/macrophages and B cells (**Table 1** and **Figure 1**). Little data exist on B cells functioning as APCs in food tolerance or sensitivity; therefore, this review will primarily focus on DCs and monocytes/macrophages in the response to food antigens, starting with a brief introduction on gut APCs.

Dendritic Cell Populations in the Gut

DCs are professional antigen-presenting cells that control both T cell tolerance and priming. Based on ontogeny, phenotype and function, DCs can be divided into conventional/classical DCs (cDCs) and plasmacytoid DCs (pDCs) [for review see (23)]. cDCs are further separated into two subsets, cDC1s and cDC2s (24).

Lamina Propria (LP)

Mouse LP is populated by $CD103^+CD11b^-CLEC9A^+XCR1^+$ cDC1s, $CD103^+CD11b^+SIRP\alpha^+$ cDC2s and then a population of cells that are $CD103^-$ $CD11b^+$DCs (25–29). Human LP have analogous cDC populations with $CD103^+CD141^+CLEC9A^+XCR1^+$ cDC1s and $CD103^+CD1c^+Sirp\alpha^+$ cDC2s (21, 30, 31). Recently, new cDC2 subsets were identified in both human and mouse (32, 33). Since these new DC subsets have not yet been studied in food allergy or tolerance, we will not discuss them. cDC subsets in the LP can migrate into mesenteric lymph nodes (MLNs) *via* CCR7-driven chemotaxis (21, 34, 35). The LP contains a fourth population of CD11b+CX3CR1 + cells; whether these cells migrate to MLNs and prime T cells *in vivo* has been debated (28, 36–39). This is partly due to the mixed origin of $CX3CR1^+$ cells in the LP (40). One Ly6C- and cDC-derived subset requires CCR2 for seeding the LP and subsequent CCR7-dependent migration to the MLN (27, 37). In contrast, a $Ly6C^+$ monocyte-derived DC (mo-DC) subset, which is also CCR2-dependent, fails to express CCR7 or migrate to MLNs and therefore is not involved in naïve T cell priming in MLN (28, 38, 41, 42). A small population of $CD103^-CD11b^-$ DCs are also present in the LP but are likely cDC1s and cDC2s as they have been shown to either express XCR1 or SIRPα (25). Finally, $PDCA1^+$ pDCs responsible for regulating intestinal cDC mobilization towards the MLNs are also present in the LP (21, 43, 44).

Mesenteric Lymph Node (MLN)

In the MLN, four populations of $CD11c^+$ $MHCII^+$ cells are observed using CD11b and CD103 surface staining: 1, cDC1s, which encompass both migratory $CD103^+$ $CD11b^-$cDC1s from the LP and some $CD11b^-CD8\alpha^+$ resident cDC1s (all are $XCR1^+$ and $CLEC9A^+$); 2, cDC2, which encompass $CD103^+CD11b^+$ migratory cDC2s and $CD11b^+$ resident cDC2s (all are $SIRP\alpha^+$); 3, $CD11b^+CD103^-$ cDC2s; and 4, depending on the inflammatory state, a monocyte-derived $CD11b^+CX3CR1^+$ population (25, 27–29). The expression of F4/80, Ly6C, CD64, Zbtb46, and CX3CR1 levels have been used to differentiate populations 3 and 4.

Peyer's Patch (PP)

PP DC subsets have been classically defined in a manner distinct from LP and MLN DCs as $CD8\alpha^+$, $CD11b^+$, or $CD8\alpha^-CD11b^-$ "double negative" (DN) (45). However, more recent work has

TABLE 1 | Antigen presenting cells in the gut.

	Location	Human	Mouse
cDC1	MLN	Resident: HLA-DR^{int} CD11c^{hi} CD11b^{-} CD8α^{+} XCR1^{+} SIRPα^{-}CD141^{+} DNGR1^{+} Migratory: HLA-DR^{hi} CD11c^{int} CD103^{+} CD11b^{-} XCR1^{+} SIRPα^{-}CD141^{+} DNGR1^{+}	Resident: MHC-II^{int} CD11c^{hi}CD11b^{-} CD8α^{+} XCR1^{+} SIRPα^{-} DNGR1^{+} Migratory: MHC-II^{hi} CD11c^{int} CD103^{+} CD11b^{-} XCR1^{+} SIRPα^{-} DNGR1^{+}
	LP	CD103^{+} CD11b^{-} XCR1^{+} SIRPα^{-}CD141^{+} DNGR1^{+}	CD103^{+} CD11b^{-} XCR1^{+} SIRPα^{-} DNGR1^{+}
	PP	CD103^{+} CD11b^{-} CD8α^{+} XCR1^{+} SIRPα^{-}CD141^{+} DNGR1^{+}	CD103^{+} CD11b^{-} CD8α^{+} XCR1^{+} SIRPα^{-} DNGR1^{+}
cDC2	MLN	Resident: HLA-DR^{int} CD11c^{hi} CD103^{+} CD11b^{+} XCR1^{-}CD1c^{+} SIRPα^{+} CD141^{-} DNGR1^{-} Migratory: HLA-DR^{hi} CD11c^{int} CD103^{+} CD11b^{+} XCR1^{-}CD1c^{+} SIRPα^{+} CD141^{-} DNGR1^{-}	Resident: MHC-II^{int} CD11c^{hi} CD103^{+} CD11b^{+} XCR1^{-} SIRPα^{+} Migratory: MHC-II^{hi} CD11c^{int} CD103^{+} CD11b^{+} XCR1^{-} SIRPα^{+}
	LP	1.CD103^{+} CD11b^{+} XCR1^{-}CD1c^{+} SIRPα^{+} CD141^{-} DNGR1^{-} 2. CD103^{-} CD11b^{+} XCR1^{-}CD1c^{+} SIRPα^{+} CD141^{-} DNGR1^{-}	1.CD103^{+} CD11b^{+} XCR1^{-} SIRPα^{+} 2.CD103^{-} CD11b^{+} XCR1^{-} SIRPα^{+}
	PP	HLA-DR^{+} CD11C^{+} CD1c^{+}(?) XCR1^{-}	CD103^{-} CD11b^{+} XCR1^{-} SIRPα^{+}
pDC	MLN, LP, PP	CD11c^{-} CD123^{+} BDCA2^{+}(?) BDCA4^{+}(?)	CD11c^{mid}B220^{+} PDCA1^{+} LY6C^{+}CCR9^{+} Siglec-H^{+}
Monocyte	MLN, LP, PP	Classical: CCR2^{hi}M-CSFR^{+} CD14^{hi}CD11b^{+} non-classical monocytes: CCR2^{low} M-CSFR^{+} CD14^{low}	Classical: CCR2^{hi}M-CSFR^{+} Ly6C^{hi} non-classical monocytes: CCR2^{low} M-CSFR^{+} Ly6C^{low}
monocyte-derived cells	MLN, LP, PP	CD14^{+} CD11b^{+}SIRPα^{+} /CD172^{+}BDCA1/CD1c^{+}CD226^{+}	CD209a^{+} CD11b^{+} CD64^{+}CCR2^{+} Ly6C^{+} CD88^{+} SIRPα^{+} /CD172^{+}CX3CR1^{mid}
Macrophage	MLN, LP, PP	HLA-DR^{+} CD68^{+} CD64^{+} CD209^{+} MerTK^{+} CD14^{+} CD206^{+}CD163^{+}	CX3CR1^{hi} CD11b^{+}CD64^{+} F4/80^{+}MerTK^{+} SIRPα^{+} CD163^{+}

Commonly used markers for defining APC subsets in the gut of humans or mice are summarized based on location and subset. "?" indicates that well-accepted markers have not yet been established.

united the subsets across a variety of tissues and secondary lymphoid organs (SLOs) using the cDC1 and cDC2 nomenclature (24), including in the gut (25). Using the new classification system, PP DCs fall into two subsets: 1, cDC1s, which includes both CD8α^{+}XCR1^{+} and DN XCR1^{+} DCs; and 2, cDC2s, which includes both CD11b^{+} SIRPα^{+} and DN SIRPα^{+} DCs. It is also helpful to maintain the classification of migratory and resident DC subsets in all SLOs, including those without afferent lymphatics like the spleen and PPs, as migration after antigen acquisition occurs between different tissue regions within these sites (23). Resident CD8α^{+}XCR1^{+} cDC1s are primarily found in the T cell-rich interfollicular zone (IFZ) of the PP. The heterogeneous populations of DN DCs in PPs have been identified by immunofluorescence staining in the subepithelial dome (SED) and IFZ of the PP (46). With microbial or adjuvant stimulation, SIRPα^{+} cDC2s, including DN DCs and CD11b^{+}DCs, can migrate from the SED into adjacent IFZs (47, 48). CLEC9A^{+} cDC1s were noted in the SED of human PPs by immunofluorescence (31). In addition, CD103^{+} cDCs were observed in the SED in rat PPs at steady state but were concentrated in the IFZ after activation (43); these could represent a migratory cDC1 population within the PP, though more work is needed to confirm this. Therefore, we propose to classify PP DC subsets as IFZ-resident cDC1s and cDC2s and SED migratory cDC2s and possibly migratory cDC1s (**Figure 1**). This mirrors the nomenclature in the spleen and LNs. PP PDCA1^{+} pDCs are also found in the SED and IFZ (49). It should be noted that there is little evidence for any of these DC subsets emigrating into PPs from the gut. Therefore, they likely seed the PPs from the blood and then migrate within the PP upon activation.

Monocytes/Macrophage Populations in the Gut

Monocytes include three main subsets, Ly6C^{hi} (mouse)/ CD14^{+}(human) classical monocytes and Ly6C^{low} (mouse)/CD14^{-} (human) non-classical monocytes and Ly6C^{int}(mouse)/CD14^{int} (human) intermediate monocytes (50). Classical monocytes express higher CCR2 and require CCR2 for bone marrow egress (51). Monocytes can differentiate into DC-like populations (mo-DCs) or macrophages according to the context, which are difficult to discriminate, and therefore, we will refer to both under the umbrella term, monocyte-derived cells (MCs) (36, 40, 52). A population of CD11c^{+} CD11b^{+} SIRPα^{+} MCs exists in the PP dome that expresses lysozyme and CX3CR1 and can activate T cells *in vitro* (53). A similar population of CD11b^{+}CX3CR1^{+} MCs exists in the LP (28, 38–40).

Macrophages in the LP are identified as MHCII^{+}F4/ 80^{+}CD11b^{+} CX3CR1^{+} MerTK^{+} in mice (54, 55). MerTK, CD64, CD163, and Sirpα are conserved features of human intestinal macrophages, although at varying levels for macrophage subset (56, 57). Gut macrophages are distinguished from DCs by CD64 expression (38). Although macrophages in most tissues have a dual origin involving both embryonic liver and hematopoietic bone marrow ontogeny, intestinal LP macrophages need continual replenishment from circulating Ly6C^{hi} monocytes in adult mice (38, 39, 58, 59). The function and phenotype of the macrophages that differentiate from these monocyte precursors vary based on the state of inflammation in the gut (40, 54). Although *in vitro* gut macrophages are capable of antigen presentation to naïve T cells, both macrophages and monocytes are rarely observed

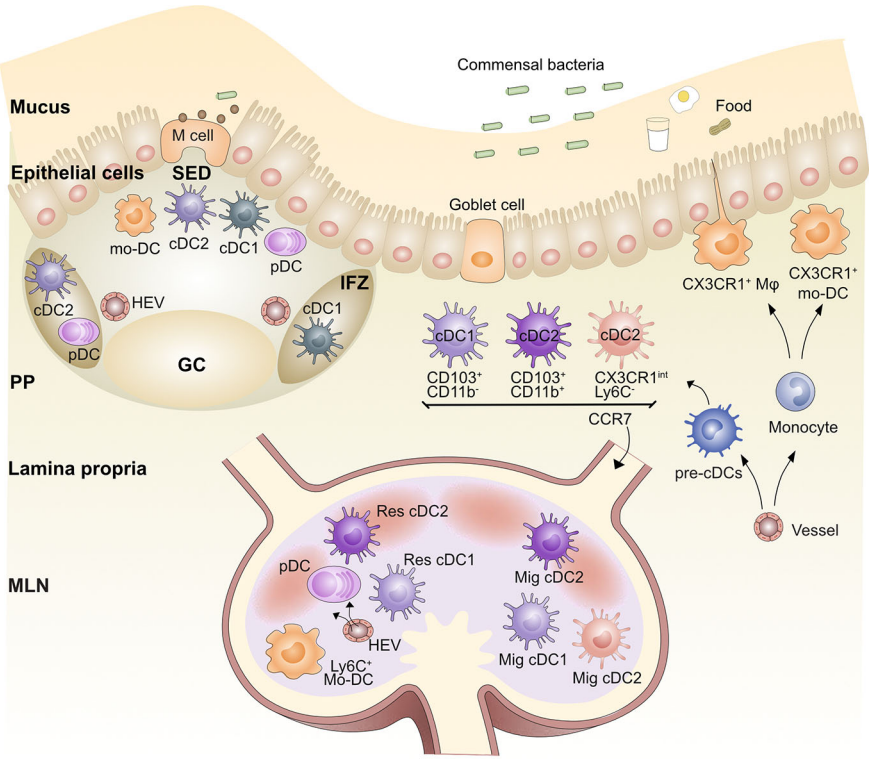


FIGURE 1 | Organization of the gut antigen presenting cell network. Blood pre-cDCs populate the lamina propria (LP), Peyer's Patches (PP), and mesenteric lymph node (MLN) and differentiate into cDC1s and cDC2s. After being activated by antigen, LP cDC1s and cDC2s are able to migrate *via* afferent lymphatics to the gut-draining MLN *via* CCR7; these DCs are called migratory DCs (Mig DC). Similarly, cDC2s and possibly cDC1s in the subepithelial dome (SED) of the PPs are able to migrate to the intrafollicular zone (IFZ). Lysozyme^{+}CX3CR1^{+} monocyte-derived DCs (mo-DC) also populate the SED. Pre-cDCs travel through the blood and seed the MLN and PP, where they differentiate into resident (Res) cDC1 and Res cDC2. Plasmacytoid DCs (pDCs) also populate the LP, PP, and MLN. Blood-derived monocytes differentiate into LP and PP macrophages (Mφ) as well as mo-DCs. Germinal center (GC), Microfold (M) cell, High endothelial venule (HEV).

transporting gut antigens from tissue to LNs to prime naïve T cells, so their primary function is within the gut itself. Inflammatory monocytes enter the LN primarily from the blood rather than migrating from the tissue using CCR2 rather than CCR7 homing signals (60, 61).

APCS AND ORAL TOLERANCE

In the steady state, ingestion of innocuous antigens generally results in oral tolerance. Long-lasting oral tolerance is enforced by Foxp3^{+} pTreg cells induced in MLNs that home to the gut by expressing the integrin α4β7 and the chemokine receptor CCR9 along with T effector cell clonal deletion or anergy (62–65). By raising mice with a diet devoid of dietary antigens, a recent study demonstrated that the majority of small intestinal pTreg cells are induced by dietary food antigens (66). These Tregs suppress CD4^{+} and CD8^{+} T cells, alter mast cell function and re-direct IgE B cell responses (65, 67, 68). Although Tregs can directly promote IgA production through production or activation of TGFβ (69), little is known about the mechanisms or relevance of humoral tolerance in the gut to food antigens. Type 2 inflammation, including IL-4 production from ILC2s, can inhibit the generation and function of these Tregs and can even reprogram them into pathogenic Th2 cells (12, 70), which has been shown in animal models to prevent tolerance and confer a food allergy phenotype.

Conventional Dendritic Cells

Intestinal APCs, including cDCs, macrophages and pDCs, play pivotal roles in oral tolerance induction (**Figure 2**). DCs have been implicated in inducing pTreg cell differentiation through multiple mechanisms. After ingestion of foreign dietary antigens, DCs acquire antigen through several routes, including transfer from M cells, macrophages or goblet cell-associated antigen passages but also by sampling the gut lumen using trans-epithelial dendrites (19, 20, 71). However, this latter function may primarily be accomplished by LP CX3CR1^{+} macrophages. MLNs are the primary site of oral tolerance induction (35, 72, 73), although PPs may contribute depending on the nature of the antigen (74). Ablation of cDCs results in the reduction of gut pTreg cells in response to dietary antigen ingestion (75). Gut CD103^{+} cDCs, carrying antigens that are critical for the development of oral tolerance, migrate from the LP to the MLNs in a CCR7-dependent manner (20, 28, 35, 41). Unlike other sites, both cDC1s and cDC2s express CD103 in the gut, and

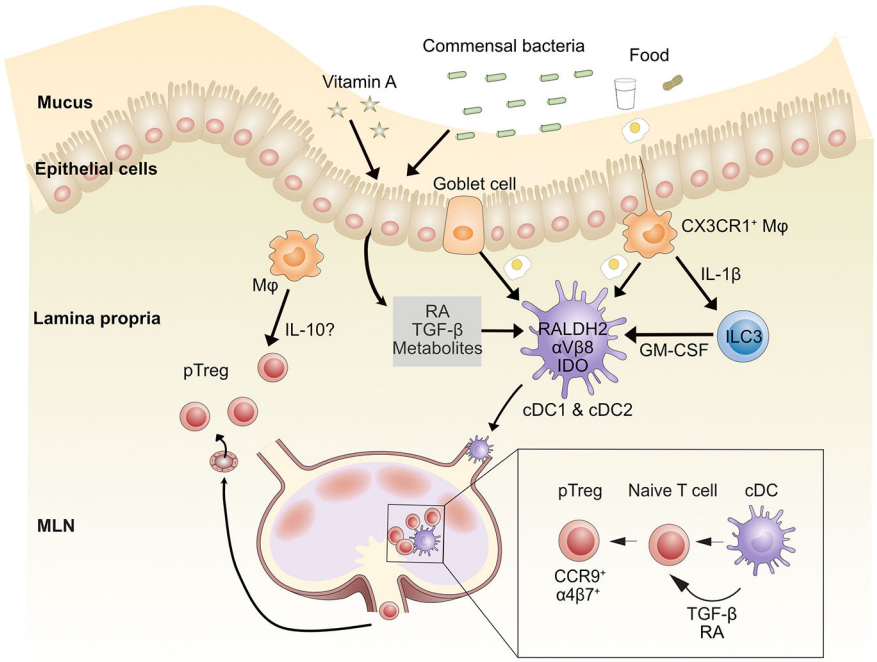


FIGURE 2 | Mechanisms by which gut-associated dendritic cells contribute to oral tolerance. After food ingestion, goblet cells and intestinal resident macrophages sample luminal food antigens and deliver them to LP CD103$^+$ cDCs (including CD103$^+$CD11b$^-$cDC1s and CD103$^+$CD11b$^+$ cDC2s). Commensal bacterial metabolites, dietary components such as vitamin A, and epithelial cell-derived TGF-β and retinoic acid (RA) imprint tolerogenic properties on cDCs. These cDCs migrate to MLNs through afferent lymphatic vessels and induce naïve CD4$^+$ T cells to differentiate into peripheral regulatory T (pTreg) cells through TGF-β and RA. CD103$^+$ cDCs induce gut homing molecules CCR9 and α4β7 on pTreg cells, which directs them to recirculate to intestinal tissue. Once in the lamina propria, pTreg cells can be further expanded by macrophages (Mφ), possibly *via* IL-10 production. MLN, mesenteric lymph node; RA, retinoic acid. Conventional dendritic cell (cDC), Innate lymphoid cell type 3 (ILC3).

therefore in many studies it is difficult to know which cDC subset is responsible for tolerance. More recent work has distinguished the two cDC populations and found that although murine CD103$^+$CD11b$^-$ cDC1s are more efficient pTreg cell inducers compared with CD103$^+$CD11b$^+$ cDC2s, the two subsets may play redundant roles in gut pTreg cell induction and oral tolerance (75, 76).

Several mechanisms have been identified for pTreg cell induction by cDCs. In the intestine, CD103$^+$ cDCs express the RALDH2 enzyme, which metabolizes vitamin A to retinoic acid (RA) (41, 75, 77, 78). RA induces the expression of gut-homing molecules CCR9 and α4β7 integrin on T cells (62, 79–82). Human intestinal cDC2s express higher RALDH2 and αVβ8 and induce more Treg cells than cDC1s *in vitro* (30, 83). Murine cDC1s and cDC2s also activate latent TGF-β through integrin αVβ8 (20, 75, 82, 84–86). RA synergizes with TGF-β to induce pTreg cell differentiation *in vitro* (78, 79, 87–90) and IgA class switching of B cells in PPs (86). Mice lacking αVβ8 on DCs have reduced Treg cells in colonic tissue (91). Moreover, TGF-β along with RA can increase β8 expression on cDC1s, thereby creating a positive feedback loop and strengthening the regulatory function of cDC1s (92). Human and mouse gut CD103$^+$ cDCs also express indoleamine 2,3-dioxygenase (IDO), an enzyme involved in tryptophan catabolism, which can reduce local tryptophan concentrations and produce immunomodulatory tryptophan metabolites. This can induce Foxp3$^+$ Treg cell conversion and oral tolerance (93, 94). An earlier study showed that programmed death ligand 1 (PD-L1, B7-H1) and PD-L2 (B7-DC) expressed on MLN DCs were required for the generation of antigen-specific CD4$^+$Foxp3$^+$ Treg cells (95). A more recent study instead found that CD11b$^-$CD103$^+$PD-L1^{high} cDC1s induce Treg cells through RA production and/or activation of TGF-β but that expression of PD-L1 or PD-L2 were dispensable (80). It is unclear whether specific culture conditions explain these inconsistencies, so more work needs to be done to clarify the function of PD-L1 and PD-L2 on DCs in Treg cell induction.

Many aspects of the gut microenvironment promote DC induction of Tregs. MUC2, the building block of gut mucus, imprints DCs to deliver tolerogenic signals promoting pTreg cells and oral tolerance (96). Both mouse and human intestinal epithelial cells can also directly promote the differentiation of tolerogenic DCs and *in vitro* generation of Tregs (87, 88). Finally, we will discuss the effect of the microbiome on gut DC function below.

Plasmacytoid Dendritic Cells

pDCs can also mediate oral tolerance. Infants who are tolerant to peanut ingestion, but possess peanut IgE, a state called sensitized tolerance, display an increased frequency of pDCs in the blood (97). In cholera toxin (CT)-induced peanut sensitization in mice, expansion of DC numbers by Flt3L, in particular pDCs, inhibits

allergic manifestations in the intestine (98). Mucosal pDCs promote the induction of antigen-specific pTreg cells through an autocrine loop involving TGF-β; pDC-ablated mice partly reduce pTreg cell generation in the MLNs after OVA feeding (99). After protein or hapten antigen ingestion, pDCs in the liver and MLN delete antigen-specific $CD8^+$ T cells and efficiently induce oral tolerance through an unknown mechanism (100, 101).

Macrophages

Although gut-resident $CX3CR1^+$ macrophages do not migrate to MLNs (28, 41), they contribute to pTreg cell generation and oral tolerance by transferring gut lumen antigens to migratory $CD103^+$ cDCs, *via* a mechanism that was shown to be Connexin 43-dependent and required membrane transfer (20). LP macrophages can also maintain $Foxp3^+$ Treg cells by a mechanism dependent on IL-10 (102). CX3CR1-deficient mice, which have reduced IL-10-producing $F4/80^+CD11b^+MHC\text{-}II^{int}$ macrophages, have impaired accumulation of $FoxP3^+$ Treg cells in the LP and oral tolerance (62). However, in two colitis studies, $CX3CR1^+$ macrophage-derived IL-10 was dispensable for maintenance of colonic Tregs; instead, loss of IL-10 receptor expression on the macrophages themselves impaired mucosal homeostasis (103, 104).

APCS AND THE MICROBIOME IN ORAL TOLERANCE

The gastrointestinal tract is colonized by large numbers of commensal microbes that contribute to the maintenance of intestinal homeostasis including protection from food allergy (105). Early life colonization is important for suppressing inappropriate IgE induction (106). Both food-allergic infants and mice demonstrate dysbiosis, and restoring particular bacterial classes such as Clostridium species reduced susceptibility to food allergy and was associated with enhanced Tregs (107–109). The microbiome can promote barrier integrity, which can preclude APCs from encountering food antigen in an inflammatory context. Clostridium species have been shown to promote the production of IL-22, which led to decreased systemic absorption of peanut allergens by increasing intestinal barrier integrity *via* the production of antimicrobial peptides and mucus (109). In addition, bacterially produced SCFA can promote inflammasome activation and IL-18 release in colonic epithelial cells, which then help maintain gut homeostasis in a chemically-induced colitis mouse model; a similar mechanism could be at play in food tolerance as well.

There are several mechanisms by which bacteria may act on APCs to protect against food allergy. First, when certain strains of bacteria like Clostridia metabolize dietary fiber in the gut, they produce short chain fatty acids (SCFA), such as butyrate and acetate, which promote the development of Tregs. SCFA bind to the receptors GPR43 and GPR109A to enhance MLN $CD103^+$ DC activity by upregulation of RALDH2, which prevents food allergy development in a murine model (110). A study in milk-allergic children found that children fed with extensively hydrolyzed formula and *Lactobacillus rhamnosus* GG supplements were more likely to outgrow their milk allergy in part because of changes in their microbiome that led to more butyrate in the stool (111), suggesting a possible role for SCFA on human DCs. Recently, metabolism of bile acid by the microbiota has also been shown to promote Treg generation. Bacterial bile acid metabolism generates biologically active steroids. One such product, 3β-hydroxydeoxycholic acid (isoDCA), acts on DCs through the farnesoid X receptor to promote Treg formation (112). It is feasible that these bile metabolism products may play a role in food tolerance as well, but that remains to be tested. Finally, microbiota can help regulate the myeloid cell populations within the gut. Mortha and colleagues showed that microbiota promoted the release of GM-CSF by ILC3s by driving macrophage IL-1β production (113). GM-CSF locally enhanced DC and macrophage numbers and their ability to produce regulatory factors like RA, TGF-β, and IL-10; ablation of GM-CSF reduced Treg cell numbers and impaired oral tolerance (113, 114).

APCS AND TOLERANCE INDUCTION *VIA* IMMUNOTHERAPY

Various forms of immunotherapy are being studied for the treatment of food allergy—these include oral, sublingual and epicutaneous applications of low amounts of food allergens. Immunotherapy alters the cellular and humoral arms of allergy, reducing IgE and enhancing IgG4 (in humans) as well as suppressing T cell, mast cell and basophil reactivity to the target allergen. Immunotherapy has been shown to capitalize on many of the tolerogenic pathways of APCs described above. In particular, cDCs and pDCs from the blood have been shown to adopt, at least transiently, a less inflammatory state after immunotherapy and promote Treg properties *in vitro* (115, 116). Successful food allergen immunotherapy is also associated with increased levels of circulating Tregs (116). In murine studies, immunotherapy with allergens induces TGF-β-producing Tregs in draining LNs capable of homing to the gut, suppressing the allergic response to food challenge and redirecting $CD4^+$ effector T cell differentiation away from a Th2 phenotype (68, 117). Looking at sites draining sublingual allergen exposure, migratory cDC2s were proposed to be the dominant APC responsible for Treg induction through a mechanism that, *in vitro*, required RA and TGF-β (118).

APCS IN FOOD ALLERGY PATHOGENESIS

The gut immune system must continuously distinguish innocuous dietary antigens and commensal microbes from pathogens. A breakdown of the default oral tolerance to food leads to abnormal immune responses that manifest as diverse pathologies, such as IgE-mediated food allergy, celiac disease, and eosinophilic gastrointestinal disease, among many others (119). In each of these conditions, adaptive immunity is targeted at a food antigen, presumably all *via* presentation on an APC, but through distinct mechanisms. For example, IgE induction is implicated in IgE-mediated food allergy, but not in celiac disease, which is instead a cell-mediated disease initiated by the presentation of modified gluten on APCs (120). Elucidating

the conditions under which APCs are activated in each type of adverse food reactions may provide insight into the differing responses. Here we describe what is known about APCs in the pathogenesis of IgE-mediated food allergy.

Food as an Innate Immune Stimulus for DCs

APCs are important in tolerance induction, but they also play a pivotal role in the induction of food allergy (**Figure 3**). Of all the APCs, DCs have the best-defined role in the initiation of food allergy. DCs reside in tissues, where they serve as sentinels that are activated by innate stimuli. The identity of the innate stimuli that can activate DCs to initiate food allergy is unclear, but both intrinsic food components and extrinsic adjuvants are potential innate stimuli currently under investigation.

There is evidence that certain foods can act as auto-adjuvants. Many of these innately immunostimulatory foods are glycoproteins that bind to dendritic cell C-type lectin receptors (CLR), a family of proteins that traditionally bind carbohydrate residues in a calcium-dependent manner (121). One group identified that the glycans on the allergenic peanut protein Ara h 1 bind to the CLR dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) on human monocyte-derived DCs and subsequently activate the DCs; these DCs then promote Th2 activation *in vitro* (122). Another group tested the ability of various food allergens and aeroallergens to bind DC-SIGN and the related DC-SIGNR on human monocyte-derived DCs and found that among other allergens, hazelnut, walnut, and egg white could also bind these CLRs. Downstream of these CLRs, the kinases ERK and Raf-1 are upregulated and TNF-α, which is important for DC activation (123), is produced in a partially Raf-1 dependent manner (124).

It has been observed that high-temperature roasting of peanuts increases the allergenicity of peanut proteins (125). Roasting causes peanut protein to undergo the Maillard reaction, which leads to more heat- and digestion-resistant peanut antigens, perhaps allowing for more antigen to reach the relevant sites of IgE induction (126). However, roasting can also lead to the generation of glycoproteins that bind the mannose receptor, a CLR that mediates antigen uptake and appears to play an important role in DC activation. One group has shown that human monocyte-derived DCs take up more roasted peanut protein Ara h 3 than raw Ara h 3, through a mechanism that is partially dependent on the mannose receptor (127). The mannose receptor has also been shown to play a role in peanut protein Ara h 2 uptake by human monocyte-derived DCs *in vitro* (128). Additionally, treating mouse bone marrow-derived DCs with mannose receptor RNAi reduced ovalbumin uptake and DC activation (129). Whether these CLRs are necessary for food allergen sensitization is still unknown.

Food may also act as an intrinsic adjuvant for DC activation by activating invariant natural killer T cells (iNKT). iNKT cells are a population of innate-like cells that display a semi-invariant T-cell receptor that binds lipid antigens presented on the MHC-I-like molecule CD1d on DCs and can in turn promote DC activation (130–132). One group found that sensitization to Brazil nuts is dependent on CD1d lipid presentation to iNKT cells in mice, and human iNKT cells are stimulated by the lipid fraction of Brazil nuts (133). Additionally, lipids from the respiratory allergen *Olea europea* (olive) pollen increase CD1d expression on DCs to activate iNKT cells and also upregulate DC activation marker CD86 (134), a mechanism that could also apply food lipids. Indeed, food lipids from cow's milk (135, 136), soy (136), and human milk (136) have also been shown to activate human iNKT cells *in vitro*. However, it remains unknown whether iNKT cells orchestrate food allergen sensitization and their exact *in vivo* role in food allergy pathogenesis.

Gut Adjuvants for Food IgE Sensitization

While the innate immunostimulatory activity of food may bias which foods can act as allergens, it is unlikely to be the only factor affecting the development of food allergy. The innate properties of food cannot explain differential responses to allergens between people, i.e. why most people tolerate food while some develop allergy. Genetic differences can influence some of this susceptibility (137), but the rapidly growing rate of food allergy does not support a solely genetic cause either. Instead, it is likely that there are increasingly prevalent extrinsic factors, such as external adjuvants, that influence activation of DCs to initiate sensitization to food. Accordingly, mouse models have demonstrated that breaking oral tolerance to food antigens, including both cellular and humoral immunity, requires the presence of an adjuvant (138–140). Adjuvants such as cholera toxin (CT) or staphylococcal enterotoxin B (SEB), are most often co-administered orally with food antigens to induce IgE and Th2 cells while inhibiting Tregs. Aluminum hydroxide is another commonly used adjuvant in allergy models that has potent immunostimulatory properties on DCs but is administered in the peritoneum and cannot directly interact with the gut immune system. Therefore, this adjuvant will not be further discussed.

CT is a potent oral adjuvant because it induces both human and mouse DC activation and migration (47, 141–143). CT enters DCs and other cells using the GM1-ganglioside receptor (144, 145). Though the mechanism of action of CT is not completely known, it activates adenylate cyclase, which increases intracellular cyclic adenosine monophosphate (cAMP) levels, which in turn leads to DC activation (146, 147). These CT-activated DCs have been shown to promote Th1, Th2, and Th17 responses (147–149), and they are effective at generating both IgE and IgA in food allergy models. CT is a member of the AB5 toxin family, which includes toxins with similar structures and mechanisms of action such as shigatoxin (*Shigella dysenteriae*), labile toxin (enterotoxigenic *E. coli*), and pertussis toxin. Exposure to other members of the AB5 family may also activate DCs and induce IgE in a similar manner as CT (143, 150). While exposure to CT is an unlikely mechanism of allergy induction in humans, data gleaned using adjuvants can give clues to the broader mechanisms by which innate stimuli initiate IgE responses to food.

SEB is a superantigen made by *Staphylococcus aureus*, which is a common microbial colonizer and determinant of disease severity in people with atopic dermatitis (151). Mouse models of food allergy have used SEB as an adjuvant, both epicutaneously, intragastrically, and intraperitoneally (140, 152, 153). Human

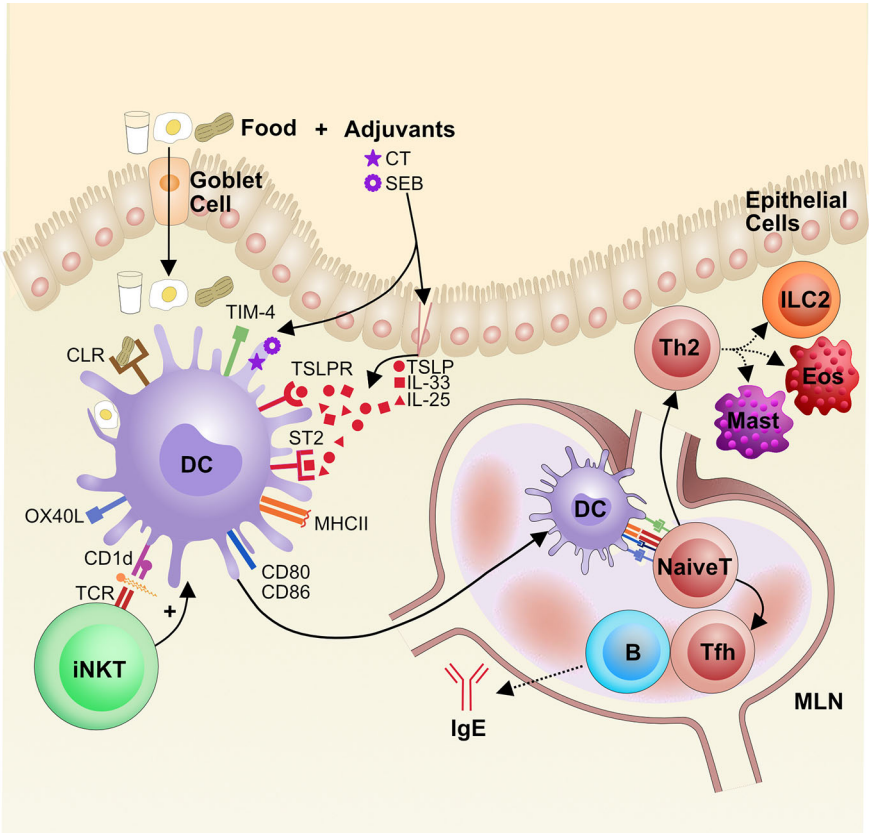


FIGURE 3 | The role of dendritic cells in the pathogenesis of food allergy. Food antigens are taken up from the gut lumen by goblet cells, which shuttle the antigens across the epithelial layer to the LP, where local dendritic cells (DCs) sample the food antigens. If DCs sense innate immune signals, adjuvants that are either extrinsic or intrinsic to the food antigen, they become activated. Some adjuvants damage the epithelial barrier and trigger the release of alarmins, like TSLP and IL-33, that can activate DCs *via* their receptors TSLPR and ST2, respectively. Additionally, food glycoproteins, such as from peanut, can bind to C-type lectin receptors (CLRs) and activate DCs. Lipids from foods can be presented on CD1d to iNKT cells that then reciprocally activate DCs *via* cytokine release. Activation of DCs leads to increased CCR7 for migration to mesenteric lymph nodes (MLN) along with presentation of food antigens on MHCII and increased expression of costimulatory molecules CD80, CD86, OX40L, and TIM-4. Altogether this promotes naïve $CD4^+$ T cell priming and differentiation into Th2 cells and T follicular helper (Tfh) cells, which drive cellular and IgE responses in food allergy, respectively. Eos, eosinophils (Eos), Mast cell (Mast), Innate lymphoid cells type 2 (ILC2).

monocyte-derived DCs are activated by SEB, at least in part through Toll-like receptor 2 (TLR2), but do not upregulate IL-12 production; accordingly, *in vitro* culture of these DCs with T cells leads to Th2 polarization (154). Using mouse mucosal DCs, SEB was also shown to promote DC activation through the cell surface molecule T-cell immunoglobulin-domain and mucin-domain-4 (TIM-4) and promote T cell activation *in vitro* (153).

Alarmins and damage-associated molecular patterns (DAMPs) are self-molecules that the immune system recognizes as distress signals; they are often released during cell death or damage and are important triggers for DC activation. Uric acid is a DAMP that can activate pattern recognition receptors and thereby initiate adaptive immunity in multiple immunization models; it has also been implicated as an adjuvant for food IgE production (155). Similarly, eosinophil peroxidase released by activated eosinophils activates DCs, which migrate to the MLNs and promote the induction of peanut IgE after immunization with peanut and CT (156). Many studies have focused on cytokine alarmins as part of the innate immune response that initiates food allergy. IL-25, IL-33, and thymic stromal lymphopoietin (TSLP) are cytokines released by damaged epithelium and promote type 2 responses across most tissues (157–159). The role of these cytokines in gut IgE induction with peanut and CT was examined, and IL-33, in particular, was found to be necessary for IgE production, whereas IL-25 and TSLP were dispensable. Mechanistically, this was proposed to work through upregulation of OX40L on activated DCs (160). In a different model using egg-derived ovalbumin and medium-chain triglycerides, dietary lipids that stimulate the release of alarmins from the intestinal epithelium (161), IL-25, IL-33, and TSLP were each necessary for the development of allergy (162). In another model of ovalbumin-directed food allergy, IL-25 activated ILC2s in the gut to produce IL-5 and -13 and, in concert with activated Th2 cells, promoted anaphylaxis (10). As will be discussed later in this section, IL-33 and TSLP have also been implicated in food sensitization through the skin. Therefore, alarmins can trigger type 2 immunity, but whether one alarmin has a dominant role in the initiation of food allergy likely depends on the nature of the antigen, adjuvant and route of exposure.

Gut DC Populations Involved in Food Allergy

Activated DCs are sufficient to induce food IgE, as evidenced by a study showing that adoptive transfer of splenic and Peyer's patch DCs from mice sensitized with milk and CT led to milk IgE production in naïve mice (163). Because different DC populations have different functions, it is plausible that food allergens or adjuvants activate a common DC subset that is efficient at priming the requisite T cell populations for IgE responses. Several groups have used mouse models to examine particular subpopulations of DCs activated in food allergy. One group reported that mice sensitized orally with peanut and CT experienced global changes to DC populations in the gut. $CD11b^+$ cDCs were increased, and $CD103^+$ cDCs were decreased in Peyer's patches and among intraepithelial lymphocytes and lamina propria lymphocytes; both populations of cDCs were increased in the MLNs, which may represent a net migration of $CD103^+$ DC to the MLN (98). Another group also demonstrated that mice orally sensitized with ovalbumin and CT had increased total DC numbers in the MLN. Among these MLN DCs, the $CD103^+CD11b^-CD8^-$ population was selectively increased (164). These findings were corroborated by a study showing that $CD103^+$ MLN DCs activate and migrate in an eosinophil-dependent manner after oral peanut and CT immunization (156). These data suggest that a migratory cDC population in the MLN is important for gut IgE induction when using CT as an adjuvant. However, the exact nature of the DC subsets essential for sensitization remains unclear; specifically, whether DC subsets are redundant for sensitization or operate differently depending on the nature of the allergen and adjuvant is unknown.

Mechanisms of DC Induction of Food Allergy in the Gut

DCs have been shown to use multiple pathways to induce IgE sensitization to food antigens. First, OX40 ligand (OX40L) on DCs has an important role in Th2 sensitization to food. OX40L is a costimulatory molecule present on activated DCs and important in Th2 development (165). In a mouse model of oral ovalbumin and CT sensitization, activated DCs expressed increased levels of OX40L mRNA, while blocking OX40L with an anti-OX40L antibody in an *in vitro* DC-T cell co-culture reduced type 2 cytokine production (164). Another group showed that OX40L is upregulated after intragastric immunization with peanut and CT in an IL-33 dependent manner and that blocking OX40L *in vivo* in mice reduced peanut IgE and IgG1 levels post-immunization (160).

Another important DC pathway for priming allergic responses involves TIM-4, which is expressed on DCs and binds TIM-1 on T cells to influence Th2 cell development (153, 166). When treated with SEB, primary human DCs upregulate TIM-4 and can drive Th2 differentiation *in vitro* (167). Immunization with peanut and CT similarly led to increased TIM-4 expression that was necessary for peanut IgE production in mice (168). Another group investigated the stimuli for TIM-4 production and found that mast cell tryptase stimulates human intestinal epithelial cells to make galectin-9, a carbohydrate-binding lectin protein. Galectin-9 binds to TIM-3 on DCs and is associated with the production of TIM-4, which is needed for sustaining ovalbumin IgE levels after immunization (169, 170). Increased expression of TIM-4 on DCs may also be mediated by STAT6 and p300 (171).

There has also been interest in the Notch pathway in food allergy. Signaling through Notch receptors on $CD4^+$ T cells is important for T cell differentiation; different ligands promote different T cell fates (172). In particular, the Notch ligands Jagged 1 and Jagged 2 are expressed on DCs and promote Th2 differentiation (172, 173). Of note, treatment with CT increases Jagged2 expression on DCs (173), which is consistent with the increased Jagged 2 mRNA seen after ovalbumin and CT immunization in mice (164). Even though Jagged2 expression on DCs is needed for Th2 differentiation *in vitro*, it appears to be dispensable *in vivo* (174).

DCs in Food Allergy Induction Through the Skin

Systemic IgE can be induced through antigen exposure at sites where the body interfaces with the environment, including the gut, respiratory tract, and skin (175). In particular, defects in the skin barrier are associated with the development of food allergy (22). While there is evidence pointing to the skin as an important site of food IgE induction, the APC subsets and mechanism of action underlying cutaneous sensitization remain only partially understood.

As with gut models of food allergy, adjuvants are used in cutaneous models of food sensitization in mice. CT and SEB have been used topically to induce food IgE (175, 176). Additionally, skin damage leading to alarmin (IL-33, TSLP, and IL-25) release can act as an innate stimulus for DC activation in mouse models of cutaneous allergy; this may mirror the skin barrier break down in people with eczema, who are more susceptible to food allergy (177). There are various methods of incurring or mimicking damage to mouse skin, including by mechanical tape stripping, application of large doses of vitamin D analogs, treatment with proteases, or by directly administering TSLP. These methods have all been used as adjuvants with cutaneous application of food to induce food IgE (139, 178, 179).

In murine models of food allergy, IgE can be induced epicutaneously without extrinsic adjuvants (180–182), differing from most gut sensitization models. In an external adjuvant-free model of peanut allergy, application of peanut extract to depilated mouse skin was able to induce peanut IgE. Peanut extract and Ara h 2 extract had intrinsic adjuvant activity and were capable of initiating IgE to a co-administered milk antigen, alpha-lactalbumin. In response to peanut extract, mouse skin cells made IL-33, which presumably binds to the IL-33 receptor, ST2, on DCs; indeed, the subsequent production of type 2 cytokines in this model was dependent on ST2 signaling (180). The mechanism of innate sensing of peanut leading to IL-33 production by keratinocytes is unclear, but perhaps the ability of glycoproteins on peanut to bind CLRs plays a role, as described in the gut. It is possible that peanut auto-adjuvanticity observed in the skin is stronger than in the gut because the immunostimulatory portion of peanut is sensitive to digestion.

IL-33 also plays an important role in adjuvanted models of cutaneous food sensitization because it can act on DCs and promote food sensitization. In an intradermal ovalbumin and TSLP model of atopic dermatitis and food sensitization, keratinocyte derived IL-33 was necessary for ovalbumin IgE production (183). Similarly, in a model of skin sensitization with peanut and tape stripping to disrupt the skin barrier, IL-33 is increased and contributes to the allergic phenotype (184). The allergic responses from IL-33 in these models likely depend on DC expression of ST2, as in the adjuvant-free epicutaneous model and in other models of IL-33 activated DCs (185, 186).

TSLP, a keratinocyte cytokine that is important in atopic dermatitis pathogenesis, also plays a key role in sensitization through the skin (187). In a mouse model of intradermal ovalbumin sensitization using TSLP as an adjuvant, TSLP signaling in DCs was required for ovalbumin IgE production and subsequent anaphylaxis (188). This response may be mediated by TSLP-induced upregulation of OX40L, which promotes type 2 responses both *in vitro* using human DCs and in mice *in vivo* (189, 190). However, in a mouse tape stripping model of skin injury, while TSLP signaling on DCs was needed for Th2 differentiation, OX40L was not upregulated in skin DCs; however, the Th2 inhibitory cytokine IL-12 was suppressed in skin DCs (191). The different data for the role of OX40L in Th2 differentiation may be due to use of different species and models of investigation between studies. In another mouse model of epicutaneous sensitization using ovalbumin with the vitamin D analog MC903 to induce a skin barrier defect, TSLP-induced basophils were necessary for the development of gut allergy (192). *In vitro*, these TSLP-induced basophils interacted with DCs to increase OX40L expression, which in turn, increased IL-4 production by basophils (178).

The cell types and mechanisms of DC initiation of epicutaneous skin allergy have also been examined. In a tape stripping mouse model of allergic inflammation, skin injury led to DC activation and migration, as evidenced by a population of $CCR7^+MHCII^+$ DCs was found in the skin draining LNs 24 h after tape stripping. These DCs were able to prime T cells to produce type 2 cytokines *in vitro* (191). Similarly, in another model of epicutaneous sensitization, after the application of milk protein alpha-lactalbumin (ALA) and CT, Langerin-negative skin DCs increased expression of MHCII and migrated to skin draining LNs, where they promoted type 2 cytokine production and systemic ALA IgE production (175). In a model of subcutaneous ovalbumin and papain skin sensitization, this migratory skin DC population was also required for ovalbumin IgE production and was found to be $PDL2^+$ and dependent on the transcription factor IRF4 (193). It is clear that sensitization through the skin induces a systemic T cell response, that can home to the gut to orchestrate a food-specific response. But skin-derived cues can have gut-specific effects as well. Recent work showed that keratinocyte-derived IL-33 induced by skin damage communicates with cells in the gut to promote IL-25 production and ILC2 activation; this enhanced mast cell numbers and anaphylaxis following oral antigen challenge (194). Therefore, it is clear that a unique skin-gut axis exists that can promote food allergy through numerous mechanisms.

The Role of Monocytes in Food Allergy

Monocytes have also been implicated in food allergy. Infants who develop food allergy were found to have a higher number of cord blood monocytes at birth; when treated with the TLR4 agonist lipopolysaccharide (LPS) *in vitro*, the $CD14^+$ monocytes from allergic children produced more inflammatory cytokines IL-1β, IL-6, and TNF-α that promoted the development of a Th2-like population at the expense of a tolerogenic Treg population (195). Another study similarly revealed that the peripheral blood mononuclear cells (PBMCs) of 1-year-old infants that ended up with persistent egg allergy had more monocytes and DCs that made more inflammatory cytokines upon *in vitro* stimulation than those from children who outgrew their egg allergy (196). It is possible that these blood monocytes are precursors for macrophages or monocyte-derived DCs that participate in antigen presentation of food antigens (197). Altogether, these studies suggest that monocytes are biased to respond differently to inflammatory stimuli in those with food allergy; whether this is a cause of or caused by the food allergic state is unclear. There is also little mechanistic data from animal studies implicating monocytes in IgE sensitization. Future studies would be beneficial for a deeper understanding of the topic.

The Role of Macrophages in Food Allergy

Contrary to their well-established role in food tolerance, the role of macrophages in food IgE priming is not well understood, and there is scant literature on the topic. Macrophages express DC-SIGN (198) and TIM-4 (199), both of which may participate in the priming of food-specific IgE. Additionally, macrophages are found in tissues throughout the body, including the skin and gut (200, 201), so they are poised to potentially play a role in food antigen presentation. Macrophages that are found in Th2 conditions appear to play an IL-33 dependent role in allergic asthma (202–205). However, given the importance of macrophages within tissues both for tissue homeostasis and presenting antigen to primed effector T cells, rather than as APCs for naïve T cells, it is likely that macrophages will be required for different phases of food allergy pathogenesis than DCs. Therefore, more information is needed to elucidate the exact function of macrophages in food sensitization.

CONCLUSION

While much work has been done to examine the role of APCs in priming food IgE, there are still many unanswered questions. In particular, the specific population of APCs that lead to food IgE production in the skin and gut should be identified, ideally using several adjuvants to home in on common mechanisms of food IgE production. It would also be useful to study the APC requirements for other nonpathogenic antibody isotypes to food such as IgA and IgG4 to better understand what APC conditions separate tolerance from allergy. Another fundamental question is the identity of innate immune stimuli that lead to DC activation in human food allergy; an understanding of what natural skin or gut adjuvants lead to food IgE induction would be

a significant advance in understanding pathophysiology and potential treatments for food allergy. Additionally, the relevance of monocytes and macrophages to food allergy induction needs clarification. Research on these and many other questions in food allergy are revealing new, unexpected pathways unique to the gut immune system and suggesting exciting new approaches for the diagnosis, prevention and treatment of food allergy.

AUTHOR CONTRIBUTIONS

EL, XY, AS, and SE wrote the manuscript and designed the figures. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by Food Allergy Research & Education (FARE)- The Ira & Diana Riklis Family Research Award in Food Allergy (SE), a gift from the Colton Foundation (SE), R01 AI136942 (SE), the Sean N Parker Center for Allergy and Asthma Research (SE), 5T32AR007107 (EL), and NCATS Grant UL1TR001863 (EL). Yale University provides funds for open access publication in Frontiers.

ACKNOWLEDGMENTS

We thank all Eisenbarth lab members for helpful discussion and review of this manuscript.

REFERENCES

1. Gupta RS, Springston EE, Warrier MR, Smith B, Kumar R, Pongracic J, et al. The Prevalence, Severity, and Distribution of Childhood Food Allergy in the United States. *Pediatrics* (2011) 128(1):e9–17. doi: 10.1542/peds.2011-0204
2. Sicherer SH. Epidemiology of food allergy. *J Allergy Clin Immunol* (2011) 127(3):594–602. doi: 10.1016/j.jaci.2010.11.044
3. Tang MLK, Mullins RJ. Food allergy: is prevalence increasing? *Internal Med J* (2017) 47(3):256–61. doi: 10.1111/imj.13362
4. Branum, Lukacs SL. Food Allergy Among Children in the United States. *Pediatrics* (2009) 124(6):1549–55. doi: 10.1542/peds.2009-1210
5. Sicherer SH, Sampson HA. 9. Food allergy. *J Allergy Clin Immunol* (2006) 117(2, Supplement 2):S470–5. doi: 10.1016/j.jaci.2005.05.048
6. Sampson HA, Aceves S, Bock SA, James J, Jones S, Lang D, et al. Food allergy: a practice parameter update-2014. *J Allergy Clin Immunol* (2014) 134(5):1016–25.e43. doi: 10.1016/j.jaci.2014.05.013
7. Sicherer SH, Noone SA, Muñoz-Furlong A. The impact of childhood food allergy on quality of life. *Ann Allergy Asthma Immunol* (2001) 87(6):461–4. doi: 10.1016/S1081-1206(10)62258-2
8. Anvari S, Miller J, Yeh CY, Davis CM. IgE-Mediated Food Allergy. *Clin Rev Allergy Immunol* (2019) 57(2):244–60. doi: 10.1007/s12016-018-8710-3
9. Yu W, Freeland DMH, Nadeau KC. Food allergy: immune mechanisms, diagnosis and immunotherapy. *Nat Rev Immunol* (2016) 16(12):751–65. doi: 10.1038/nri.2016.111
10. Lee JB, Chen CY, Liu B, Mugge L, Angkasekwinai P, Facchinetti V, et al. IL-25 and CD4(+) TH2 cells enhance type 2 innate lymphoid cell-derived IL-13 production, which promotes IgE-mediated experimental food allergy. *J Allergy Clin Immunol* (2016) 137(4):1216–25.e5. doi: 10.1016/j.jaci.2015.09.019
11. Chen C-Y, Lee J-B, Liu B, Ohta S, Wang P-Y, Kartashov AV, et al. Induction of Interleukin-9-Producing Mucosal Mast Cells Promotes Susceptibility to IgE-Mediated Experimental Food Allergy. *Immunity* (2015) 43(4):788–802. doi: 10.1016/j.immuni.2015.08.020
12. Noval Rivas M, Burton OT, Oettgen HC, Chatila T. IL-4 production by group 2 innate lymphoid cells promotes food allergy by blocking regulatory T-cell function. *J Allergy Clin Immunol* (2016) 138(3):801–11.e9. doi: 10.1016/j.jaci.2016.02.030
13. Burton OT, Noval Rivas M, Zhou JS, Logsdon SL, Darling AR, Koleoglou KJ, et al. Immunoglobulin E signal inhibition during allergen ingestion leads to reversal of established food allergy and induction of regulatory T cells. *Immunity* (2014) 41(1):141–51. doi: 10.1016/j.immuni.2014.05.017
14. Gowthaman U, Chen JS, Eisenbarth SC, Zhang B, Flynn WF, Lu Y, Song W, et al. Identification of a T follicular helper cell subset that drives anaphylactic IgE. *Science* (2019) 365(6456):eaaw6433. doi: 10.1126/science.aaw6433
15. Reber LL, Marichal T, Mukai K, Kita Y, Tokuoka SM, Roers A, et al. Selective ablation of mast cells or basophils reduces peanut-induced anaphylaxis in mice. *J Allergy Clin Immunol* (2013) 132(4):881–8.e11. doi: 10.1016/j.jaci.2013.06.008
16. Brandt EB, Strait RT, Hershko D, Wang Q, Muntel EE, Scribner TA, et al. Mast cells are required for experimental oral allergen-induced diarrhea. *J Clin Invest* (2003) 112(11):1666–77. doi: 10.1172/JCI19785
17. Finkelman FD, Khodoun MV, Strait R. Human IgE-independent systemic anaphylaxis. *J Allergy Clin Immunol* (2016) 137(6):1674–80. doi: 10.1016/j.jaci.2016.02.015
18. Noah TK, Knoop KA, McDonald KG, Gustafsson JK, Waggoner L, Vanoni S, et al. IL-13-induced intestinal secretory epithelial cell antigen passages are required for IgE-mediated food-induced anaphylaxis. *J Allergy Clin Immunol* (2019) 144(4):1058–73.e3. doi: 10.1016/j.jaci.2019.04.030
19. McDole JR, Wheeler LW, McDonald KG, Wang B, Konjufca V, Knoop KA, et al. Goblet cells deliver luminal antigen to CD103+ dendritic cells in the small intestine. *Nature* (2012) 483(7389):345–9. doi: 10.1038/nature10863
20. Mazzini E, Massimiliano L, Penna G, Rescigno M. Oral tolerance can be established via gap junction transfer of fed antigens from CX3CR1$^+$ macrophages to CD103$^+$ dendritic cells. *Immunity* (2014) 40(2):248–61. doi: 10.1016/j.immuni.2013.12.012
21. Worbs T, Hammerschmidt SI, Förster R. Dendritic cell migration in health and disease. *Nat Rev Immunol* (2017) 17(1):30–48. doi: 10.1038/nri.2016.116
22. Lack G. Update on risk factors for food allergy. *J Allergy Clin Immunol* (2012) 129(5):1187–97. doi: 10.1016/j.jaci.2012.02.036
23. Eisenbarth SC. Dendritic cell subsets in T cell programming: location dictates function. *Nat Rev Immunol* (2019) 19(2):89–103. doi: 10.1038/s41577-018-0088-1
24. Guilliams M, Ginhoux F, Jakubzick C, Naik SH, Onai N, Schraml BU, et al. Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. *Nat Rev Immunol* (2014) 14(8):571–8. doi: 10.1038/nri3712
25. Becker M, Güttler S, Bachem A, Hartung E, Mora A, Jäkel A, et al. Ontogenic, Phenotypic, and Functional Characterization of XCR1(+) Dendritic Cells Leads to a Consistent Classification of Intestinal Dendritic Cells Based on the Expression of XCR1 and SIRPα. *Front Immunol* (2014) 5:326–6. doi: 10.3389/fimmu.2014.00326
26. Cerovic V, Houston SA, Scott CL, Aumeunier A, Yrlid U, Mowat AM, et al. Intestinal CD103(-) dendritic cells migrate in lymph and prime effector T cells. *Mucosal Immunol* (2013) 6(1):104–13. doi: 10.1038/mi.2012.53
27. Scott CL, Bain CC, Wright PB, Sichien D, Kotarsky K, Persson EK, et al. CCR2(+)CD103(-) intestinal dendritic cells develop from DC-committed precursors and induce interleukin-17 production by T cells. *Mucosal Immunol* (2015) 8(2):327–39. doi: 10.1038/mi.2014.70
28. Bogunovic M, Ginhoux F, Helft J, Shang L, Hashimoto D, Greter M, et al. Origin of the lamina propria dendritic cell network. *Immunity* (2009) 31(3):513–25. doi: 10.1016/j.immuni.2009.08.010
29. Muzaki ARBM, Tetlak P, Sheng J, Loh SC, Setiagani YA, Poidinger M, et al. Intestinal CD103+CD11b– dendritic cells restrain colitis via IFN-γ-induced anti-inflammatory response in epithelial cells. *Mucosal Immunol* (2016) 9(2):336–51. doi: 10.1038/mi.2015.64
30. Watchmaker PB, Lahl K, Lee M, Baumjohann D, Morton J, Kim SJ, et al. Comparative transcriptional and functional profiling defines conserved

programs of intestinal DC differentiation in humans and mice. *Nat Immunol* (2014) 15(1):98–108. doi: 10.1038/ni.2768
31. Poulin LF, Reyal Y, Uronen-Hansson H, Schraml BU, Sancho D, Murphy KM, et al. DNGR-1 is a specific and universal marker of mouse and human Batf3-dependent dendritic cells in lymphoid and nonlymphoid tissues. *Blood* (2012) 119(25):6052–62. doi: 10.1182/blood-2012-01-406967
32. Brown CC, Gudjonson H, Pritykin Y, Deep D, Lavallée VP, Mendoza A, et al. Transcriptional Basis of Mouse and Human Dendritic Cell Heterogeneity. *Cell* (2019) 179(4):846–63.e24. doi: 10.1016/j.cell.2019.09.035
33. Heger L, Hofer TP, Bigley V, de Vries IJM, Dalod M, Dudziak D, et al. Subsets of CD1c+ DCs: Dendritic Cell Versus Monocyte Lineage. *Front Immunol* (2020) 11(2575). doi: 10.3389/fimmu.2020.559166
34. Jang MH, Sougawa N, Tanaka T, Hirata T, Hiroi T, Tohya K, et al. CCR7 Is Critically Important for Migration of Dendritic Cells in Intestinal Lamina Propria to Mesenteric Lymph Nodes. *J Immunol* (2006) 176(2):803. doi: 10.4049/jimmunol.176.2.803
35. Worbs T, Bode U, Yan S, Hoffmann MW, Hintzen G, Bernhardt G, et al. Oral tolerance originates in the intestinal immune system and relies on antigen carriage by dendritic cells. *J Exp Med* (2006) 203(3):519–27. doi: 10.1084/jem.20052016
36. Rivollier A, He J, Kole A, Valatas V, Kelsall BL. Inflammation switches the differentiation program of Ly6Chi monocytes from antiinflammatory macrophages to inflammatory dendritic cells in the colon. *J Exp Med* (2012) 209(1):139–55. doi: 10.1084/jem.20101387
37. Diehl GE, Longman RS, Zhang J-X, Breart B, Galan C, Cuesta A, et al. Microbiota restricts trafficking of bacteria to mesenteric lymph nodes by CX3CR1hi cells. *Nature* (2013) 494(7435):116–20. doi: 10.1038/nature11809
38. Tamoutounour S, Henri S, Lelouard H, de Bovis B, de Haar C, van der Woude CJ, et al. CD64 distinguishes macrophages from dendritic cells in the gut and reveals the Th1-inducing role of mesenteric lymph node macrophages during colitis. *Eur J Immunol* (2012) 42(12):3150–66. doi: 10.1002/eji.201242847
39. Varol C, Vallon-Eberhard A, Elinav E, Aychek T, Shapira Y, Luche H, et al. Intestinal Lamina Propria Dendritic Cell Subsets Have Different Origin and Functions. *Immunity* (2009) 31(3):502–12. doi: 10.1016/j.immuni.2009.06.025
40. Bain CC, Scott CL, Uronen-Hansson H, Gudjonsson S, Jansson O, Grip O, et al. Resident and pro-inflammatory macrophages in the colon represent alternative context-dependent fates of the same Ly6Chi monocyte precursors. *Mucosal Immunol* (2013) 6(3):498–510. doi: 10.1038/mi.2012.89
41. Schulz O, Jaensson E, Persson EK, Liu X, Worbs T, Agace WW, et al. Intestinal CD103+, but not CX3CR1+, antigen sampling cells migrate in lymph and serve classical dendritic cell functions. *J Exp Med* (2009) 206 (13):3101–14. doi: 10.1084/jem.20091925
42. Siddiqui KRR, Laffont S, Powrie F. E-Cadherin Marks a Subset of Inflammatory Dendritic Cells that Promote T Cell-Mediated Colitis. *Immunity* (2010) 32(4):557–67. doi: 10.1016/j.immuni.2010.03.017
43. Yrlid U, Milling SW, Miller JL, Cartland S, Jenkins CD, MacPherson GG. Regulation of intestinal dendritic cell migration and activation by plasmacytoid dendritic cells, TNF-alpha and type 1 IFNs after feeding a TLR7/8 ligand. *J Immunol* (2006) 176(9):5205–12. doi: 10.4049/jimmunol.176.9.5205
44. Wendland M, Czeloth N, Mach N, Malissen B, Kremmer E, Pabst O, et al. CCR9 is a homing receptor for plasmacytoid dendritic cells to the small intestine. *Proc Natl Acad Sci* (2007) 104(15):6347. doi: 10.1073/pnas.0609180104
45. Iwasaki A, Kelsall BL. Unique functions of CD11b+, CD8 alpha+, and double-negative Peyer's patch dendritic cells. *J Immunol* (2001) 166 (8):4884–90. doi: 10.4049/jimmunol.166.8.4884
46. Iwasaki A, Kelsall BL. Localization of distinct Peyer's patch dendritic cell subsets and their recruitment by chemokines macrophage inflammatory protein (MIP)-3alpha, MIP-3beta, and secondary lymphoid organ chemokine. *J Exp Med* (2000) 191(8):1381–94. doi: 10.1084/jem.191.8.1381
47. Shreedhar VK, Kelsall BL, Neutra MR. Cholera toxin induces migration of dendritic cells from the subepithelial dome region to T- and B-cell areas of Peyer's patches. *Infect Immun* (2003) 71(1):504–9. doi: 10.1128/IAI.71.1.504-509.2003
48. Bonnardel J, Da Silva C, Wagner C, Bonifay R, Chasson L, Masse M, et al. Distribution, location, and transcriptional profile of Peyer's patch conventional DC subsets at steady state and under TLR7 ligand stimulation. *Mucosal Immunol* (2017) 10(6):1412–30. doi: 10.1038/mi.2017.30
49. Contractor N, Louten J, Kim L, Biron CA, Kelsall BL. Cutting Edge: Peyer's Patch Plasmacytoid Dendritic Cells (pDCs) Produce Low Levels of Type I Interferons: Possible Role for IL-10, TGFβ, and Prostaglandin E_2 in Conditioning a Unique Mucosal pDC Phenotype. *J Immunol* (2007) 179 (5):2690. doi: 10.4049/jimmunol.179.5.2690
50. Ziegler-Heitbrock L, Ancuta P, Crowe S, Dalod M, Grau V, Hart DN, et al. Nomenclature of monocytes and dendritic cells in blood. *Blood* (2010) 116 (16):E74–80. doi: 10.1182/blood-2010-02-258558
51. Serbina NV, Pamer EG. Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. *Nat Immunol* (2006) 7(3):311–7. doi: 10.1038/ni1309
52. Zigmond E, Varol C, Farache J, Elmaliah E, Satpathy AT, Friedlander G, et al. Ly6C hi monocytes in the inflamed colon give rise to proinflammatory effector cells and migratory antigen-presenting cells. *Immunity* (2012) 37 (6):1076–90. doi: 10.1016/j.immuni.2012.08.026
53. Bonnardel J, Da Silva C, Henri S, Tamoutounour S, Chasson L, Montanana-Sanchis F, et al. Innate and adaptive immune functions of peyer's patch monocyte-derived cells. *Cell Rep* (2015) 11(5):770–84. doi: 10.1016/j.celrep.2015.03.067
54. Joeris T, Muller-Luda K, Agace WW, Mowat AM. Diversity and functions of intestinal mononuclear phagocytes. *Mucosal Immunol* (2017) 10(4):845–64. doi: 10.1038/mi.2017.22
55. Denning TL, Norris BA, Medina-Contreras O, Manicassamy S, Geem D, Madan R, et al. Functional specializations of intestinal dendritic cell and macrophage subsets that control Th17 and regulatory T cell responses are dependent on the T cell/APC ratio, source of mouse strain, and regional localization. *J Immunol* (2011) 187(2):733–47. doi: 10.4049/jimmunol.1002701
56. Bujko A, Atlasy N, Landsverk OJB, Richter L, Yaqub S, Horneland R, et al. Transcriptional and functional profiling defines human small intestinal macrophage subsets. *J Exp Med* (2018) 215(2):441–58. doi: 10.1084/jem.20170057
57. Bain CC, Schridde A. Origin, Differentiation, and Function of Intestinal Macrophages. *Front Immunol* (2018) 9:2733. doi: 10.3389/fimmu.2018.02733
58. Bain CC, Bravo-Blas A, Scott CL, Gomez Perdiguero E, Geissmann F, Henri S, et al. Constant replenishment from circulating monocytes maintains the macrophage pool in the intestine of adult mice. *Nat Immunol* (2014) 15 (10):929–37. doi: 10.1038/ni.2967
59. Epelman S, Lavine KJ, Randolph GJ. Origin and functions of tissue macrophages. *Immunity* (2014) 41(1):21–35. doi: 10.1016/j.immuni.2014.06.013
60. Nakano H, Lin KL, Yanagita M, Charbonneau C, Cook DN, Kakiuchi T, et al. Blood-derived inflammatory dendritic cells in lymph nodes stimulate acute T helper type 1 immune responses. *Nat Immunol* (2009) 10(4):394–402. doi: 10.1038/ni.1707
61. De Koker S, Van Hoecke L, De Beuckelaer A, Roose K, Deswarte K, Willart MA, et al. Inflammatory monocytes regulate Th1 oriented immunity to CpG adjuvanted protein vaccines through production of IL-12. *Sci Rep* (2017) 7 (1):5986. doi: 10.1038/s41598-017-06236-6
62. Hadis U, Wahl B, Schulz O, Hardtke-Wolenski M, Schippers A, Wagner N, et al. Intestinal Tolerance Requires Gut Homing and Expansion of FoxP3+ Regulatory T Cells in the Lamina Propria. *Immunity* (2011) 34(2):237–46. doi: 10.1016/j.immuni.2011.01.016
63. Dubois B, Chapat L, Goubier A, Papiernik M, Nicolas JF, Kaiserlian D. Innate CD4+CD25+ regulatory T cells are required for oral tolerance and inhibition of CD8+ T cells mediating skin inflammation. *Blood* (2003) 102 (9):3295–301. doi: 10.1182/blood-2003-03-0727
64. Cassani B, Villablanca EJ, Quintana FJ, Love PE, Lacy–Hulbert A, Blaner WS, et al. Gut-Tropic T Cells That Express Integrin α4β7 and CCR9 Are Required for Induction of Oral Immune Tolerance in Mice. *Gastroenterology* (2011) 141(6):2109–18. doi: 10.1053/j.gastro.2011.09.015
65. Curotto de Lafaille MA, Kutchukhidze N, Shen S, Ding Y, Yee H, Lafaille JJ. Adaptive Foxp3+ Regulatory T Cell-Dependent and -Independent Control of Allergic Inflammation. *Immunity* (2008) 29(1):114–26. doi: 10.1016/j.immuni.2008.05.010
66. Kim KS, Hong S-W, Han D, Yi J, Jung J, Yang B-G, et al. Dietary antigens limit mucosal immunity by inducing regulatory T cells in the small intestine. *Science* (2016) 351(6275):858. doi: 10.1126/science.aac5560

67. Ganeshan K, Bryce PJ. Regulatory T Cells Enhance Mast Cell Production of IL-6 via Surface-Bound TGF-β. *J Immunol* (2012) 188(2):594. doi: 10.4049/jimmunol.1102389
68. Tordesillas L, Mondoulet L, Blazquez AB, Benhamou P-H, Sampson HA, Berin MC. Epicutaneous immunotherapy induces gastrointestinal LAP+ regulatory T cells and prevents food-induced anaphylaxis. *J Allergy Clin Immunol* (2017) 139(1):189–201.e4. doi: 10.1016/j.jaci.2016.03.057
69. Cong Y, Feng T, Fujihashi K, Schoeb TR, Elson CO. A dominant, coordinated T regulatory cell-IgA response to the intestinal microbiota. *Proc Natl Acad Sci* (2009) 106(46):19256–61. doi: 10.1073/pnas.0812681106
70. Noval Rivas M, Burton OT, Wise P, Charbonnier LM, Georgiev P, Oettgen HC, et al. Regulatory T cell reprogramming toward a Th2-cell-like lineage impairs oral tolerance and promotes food allergy. *Immunity* (2015) 42 (3):512–23. doi: 10.1016/j.immuni.2015.02.004
71. Kulkarni DH, Gustafsson JK, Knoop KA, McDonald KG, Bidani SS, Davis JE, et al. Goblet cell associated antigen passages support the induction and maintenance of oral tolerance. *Mucosal Immunol* (2020) 13(2):271–82. doi: 10.1038/s41385-019-0240-7
72. Spahn TW, Weiner HL, Rennert PD, Lügering N, Fontana A, Domschke W, et al. Mesenteric lymph nodes are critical for the induction of high-dose oral tolerance in the absence of Peyer's patches. *Eur J Immunol* (2002) 32 (4):1109–13. doi: 10.1002/1521-4141(200204)32:4<1109::AID-IMMU1109>3.0.CO;2-K
73. Kraus TA, Brimnes J, Muong C, Liu JH, Moran TM, Tappenden KA, et al. Induction of mucosal tolerance in Peyer's patch-deficient, ligated small bowel loops. *J Clin Invest* (2005) 115(8):2234–43. doi: 10.1172/JCI19102
74. Fujihashi K, Dohi T, Rennert PD, Yamamoto M, Koga T, Kiyono H, et al. Peyer's patches are required for oral tolerance to proteins. *Proc Natl Acad Sci USA* (2001) 98(6):3310–5. doi: 10.1073/pnas.061412598
75. Esterhazy D, Loschko J, London M, Jove V, Oliveira TY, Mucida D. Classical dendritic cells are required for dietary antigen-mediated induction of peripheral T(reg) cells and tolerance. *Nat Immunol* (2016) 17(5):545–55. doi: 10.1038/ni.3408
76. Welty NE, Staley C, Ghilardi N, Sadowsky MJ, Igyarto BZ, Kaplan DH. Intestinal lamina propria dendritic cells maintain T cell homeostasis but do not affect commensalism. *J Exp Med* (2013) 210(10):2011–24. doi: 10.1084/jem.20130728
77. Scott CL, Aumeunier AM, Mowat AM. Intestinal CD103+ dendritic cells: master regulators of tolerance? *Trends Immunol* (2011) 32(9):412–9. doi: 10.1016/j.it.2011.06.003
78. Bakdash G, Vogelpoel LT, van Capel TM, Kapsenberg ML, de Jong EC. Retinoic acid primes human dendritic cells to induce gut-homing, IL-10-producing regulatory T cells. *Mucosal Immunol* (2015) 8(2):265–78. doi: 10.1038/mi.2014.64
79. Hall JA, Grainger JR, Spencer SP, Belkaid Y. The role of retinoic acid in tolerance and immunity. *Immunity* (2011) 35(1):13–22. doi: 10.1016/j.immuni.2011.07.002
80. Shiokawa A, Kotaki R, Takano T, Nakajima-Adachi H, Hachimura S. Mesenteric lymph node CD11b(-) CD103(+) PD-L1(High) dendritic cells highly induce regulatory T cells. *Immunology* (2017) 152(1):52–64. doi: 10.1111/imm.12747
81. Iwata M, Hirakiyama A, Eshima Y, Kagechika H, Kato C, Song S-Y. Retinoic Acid Imprints Gut-Homing Specificity on T Cells. *Immunity* (2004) 21 (4):527–38. doi: 10.1016/j.immuni.2004.08.011
82. Benson MJ, Pino-Lagos K, Rosemblatt M, Noelle RJ. All-trans retinoic acid mediates enhanced T reg cell growth, differentiation, and gut homing in the face of high levels of co-stimulation. *J Exp Med* (2007) 204(8):1765–74. doi: 10.1084/jem.20070719
83. Fenton TM, Kelly A, Shuttleworth EE, Smedley C, Atakilit A, Powrie F, et al. Inflammatory cues enhance TGFβ activation by distinct subsets of human intestinal dendritic cells via integrin αvβ8. *Mucosal Immunol* (2017) 10 (3):624–34. doi: 10.1038/mi.2016.94
84. Worthington JJ, Czajkowska BI, Melton AC, Travis MA. Intestinal dendritic cells specialize to activate transforming growth factor-beta and induce Foxp3 + regulatory T cells via integrin alphavbeta8. *Gastroenterology* (2011) 141 (5):1802–12. doi: 10.1053/j.gastro.2011.06.057
85. Paidassi H, Acharya M, Zhang A, Mukhopadhyay S, Kwon M, Chow C, et al. Preferential expression of integrin alphavbeta8 promotes generation of regulatory T cells by mouse CD103+ dendritic cells. *Gastroenterology* (2011) 141(5):1813–20. doi: 10.1053/j.gastro.2011.06.076
86. Reboldi A, Arnon TI, Rodda LB, Atakilit A, Sheppard D, Cyster JG. IgA production requires B cell interaction with subepithelial dendritic cells in Peyer's patches. *Science* (2016) 352(6287):aaf4822. doi: 10.1126/science.aaf4822
87. Iliev ID, Spadoni I, Mileti E, Matteoli G, Sonzogni A, Sampietro GM, et al. Human intestinal epithelial cells promote the differentiation of tolerogenic dendritic cells. *Gut* (2009) 58(11):1481. doi: 10.1136/gut.2008.175166
88. Iliev ID, Mileti E, Matteoli G, Chieppa M, Rescigno M. Intestinal epithelial cells promote colitis-protective regulatory T-cell differentiation through dendritic cell conditioning. *Mucosal Immunol* (2009) 2(4):340–50. doi: 10.1038/mi.2009.13
89. Coombes JL, Siddiqui KR, Arancibia-Carcamo CV, Hall J, Sun CM, Belkaid Y, et al. A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. *J Exp Med* (2007) 204(8):1757–64. doi: 10.1084/jem.20070590
90. Sun CM, Hall JA, Blank RB, Bouladoux N, Oukka M, Mora JR, et al. Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. *J Exp Med* (2007) 204(8):1775–85. doi: 10.1084/jem.20070602
91. Travis MA, Reizis B, Melton AC, Masteller E, Tang Q, Proctor JM, et al. Loss of integrin alpha(v)beta8 on dendritic cells causes autoimmunity and colitis in mice. *Nature* (2007) 449(7160):361–5. doi: 10.1038/nature06110
92. Boucard-Jourdin M, Kugler D, Endale Ahanda ML, This S, De Calisto J, Zhang A, et al. beta8 Integrin Expression and Activation of TGF-beta by Intestinal Dendritic Cells Are Determined by Both Tissue Microenvironment and Cell Lineage. *J Immunol* (2016) 197(5):1968–78. doi: 10.4049/jimmunol.1600244
93. Matteoli G, Mazzini E, Iliev ID, Mileti E, Fallarino F, Puccetti P, et al. Gut CD103+ dendritic cells express indoleamine 2,3-dioxygenase which influences T regulatory/T effector cell balance and oral tolerance induction. *Gut* (2010) 59:595–604. doi: 10.1136/gut.2009.185108
94. Munn DH, Mellor AL. IDO in the Tumor Microenvironment: Inflammation, Counter-Regulation, and Tolerance. *Trends Immunol* (2016) 37(3):193–207. doi: 10.1016/j.it.2016.01.002
95. Fukaya T, Takagi H, Sato Y, Sato K, Eizumi K, Taya H, et al. Crucial roles of B7-H1 and B7-DC expressed on mesenteric lymph node dendritic cells in the generation of antigen-specific CD4+Foxp3+ regulatory T cells in the establishment of oral tolerance. *Blood* (2010) 116(13):2266–76. doi: 10.1182/blood-2009-10-250472
96. Shan M, Gentile M, Yeiser JR, Walland AC, Bornstein VU, Chen K, et al. Mucus enhances gut homeostasis and oral tolerance by delivering immunoregulatory signals. *Science* (2013) 342(6157):447–53. doi: 10.1126/science.1237910
97. Neeland MR, Andorf S, Manohar M, Dunham D, Lyu S-C, Dang TD, et al. Mass cytometry reveals cellular fingerprint associated with IgE+ peanut tolerance and allergy in early life. *Nat Commun* (2020) 11(1):1091. doi: 10.1038/s41467-020-14919-4
98. Smit JJ, Bol-Schoenmakers M, Hassing I, Fiechter D, Boon L, Bleumink R, et al. The role of intestinal dendritic cells subsets in the establishment of food allergy. *Clin Exp Allergy* (2011) 41(6):890–8. doi: 10.1111/j.1365-2222.2011.03738.x
99. Uto T, Takagi H, Fukaya T, Nasu J, Fukui T, Miyanaga N, et al. Critical role of plasmacytoid dendritic cells in induction of oral tolerance. *J Allergy Clin Immunol* (2018) 141(6):2156–67.e9. doi: 10.1016/j.jaci.2017.11.048
100. Dubois B, Joubert G, Gomez de Agüero M, Gouanvic M, Goubier A, Kaiserlian D. Sequential Role of Plasmacytoid Dendritic Cells and Regulatory T Cells in Oral Tolerance. *Gastroenterology* (2009) 137 (3):1019–28. doi: 10.1053/j.gastro.2009.03.055
101. Goubier A, Dubois B, Gheit H, Joubert G, Villard-Truc F, Asselin-Paturel C, et al. Plasmacytoid dendritic cells mediate oral tolerance. *Immunity* (2008) 29(3):464–75. doi: 10.1016/j.immuni.2008.06.017
102. Denning TL, Wang YC, Patel SR, Williams IR, Pulendran B. Lamina propria macrophages and dendritic cells differentially induce regulatory and interleukin 17-producing T cell responses. *Nat Immunol* (2007) 8 (10):1086–94. doi: 10.1038/ni1511

103. Zigmond E, Bernshtein B, Friedlander G, Walker CR, Yona S, Kim K-W, et al. Macrophage-Restricted Interleukin-10 Receptor Deficiency, but Not IL-10 Deficiency, Causes Severe Spontaneous Colitis. *Immunity* (2014) 40 (5):720–33. doi: 10.1016/j.immuni.2014.03.012
104. Shouval DS, Biswas A, Goettel JA, McCann K, Conaway E, Redhu NS, et al. Interleukin-10 Receptor Signaling in Innate Immune Cells Regulates Mucosal Immune Tolerance and Anti-Inflammatory Macrophage Function. *Immunity* (2014) 40(5):706–19. doi: 10.1016/j.immuni.2014.03.011
105. Wesemann DR, Nagler CR. The Microbiome, Timing, and Barrier Function in the Context of Allergic Disease. *Immunity* (2016) 44(4):728–38. doi: 10.1016/j.immuni.2016.02.002
106. Cahenzli J, Köller Y, Wyss M, Geuking MB, McCoy KD. Intestinal microbial diversity during early-life colonization shapes long-term IgE levels. *Cell Host Microbe* (2013) 14(5):559–70. doi: 10.1016/j.chom.2013.10.004
107. Abdel-Gadir A, Stephen-Victor E, Gerber GK, Noval Rivas M, Wang S, Harb H, et al. Microbiota therapy acts via a regulatory T cell MyD88/RORγt pathway to suppress food allergy. *Nat Med* (2019) 25(7):1164–74. doi: 10.1038/s41591-019-0461-z
108. Feehley T, Plunkett CH, Bao R, Choi Hong SM, Culleen E, Belda-Ferre P, et al. Healthy infants harbor intestinal bacteria that protect against food allergy. *Nat Med* (2019) 25(3):448–53. doi: 10.1038/s41591-018-0324-z
109. Stefka AT, Feehley T, Tripathi P, Qiu J, McCoy K, Mazmanian SK, et al. Commensal bacteria protect against food allergen sensitization. *Proc Natl Acad Sci* (2014) 111(36):13145. doi: 10.1073/pnas.1412008111
110. Tan J, McKenzie C, Vuillermin PJ, Goverse G, Vinuesa CG, Mebius RE, et al. Dietary Fiber and Bacterial SCFA Enhance Oral Tolerance and Protect against Food Allergy through Diverse Cellular Pathways. *Cell Rep* (2016) 15 (12):2809–24. doi: 10.1016/j.celrep.2016.05.047
111. Berni Canani R, Sangwan N, Stefka AT, Nocerino R, Paparo L, Aitoro R, et al. Lactobacillus rhamnosus GG-supplemented formula expands butyrate-producing bacterial strains in food allergic infants. *Isme J* (2016) 10 (3):742–50. doi: 10.1038/ismej.2015.151
112. Campbell C, McKenney PT, Konstantinovsky D, Isaeva OI, Schizas M, Verter J, et al. Bacterial metabolism of bile acids promotes generation of peripheral regulatory T cells. *Nature* (2020) 581(7809):475–9. doi: 10.1038/s41586-020-2193-0
113. Mortha A, Chudnovskiy A, Hashimoto D, Bogunovic M, Spencer SP, Belkaid Y, et al. Microbiota-dependent crosstalk between macrophages and ILC3 promotes intestinal homeostasis. *Science (New York NY)* (2014) 343 (6178):1249288–1249288. doi: 10.1126/science.1249288
114. Yokota A, Takeuchi H, Maeda N, Ohoka Y, Kato C, Song SY, et al. GM-CSF and IL-4 synergistically trigger dendritic cells to acquire retinoic acid-producing capacity. *Int Immunol* (2009) 21(4):361–77. doi: 10.1093/intimm/dxp003
115. Gorelik M, Narisety SD, Guerrerio AL, Chichester KL, Keet CA, Bieneman AP, et al. Suppression of the immunologic response to peanut during immunotherapy is often transient. *J Allergy Clin Immunol* (2015) 135 (5):1283–92. doi: 10.1016/j.jaci.2014.11.010
116. Syed A, Garcia MA, Lyu S-C, Bucayu R, Kohli A, Ishida S, et al. Peanut oral immunotherapy results in increased antigen-induced regulatory T-cell function and hypomethylation of forkhead box protein 3 (FOXP3). *J Allergy Clin Immunol* (2014) 133(2):500–510.e11. doi: 10.1016/j.jaci.2013.12.1037
117. O'Konek JJ, Landers JJ, Janczak KW, Goel RR, Mondrusov AM, Wong PT, et al. Nanoemulsion adjuvant–driven redirection of TH2 immunity inhibits allergic reactions in murine models of peanut allergy. *J Allergy Clin Immunol* (2018) 141(6):2121–31. doi: 10.1016/j.jaci.2018.01.042
118. Tanaka Y, Nagashima H, Bando K, Lu L, Ozaki A, Morita Y, et al. Oral CD103(-)CD11b(+) classical dendritic cells present sublingual antigen and induce Foxp3(+) regulatory T cells in draining lymph nodes. *Mucosal Immunol* (2017) 10(1):79–90. doi: 10.1038/mi.2016.46
119. Nowak-Węgrzyn A, Katz Y, Mehr SS, Koletzko S. Non–IgE-mediated gastrointestinal food allergy. *J Allergy Clin Immunol* (2015) 135(5):1114–24. doi: 10.1016/j.jaci.2015.03.025
120. Gujral N, Freeman HJ, Thomson ABR. Celiac disease: prevalence, diagnosis, pathogenesis and treatment. *World J Gastroenterol* (2012) 18(42):6036–59. doi: 10.3748/wjg.v18.i42.6036
121. Brown GD, Willment JA, Whitehead L. C-type lectins in immunity and homeostasis. *Nat Rev Immunol* (2018) 18(6):374–89. doi: 10.1038/s41577-018-0004-8
122. Shreffler WG, Castro RR, Kucuk ZY, Charlop-Powers Z, Grishina G, Yoo S, et al. The major glycoprotein allergen from Arachis hypogaea, Ara h 1, is a ligand of dendritic cell-specific ICAM-grabbing nonintegrin and acts as a Th2 adjuvant in vitro. *J Immunol* (2006) 177(6):3677–85. doi: 10.4049/jimmunol.177.6.3677
123. Schnurr M, Then F, Galambos P, Scholz C, Siegmund B, Endres S, et al. Extracellular ATP and TNF-α Synergize in the Activation and Maturation of Human Dendritic Cells. *J Immunol* (2000) 165(8):4704–9. doi: 10.4049/jimmunol.165.8.4704
124. Hsu SC, Chen CH, Tsai SH, Kawasaki H, Hung CH, Chu YT, et al. Functional interaction of common allergens and a C-type lectin receptor, dendritic cell-specific ICAM3-grabbing non-integrin (DC-SIGN), on human dendritic cells. *J Biol Chem* (2010) 285(11):7903–10. doi: 10.1074/jbc.M109.058370
125. Beyer K, Morrow E, Li XM, Bardina L, Bannon GA, Burks AW, et al. Effects of cooking methods on peanut allergenicity. *J Allergy Clin Immunol* (2001) 107(6):1077–81. doi: 10.1067/mai.2001.115480
126. Maleki SJ, Chung SY, Champagne ET, Raufman JP. The effects of roasting on the allergenic properties of peanut proteins. *J Allergy Clin Immunol* (2000) 106(4):763–8. doi: 10.1067/mai.2000.109620
127. Cabanillas B, Maleki SJ, Cheng H, Novak N. Differences in the Uptake of Ara h 3 from Raw and Roasted Peanut by Monocyte-Derived Dendritic Cells. *Int Arch Allergy Immunol* (2018) 177(1):35–9. doi: 10.1159/000489277
128. Novak N, Maleki SJ, Cuadrado C, Crespo JF, Cabanillas B. Interaction of Monocyte-Derived Dendritic Cells with Ara h 2 from Raw and Roasted Peanuts. *Foods (Basel Switzerland)* (2020) 9(7):863. doi: 10.3390/foods9070863
129. Li J, Jiang H, Wen W, Zheng J, Xu G. The dendritic cell mannose receptor mediates allergen internalization and maturation involving notch 1 signalling. *Clin Exp Immunol* (2010) 162(2):251–61. doi: 10.1111/j.1365-2249.2010.04244.x
130. Bendelac A, Savage PB, Teyton L. The biology of NKT cells. *Annu Rev Immunol* (2007) 25:297–336. doi: 10.1146/annurev.immunol.25.022106.141711
131. Fujii S-I, Shimizu K, Hemmi H, Steinman RM. Innate Vα14+ natural killer T cells mature dendritic cells, leading to strong adaptive immunity. *Immunol Rev* (2007) 220(1):183–98. doi: 10.1111/j.1600-065X.2007.00561.x
132. Gillessen S, Naumov YN, Nieuwenhuis EES, Exley MA, Lee FS, Mach N, et al. CD1d-restricted T cells regulate dendritic cell function and antitumor immunity in a granulocyte–macrophage colony-stimulating factor-dependent fashion. *Proc Natl Acad Sci* (2003) 100(15):8874–9. doi: 10.1073/pnas.1033098100
133. Mirotti L, Florsheim E, Rundqvist L, Larsson G, Spinozzi F, Leite-de-Moraes M, et al. Lipids are required for the development of Brazil nut allergy: the role of mouse and human iNKT cells. *Allergy* (2013) 68(1):74–83. doi: 10.1111/all.12057
134. Abós-Gracia B, del Moral MG, López-Relaño J, Viana-Huete V, Castro L, Villalba M, et al. Olea europaea pollen lipids activate invariant natural killer T cells by upregulating CD1d expression on dendritic cells. *J Allergy Clin Immunol* (2013) 131(5):1393–9.e5. doi: 10.1016/j.jaci.2012.11.014
135. Jyonouchi S, Abraham V, Orange JS, Spergel JM, Gober L, Dudek E, et al. Invariant natural killer T cells from children with versus without food allergy exhibit differential responsiveness to milk-derived sphingomyelin. *J Allergy Clin Immunol* (2011) 128(1):102–9.e13. doi: 10.1016/j.jaci.2011.02.026
136. Brennan PJ, Tatituri RVV, Heiss C, Watts GFM, Hsu F-F, Veerapen N, et al. Activation of iNKT cells by a distinct constituent of the endogenous glucosylceramide fraction. *Proc Natl Acad Sci* (2014) 111(37):13433. doi: 10.1073/pnas.1415357111
137. Hong X, Tsai H-J, Wang X. Genetics of food allergy. *Curr Opin Pediatr* (2009) 21(6):770–6. doi: 10.1097/MOP.0b013e32833252dc
138. Liu T, Navarro S, Lopata AL. Current advances of murine models for food allergy. *Mol Immunol* (2016) 70:104–17. doi: 10.1016/j.molimm.2015.11.011
139. Kanagaratham C, Sallis BF, Fiebiger E. Experimental Models for Studying Food Allergy. *Cell Mol Gastroenterol Hepatol* (2018) 6(3):356–69.e1. doi: 10.1016/j.jcmgh.2018.05.010

140. Ganeshan K, Neilsen CV, Hadsaitong A, Schleimer RP, Luo X, Bryce PJ. Impairing oral tolerance promotes allergy and anaphylaxis: a new murine food allergy model. *J Allergy Clin Immunol* (2009) 123(1):231–8.e4. doi: 10.1016/j.jaci.2008.10.011
141. Gagliardi MC, Sallusto F, Marinaro M, Langenkamp A, Lanzavecchia A, De Magistris MT. Cholera toxin induces maturation of human dendritic cells and licences them for Th2 priming. *Eur J Immunol* (2000) 30(8):2394–403. doi: 10.1002/1521-4141(2000)30:8<2394::AID-IMMU2394>3.0.CO;2-Y
142. Anjuère F, Luci C, Lebens M, Rousseau D, Hervouet C, Milon G, et al. In vivo adjuvant-induced mobilization and maturation of gut dendritic cells after oral administration of cholera toxin. *J Immunol* (2004) 173(8):5103–11. doi: 10.4049/jimmunol.173.8.5103
143. Anosova NG, Chabot S, Shreedhar V, Borawski JA, Dickinson BL, Neutra MR. Cholera toxin, E. coli heat-labile toxin, and non-toxic derivatives induce dendritic cell migration into the follicle-associated epithelium of Peyer's patches. *Mucosal Immunol* (2008) 1(1):59–67. doi: 10.1038/mi.2007.7
144. Kawamura YI, Kawashima R, Shirai Y, Kato R, Hamabata T, Yamamoto M, et al. Cholera toxin activates dendritic cells through dependence on GM1-ganglioside which is mediated by NF-κB translocation. *Eur J Immunol* (2003) 33(11):3205–12. doi: 10.1002/eji.200324135
145. Gustafsson T, Hua Y-J, Dahlgren MW, Livingston M, Johansson-Lindbom B, Yrlid U. Direct interaction between cholera toxin and dendritic cells is required for oral adjuvant activity. *Eur J Immunol* (2013) 43(7):1779–88. doi: 10.1002/eji.201242867
146. Bharati K, Ganguly NK. Cholera toxin: a paradigm of a multifunctional protein. *Indian J Med Res* (2011) 133(2):179–87.
147. Mattsson J, Schon K, Ekman L, Fahlen-Yrlid L, Yrlid U, Lycke NY. Cholera toxin adjuvant promotes a balanced Th1/Th2/Th17 response independently of IL-12 and IL-17 by acting on Gsalpha in CD11b(+) DCs. *Mucosal Immunol* (2015) 8(4):815–27. doi: 10.1038/mi.2014.111
148. Eriksson K, Fredriksson M, Nordström I, Holmgren J. Cholera toxin and its B subunit promote dendritic cell vaccination with different influences on Th1 and Th2 development. *Infect Immun* (2003) 71(4):1740–7. doi: 10.1128/IAI.71.4.1740-1747.2003
149. Datta SK, Sabet M, Nguyen KPL, Valdez PA, Gonzalez-Navajas JM, Islam S, et al. Mucosal adjuvant activity of cholera toxin requires Th17 cells and protects against inhalation anthrax. *Proc Natl Acad Sci* (2010) 107 (23):10638–43. doi: 10.1073/pnas.1002348107
150. Beddoe T, Paton AW, Le Nours J, Rossjohn J, Paton JC. Structure, biological functions and applications of the AB5 toxins. *Trends Biochem Sci* (2010) 35 (7):411–8. doi: 10.1016/j.tibs.2010.02.003
151. Bunikowski R, Mielke MEA, Skarabis H, Worm M, Anagnostopoulos I, Kolde G, et al. Evidence for a disease-promoting effect of Staphylococcus aureus-derived exotoxins in atopic dermatitis. *J Allergy Clin Immunol* (2000) 105(4):814–9. doi: 10.1067/mai.2000.105528
152. Laouini D, Kawamoto S, Yalcindag A, Bryce P, Mizoguchi E, Oettgen H, et al. Epicutaneous sensitization with superantigen induces allergic skin inflammation. *J Allergy Clin Immunol* (2003) 112(5):981–7. doi: 10.1016/j.jaci.2003.07.007
153. Yang PC, Xing Z, Berin CM, Soderholm JD, Feng BS, Wu L, et al. TIM-4 expressed by mucosal dendritic cells plays a critical role in food antigen-specific Th2 differentiation and intestinal allergy. *Gastroenterology* (2007) 133(5):1522–33. doi: 10.1053/j.gastro.2007.08.006
154. Mandron M, Ariès M-F, Brehm RD, Tranter HS, Acharya KR, Charveron M, et al. Human dendritic cells conditioned with Staphylococcus aureus enterotoxin B promote Th2 cell polarization. *J Allergy Clin Immunol* (2006) 117(5):1141–7. doi: 10.1016/j.jaci.2005.12.1360
155. Kong J, Chalcraft K, Mandur TS, Jimenez-Saiz R, Walker TD, Goncharova S, et al. Comprehensive metabolomics identifies the alarmin uric acid as a critical signal for the induction of peanut allergy. *Allergy* (2015) 70(5):495–505. doi: 10.1111/all.12579
156. Chu DK, Jimenez-Saiz R, Verschoor CP, Walker TD, Goncharova S, Llop-Guevara A, et al. Indigenous enteric eosinophils control DCs to initiate a primary Th2 immune response in vivo. *J Exp Med* (2014) 211(8):1657–72. doi: 10.1084/jem.20131800
157. Fort MM, Cheung J, Yen D, Li J, Zurawski SM, Lo S, et al. IL-25 Induces IL-4, IL-5, and IL-13 and Th2-Associated Pathologies In Vivo. *Immunity* (2001) 15(6):985–95. doi: 10.1016/S1074-7613(01)00243-6
158. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, et al. IL-33, an Interleukin-1-like Cytokine that Signals via the IL-1 Receptor-Related Protein ST2 and Induces T Helper Type 2-Associated Cytokines. *Immunity* (2005) 23(5):479–90. doi: 10.1016/j.immuni.2005.09.015
159. Soumelis V, Reche PA, Kanzler H, Yuan W, Edward G, Homey B, et al. Human epithelial cells trigger dendritic cell–mediated allergic inflammation by producing TSLP. *Nat Immunol* (2002) 3(7):673–80. doi: 10.1038/ni805
160. Chu DK, Llop-Guevara A, Walker TD, Flader K, Goncharova S, Boudreau JE, et al. IL-33, but not thymic stromal lymphopoietin or IL-25, is central to mite and peanut allergic sensitization. *J Allergy Clin Immunol* (2013) 131 (1):187–200.e1-8. doi: 10.1016/j.jaci.2012.08.002
161. Li J, Wang Y, Tang L, de Villiers WJS, Cohen D, Woodward J, et al. Dietary medium-chain triglycerides promote oral allergic sensitization and orally induced anaphylaxis to peanut protein in mice. *J Allergy Clin Immunol* (2013) 131(2):442–50 doi: .10.1016/j.jaci.2012.10.011
162. Khodoun MV, Tomar S, Tocker JE, Wang YH, Finkelman FD. Prevention of food allergy development and suppression of established food allergy by neutralization of thymic stromal lymphopoietin, IL-25, and IL-33. *J Allergy Clin Immunol* (2018) 141(1):171–9.e1. doi: 10.1016/j.jaci.2017.02.046
163. Chambers SJ, Bertelli E, Winterbone MS, Regoli M, Man AL, Nicoletti C. Adoptive transfer of dendritic cells from allergic mice induces specific immunoglobulin E antibody in naïve recipients in absence of antigen challenge without altering the T helper 1/T helper 2 balance. *Immunology* (2004) 112(1):72–9. doi: 10.1111/j.1365-2567.2004.01846.x
164. Blázquez AB, Berin MC. Gastrointestinal dendritic cells promote Th2 skewing via OX40L. *J Immunol* (2008) 180(7):4441–50. doi: 10.4049/jimmunol.180.7.4441
165. Jenkins SJ, Perona-Wright G, Worsley AG, Ishii N, MacDonald AS. Dendritic cell expression of OX40 ligand acts as a costimulatory, not polarizing, signal for optimal Th2 priming and memory induction in vivo. *J Immunol* (2007) 179(6):3515–23. doi: 10.4049/jimmunol.179.6.3515
166. Meyers JH, Chakravarti S, Schlesinger D, Illes Z, Waldner H, Umetsu SE, et al. TIM-4 is the ligand for TIM-1, and the TIM-1–TIM-4 interaction regulates T cell proliferation. *Nat Immunol* (2005) 6(5):455–64. doi: 10.1038/ni1185
167. Liu T, He S-H, Zheng P-Y, Zhang T-Y, Wang B-Q, Yang P-C. Staphylococcal enterotoxin B increases TIM4 expression in human dendritic cells that drives naïve CD4 T cells to differentiate into Th2 cells. *Mol Immunol* (2007) 44 (14):3580–7. doi: 10.1016/j.molimm.2007.03.004
168. Feng BS, Chen X, He SH, Zheng PY, Foster J, Xing Z, et al. Disruption of T-cell immunoglobulin and mucin domain molecule (TIM)-1/TIM4 interaction as a therapeutic strategy in a dendritic cell-induced peanut allergy model. *J Allergy Clin Immunol* (2008) 122(1):55–61, 61.e1-7. doi: 10.1016/j.jaci.2008.04.036
169. Zhu C, Anderson AC, Schubart A, Xiong H, Imitola J, Khoury SJ, et al. The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity. *Nat Immunol* (2005) 6(12):1245–52. doi: 10.1038/ni1271
170. Chen X, Song CH, Liu ZQ, Feng BS, Zheng PY, Li P, et al. Intestinal epithelial cells express galectin-9 in patients with food allergy that plays a critical role in sustaining allergic status in mouse intestine. *Allergy* (2011) 66(8):1038–46. doi: 10.1111/j.1398-9995.2011.02585.x
171. Yang B, Li L-J, Xu L-Z, Liu J-Q, Zhang H-P, Geng X-R, et al. Histone acetyltransferase p300 modulates TIM4 expression in dendritic cells. *Sci Rep* (2016) 6:21336–6. doi: 10.1038/srep21336
172. Fang TC, Yashiro-Ohtani Y, Del Bianco C, Knoblock DM, Blacklow SC, Pear WS. Notch Directly Regulates Gata3 Expression during T Helper 2 Cell Differentiation. *Immunity* (2007) 27(1):100–10. doi: 10.1016/j.immuni.2007.04.018
173. Amsen D, Blander JM, Lee GR, Tanigaki K, Honjo T, Flavell RA. Instruction of Distinct CD4 T Helper Cell Fates by Different Notch Ligands on Antigen-Presenting Cells. *Cell* (2004) 117(4):515–26. doi: 10.1016/S0092-8674(04)00451-9
174. Worsley AGF, LeibundGut-Landmann S, Slack E, Phng L-K, Gerhardt H, Sousa CRe, et al. Dendritic cell expression of the Notch ligand jagged2 is not essential for Th2 response induction in vivo. *Eur J Immunol* (2008) 38 (4):1043–9. doi: 10.1002/eji.200737335
175. Dunkin D, Berin MC, Mayer L. Allergic sensitization can be induced via multiple physiologic routes in an adjuvant-dependent manner. *J Allergy Clin Immunol* (2011) 128(6):1251–8.e2. doi: 10.1016/j.jaci.2011.06.007

176. Savinko T, Lauerma A, Lehtimäki S, Gombert M, Majuri ML, Fyhrquist-Vanni N, et al. Topical superantigen exposure induces epidermal accumulation of CD8 + T cells, a mixed Th1/Th2-type dermatitis and vigorous production of IgE antibodies in the murine model of atopic dermatitis. *J Immunol* (2005) 175 (12):8320–6. doi: 10.4049/jimmunol.175.12.8320
177. Martin PE, Eckert JK, Koplin JJ, Lowe AJ, Gurrin LC, Dharmage SC, et al. Which infants with eczema are at risk of food allergy? Results from a population-based cohort. *Clin Exp Allergy* (2015) 45(1):255–64. doi: 10.1111/cea.12406
178. Hussain M, Borcard L, Walsh KP, Pena Rodriguez M, Mueller C, Kim BS, et al. Basophil-derived IL-4 promotes epicutaneous antigen sensitization concomitant with the development of food allergy. *J Allergy Clin Immunol* (2018) 141(1):223–234.e5. doi: 10.1016/j.jaci.2017.02.035
179. Shimura S, Takai T, Iida H, Maruyama N, Ochi H, Kamijo S, et al. Epicutaneous Allergic Sensitization by Cooperation between Allergen Protease Activity and Mechanical Skin Barrier Damage in Mice. *J Invest Dermatol* (2016) 136(7):1408–17. doi: 10.1016/j.jid.2016.02.810
180. Tordesillas L, Goswami R, Benedé S, Grishina G, Dunkin D, Järvinen KM, et al. Skin exposure promotes a Th2-dependent sensitization to peanut allergens. *J Clin Invest* (2014) 124(11):4965–75. doi: 10.1172/JCI75660
181. Parvataneni S, Gonipeta B, Tempelman RJ, Gangur V. Development of an Adjuvant-Free Cashew Nut Allergy Mouse Model. *Int Arch Allergy Immunol* (2009) 149(4):299–304. doi: 10.1159/000205575
182. Gonipeta B, Parvataneni S, Tempelman RJ, Gangur V. An adjuvant-free mouse model to evaluate the allergenicity of milk whey protein. *J Dairy Sci* (2009) 92(10):4738–44. doi: 10.3168/jds.2008-1927
183. Han H, Roan F, Johnston LK, Smith DE, Bryce PJ, Ziegler SF. IL-33 promotes gastrointestinal allergy in a TSLP-independent manner. *Mucosal Immunol* (2018) 11(2):394–403. doi: 10.1038/mi.2017.61
184. Galand C, Leyva-Castillo JM, Yoon J, Han A, Lee MS, McKenzie ANJ, et al. IL-33 promotes food anaphylaxis in epicutaneously sensitized mice by targeting mast cells. *J Allergy Clin Immunol* (2016) 138(5):1356–66. doi: 10.1016/j.jaci.2016.03.056
185. Rank MA, Kobayashi T, Kozaki H, Bartemes KR, Squillace DL, Kita H. IL-33-activated dendritic cells induce an atypical TH2-type response. *J Allergy Clin Immunol* (2009) 123(5):1047–54. doi: 10.1016/j.jaci.2009.02.026
186. Besnard A-G, Togbe D, Guillou N, Erard F, Quesniaux V, Ryffel B. IL-33-activated dendritic cells are critical for allergic airway inflammation. *Eur J Immunol* (2011) 41(6):1675–86. doi: 10.1002/eji.201041033
187. Wilson SR, Thé L, Batia LM, Beattie K, Katibah GE, McClain SP, et al. The Epithelial Cell-Derived Atopic Dermatitis Cytokine TSLP Activates Neurons to Induce Itch. *Cell* (2013) 155(2):285–95. doi: 10.1016/j.cell.2013.08.057
188. Han H, Thelen TD, Comeau MR, Ziegler SF. Thymic stromal lymphopoietin-mediated epicutaneous inflammation promotes acute diarrhea and anaphylaxis. *J Clin Invest* (2014) 124(12):5442–52. doi: 10.1172/JCI77798
189. Ito T, Wang Y-H, Duramad O, Hori T, Delespesse GJ, Watanabe N, et al. TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. *J Exp Med* (2005) 202(9):1213–23. doi: 10.1084/jem.20051135
190. Seshasayee D, Lee WP, Zhou M, Shu J, Suto E, Zhang J, et al. In vivo blockade of OX40 ligand inhibits thymic stromal lymphopoietin driven atopic inflammation. *J Clin Invest* (2007) 117(12):3868–78. doi: 10.1172/JCI33559
191. Oyoshi MK, Larson RP, Ziegler SF, Ziegler RS. Mechanical injury polarizes skin dendritic cells to elicit a T(H)2 response by inducing cutaneous thymic stromal lymphopoietin expression. *J Allergy Clin Immunol* (2010) 126 (5):976–84.e9845. doi: 10.1016/j.jaci.2010.08.041
192. Noti M, Kim BS, Siracusa MC, Rak GD, Kubo M, Moghaddam AE, et al. Exposure to food allergens through inflamed skin promotes intestinal food allergy through the thymic stromal lymphopoietin-basophil axis. *J Allergy Clin Immunol* (2014) 133(5):1390–9, 1399.e1-6. doi: 10.1016/j.jaci.2014.01.021
193. Gao Y, Nish SA, Jiang R, Hou L, Licona-Limón P, Weinstein JS, et al. Control of T helper 2 responses by transcription factor IRF4-dependent dendritic cells. *Immunity* (2013) 39(4):722–32. doi: 10.1016/j.immuni.2013.08.028
194. Leyva-Castillo JM, Galand C, Kam C, Burton O, Gurish M, Musser MA, et al. Mechanical Skin Injury Promotes Food Anaphylaxis by Driving Intestinal Mast Cell Expansion. *Immunity* (2019) 50(5):1262–1275.e4. doi: 10.1016/j.immuni.2019.03.023
195. Zhang Y, Collier F, Naselli G, Saffery R, Tang ML, Allen KJ, et al. Cord blood monocyte-derived inflammatory cytokines suppress IL-2 and induce nonclassic "T(H)2-type" immunity associated with development of food allergy. *Sci Transl Med* (2016) 8(321):321ra8. doi: 10.1126/scitranslmed.aad4322
196. Neeland MR, Koplin JJ, Dang TD, Dharmage SC, Tang ML, Prescott SL, et al. Early life innate immune signatures of persistent food allergy. *J Allergy Clin Immunol* (2018) 142(3):857–64.e3. doi: 10.1016/j.jaci.2017.10.024
197. Randolph GJ, Jakubzick C, Qu C. Antigen presentation by monocytes and monocyte-derived cells. *Curr Opin Immunol* (2008) 20(1):52–60. doi: 10.1016/j.coi.2007.10.010
198. Soilleux EJ, Morris LS, Leslie G, Chehimi J, Luo Q, Levroney E, et al. Constitutive and induced expression of DC-SIGN on dendritic cell and macrophage subpopulations in situ and in vitro. *J Leukoc Biol* (2002) 71 (3):445–57.
199. Shaw TN, Houston SA, Wemyss K, Bridgeman HM, Barbera TA, Zangerle-Murray T, et al. Tissue-resident macrophages in the intestine are long lived and defined by Tim-4 and CD4 expression. *J Exp Med* (2018) 215(6):1507–18. doi: 10.1084/jem.20180019
200. Weber-Matthiesen K, Sterry W. Organization of the Monocyte/Macrophage System of Normal Human Skin. *J Invest Dermatol* (1990) 95(1):83–9. doi: 10.1111/1523-1747.ep12874002
201. Bain CC, Mowat AM. The monocyte-macrophage axis in the intestine. *Cell Immunol* (2014) 291(1):41–8. doi: 10.1016/j.cellimm.2014.03.012
202. Peter JM. Macrophage Polarization. *Annu Rev Physiol* (2017) 79(1):541–66. doi: 10.1146/annurev-physiol-022516-034339
203. Girodet PO, Nguyen D, Mancini JD, Hundal M, Zhou X, Israel E, et al. Alternative Macrophage Activation Is Increased in Asthma. *Am J Respir Cell Mol Biol* (2016) 55(4):467–75. doi: 10.1165/rcmb.2015-0295OC
204. Robbe P, Draijer C, Borg TR, Luinge M, Timens W, Wouters IM, et al. Distinct macrophage phenotypes in allergic and nonallergic lung inflammation. *Am J Physiol Lung Cell Mol Physiol* (2015) 308(4):L358–67. doi: 10.1152/ajplung.00341.2014
205. Nabe T, Wakamori H, Yano C, Nishiguchi A, Yuasa R, Kido H, et al. Production of interleukin (IL)-33 in the lungs during multiple antigen challenge-induced airway inflammation in mice, and its modulation by a glucocorticoid. *Eur J Pharmacol* (2015) 757:34–41. doi: 10.1016/j.ejphar.2015.03.015

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

ORIGINAL RESEARCH
published: 19 January 2021
doi: 10.3389/fimmu.2020.599637

Fecal IgA, Antigen Absorption, and Gut Microbiome Composition Are Associated With Food Antigen Sensitization in Genetically Susceptible Mice

Johanna M. Smeekens[1,2], Brandi T. Johnson-Weaver[3], Andrew L. Hinton[2,4], M. Andrea Azcarate-Peril[5,6], Timothy P. Moran[1], Robert M. Immormino[1], Janelle R. Kesselring[1,2], Erin C. Steinbach[1], Kelly A. Orgel[1,2], Herman F. Staats[3,7,8], A. Wesley Burks[1,2], Peter J. Mucha[4,9], Martin T. Ferris[10] and Michael D. Kulis[1,2]*

[1] Department of Pediatrics, Division of Rheumatology, Allergy and Immunology, School of Medicine, University of North Carolina, Chapel Hill, NC, United States, [2] UNC Food Allergy Initiative, School of Medicine, University of North Carolina, Chapel Hill, NC, United States, [3] Department of Pathology, Duke University School of Medicine, Durham, NC, United States, [4] Curriculum in Bioinformatics and Computational Biology, University of North Carolina, Chapel Hill, NC, United States, [5] Department of Medicine, Division of Gastroenterology and Hepatology, University of North Carolina, Chapel Hill, NC, United States, [6] UNC Microbiome Core, Center for Gastrointestinal Biology and Disease, University of North Carolina, Chapel Hill, NC, United States, [7] Duke Human Vaccine Institute, Duke University School of Medicine, Durham, NC, United States, [8] Department of Immunology, Duke University School of Medicine, Durham, NC, United States, [9] Department of Mathematics and Department of Applied Physical Sciences, University of North Carolina, Chapel Hill, NC, United States, [10] Department of Genetics, School of Medicine, University of North Carolina, Chapel Hill, NC, United States

OPEN ACCESS

Edited by:
Simon Patrick Hogan, University of Michigan, United States

Reviewed by:
Gary B. Huffnagle, University of Michigan, United States
Gerard Kaiko, The University of Newcastle, Australia

***Correspondence:**
Johanna M. Smeekens
smeeken3@email.unc.edu

Specialty section:
This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology

Received: *27 August 2020*
Accepted: *25 November 2020*
Published: *19 January 2021*

Citation:
Smeekens JM, Johnson-Weaver BT, Hinton AL, Azcarate-Peril MA, Moran TP, Immormino RM, Kesselring JR, Steinbach EC, Orgel KA, Staats HF, Burks AW, Mucha PJ, Ferris MT and Kulis MD (2021) Fecal IgA, Antigen Absorption, and Gut Microbiome Composition Are Associated With Food Antigen Sensitization in Genetically Susceptible Mice. Front. Immunol. 11:599637. doi: 10.3389/fimmu.2020.599637

Food allergy is a potentially fatal disease affecting 8% of children and has become increasingly common in the past two decades. Despite the prevalence and severe nature of the disease, the mechanisms underlying sensitization remain to be further elucidated. The Collaborative Cross is a genetically diverse panel of inbred mice that were specifically developed to study the influence of genetics on complex diseases. Using this panel of mouse strains, we previously demonstrated CC027/GeniUnc mice, but not C3H/HeJ mice, develop peanut allergy after oral exposure to peanut in the absence of a Th2-skewing adjuvant. Here, we investigated factors associated with sensitization in CC027/GeniUnc mice following oral exposure to peanut, walnut, milk, or egg. CC027/GeniUnc mice mounted antigen-specific IgE responses to peanut, walnut and egg, but not milk, while C3H/HeJ mice were not sensitized to any antigen. Naïve CC027/GeniUnc mice had markedly lower total fecal IgA compared to C3H/HeJ, which was accompanied by stark differences in gut microbiome composition. Sensitized CC027/GeniUnc mice had significantly fewer $CD3^+$ T cells but higher numbers of $CXCR5^+$ B cells and T follicular helper cells in the mesenteric lymph nodes compared to C3H/HeJ mice, which is consistent with their relative immunoglobulin production. After oral challenge to the corresponding food, peanut- and walnut-sensitized CC027/GeniUnc mice experienced anaphylaxis, whereas mice exposed to milk and egg did not. Ara h 2 was detected in serum collected post-challenge from peanut-sensitized mice, indicating increased

absorption of this allergen, while Bos d 5 and Gal d 2 were not detected in mice exposed to milk and egg, respectively. Machine learning on the change in gut microbiome composition as a result of food protein exposure identified a unique signature in CC027/GeniUnc mice that experienced anaphylaxis, including the depletion of *Akkermansia*. Overall, these results demonstrate several factors associated with enteral sensitization in CC027/GeniUnc mice, including diminished total fecal IgA, increased allergen absorption and altered gut microbiome composition. Furthermore, peanuts and tree nuts may have inherent properties distinct from milk and eggs that contribute to allergy.

Keywords: food allergy, peanut allergy, IgE, microbiome, Tfh cells, fecal IgA, antigen absorption

INTRODUCTION

Food allergy is a potentially fatal disease that has increased in prevalence in the past two decades, now affecting an estimated 4%–8% of children and 10% of adults (1, 2). The most common foods to trigger allergic reactions are peanuts, tree nuts, milk, egg, soy, shellfish, fish, and wheat. Even trace amounts of an allergen can elicit a severe anaphylactic reaction. Therefore, allergic individuals must strictly avoid the offending allergen in order to prevent accidental exposure and reactions. As a result, the quality of life of allergic individuals and their caretakers is diminished (3). Of the eight most common food allergens, reactions to peanuts and tree nuts are most severe and account for 70%–90% of fatal allergic reactions (4, 5). Some food allergies, such as milk and egg, are often outgrown; however, peanut and tree nut allergies are only outgrown in an estimated 10% of individuals (6). The underlying characteristics and mechanisms that make peanut and tree nut allergies longer lasting and more severe are widely unknown.

Typically, oral ingestion of antigen leads to immunologic suppression of immune responses to the antigen, a process termed oral tolerance. These active processes in the gut prevent harmful immune responses to dietary antigens and commensal bacteria, while protecting against pathogens and toxins (7). $CD4^+CD25^+Foxp3^+$ regulatory T cells (Tregs) are necessary for oral tolerance (8). $CD103^+$ dendritic cells capture antigen in the lamina propria and then migrate to the mesenteric lymph nodes (MLNs) where they induce differentiation of naïve T cells to Tregs. These antigen-specific Tregs then migrate back to the lamina propria and expand (9). Failure in oral tolerance mechanisms to dietary antigens leads to T helper type 2 (Th2) immune responses, resulting in food allergy.

Secretory IgA in the gut also plays a crucial role in tolerance. IgA acts as a neutralizing antibody on epithelial surfaces and is hypothesized to prevent allergic immune responses by immune exclusion of luminal food proteins (10). Secretory IgA also regulates commensal bacteria composition in the gut, which in turn prevents uptake of toxins and pathogens (11). Altered microbiota composition is associated with oral tolerance failure and development of food allergy (12), and previous studies have demonstrated that several microbiota species, including the *Clostridia* class, are related to the mitigation of food allergy in mouse studies (13). Overall, the gut is a dynamic environment that involves regulation of microbiota by secretory IgA, which in turn can induce Treg production, leading to oral tolerance.

Murine models provide a platform to investigate the underlying mechanisms that lead to sensitization to food antigens. The Collaborative Cross is a genetically diverse panel of inbred mice that were specifically developed to study the influence of genetics on complex diseases (14, 15). Previously, we screened strains from the Collaborative Cross to identify an orally reactive model of peanut allergy (16). Specifically, CC027/GeniUnc mice were orally sensitized to peanut in the presence of cholera toxin and reacted on oral challenge. These mice had fewer Tregs and more intestinal mast cells compared to C3H/HeJ and C57BL/6J mice. Additionally, the CC027/GeniUnc mice could be sensitized to peanut in the absence of an adjuvant. Here, we aimed to explore the mechanisms of gut sensitization in this adjuvant-free model through exposure to peanut, walnut, milk, or egg, with a focus on fecal IgA, antigen absorption and gut microbiome. We utilized the C3H/HeJ strain as a comparator to CC027/GeniUnc because they are known to produce high quantities of IgE and are Th2-skewed, and are therefore used as a standard model of food allergy in the field (17–20).

MATERIALS AND METHODS

Mice

CC027/GeniUnc mice were obtained from the UNC Systems Genetics Core Facility. A colony of C3H/HeJ mice originally obtained from the Jackson Labs was kept in the same facility where the CC027/GeniUnc mice were born, to standardize their environments. Mice were received for experiments starting at 4–6 weeks of age. Mice were kept on a 12:12 light:dark cycle and fed standard chow free of peanut, walnut, milk, and egg ingredients. All animal experiments were approved by the Institutional Animal Care and Use Committee at the University of North Carolina at Chapel Hill under protocol 17-286.

Peanut, Walnut, Milk, and Egg Protein Extractions

Protein extractions were performed as reported previously (21). Briefly, proteins were extracted from roasted, defatted peanut flour (Golden Peanut, Alpharetta, GA), roasted, defatted walnut

flour (Holmquist Hazelnut Orchards, Lynden, WA), nonfat dry milk powder (The Milky Whey, Missoula, MT) or egg white powder (Deb El Foods, Elizabethport, NJ) in PBS (supplemented with 1 M NaCl for peanut and walnut extractions). Protein concentrations were measured by BCA (Pierce, Waltham, MA) and extracts were determined to contain all major allergens by SDS-PAGE gel.

Sensitization and Oral Food Challenges

Mice were exposed to food protein by oral gavage once per week for 4 weeks as follows: peanut and egg (2 mg the first 3 weeks, 5 mg the final week), walnut and milk (2 mg all 4 weeks). The following week, fecal pellets and serum were collected from mice. Mice were then challenged to the corresponding food *via* oral gavage (peanut and egg: 10 mg; milk and walnut: 5 mg). Core body temperatures were recorded every 15 min for 1 h post-challenge using a rectal probe (Physitemp, Clifton, NJ). Anaphylaxis was defined as a greater than 3°C decrease in body temperature. Mice were rested for several weeks, re-challenged and serum was collected 30 min post-challenge for allergen quantification. Several weeks after challenges, mice were euthanized for MLN isolation.

Food protein exposure resulted in 10 strain-food protein groups of mice: CC027-peanut, CC027-walnut, CC027-milk, CC027-egg, CC027-PBS, C3H-peanut, C3H-walnut, C3H-milk, C3H-egg, and C3H-PBS.

Antigen-Specific IgE, IgG1, and IgG2a ELISAs

Antigen-specific IgE, IgG1 and IgG2a were quantified by ELISA as described previously (21). Briefly, 96-well plates were coated with 20 μg/ml peanut, walnut, milk, or egg extract for samples, or 20 μg/ml HSA-DNP (Millipore Sigma, St. Louis, MO) for standard curves, and blocked with 2% BSA in PBS-0.5% Tween. Samples were diluted 1:100 for IgE, 1:20,000 for IgG1, and 1:1,000 for IgG2a. Standard curves were prepared using mouse IgE anti-DNP, IgG1 anti-DNP or IgG2a anti-DNP (Accurate Chemicals, Westbury, NY) ranging from 0.002–2 μg/ml. The following antibodies were added to IgE plates in succession: 0.5 μg/ml sheep IgG anti-mouse IgE (The Binding Site, Birmingham, UK), 0.5 μg/ml biotinylated donkey anti-sheep IgG (Accurate Chemicals), and 0.5 μg/ml NeutrAvidin-HRP (Pierce Biotechnology, Rockford, IL). For IgG1 and IgG2a plates, HRP goat anti-mouse IgG1 (1:40,000, Southern Biotech, Birmingham, AL) or IgG2a (1:20,000, Southern Biotech) were added to plates. All plates were developed with TMB (SeraCare, Milford, MA), stopped with 1% HCl (SeraCare), and read at 450 nm using a spectrophotometer (BioTek Instruments, Winooski, VT).

Allergen ELISAs (Ara h 2, Gal d 2, Bos d 5)

Serum collected 30 min post-challenge was diluted 1:10 and Ara h 2, Gal d 2, and Bos d 5 were quantified *via* ELISA according to the manufacturer's instructions (Indoor Biotechnologies, Charlottesville, VA).

Fecal IgA and IgG ELISAs

Fecal pellets were collected before and after the 4-week food protein exposure period, snap-frozen in liquid nitrogen, and stored at −80°C until analysis. Fecal pellets were resuspended in protein extraction buffer [10% goat serum (Fisher Scientific, Waltham, MA) in PBS] at a ratio of 10 mg per 100 μl, and vortexed for 20 min to disrupt pellets. Extracted samples were centrifuged for 10 min at 13,000 x g, and supernatants were collected for subsequent analysis.

ELISA was used to determine total IgG and IgA antibody concentrations present in mouse fecal extracts collected before and after the exposure period. Maxisorp 384-well black plates were coated with unlabeled goat anti-mouse IgG or IgA capture antibodies (Southern Biotech) at 5 μg/ml in carbonate/bicarbonate buffer and incubated at 4°C overnight. A similar ELISA method was used to detect allergen-specific IgG and IgA in fecal samples as described for total IgG and IgA ELISA with minor modifications. Maxisorp 384-well plates were coated with an individual allergen at a concentration of 2 μg/ml. Plates were blocked with non-fat dry milk blocking buffer for 2 h at room temperature. After blocking, ELISA plates were washed four times using PBS/0.1% Tween 20. Fecal samples and purified mouse IgG and IgA positive control standards were diluted two folds in complete sample diluent. The starting dilution for fecal samples was 1:8 and the starting concentration for the IgG and IgA standards was 1,000 ng/ml. Diluted samples and standards were added to the ELISA plates and incubated overnight at 4°C. After incubation, plates were washed to remove unbound antibodies prior to adding AP-labeled goat anti-mouse IgG or IgA secondary antibodies at a 1:8,000 dilution. Secondary antibodies were incubated at room temperature for 2 h. The fluorescent-based AttoPhos substrate system (Promega; Madison, WI) was used to develop the plates by incubating at room temperature for 15 min. Relative light units (RLU) for each sample were measured using a Biotek Synergy 2 plate reader (Winooski, VT).

The total IgG and IgA concentration for each fecal sample was determined using a standard curve calculator. The RLU values for the IgG and IgA standards and corresponding ng/ml concentration were used to develop a standard curve in GraphPad PRISM (San Diego, CA). The RLU values that fell within an acceptable range of the standard curves (80%–120% standard recovery) were used to interpolate an ng/ml concentration for each sample. The interpolated values for each sample were multiplied by the sample dilution factor. The final ng/ml concentration for each sample is the average of the interpolated values multiplied by the sample dilution factor.

The end-point titer was determined by the last sample dilution that provided a positive RLU value three-times greater than a naive reference sample at the same dilution as the sample. End-point titers are reported as geometric mean titers (GMTs).

Mesenteric Lymph Node Analysis

MLNs were harvested, and lymphocytes were isolated from a subset of allergen-exposed mice. MLNs were homogenized and passed through a 70 μm strainer to obtain a single cell suspension

and centrifuged at 1,500 rpm for 10 min at room temperature. Lymphocytes from MLNs were washed once with RPMI and centrifuged at 1,500 rpm for 10 min. Lymphocytes were resuspended in RPMI and counted using a hemocytometer.

Flow cytometric analysis of MLNs was performed as previously described (22). Briefly, cells were resuspended in FACS buffer (2 mM EDTA and 0.5% BSA in PBS) and incubated with anti-mouse CD16/CD32 (2.4G2) and 5% rat serum for 5 min to block Fc receptors. Cells were then incubated with fluorochrome-conjugated antibodies against murine CD3ϵ (145-2C11), CD4 (GK1.5), CD19 (6D5), CXCR5 (L138D7), and PD-1 (29F.1A12) for 30 min on ice. Cells were also concurrently stained with Zombie Aqua (BioLegend, San Diego, CA) for live cell/dead cell discrimination. Flow cytometry data were acquired with a four-laser LSRII (BD Biosciences, San Jose, CA) and analyzed using FlowJo (Ashland, OR) software. Only single cells were analyzed. Tfh cells were identified as $CXCR5^+PD\text{-}1^+CD4^+$ T cells. All antibodies were purchased from BioLegend or BD Biosciences.

Microbiome Analysis

Fecal pellets were collected before and after the 4-week food protein exposure period, snap-frozen in liquid nitrogen, and stored at −80°C until analysis.

DNA Isolation

DNA isolation was performed as previously described (23–25). Mouse stool samples were transferred to a 2 ml tube containing 200 mg of ≤106 µm glass beads (Sigma, St. Louis, MO) and 0.5 ml of Qiagen PM1 buffer (Valencia, CA). Bead beating was performed for 5 min in a Qiagen TissueLyser II at 30Hz. After a 5-min centrifugation, 0.45 ml of supernatants were aspirated and transferred to a new tube containing 0.15 ml of Qiagen IRS solution. The suspension was incubated at 4°C overnight. After a brief centrifugation, supernatants were aspirated and transferred to deep well plates containing 0.45 ml of Qiagen binding buffer supplemented with Qiagen ClearMag Beads. DNA was purified using the automated KingFisher™ Flex Purification System and eluted in DNase-free water.

16S rRNA Amplicon Sequencing

Barcoding and library preparation were carried out as described (23, 24, 26). A total of 12.5 ng of DNA was amplified using universal primers targeting the V4 region of the bacterial 16S rRNA gene. Primer sequences contained overhang adapters appended to the 5' end of each primer for compatibility with Illumina sequencing platform (27). The complete sequences of the primers were:

515F - 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGCCAGCMGCCGCGGTAA 3'

806R - 5'GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACTACHVGGGTWTCTAAT 3'.

Master mixes contained 12.5 ng of total DNA, 0.5 µM of each primer and 2× KAPA HiFi HotStart ReadyMix (KAPA Biosystems, Wilmington, MA). The thermal profile for the amplification of each sample had an initial denaturing step at 95°C for 3 min, followed by a cycling of denaturing of 95°C for 30 s, annealing at 55°C for 30 s and a 30-s extension at 72°C (25 cycles), a 5 min extension at 72°C and a final hold at 4°C. Each 16S rRNA gene amplicon was purified using the AMPure XP reagent (Beckman Coulter, Indianapolis, IN). In the next step each sample was amplified using a limited cycle PCR program, adding Illumina sequencing adapters and dual-index barcodes [index 1(i7) and index 2(i5), Illumina, San Diego, CA] to the amplicon target. The thermal profile for the amplification of each sample had an initial denaturing step at 95°C for 3 min, followed by a denaturing cycle of 95°C for 30 s, annealing at 55°C for 30 s and a 30-s extension at 72°C (8 cycles), a 5 min extension at 72°C and a final hold at 4°C. The final libraries were again purified using the AMPure XP reagent (Beckman Coulter), quantified and normalized prior to pooling. The DNA library pool was then denatured with NaOH, diluted with hybridization buffer and heat denatured before loading on the MiSeq reagent cartridge (Illumina) and on the MiSeq instrument (Illumina). Automated cluster generation and paired-end sequencing with dual reads were performed according to the manufacturer's instructions.

Bioinformatics Analysis

Sequencing output from the Illumina MiSeq platform were converted to fastq format and demultiplexed using Illumina Bcl2Fastq 2.18.0.12. The resulting paired-end reads were processed using QIIME 2 2018.11 (28). Index and linker primer sequences were trimmed using the QIIME 2 invocation of cutadapt. The resulting paired-end reads were processed with DADA2 through QIIME 2 including merging paired ends, quality filtering, error correction, and chimera detection (29). Amplicon sequencing units from DADA2 were assigned taxonomic identifiers with respect to Greengenes release 13_08 using the QIIME 2 q2-featureclassifier (30). All sequencing data has been submitted to the NCBI repository and can be accessed *via* the following accession numbers: PRJNA674375.

Compositional Data Analysis

Compositional data analyses were performed on taxonomic count data from the ASV table after imputing values to replace zeroes. The taxa count matrices of the n = 78 samples and P = 230 taxa of the pre-exposure, $\mathbf{X}\in\mathbb{R}^{n\times P}$, and post-exposure, $\mathbf{Y}\in\mathbb{R}^{n\times P}$, gut microbiome profiles were processed as follows, respecting the compositional nature of the data (31, 32). First, both matrices were row-sum normalized, $\mathbf{X}' = C[\mathbf{X}]$, $\mathbf{Y}' = C[\mathbf{Y}]$, to map each row onto the corresponding coordinates in the unit-sum simplex, by the closure operator defined in terms of the matrix elements x_{ij} of $\mathbf{X}$ by

$$x'_{ij} = (C[X])_{ij} = \frac{x_{ij}}{\sum_{k=1}^{P} x_{ik}}$$

and similarly for $\mathbf{Y}$. We here imputed nonzero values to replace zeroes using the multiplicative approach previously described (33), setting the δ imputed values to a single constant equal to the smallest nonzero element encountered across all of $\mathbf{X}'$ and $\mathbf{Y}'$. That is, given δ, we replaced $\mathbf{X}'$ with $\mathbf{X}''$ defined as

$$x''_{ij} = \begin{cases} \delta, & |x'_{ij} = 0 \\ \left(1 - \sum_{k|x_{ik}=0} \delta\right) x'_{ij} & |x'_{ij} > 0 \end{cases}$$

and similarly replaced $\mathbf{Y}'$ with $\mathbf{Y}''$ in terms of the elements $\mathbf{Y}'$. We found this approach to be more stable across various δ imputed values and more robust than adding pseudo counts. Given sequencing instrument limitations, this approach treats all zero counts as rounded zeroes where each taxon is assumed to be present but below detection level.

To analyze the overall gut microbiome compositional change in response to treatment, we quantified the relative change from the pre-exposure ($\mathbf{X}'' \in \mathbb{R}^{n \times P}$) to the post-exposure ($\mathbf{Y}'' \in \mathbb{R}^{n \times P}$) gut microbiome profile by the compositional perturbation operation $\mathbf{Z}'' = \mathbf{Y}'' \ominus \mathbf{X}'' = C[\mathbf{Y}''/\mathbf{X}'']$ where the input to the closure operator C[] is the array of element-wise division of $\mathbf{Y}''$ by the corresponding elements of $\mathbf{X}''$ (34). To robustly handle compositional constraints on the perturbations encoded in $\mathbf{Z}''$, simplify interpretation, and to avoid using a stepwise procedure on the high dimensional set of $(P2) = 26{,}335$ pairwise log-ratios (PLR), an additive log-ratio (ALR) transformation was then applied to each sample (34): $\mathbf{X}''' = \text{ALR}[\mathbf{X}'']$, $\mathbf{Y}''' \in \mathbb{R}^{n \times (P-1)}$ and $\mathbf{Z}''' = \text{ALR}[\mathbf{Z}'']$, $\mathbf{Z}''' \in \mathbb{R}^{n \times (P-1)}$, defined in terms of selected reference taxa x_{ij}'' or z_{ip} by

$$x'''_{ij} = \left[ALR\left(X''\right)\right]_{ij} = \ln\left(\frac{x''_{ij}}{x''_{ip}}\right), j \neq p$$

$$z'''_{ij} = \left[ALR\left(Z''\right)\right]_{ij} = \ln\left(\frac{z''_{ij}}{z''_{ip}}\right), j \neq p$$

and with the j = p column removed. To ensure the lower dimensional sets of ALRs ($P - 1 = 229$) adequately preserved the distance between samples using all PLR, the reference denominators x_{ip}'' or z_{ip}'' were selected such that the Procrustes matching to the higher-dimensional all-PLR configuration was maximized (35). Results from the selection of the top ALR denominator for the Procrustes correlation between the full PLR inter-sample distances for the pre-exposure and perturbation compositions are shown in **Figure S1**. To reduce computational complexity, the Aitchison distance was computed with a weighted centered log-ratio transformation in lieu of pairwise distances using all PLRs (36, 37). Procrustes analysis and Permutational multivariate ANOVA (PERMANOVA) were implemented using the vegan package in R (38). To identify a minimal taxa log-ratio signature, feature selection using machine learning was applied separately to $\mathbf{X}'''$ and to $\mathbf{Z}'''$, where the target outcome of interest was either strain or strain-food protein respectively (see *Machine Learning Analysis*). This resulted in the pre-exposure log-ratio $\hat{X}$ signature and perturbation log-ratio signature $\hat{Z}$. The pairwise distance matrix $\mathbf{A}_{pert}$ between samples in $\hat{Z}$ were then calculated as

$$\mathbf{A}_{pert} \sum_k \left(\hat{z}_{ik} - \hat{z}_{jk}\right)^2$$

and $\mathbf{A}_{presen}$ was similarly defined in terms $\hat{X}$ of Principal coordinate analysis using $\mathbf{A}$ was employed to visualize the composition of the pre-exposure or compositional perturbation gut microbiome between samples. PERMANOVA (39) was applied using $\mathbf{A}$ to compare: (1) the pre-exposure composition between strains (CC027/GeniUnc, C3H/HeJ), and (2) the compositional perturbation between strain-food protein groups (g = 10). Next, the vegan implementation of the PERMDISP2 procedure was applied to assess heterogeneity of variance between groups (39) and to confirm centroid and/or dispersion differences. Finally, pairwise multiple comparisons (compositional perturbation) using PERMANOVA with Benjamini-Hochberg (BH) multiple testing corrections were applied to confirm significant differences and to control the false discovery rate (FDR). The FDR was estimated using $\alpha = 0.06$. Finally, the mean taxa log-ratio signature $\bar{S} \in \mathbb{R}^{g \times d}$ where $d < P$ for each strain-food protein group in $\hat{Z}$ was computed.

Cluster analysis was performed on the strain-food protein groups with the Leiden algorithm (40) for community detection on graphs, and applied to a strain-food protein similarity graph G to model the similarity between samples within each strain-food protein group. To do so, $G = (V, E, W)$ where V represents the set of strain-food protein groups, E represents the edge set formed by non-significant Pseudo-F values (from pairwise PERMANOVAs using BH corrected values, thereby indicating some possibility of similarity) with edge weights W given by corresponding pairwise Pseudo-F value between strain-food protein groups. To identify a biologically relevant set of clusters we tuned the resolution parameter used in the algorithm to satisfy two objectives: (1) minimize entropy between numbers of mice experiencing anaphylaxis (reactors) within each cluster and (2) maximize the number of clusters returned (which is achieved in the present case by maximizing the resolution parameter). With clusters defined in this way, the taxa log-ratios in were compared using the Kruskal-Wallis rank sum test to determine differences between clusters. Kruskall-Wallis p-values were adjusted for multiple comparison using BH (**Supplemental Table 1**).

Machine Learning Analysis

To reduce the number of features, we used a machine learning approach to select a reduced number of features while simultaneously assessing both the performance [repeated cross-validation (CV)] of the model and statistical significance (permutation testing) of the association between labels and features. To do this, all machine learning models used were trained with the random forest algorithm as implemented by the randomForest and caret packages in R (41, 42). Feature selection was cross-validated within the training folds and was done using the Boruta (43) algorithm in R (pre-exposure microbiome profile; binary outcome), or an implementation of the random forest recursive feature elimination (perturbation microbiome profile; mutli-classification). Model performance was computed using area under the receiver operating characteristic curve (AUROC) for both binary and multi-class classification metrics and was implemented using the pROC package in R (44). Performance was estimated using stratified 10 × 10-fold CV for binary classification and stratified 10 × 5-fold CV for mutli-classification. Given the small sample size and to ensure models were finding true associations between the labels and features, we

performed additional permutation testing using the previously described procedure (45): we trained 100 additional models with permutated labels for each test and the empirical p-value was calculated as previously described as definition 1 (45). Feature importance metrics were calculated using the permutational accuracy metric during the final model training process. All log-ratio signatures were calculated using the full dataset after CV and permutation testing by first performing feature selection and then training the final model. All analyses were performed in R software 4.0.0 and are publicly available at https://github.com/andrew84830813/food_antigen_sensitization-mircobiome-genetically_susceptible_mice.git.

RESULTS

CC027/GeniUnc Mice Become Sensitized to Peanut, Walnut, and Egg, but Not Milk, in the Absence of a Th2-Skewing Adjuvant

CC027/GeniUnc and C3H/HeJ mice differentially respond to oral peanut exposure (16). Here, we aimed to determine how these two strains of mice would react to various food proteins in the absence of a Th2-skewing adjuvant. To do this, mice were orally gavaged with peanut, walnut, milk, or egg protein once weekly for 4 weeks, and serum was collected after the exposure period to measure immunoglobulin production (**Figure 1A**). CC027/GeniUnc mice orally exposed to peanut, walnut, or egg produced high levels of antigen-specific IgE, IgG1, and IgG2a, indicating sensitization, while CC027/GeniUnc mice exposed to milk did not mount an IgE response. C3H/HeJ mice produced significantly lower quantities of serum peanut-, walnut-, and egg-specific IgE compared to CC027/GeniUnc, and near-undetectable milk-specific IgE (**Figures 1B–D**). Thus, CC027/GeniUnc mice were highly sensitized to peanut, walnut and egg, whereas C3H/HeJ mice were not.

CC027/GeniUnc Mice Have Significantly Lower Total Fecal IgA Quantities and a Distinct Gut Microbiome Compared to C3H/HeJ Mice

Sensitization through the GI tract can be influenced by luminal IgA and the consortia of microbiota in the gut (10, 11, 13). Fecal pellets were collected from mice before the 4-week food protein exposure period to compare fecal IgA quantities and gut microbiome composition in naïve mice. CC027/GeniUnc mice had significantly lower total fecal IgA quantities compared to C3H/HeJ mice (**Figure 2A**). The vast majority of CC027/GeniUnc mice (34 of 39 mice) had <15 ng/ml of IgA, while IgA quantities in C3H/HeJ mice average 127 ng/ml (range: 18–509 ng/ml).

Increased gut microbiome diversity was observed in CC027/GeniUnc mice, as demonstrated by the Shannon index (**Figure 2B**). Notably, both strains had similar numbers of unique assigned taxa (**Figure 2C**, a complete list of taxa is in **Supplemental Table 2**). Compositional data analysis revealed the gut microbiome composition was significantly different between strains at baseline, using a combination of machine learning and beta diversity analysis (**Figures 2D, E**). Indeed, our machine learning model with feature selection was able to perfectly classify strains (AUROC = 1, 10 times repeated 10-fold CV, see *Materials and Methods*) when trained on the baseline gut microbiome composition. Marked visual differences in the average gut microbiome composition of CC027/GeniUnc and C3H/HeJ mice at the order level are shown in **Figure 2F**. Since there were clear gut microbiome differences between strains, we next sought to identify the specific taxa responsible for these strain differences.

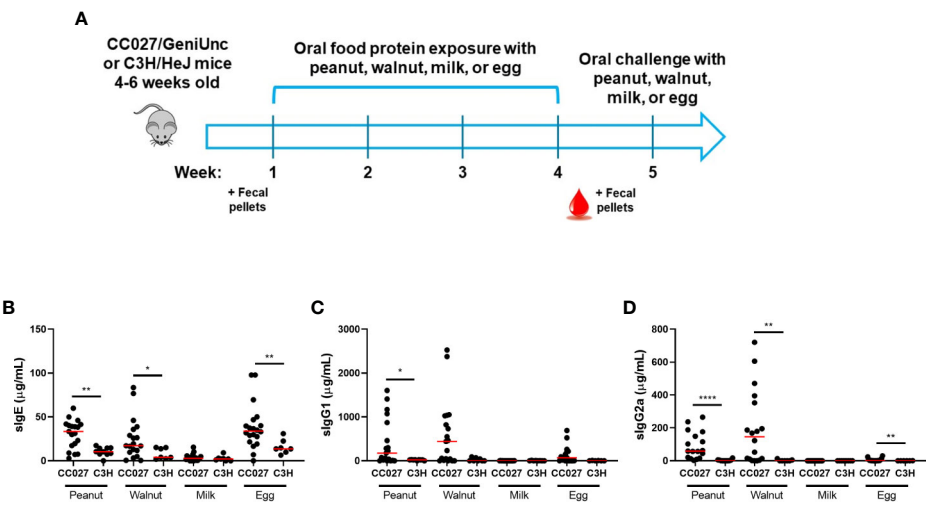


FIGURE 1 | Antibody responses in CC027/GeniUnc and C3H/HeJ mice following oral exposure to peanut, walnut, milk, or egg. **(A)** Experimental design. **(B–D)**. Serum antigen-specific IgE **(B)**, IgG1 **(C)** and IgG2a **(D)** production in CC027/GeniUnc (n = 16–20 per food) and C3H/HeJ (n = 6–10 per food) mice at week 5. *p < 0.05, **p < 0.01, ****p < 0.0001, Mann Whitney U test, individual data are shown with a red line representing the median.

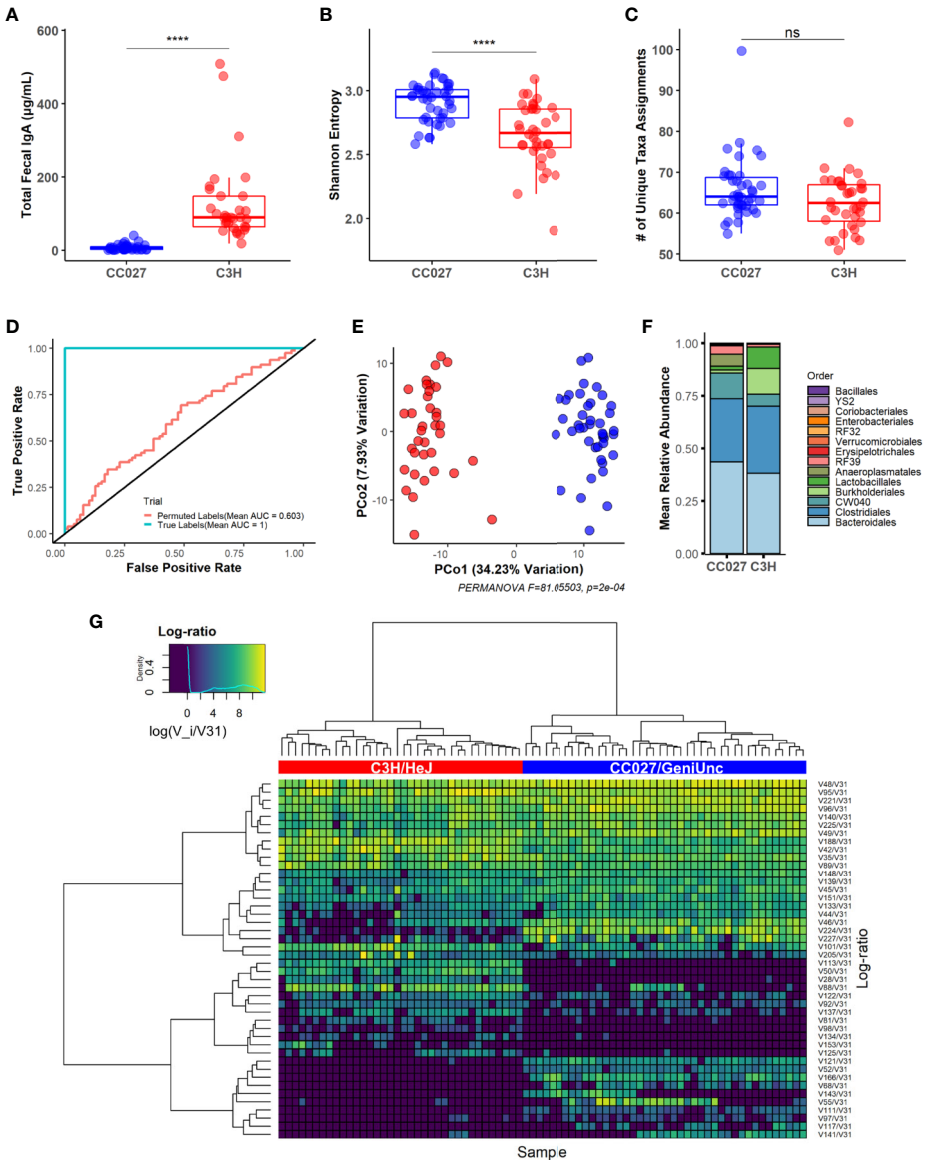


FIGURE 2 | Fecal IgA and gut microbiome of naive CC027/GeniUnc and C3H/HeJ mice. **(A)** Total fecal IgA (CC027/GeniUnc: n = 39; C3H/HeJ: n = 32). **(B)** Shannon diversity between strains (CC027/GeniUnc: n = 42; C3H/HeJ: n = 36; used for all naive microbiome analysis). **(C)** Number of unique taxa. **(D)** Pooled Receiver Operating Characteristic (ROC) curve showing model performance with feature selection under true and permuted labels (strain). **(E)** Principal coordinate analysis plot of gut microbiome between CC027/GeniUnc (blue) and C3H/HeJ (red) mice. **(F)** Relative abundances based on order. **(G)** Heatmap showing between-strain log-ratio signature clustered with ward.D2 with Euclidean dissimilarity (CC027/GeniUnc: blue, C3H/HeJ: red; x-axis). **(A–C)** Wilcoxon rank-sum test, individual data are shown within a box plot showing median with IQ range. ****$p < 0.0001$.

While our model was able to easily classify strains, we used permutation testing to ensure the model learned true associations between microbiome compositions and strain (**Figure S2**, see *Machine Learning Analysis*). To identify a strain-specific gut microbiome signature, we applied our cross-validated feature selection process using all samples. Indeed, we found log-ratios formed between the abundance of 44 taxa relative to a reference taxa, *Atopobium vaginae* (V31, used as the denominator in log-ratios, see *Compositional Data Analysis* for selection), to be important for explaining the difference between strains (**Figure 2G**). Specifically, random forest feature importance derived from the final model trained on the strain-specific signature revealed log-ratios of taxa from the *Anaeroplasma* (V224) genus and *Lachnospiraceae* (V113) family relative to *A. vaginae* were the top 2 among 44 features most important for classifying strain differences (**Figure S3**). In particular, univariate statistical analysis confirmed taxa from the *Anaeroplasma* genus were significantly more enriched relative to *A. vaginae* in CC027/GeniUnc mice than C3H/HeJ mice (all pairwise comparisons in **Figure S4A**). Conversely, univariate statistical analysis confirmed taxa from

the *Lachnospiraceae* family were significantly more enriched relative to *A. vaginae* in C3H/HeJ mice rather than CC027/GeniUnc mice (**Figure S4B**). Together, these results indicate that differences in IgA levels between these two strains, as well as the composition and abundances of a subset of the gut microbiota distinguish each strain's relative susceptibilities to sensitization.

A Higher Number of T Follicular Helper and CXCR5$^+$ B Cells in CC027/GeniUnc Mice Are Consistent With Higher Immunoglobulin Production Compared to C3H/HeJ

Because the immunologic process of oral tolerance occurs in MLNs, we investigated T and B cells in the MLNs. Specifically, Tfh cells are integral in driving IgE production (46, 47), and their presence may help explain why CC027/GeniUnc mice become sensitized to peanut, walnut and egg. Following food protein oral exposure and challenge, MLNs were collected from mice for T and B cell analysis by flow cytometry (gating shown in **Figure S5**). CC027/GeniUnc mice had lower frequency of T cells overall, but similar frequency of CD4$^+$ T cells compared to C3H/HeJ mice (**Figures 3A, B**). CC027/GeniUnc mice also had higher frequency of antigen-experienced, PD-1$^+$CD4$^+$ T cells (**Figure 3C**). Importantly, the frequency of Tfh cells was higher in CC027/GeniUnc mice compared to C3H/HeJ mice (**Figure 3D**). CC027/GeniUnc mice also had higher frequency of B cells and CXCR5$^+$ B cells (**Figures 3E, F**), suggesting that the number of germinal center B cells is higher, resulting in higher immunoglobulin production in CC027/GeniUnc mice. Overall, the increased abundances of Tfh cells and CXCR5$^+$ B cells may lead to higher IgE production in CC027/GeniUnc mice.

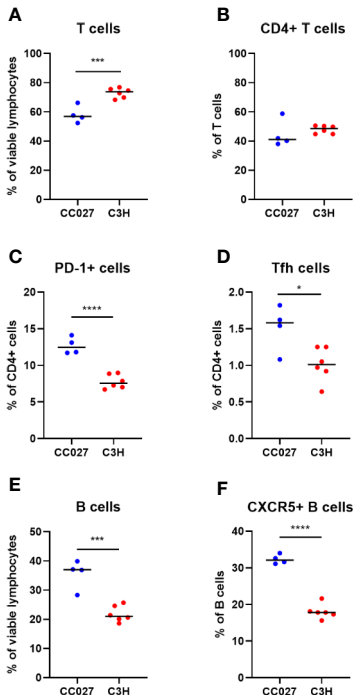


FIGURE 3 | Lymphocyte subsets in mesenteric lymph nodes from CC027/GeniUnc (n = 4) and C3H/HeJ mice (n = 6). **(A)** T cells, **(B)** CD4+ T cells, **(C)** PD-1+ CD4+ cells, **(D)** T follicular helper cells, **(E)** B cells, and **(F)** CXCR5+ B cells. *p < 0.05, ***p < 0.001, ****p < 0.0001, Unpaired t test, individual data are shown with a black line representing the median.

CC027/GeniUnc Mice Experience Anaphylaxis Upon Oral Challenge to Peanut and Walnut, but Not Milk or Egg

While IgE production indicates that CC027/GeniUnc mice were sensitized to peanut, walnut and egg, oral food challenges are required to confirm an allergy to these foods. Mice were challenged to either peanut, walnut, milk or egg proteins *via* oral gavage following the exposure period. CC027/GeniUnc mice experienced severe anaphylaxis (>5°C decrease) to peanut (**Figure 4A**), and mild anaphylaxis (>3°C decrease) to walnut (**Figure 4B**), but did not experience allergic reactions (<3°C decrease) to milk, egg or PBS (**Figures 4C–E**). C3H/HeJ mice did not experience reactions to any oral challenge. Although CC027/GeniUnc mice had increased IgE production in response to peanut, walnut and egg, mice only experienced anaphylaxis to peanut and walnut, suggesting that there are additional factors besides IgE production that contribute to reactivity.

We previously reported that CC027/GeniUnc mice sensitized with peanut and cholera toxin have detectable peanut allergen, Ara h 2, in their serum following oral peanut challenge (16), suggesting increased antigen absorption may contribute to anaphylaxis to peanut. To determine whether cholera toxin-free exposure to each food led to antigen absorption post-challenge, we quantified major serum allergens in peanut (Ara h 2), milk (Bos d 5), and egg (Gal d 2) *via* ELISA in peanut-, milk-, and egg-exposed mice, respectively (**Figure 4F**). Consistent with our previous results, CC027/GeniUnc mice sensitized to peanut had detectable levels of Ara h 2 in serum following oral peanut challenge. Contrarily, Bos d 5 and Gal d 2 were not detected in mice following milk and egg challenge, respectively. Validated ELISA reagents were not available for any major walnut allergen, and allergens were not quantified in walnut-sensitized mice. Together these results indicate that CC027/GeniUnc mice have increased antigen absorption in the gut following peanut, but not milk or egg, challenge, which is likely an important factor leading to anaphylaxis.

Fecal Immunoglobulin Quantities in CC027/GeniUnc Mice Are Associated With Anaphylaxis Upon Oral Challenge

To further investigate potential gut absorption differences between groups, fecal pellets collected after the food protein exposure period were analyzed for total fecal IgA. CC027/GeniUnc mice have very low total fecal IgA, while C3H/HeJ mice have higher total fecal IgA regardless of antigen exposure (**Figure 5A**). Antigen-specific IgA was not detected, regardless of strain or food protein exposure. Antigen-specific IgG was also

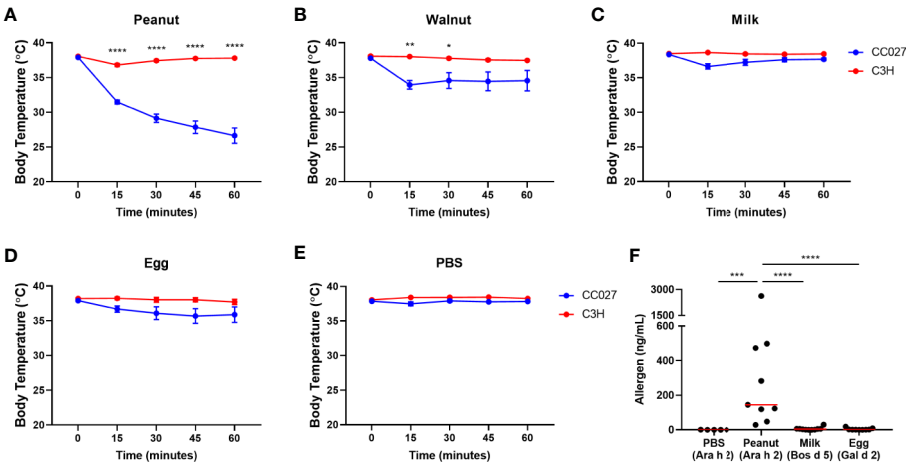


FIGURE 4 | Oral challenge results. **(A–E)** Body temperature changes in CC027/GeniUnc (blue, n = 10 per food) and C3H/HeJ (red, n = 7–9 per food) mice challenged to peanut **(A)**, walnut **(B)**, milk **(C)**, egg **(D)**, and PBS **(E)**. **(F)** Serum allergen quantities post-challenge. Allergens in parentheses represent the allergen quantified for each group. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, two-way ANOVA, data is represented by the mean ± SEM for each time point. Statistics are shown for groups that reacted upon challenge (>3°C decrease). For allergen quantities: Mann Whitney U test, individual data are shown with a red line representing the median.

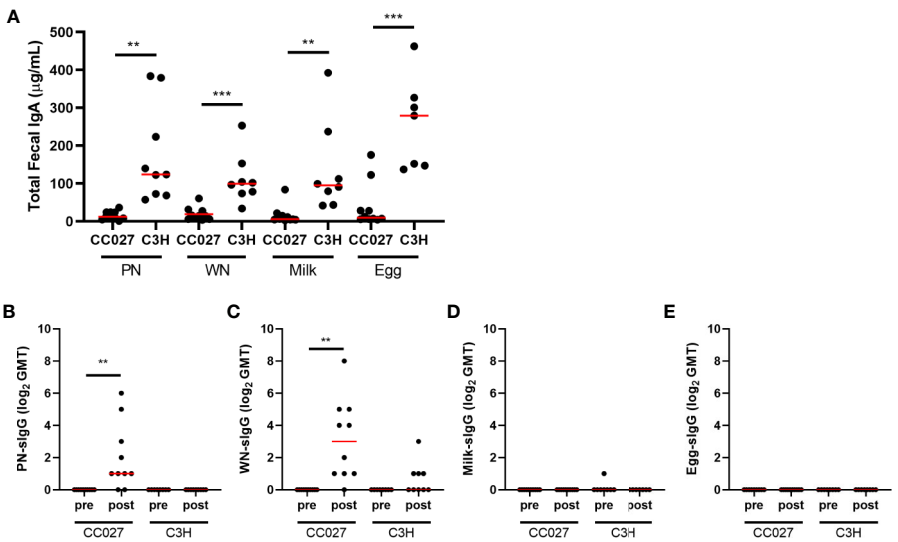


FIGURE 5 | Fecal immunoglobulin quantities. **(A)** Total fecal IgA measured in peanut-, walnut-, milk-, or egg-exposed CC027/GeniUnc (n = 10 per food) and C3H/HeJ (n = 7–9 per food) mice. **(B–E)** Fecal peanut- **(B)**, walnut- **(C)**, milk- **(D)**, and egg- **(E)** specific IgG (represented as $\log_2$ of the geometric mean titer (GMT)) in food protein-exposed CC027/GeniUnc (n = 9–10 per food) or C3H/HeJ (n = 7–9 per food) mice. **p < 0.01, ***p < 0.001, Unpaired t test (IgA) and Wilcoxon test (IgG), individual data are shown with a red line representing the median.

measured in fecal pellets. Interestingly, CC027/GeniUnc mice sensitized to peanut or walnut had increased fecal antigen-specific IgG following exposure, while CC027/GeniUnc mice exposed to milk and egg had no detectable antigen-specific fecal IgG (**Figures 5B–E**). These results associated with the CC027/GeniUnc anaphylaxis outcomes and may suggest increased antibody production in the gut or the translocation of antibody across the gut barrier to the lumen.

Microbial Compositions Differ Among CC027/GeniUnc Mice Exposed to Peanut, Walnut, Milk, or Egg

Given that gut microbial composition is affected by dietary antigens, we hypothesized that enteral exposure to different foods in CC027/GeniUnc mice would lead to distinct changes of microbial compositions. To compare the effects of different food proteins, the relative changes in gut microbiome before and

after exposure (i.e., perturbation, see *Materials and Methods*) were further analyzed. Accordingly, a multi-classification model with feature selection was trained on the microbiome perturbation composition in response to food protein exposure, and a statistically significant association (AUROC = 0.84, p = 0.009, see *Materials and Methods*, **Figure S6**) was identified between the strain-food protein interaction (i.e., CC027-peanut, C3H-peanut, etc.) and the microbiome perturbation. Using this feature selection process on all samples we identified a reduced signature of 23 taxa log-ratios representative of how each strain's gut microbiome responded to the food exposure. A Principal Coordinate Analysis (PCoA) plot using the log-ratio signature shows perturbation dissimilarities based on food exposure and strain (**Figure 6A**). PERMANOVA analysis confirmed there were some significantly distinct gut microbiome responses to food exposure based on strain (Pseudo-F = 2.7006, p < 0.0001). Multivariate dispersions between strain-food protein interactions were analyzed with PERMDISP2 (**Figure S7**), and the results indicated differences in multivariate dispersion were not significant (Pseudo-F = 1.0100, p = 0.4409) and instead that there were only differences among the location of group centroids.

To compare perturbation responses, we used similarity graphs (see *Compositional Data Analysis*) to cluster each strain-food protein group. From this, we identified five clusters (**Figure 6B** and **Figure S8**) of responses to food protein exposure. Notably, our clusters resulted in a disproportionate number of anaphylactic mice (90%, n = 16) being concentrated in cluster 1 (**Figure S9A**). Further, permutation testing revealed that our five cluster assignments clustered anaphylactic mice significantly better than random clustering (p = 0.0146, **Figure S9B**). These results indicate our log-ratio signature not only distinguishes differences between strain-food protein perturbations but also may explain key changes in the gut microbiome associated with increased risk of anaphylaxis.

To explore the location-only effects, principal component analysis (PCA) of the mean log-ratio signature of each strain-food protein group revealed strong separation between clusters (**Figure S10**). PCA biplot analysis revealed key log-ratios responsible for cluster separation (**Figure 6B**) defined primarily by *Bifidobacterium* (V26), *Akkermansia* (V227), and *Candidatus Arthromitus* (V101) relative to *Listeria* (V79, see *Compositional Data Analysis* for selection). We next sought to understand how each cluster differed based on each log-ratio of the perturbation signature. Kruskal-Wallis testing revealed 13 log-ratios had some statistically significant difference in values across clusters (**Supplemental Table 3**). Additionally, random forest variable importance shows the relative importance of each log-ratio for associating strain-food protein to microbiome response (**Figure S11**). Specific taxa responsible for differences between groups are shown in **Figure 6C**, which demonstrates an average depletion or enrichment of taxa relative to *Listeria* (V79) in each group. These results indicate key taxa may be critical for distinguishing groups of mice that react upon oral food challenge.

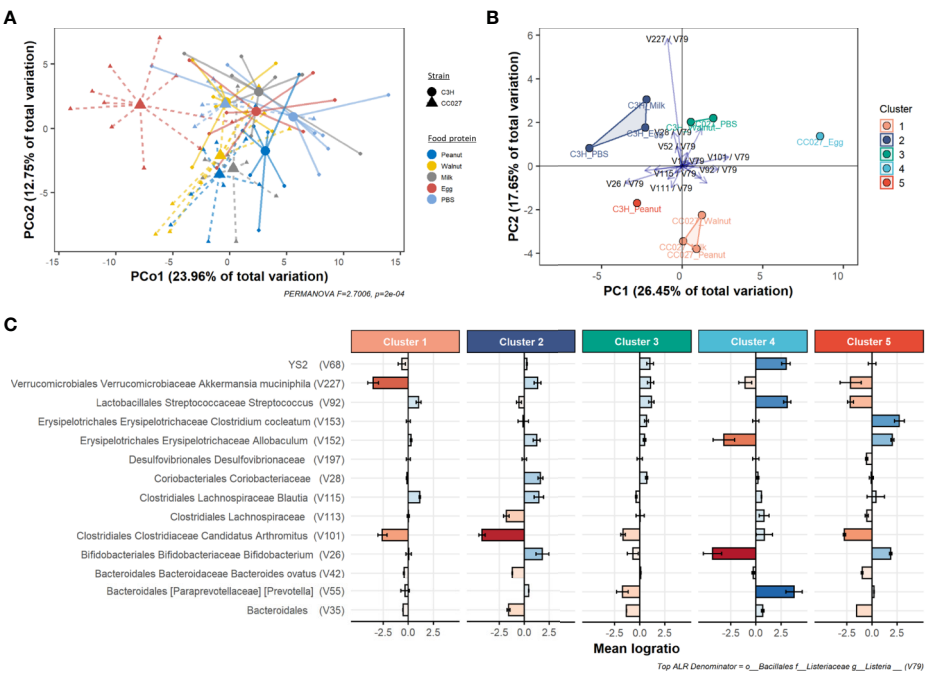


FIGURE 6 | Gut microbiome changes after food protein exposure in CC027/GeniUnc (n = 7–10 per food) and C3H/HeJ (n = 7–8 per food) mice. **(A)** PCoA plot of compositional perturbation explaining 36.71% of total variation grouped by strain-food protein. **(B)** PCA biplot showing relative location of clusters and how the mean strain-food protein log-ratios within the perturbation signature contribute to differences between groups. **(C)** Depletion or enrichment of specific taxa relative to *Listeria* within each cluster. Error bars reflect 95% confidence intervals of the mean log-ratio value for all samples within each cluster. All taxa information is included in the following sequence: order, family, genus, species.

DISCUSSION

Food allergies are thought to develop from oral tolerance failure, but the underlying mechanisms leading to sensitization are widely unknown. In mouse models, oral tolerance is typically broken by using mucosal adjuvants like cholera toxin or Staphylococcal enterotoxin B. However, we recently identified a genetically susceptible strain (CC027/GeniUnc) that does not require an adjuvant for peanut sensitization (16). Here, we focused on understanding the mucosal immunology in this CC027/GeniUnc strain that allows for enteric sensitization by analysing fecal IgA, antigen absorption, microbiome composition and MLN lymphocyte subsets after exposure to peanut, walnut, milk, or egg.

CC027/GeniUnc mice were orally sensitized to peanut, walnut and egg based on their immunoglobulin production, but only reacted upon oral challenge to peanut and walnut. Furthermore, CC027/GeniUnc mice had detectable serum Ara h 2 post-oral peanut challenge, while Bos d 5 and Gal d 2 were not detected in milk- or egg-challenged mice. Together, these results suggest that antigen absorption may play a role in reactivity in sensitized mice. Systemic absorption was previously shown to be required for anaphylaxis (48), which is consistent with our findings. Prior to absorption, antigens encounter enzymes and an acidic environment, which can lead to extensive hydrolysis of the antigen. Therefore, the biochemical properties of allergens are critical in maintaining the capacity to cross-link IgE. The major peanut allergen, Ara h 2, is a 2S albumin and is resistant to proteolysis, which likely contributes to its absorption and reaction susceptibility (49, 50). Similarly, the 2S albumins that are major allergens in tree nuts are also quite stable, which may explain walnut-induced reactions in this model.

With enteral exposure, antigen presentation occurs in gut-draining MLNs. These immunologic sites can lead to tolerance or allergy. Mounting evidence demonstrates that Tfh cells are the main source of IL-4 leading to IgE production (51). Since CC027/GeniUnc but not C3H/HeJ mice produced IgE against enteral antigens, we hypothesized that there may be more Tfh cells in MLNs. Indeed, CC027/GeniUnc mice had a higher frequency of Tfh cells compared to C3H/HeJ, despite having lower T cell frequencies. Furthermore, CC027/GeniUnc mice had more $CD4^+$ T cells expressing PD-1, indicating more antigen presentation leading to antigen-experienced T cells occurs compared to C3H/HeJ mice. CC027/GeniUnc mice also had a higher frequency of MLN $CXCR5^+$ B cells compared to C3H/HeJ. CXCR5 is critical for homing to lymph node follicles and is therefore important for class-switching and IgE production (52). These results suggest increased frequencies of MLN Tfh and $CXCR5^+$ B cells in the CC027/GeniUnc mice may lead to higher IgE production compared to C3H/HeJ mice.

Mucosal IgA is thought to be protective against food allergies by preventing translocation of allergen through the gut barrier. In humans, selective IgA deficiency has been associated with allergic diseases, including food allergy (53, 54). Here, CC027/GeniUnc mice have very low levels of total fecal IgA compared to C3H/HeJ mice prior to food protein exposure, which may lead to less neutralization of allergen and more allergen absorption in the gut. Of note, fecal antigen-specific IgA was undetectable in either strain. Together, our results are consistent with a recent report demonstrating that cholera toxin was required for fecal antigen-specific IgA production, and that Tfh cells were not required for mucosal IgA production (11).

The main contributor to the gut mucosa is the microbiome, which has been linked to susceptibility to food allergy. We rigorously analyzed the gut microbiome with a combination of compositional data and machine learning approaches and found marked compositional differences between CC027/GeniUnc and C3H/HeJ mice at baseline. Furthermore, we found the gut microbiome of CC027/GeniUnc mice responded differently when exposed to peanut, walnut, milk, and egg when compared to C3H/HeJ mice. Importantly, separation of the five clusters in response to food protein exposure was driven primarily by specific changes in relative abundance of *Bifidobacterium*, *Akkermansia*, and *Candidatus Arthromitus* relative to *Listeria*. In our analysis, the peanut-, walnut- and milk-exposed CC027/GeniUnc mice (cluster 1 in **Figures 6B, C**), were defined by a depletion in *Akkermansia* bacteria relative to *Listeria*. *Akkermansia*, in particular, is known to play a critical role in gut barrier integrity through mucus layer production (55, 56); the relative depletion of *Akkermansia* is consistent with the presumed decreased gut barrier function in peanut- and walnut-sensitized CC027/GeniUnc mice. Moreover, given the clustering assignments, we found that cluster 1 (peanut-, walnut-, and milk-exposed CC027/GeniUnc mice) disproportionately contained ~90% of all mice that reacted upon challenge. Although milk-exposed CC027/GeniUnc mice did not demonstrate similar gut barrier permeability, they also did not produce any milk-specific immunoglobulins, which is a prerequisite component of allergy. Similar to cluster 1, peanut-exposed C3H/HeJ mice (cluster 5) had a notable decrease in *Akkermansia*, but also an increase in *Bifidobacterium*, which together with IgA production, may be protective against peanut allergy. The gut microbiome of egg-sensitized CC027/GeniUnc mice was characterized by the enrichment of taxa from the order YS2, and *Prevotella* and *Streptococcus* genera, and depletion of taxa from the *Bifidobacterium* and *Allobaculum* genera relative to *Listeria*. Since egg-sensitized CC027/GeniUnc mice did produce increased egg-specific IgE, perhaps the egg-induced microbiome changes did not further facilitate an *Akkermansia*-depleted, and therefore weaker, gut barrier that we believe is associated with the reactions in the peanut- and walnut-sensitized CC027/GeniUnc mice. Together these results suggest increased production of specific IgE, decreased total fecal IgA, and alterations of *Bifidobacterium* and *Akkermansia* communities in the gut are all associated with sensitization of food proteins and risk of anaphylaxis on exposure.

Due to the dynamic nature of the gut environment, multiple factors are known to contribute to sensitization susceptibility. The results presented here suggest an interactive role between food antigens and the local gut environment, including IgA and microbiome, leading to tolerance or sensitization. Specifically, CC027/GeniUnc mice have very low levels of total fecal IgA and a unique microbiome, which together may lead to increased

antigen uptake. Then, in the MLNs, Tfh cells, which are also increased in CC027/GeniUnc mice, may drive the $CXCR5^+$ B cells to class-switch into IgE-producing cells. Once animals are sensitized, increased gut permeability is a potential factor allowing for systemic absorption and anaphylaxis to occur. Based on the immunologic responses generated after food protein exposure in the absence of a Th2-skewing adjuvant, this model in CC027/GeniUnc mice provides a useful platform for future food allergy studies.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

ETHICS STATEMENT

The animal study was reviewed and approved by Institutional Animal Care and Use Committee at the University of North Carolina at Chapel Hill.

AUTHOR CONTRIBUTIONS

JS, MK, KO, MF, HS, and AB conceived project and contributed to study design. JS, MK, BJ-W, MA-P, TM, RI, JK, and ES performed experiments and analyzed data. AH performed compositional and machine learning analysis in consultation with JS, MK, and PM. JS and MK wrote the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

JS is funded by a T32 Allergy/Immunology Training Grant (AI007062) through Duke University and University of North Carolina (UNC) at Chapel Hill. Research was supported by UNC SOM Office of Research and TraCS Translational Team Science Award (grant nos. TTSA017P1 and TTSA017P2; to AB, MK, and MF), American Research Foundation for Nut Allergies (to JS and MK). ES is funded by T32 DK007737 (CGIBD T32) and Thurston Arthritis Research Center and NIAID Loan Repayment Program. AH is funded as a Howard Hughes Medical Institute Gilliam Fellow.

ACKNOWLEDGMENTS

We would like to acknowledge the UNC Systems Genetics Core Facility for maintenance and distribution of the Collaborative Cross mice.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2020.599637/full#supplementary-material

REFERENCES

1. Branum AM, Lukacs SL. Food allergy among children in the United States. *Pediatrics* (2009) 124(6):1549–55. doi: 10.1542/peds.2009-1210
2. Gupta RS, Warren CM, Smith BM, Jiang J, Blumenstock JA, Davis MM, et al. Prevalence and Severity of Food Allergies Among US Adults. *JAMA Netw Open* (2019) 2(1):e185630. doi: 10.1001/jamanetworkopen.2018.5630
3. Avery NJ, King RM, Knight S, Hourihane JO. Assessment of quality of life in children with peanut allergy. *Pediatr Allergy Immunol* (2003) 14(5):378–82. doi: 10.1034/j.1399-3038.2003.00072.x
4. Bock SA, Munoz-Furlong A, Sampson HA. Fatalities due to anaphylactic reactions to foods. *J Allergy Clin Immunol* (2001) 107(1):191–3. doi: 10.1067/mai.2001.112031
5. Bock SA, Munoz-Furlong A, Sampson HA. Further fatalities caused by anaphylactic reactions to food, 2001-2006. *J Allergy Clin Immunol* (2007) 119(4):1016–8. doi: 10.1016/j.jaci.2006.12.622
6. Fleischer DM. The natural history of peanut and tree nut allergy. *Curr Allergy Asthma Rep* (2007) 7(3):175–81. doi: 10.1007/s11882-007-0018-y
7. Tordesillas L, Berin MC. Mechanisms of Oral Tolerance. *Clin Rev Allergy Immunol* (2018) 55(2):107–17. doi: 10.1007/s12016-018-8680-5
8. Worbs T, Bode U, Yan S, Hoffmann MW, Hintzen G, Bernhardt G, et al. Oral tolerance originates in the intestinal immune system and relies on antigen carriage by dendritic cells. *J Exp Med* (2006) 203(3):519–27. doi: 10.1084/jem.20052016
9. Hadis U, Wahl B, Schulz O, Hardtke-Wolenski M, Schippers A, Wagner N, et al. Intestinal tolerance requires gut homing and expansion of FoxP3+ regulatory T cells in the lamina propria. *Immunity* (2011) 34(2):237–46. doi: 10.1016/j.immuni.2011.01.016
10. Corthesy B. Multi-faceted functions of secretory IgA at mucosal surfaces. *Front Immunol* (2013) 4:185. doi: 10.3389/fimmu.2013.00185
11. Zhang B, Liu E, Gertie JA, Joseph J, Xu L, Pinker EY, et al. Divergent T follicular helper cell requirement for IgA and IgE production to peanut during allergic sensitization. *Sci Immunol* (2020) 5(47). doi: 10.1126/sciimmunol.aay2754
12. Stefka AT, Feehley T, Tripathi P, Qiu J, McCoy K, Mazmanian SK, et al. Commensal bacteria protect against food allergen sensitization. *Proc Natl Acad Sci USA* (2014) 111(36):13145–50. doi: 10.1073/pnas.1412008111
13. Feehley T, Plunkett CH, Bao R, Choi Hong SM, Culleen E, Belda-Ferre P, et al. Healthy infants harbor intestinal bacteria that protect against food allergy. *Nat Med* (2019) 25(3):448–53. doi: 10.1038/s41591-018-0324-z
14. Srivastava A, Morgan AP, Najarian ML, Sarsani VK, Sigmon JS, Shorter JR, et al. Genomes of the Mouse Collaborative Cross. *Genetics* (2017) 206(2):537–56. doi: 10.1534/genetics.116.198838
15. Churchill GA, Airey DC, Allayee H, Angel JM, Attie AD, Beatty J, et al. The Collaborative Cross, a community resource for the genetic analysis of complex traits. *Nat Genet* (2004) 36(11):1133–7. doi: 10.1038/ng1104-1133
16. Orgel K, Smeekens JM, Ye P, Fotsch L, Guo R, Miller DR, et al. Genetic diversity between mouse strains allows identification of the CC027/GeniUnc strain as an orally reactive model of peanut allergy. *J Allergy Clin Immunol* (2019) 143(3):1027–37. doi: 10.1016/j.jaci.2018.10.009
17. Li XM, Serebrisky D, Lee SY, Huang CK, Bardina L, Schofield BH, et al. A murine model of peanut anaphylaxis: T- and B-cell responses to a major peanut allergen mimic human responses. *J Allergy Clin Immunol* (2000) 106(1 Pt 1):150–8. doi: 10.1067/mai.2000.107395
18. Tordesillas L, Goswami R, Benede S, Grishina G, Dunkin D, Jarvinen KM, et al. Skin exposure promotes a Th2-dependent sensitization to peanut allergens. *J Clin Invest* (2014) 124(11):4965–75. doi: 10.1172/JCI75660

19. Kulis M, Chen X, Lew J, Wang Q, Patel OP, Zhuang Y, et al. The 2S albumin allergens of Arachis hypogaea, Ara h 2 and Ara h 6, are the major elicitors of anaphylaxis and can effectively desensitize peanut-allergic mice. *Clin Exp Allergy* (2012) 42(2):326–36. doi: 10.1111/j.1365-2222.2011.03934.x
20. Smarr CB, Hsu CL, Byrne AJ, Miller SD, Bryce PJ. Antigen-fixed leukocytes tolerize Th2 responses in mouse models of allergy. *J Immunol* (2011) 187 (10):5090–8. doi: 10.4049/jimmunol.1100608
21. Bednar KJ, Hardy L, Smeekens J, Raghuwanshi D, Duan S, Kulis MD, et al. Antigenic Liposomes for Generation of Disease-specific Antibodies. *J Vis Exp JoVE* (2018) 140). doi: 10.3791/58285
22. Smeekens JM, Immormino RM, Balogh PA, Randell SH, Kulis MD, Moran TP. Indoor dust acts as an adjuvant to promote sensitization to peanut through the airway. *Clin Exp Allergy* (2019) 49(11):1500–11. doi: 10.1111/cea.13486
23. Azcarate-Peril MA, Butz N, Cadenas MB, Koci M, Ballou A, Mendoza M, et al. An Attenuated Salmonella enterica Serovar Typhimurium Strain and Galacto-Oligosaccharides Accelerate Clearance of Salmonella Infections in Poultry through Modifications to the Gut Microbiome. *Appl Environ Microbiol* (2018) 84(5). doi: 10.1128/AEM.02526-17
24. Carlson AL, Xia K, Azcarate-Peril MA, Goldman BD, Ahn M, Styner MA, et al. Infant Gut Microbiome Associated With Cognitive Development. *Biol Psychiatry* (2018) 83(2):148–59. doi: 10.1016/j.biopsych.2017.06.021
25. Arnold JW, Simpson JB, Roach J, Bruno-Barcena JM, Azcarate-Peril MA. Prebiotics for Lactose Intolerance: Variability in Galacto-Oligosaccharide Utilization by Intestinal Lactobacillus rhamnosus. *Nutrients* (2018) 10(10). doi: 10.3390/nu10101517
26. Jones RB, Zhu X, Moan E, Murff HJ, Ness RM, Seidner DL, et al. Inter-niche and inter-individual variation in gut microbial community assessment using stool, rectal swab, and mucosal samples. *Sci Rep* (2018) 8(1):4139. doi: 10.1038/s41598-018-22408-4
27. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, et al. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc Natl Acad Sci USA* (2011) 108 Suppl 1:4516–22. doi: 10.1073/pnas.1000080107
28. Mohsen A, Park J, Chen YA, Kawashima H, Mizuguchi K. Impact of quality trimming on the efficiency of reads joining and diversity analysis of Illumina paired-end reads in the context of QIIME1 and QIIME2 microbiome analysis frameworks. *BMC Bioinf* (2019) 20(1):581. doi: 10.1186/s12859-019-3187-5
29. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* (2016) 13(7):581–3. doi: 10.1038/nmeth.3869
30. Bokulich NA, Kaehler BD, Rideout JR, Dillon M, Bolyen E, Knight R, et al. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome* (2018) 6(1):90. doi: 10.1186/s40168-018-0470-z
31. Gloor GB, Macklaim JM, Pawlowsky-Glahn V, Egozcue JJ. Microbiome Datasets Are Compositional: And This Is Not Optional. *Front Microbiol* (2017) 8:2224. doi: 10.3389/fmicb.2017.02224
32. Quinn TP, Erb I, Richardson MF, Crowley TM. Understanding sequencing data as compositions: an outlook and review. *Bioinformatics* (2018) 34 (16):2870–8. doi: 10.1093/bioinformatics/bty175
33. Martín-Fernández JA, Barceló-Vidal C, Pawlowsky-Glahn V. Dealing with Zeros and Missing Values in Compositional Data Sets Using Nonparametric Imputation. *Math Geol* (2003) 35(3):253–78. doi: 10.1023/A:1023866030544
34. Aitchison J. The Statistical Analysis of Compositional Data. *J R Stat Soc Ser B (Methodol)* (1982) 44(2):139–77. doi: 10.1111/j.2517-6161.1982.tb01195.x
35. Greenacre M. Variable Selection in Compositional Data Analysis Using Pairwise Logratios. *Math Geosci* (2019) 51(5):649–82. doi: 10.1007/s11004-018-9754-x
36. Aitchison J, Barceló-Vidal C, Martín-Fernández JA, Pawlowsky-Glahn V. Logratio Analysis and Compositional Distance. *Math Geol* (2000) 32(3):271–5. doi: 10.1023/A:1007529726302
37. Greenacre M, Lewi P. Distributional Equivalence and Subcompositional Coherence in the Analysis of Compositional Data, Contingency Tables and Ratio-Scale Measurements. *J Classif* (2009) 26(1):29–54. doi: 10.1007/s00357-009-9027-y
38. Oksanen J, Guillaume Blanchet F, Friendly M, Kindt R, Legendre P, McGlinn D, et al. *vegan: Community Ecology Package. R package version 25-6.* (2019). Available at: https://CRAN.R-project.org/package=vegan.
39. Anderson MJ. *Permutational Multivariate Analysis of Variance (PERMANOVA)*. Wiley StatsRef (2017) p. 1–15. Statistics Reference Online.
40. Traag VA, Waltman L, van Eck NJ. From Louvain to Leiden: guaranteeing well-connected communities. *Sci Rep* (2019) 9(1):5233. doi: 10.1038/s41598-019-41695-z
41. Liaw A, Wiener M. Classification and Regression by randomForest. *R News* (2002) 2(3):18–22.
42. Kuhn M. *caret: Classification and Regression Training. R package version 60-86.* (2020). Available at: https://CRAN.R-project.org/package=caret.
43. Kursa MB, Rudnicki WR. Feature Selection with the Boruta Package. *J Stat Softw* (2010) 36: (11):13. doi: 10.18637/jss.v036.i11
44. Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez J-C, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinf* (2011) 12(1):77. doi: 10.1186/1471-2105-12-77
45. M Ojala, GC Garriga eds. Permutation Tests for Studying Classifier Performance. *J Mach Learn Res* (2010) 11:1833–63.
46. Kobayashi T, Iijima K, Dent AL, Kita H. Follicular helper T cells mediate IgE antibody response to airborne allergens. *J Allergy Clin Immunol* (2017) 139 (1):300–13. doi: 10.1016/j.jaci.2016.04.021
47. Gowthaman U, Chen JS, Zhang B, Flynn WF, Lu Y, Song W, et al. Identification of a T follicular helper cell subset that drives anaphylactic IgE. *Science* (2019) 365(6456). doi: 10.1126/science.aaw6433
48. Strait RT, Mahler A, Hogan S, Khodoun M, Shibuya A, Finkelman FD. Ingested allergens must be absorbed systemically to induce systemic anaphylaxis. *J Allergy Clin Immunol* (2011) 127(4):982–9 e1. doi: 10.1016/j.jaci.2011.01.034
49. Sen M, Kopper R, Pons L, Abraham EC, Burks AW, Bannon GA. Protein structure plays a critical role in peanut allergen stability and may determine immunodominant IgE-binding epitopes. *J Immunol* (2002) 169(2):882–7. doi: 10.4049/jimmunol.169.2.882
50. Koppelman SJ, Hefle SL, Taylor SL, de Jong GA. Digestion of peanut allergens Ara h 1, Ara h 2, Ara h 3, and Ara h 6: a comparative in vitro study and partial characterization of digestion-resistant peptides. *Mol Nutr Food Res* (2010) 54 (12):1711–21. doi: 10.1002/mnfr.201000011
51. Dolence JJ, Kobayashi T, Iijima K, Krempski J, Drake LY, Dent AL, et al. Airway exposure initiates peanut allergy by involving the IL-1 pathway and T follicular helper cells in mice. *J Allergy Clin Immunol* (2018) 142(4):1144–58 e8. doi: 10.1016/j.jaci.2017.11.020
52. Ansel KM, Ngo VN, Hyman PL, Luther SA, Forster R, Sedgwick JD, et al. A chemokine-driven positive feedback loop organizes lymphoid follicles. *Nature* (2000) 406(6793):309–14. doi: 10.1038/35018581
53. Janzi M, Kull I, Sjoberg R, Wan J, Melen E, Bayat N, et al. Selective IgA deficiency in early life: association to infections and allergic diseases during childhood. *Clin Immunol* (2009) 133(1):78–85. doi: 10.1016/j.clim.2009.05.014
54. Tuano KS, Orange JS, Sullivan K, Cunningham-Rundles C, Bonilla FA, Davis CM. Food allergy in patients with primary immunodeficiency diseases: prevalence within the US Immunodeficiency Network (USIDNET). *J Allergy Clin Immunol* (2015) 135(1):273–5. doi: 10.1016/j.jaci.2014.09.024
55. Hiippala K, Jouhten H, Ronkainen A, Hartikainen A, Kainulainen V, Jalanka J, et al. The Potential of Gut Commensals in Reinforcing Intestinal Barrier Function and Alleviating Inflammation. *Nutrients* (2018) 10(8). doi: 10.3390/nu10080988
56. Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, et al. Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. *Proc Natl Acad Sci USA* (2013) 110(22):9066–71. doi: 10.1073/pnas.1219451110

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

ORIGINAL RESEARCH
published: 03 February 2021
doi: 10.3389/fimmu.2020.603059

Regulatory T Cells Developing Peri-Weaning Are Continually Required to Restrain Th2 Systemic Responses Later in Life

Kathryn A. Knoop[1,2*], *Keely G. McDonald*[1], *Chyi-Song Hsieh*[1], *Phillip I. Tarr*[1,3†] *and Rodney D. Newberry*[1*]

[1] Department of Internal Medicine, Washington University School of Medicine, St. Louis, MO, United States, [2] Department of Immunology, Mayo Clinic, Rochester, MN, United States, [3] Department of Pediatrics and Molecular Medicine, Washington University School of Medicine, St. Louis, MO, United States

OPEN ACCESS

Edited by:
Ana Olivera,
National Institutes of Health (NIH), United States

Reviewed by:
Manolo Sambucci,
Santa Lucia Foundation (IRCCS), Italy
Alex Dent,
Purdue University Indianapolis, United States

***Correspondence:**
Kathryn A. Knoop
Knoop.Kathryn@mayo.edu
Rodney D. Newberry
rnewberry@wustl.edu

†ORCID:
Phillip I. Tarr
orcid.org/0000-0003-4078-7517

Specialty section:
This article was submitted to T Cell Biology, a section of the journal Frontiers in Immunology

Received: *09 September 2020*
Accepted: *18 December 2020*
Published: *03 February 2021*

Citation:
Knoop KA, McDonald KG, Hsieh C-S, Tarr PI and Newberry RD (2021) Regulatory T Cells Developing Peri-Weaning Are Continually Required to Restrain Th2 Systemic Responses Later in Life. Front. Immunol. 11:603059. doi: 10.3389/fimmu.2020.603059

Atopic disorders including allergic rhinitis, asthma, food allergy, and dermatitis, are increasingly prevalent in Western societies. These disorders are largely characterized by T helper type 2 (Th2) immune responses to environmental triggers, particularly inhaled and dietary allergens. Exposure to such stimuli during early childhood reduces the frequency of allergies in at-risk children. These allergic responses can be restrained by regulatory T cells (Tregs), particularly Tregs arising in the gut. The unique attributes of how early life exposure to diet and microbes shape the intestinal Treg population is a topic of significant interest. While imprinting during early life promotes the development of a balanced immune system and protects against immunopathology, it remains unclear if Tregs that develop in early life continue to restrain systemic inflammatory responses throughout adulthood. Here, an inducible deletion strategy was used to label Tregs at specified time points with a targeted mechanism to be deleted later. Deletion of the Tregs labeled peri-weaning at day of life 24, but not before weaning at day of life 14, resulted in increased circulating IgE and IL-13, and abrogated induction of tolerance towards new antigens. Thus, Tregs developing peri-weaning, but not before day of life 14 are continually required to restrain allergic responses into adulthood.

Keywords: regulatory T cells, RORγT, Foxp3, weaning, allergy

INTRODUCTION

The prevalence of food allergies, perennial rhinitis, asthma, and eczema have increased dramatically in recent decades, especially among children (1–3). These allergies are partly attributed to the loss or absence of tolerance to environmental antigens, a process largely meditated by Tregs, which express the transcription factor FoxP3. Current focus has turned to how tolerance induced in early life protects from the development of allergic disorders. Children at high risk for allergic disorders exposed to food allergens, such as peanut or egg, between 4 and 12 months of age had reduced risk

of food sensitization compared to children avoiding allergens until after 5 years of age (4–7). Along with food allergen avoidance in early life, another risk factor for allergic disorders is antibiotic use in the first year of life, implicating a role for exposure to microbes in early life, and particularly microbes in the gastrointestinal tract, as protecting against allergy (8–10).

Within the spectrum of allergic disorders, IgE-mediated food allergy is particularly concerning due to its increasing incidence and life-threatening anaphylactic response on allergen consumption (11). Such IgE driven immune responses can be initiated and promoted by type 2 helper (Th2) responses, including the hallmark cytokine IL-13, which is predominantly produce by Th2 T cells, and promotes the production of IgE (12). Such responses can be suppressed by Tregs, therefore understanding the role of Tregs in pathogenesis of food allergy is a key to understanding how prevention of allergic disorders is best maintained.

Exposure to dietary allergens in the first year of life offers protection from future food allergies, while allergen avoidance until after five years of age is linked with increased food allergies (5–7, 13–15). These epidemiologic data are consistent with oral tolerance, or suppression of systemic responses to antigens and allergens first encountered in the gastrointestinal tract (16), having unique features when induced in early life (17). Induction of oral tolerance is correlated to the initiation of regulatory T cells that can suppress Th2 responses (18–20).

A population of Tregs expressing the transcription factor RORγt+ differentiate early in life in a process driven by the microbiota, and may have unique capacities to avoid immunopathologies and restrain Th2 responses (21, 22). Intriguingly this population of Tregs are reduced in children with food allergies (23). Children with food allergies had distinct microbiotas from healthy children, which induced significantly less RORγt+ Tregs (23) suggesting specific microbiota cues during early life are necessary for the development of this population of Tregs. Specific deletion of all Foxp3+ Tregs developing in early life increased gut inflammation, though the phenotype of the Tregs depleted was not determined (24). Moreover, reduced exposure to luminal antigens in early life decreased development of RORγt+ peripheral Tregs (pTregs) and was associated with an increase in Th2 responses to oral antigens (17). Adoptive transfer of RORγt+ pTregs from isolated from the peri-weaning colon reduced Th2 responses against oral antigens suggesting early life RORγt+ pTregs are sufficient to restrain Th2 responses in an unbalanced immune system. Tregs promote tolerance through production of cytokines necessary for the tolerant environment, and therefore Tregs developing in early could contribute to the tolerogenic milieu that gives rise to future Tregs control Th2 responses. However we do not know if these Tregs are continually required to restrain Th2 responses later in life or if their role is restricted to early life to promote the development of a balanced immune system and once developed they are dispensable.

Here we show that the continued presence of "peri-weaning Tregs", Tregs developing prior to weaning, are necessary for the maintenance and development of tolerance to antigens encountered later in life. We labeled Tregs at weaning with a diphtheria toxin receptor to specifically delete adults of Tregs of early life origin which reduced RORγt+ pTregs, increased serum IgE and IL13, and abrogated tolerance to new orally administered antigens. Thus Tregs developing peri-weaning are a major source of RORγt+ pTregs and are continually required to restrain Th2 responses in later life.

MATERIALS AND METHODS

Mice

All mice were maintained on the C57BL/6 background. C57BL/6 mice, OTII T-cell receptor transgenic mice (25), Foxp3^{GFPCreERT2} mice (26), Rosa^{lslDTR} (27), were purchased from The Jackson Laboratory (Bar Harbor, ME). All mice were fed a routine chow diet. Co-housed littermates were used for experimental controls. All mice were weaned at DOL 21. Adult mice were 8 to 16 weeks of age when analyzed unless stated otherwise. Foxp3^{GFPCreERT2} mice and Rosa^{lslDTR} mice were bred for Foxp3^{GFPiDTR} mice, which were injected with 100μg tamoxifen (Sigma-Aldrich, St. Louis, MO, USA) dissolved in sunflower seed oil with 20% ethanol (Sigma-Aldrich) intraperitoneally (i.p.) on DOL 14 or 24. Mice were then aged to 8 weeks old (DOL 56), and injected with 50μg/kg diphtheria toxin (DT) (Sigma Aldrich) i.p. In some experiments to validate deletion mechanism, Foxp3^{GFPiDTR} mice were injected with tamoxifen on DOL21, and diphtheria toxin (DT) on DOL24. For controls, C57Bl/6 mice were injected with tamoxifen or vehicle (sunflower seed oil with 20% ethanol) on DOL24, and DT on DOL56 to minimize variation due to treatments. Animal procedures and protocols were performed in accordance with the Institutional Animal Care and Use Committee at Washington University School of Medicine.

Isolation of Cellular Populations and Flow Cytometry

Colons were harvested, rinsed with PBS, and colonic patches were removed. Isolation of splenic and LP cellular populations was performed as previously described (28). Colonic Treg subpopulations were identified as 7AAD$^-$, CD45$^+$, CD3$^+$, CD4$^+$, Foxp3$^+$, and RORγt$^+$. Foxp3 was identified using GFP signal. To detect intracellular antigens (RORγt, cMAF, GATA3, and Tbet) and cytokines (IL13), cells were fixed and permeabilized overnight and stained per the manufacturer's recommendations (eBioscience). Flow cytometry was performed with a FACScan cytometer (BD Biosciences, San Jose, CA) retrofitted with additional lasers, or an Attune NXT four-laser flow cytometer (Invitrogen). Data acquisition and analysis were performed using Attune NXT software and FlowJo software (Tree Star, Ashland, OR). viSNE analysis was performed with FlowJo software.

Oral Tolerance and Delayed Type Hypersensitivity Responses

Mice were given Ova 20g/L in drinking water for 10 days beginning at DOL 70. Mice were then immunized subcutaneously with 100μg Ova in incomplete Freund's

Adjuvant (Sigma Aldrich) 14 and 28 days later (i.e., DOL 84 and 98). At 16 weeks of age (DOL 112), mice were challenged with 20 µg Ova in the footpad and 24 h later the DTH response was read as the increase in footpad thickness before and after challenge as measured with micrometer calipers. Body temperature was monitored for one hour following challenge.

Adoptive T-Cell Transfer of Ova-Specific T Cells

Two days after the start of dietary Ova, mice were injected i.p. with 5 x 10^5 naïve Ova-specific T cells ($CD45.1^+CD3^+CD4^+CD62L^+V\alpha2^+$) fluorescence-activated cell sorting (FACS)-isolated from spleens of adult OTII mice on the congenic CD45.1 background. Following transfer, organs were harvested at the indicated time points for cell isolation and analyzed by flow cytometry to detect and evaluate the phenotype of the $CD45.1^+$ OTII T-cells. Mice were evaluated seven days after transfer for the phenotype of the transferred $CD45.1^+$ OTII T-cells.

Measurement of Cytokines, and Immunoglobulins

ELISAs specific for IL-13 (eBioscience), Eotaxin (R&D systems), and IgE (eBiosciences) were used per manufacturers recommendations.

Statistical Analysis

Data were assumed to be normally distributed. Analysis was performed using a Student's *t* test, one-way ANOVA with a Dunnett's post-test, or two-way ANOVA with a with GraphPad Prism (GraphPad Software Inc., San Diego, CA) and a two-tailed cutoff of P<0.05 for significance.

Study Approval

Animal procedures and protocols were performed in accordance with the Institutional Animal Care and Use Committee at Washington University School of Medicine.

RESULTS

RORγt+ Tregs Developing Peri-Weaning Persist Into Adulthood

The induction of RORγt+ pTregs begins before weaning and requires exposure to luminal antigens and microbes for their development. We have shown disruption of delivery of luminal substances to the lamina propria during early life reduced development of RORγt+ pTregs (17). This reduction remained statistically significant months later, and was associated with decreased oral tolerance induction and an increase in allergic-type responses against dietary antigens (17). We first asked if specific deletion of FoxP3 cells developing in early life was also associated with decreased oral tolerance. Mice expressing the diphtheria toxin receptor (DTR) exclusively in FoxP3+ cells ($Foxp3^{DTR}$) were injected with the diphtheria toxin (DT) on day of life (DOL) 14 or DOL24 (**Figure 1A**). FoxP3+ cells were significantly decreased on DOL28, in both the DT14 and DT24 groups (**Figure 1B**), but the FoxP3 compartment was restored by DOL120 (**Figure 1B**). Interestingly RORγt+ pTregs remained significantly decreased following DT-depletion of Foxp3 cells at DOL 24, but not at DOL 14 (**Figure 1B**). Thus RORγt+ pTregs develop after DOL 14, in agreement with previous findings (21, 24, 29).

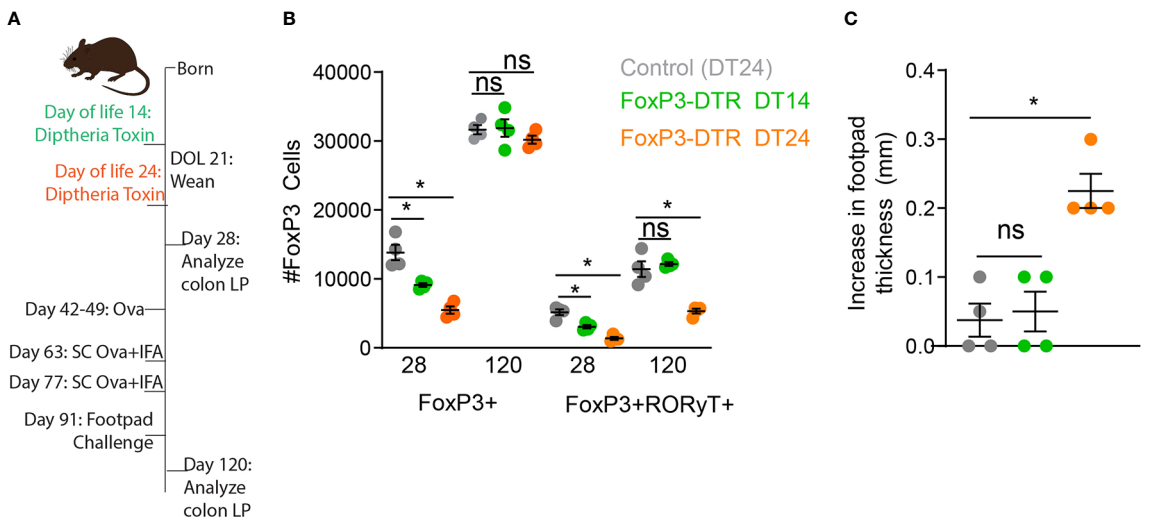


FIGURE 1 | Deletion of Tregs on DOL24, but not DOL14, abrogates oral tolerance later in life. **(A)** Schematic, FoxP3-DTR mice were injected with diphtheria toxin on DOL14 (green) or DOL24 (orange), control mice were injected with diphtheria toxin on DOL24 (gray). **(B)** Absolute number of Foxp3+ or RORγt+FoxP3+ colon LP cells analyzed on DOL 28 or DOL120. **(C)** Increase in footpad thickness following 7 days of Ova in drinking water, immunization, and footpad challenge with Ova, analyzed by one-way ANOVA with Dunnett's test for multiple comparison. n = 4 mice per group in panels b and c, *denotes statistical significance < 0.05, ns, not significant.

Following deletion of Tregs in early life, we utilized an oral tolerance and delayed-hypersensitivity challenge model by exposing mice to ovalbumin (Ova) in drinking water, immunizing mice with Ova, challenging mice with Ova in the footpad, and measuring footpad swelling as an index of lack of tolerance. Mice had significantly increased footpad swelling, indicating reduced oral tolerance systemically, following deletion of Tregs at DOL24, but not DOL14. Taken along with our previous studies (17), these data highlight the importance of antigen delivery and development of Tregs during early life, but do not address if the RORγt+ pTregs arising during early life are continually required for constraining Th2 responses in later life or if once a balanced immune system is developed these Tregs are dispensable.

Mice expressing the diphtheria toxin receptor (DTR) under the tamoxifen driven cre in Foxp3 expressing cells, (Foxp3^{iDTR}) were used to label Tregs arising at specific times in life to be deleted later. This construct permits the development of a balanced and healthy immune system, including the full Treg compartment and deletion of the Tregs developing in early life to assess their continuing role in later life. For initial validation of this system, Foxp3^{iDTR} and control mice were injected with tamoxifen on DOL21 for labeling with the DTR, which was followed by an injection with DT on DOL24 for deletion of labeled cells. A significant depletion in Foxp3+ cells was quantified on DOL28, indicating DTR was successfully expressed on Foxp3+ cells following tamoxifen labeling in the Foxp3^{iDTR} and that DTR induced deletion of this population (**Figure 2A**), without significantly affecting other T cell subsets (**Figure 2B**).

Next, Foxp3^{iDTR} mice were injected with tamoxifen on DOL 14 or 24, resulting in expression of the DTR in Foxp3+ cells present at those respective points. DT injection on DOL 56 depleted the Tregs that expressed Foxp3 on either DOL 14 or DOL 24 while leaving the Tregs developing after this time unchanged (**Figure 2C**). Depletion of early life Foxp3+ cells in adults resulted in no significant decrease in the total Foxp3+ population in the colon, as the labeled population of Tregs was a

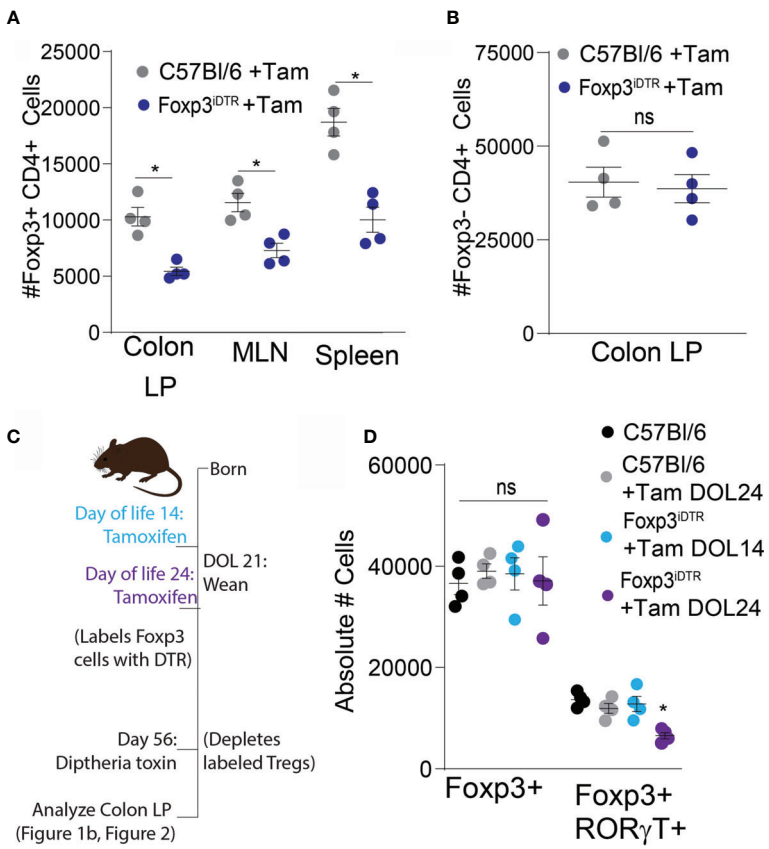


FIGURE 2 | RORγt+FoxP3+ Tregs are induced peri-weaning and are present in the adult colon lamina propria. Control mice (gray) and FoxP3iDTR mice (dark blue) were injected with tamoxifen on DOL21, diphtheria toxin on DOL24, and analyzed on DOL28: **(A)** absolute number of Foxp3+ CD4 T cells and **(B)** absolute number of T cells subsets in colon lamina propria, analyzed by two-tailed Student's t test. **(C)** Experimental schematic describing labeling of Tregs with DTR using tamoxifen by injecting FoxP3iDTR mice on DOL14 (light blue) or DOL24 (purple). C57Bl6 mice were used as controls and injected with a vehicle (black) or tamoxifen on DOL24 (gray). All groups were injected with diphtheria toxin on DOL56 for deletion of labeled cells on DOL 56. **(D)** Absolute number of Foxp3+ or RORγt+FoxP3+ colon LP cells analyzed on DOL 63 following depletion at DOL 56, analyzed by one-way ANOVA with Dunnett's test for multiple comparisons. n = 4 mice per group in panels **(A–C)** *denotes statistical significance < 0.05, ns, not significant.

small proportion of the total Treg population at DOL 56 (**Figure 2D**). However, there was a significant decrease in the number of RORγt+ pTregs following DT-depletion of Foxp3 cells developing before DOL 24, but not before DOL 14 (**Figure 2D**). Confirming the critical time for RORγt+ pTregs development is after DOL 14 (21, 24, 29), and in our colony largely do not develop after DOL 24. We term all of the FoxP3+ cells developing between DOL14 and DOL24 "peri-weaning Tregs", which does include the RORγt+ pTregs (17). Tregs developing prior to DOL 14 are predominantly natural thymic derived Tregs and supress autoimmune responses later in life (30).

Multiparameter flow cytometry was performed on the CD4+ T cell population within the colon lamina propria following depletion of Tregs developing prior to DOL14 (Tam DOL14) or peri-weaning Tregs (Tam DOL24) in adults to evaluate the expression of the transcription factors Foxp3, RORγt, cMaf, GATA3, and Tbet. Flow cytometry data was analyzed using a visualization tool for high-dimensional single-cell data based on the t-Distributed Stochastic Neighbor Embedding (t-SNE) algorithm, (viSNE), to visualize cell clusters and loss of cellular populations after deletion. No noticeable cluster was missing from following depletion of Tregs developing prior to DOL14, suggesting such cells either do not cluster into a distinct population, are not long-lived, or that a population with a similar pattern of transcription factor expression is also generated after DOL 14 (**Figure 2**). Depletion of peri-weaning Tregs, those labeled at DOL 24 and depleted in adults, greatly reduced the RORγt+Foxp3+ cluster that included GATA3, cMaf, and Tbet expression (**Figure 3**). Taken together, these data suggest RORγt+ pTregs are largely generated in early life in mice in our colony, that these cell persist into adulthood, and that the peri-weaning Tregs includes a heterogenous population of Tregs expressing diverse transcription factors.

Peri-Weaning Tregs Restrain Th2 Responses Later in Life

To determine if peri-weaning Tregs were continually required to restrain Th2 responses, mice were monitored following depletion of early life Tregs. Serum IgE and IL13 concentrations were significantly elevated following depletion of peri-weaning Tregs, but not Tregs developing prior to DOL14 (**Figures 4A, B**). Serum IL-13 concentrations initially spiked following depletion of peri-weaning Tregs, but remained significantly elevated throughout the monitoring period. Serum IgE concentrations continued to increase suggesting peri-weaning Tregs are necessary for restraining systemic Th2 cytokines and antibodies.

Failure to develop the colonic Treg population in early life spontaneously skewed Th2 profiles that persisted into adulthood and impaired oral tolerance to dietary antigens initially encountered in adulthood. We assessed whether deletion of Tregs developing prior to DOL14 or peri-weaning Tregs in adults impaired oral tolerance to dietary antigens introduced in adulthood by deleting these Tregs in adult mice, exposing mice to ovalbumin (Ova) in drinking water, immunizing mice with Ova, challenging mice with Ova in the footpad, and measuring footpad swelling as an index of lack of tolerance (**Figure 5A**). Mice lacking peri-weaning Tregs had significantly increased footpad swelling, indicating reduced oral tolerance (**Figure 5B**). Additionally, Ova injected into the footpad of mice lacking peri-weaning Tregs caused a decrease in body temperature, suggestive of a hypersensitivity response to the injected antigen (**Figure 5C**).

Ova-specific CD4+ OTII T cells were transferred into mice receiving Ova in drinking water and analysed one week later, to phenotype antigen-specific T cell responses to dietary antigens in mice lacking Tregs developing prior to DOL14 or peri-weaning Tregs. A majority of the OTII T cells became Foxp3+ in the unmanipulated mice and mice depleted of Tregs developing prior to DOL14. In contrast, mice depleted of peri-weaning Tregs had significantly more OTII T cells with reduced Foxp3 expression and increased IL13 expression, suggesting an expansion of effector T cells specific for dietary Ova in the absence of peri-weaning Tregs and a portion of these expressed Th2 cytokines (**Figure 5D**).

DISCUSSION

Our results demonstrate that continued presence of peri-weaning + Tregs, including RORγt+ pTregs, in adulthood is necessary to restrain Th2 responses and tolerance to dietary antigens encountered initially in adulthood. We have shown that RORγt+ pTregs developing in the colon are longer-lived in the absence of cognate antigen (17). Integrating these observations with prior studies suggests that there are periods in life for the development of Tregs with distinct properties. The first period occurs in the first week of life in mice during which naturally derived Tregs specific for self-antigens develop which function to protect from autoimmunity (30). The next period spanning the interval between the first week through weaning in mice is defined the formation of goblet cell-associated antigen passages (GAPs) in the colon. During this time peripherally induced Tregs (pTregs) specific for non-self-antigens develop extrathymically, a mechanism of Treg induction important for restraint of Th2 responses (31). A portion of these pTregs express the transcription factor RORγt and their development is driven by the microbiota, which is potentially delivered to the colon *via* GAPs (17, 32). The RORγt+ pTregs developing during this period are longer-lived than those developing in adulthood and have the capacity to restrain Th2 responses and promote tolerance to new antigens throughout life. While RORγt+ pTregs can develop throughout life, our data suggest that the RORγt+ pTregs developing after weaning are insufficient to restrain immunopathology.

Intriguingly, peri-weaning Tregs, which includes RORγt+ pTregs, restrained future responses in our model in a non-antigen specific manner suggesting here Tregs act in an innate manner to shape and promote a tolerogenic environment. Within the context of food allergy, the reduction or absence of RORγt+ pTregs could shift the immune environment, influencing both effector subsets and innate cells, within the intestine, allowing for pathogenesis of allergic reactions.

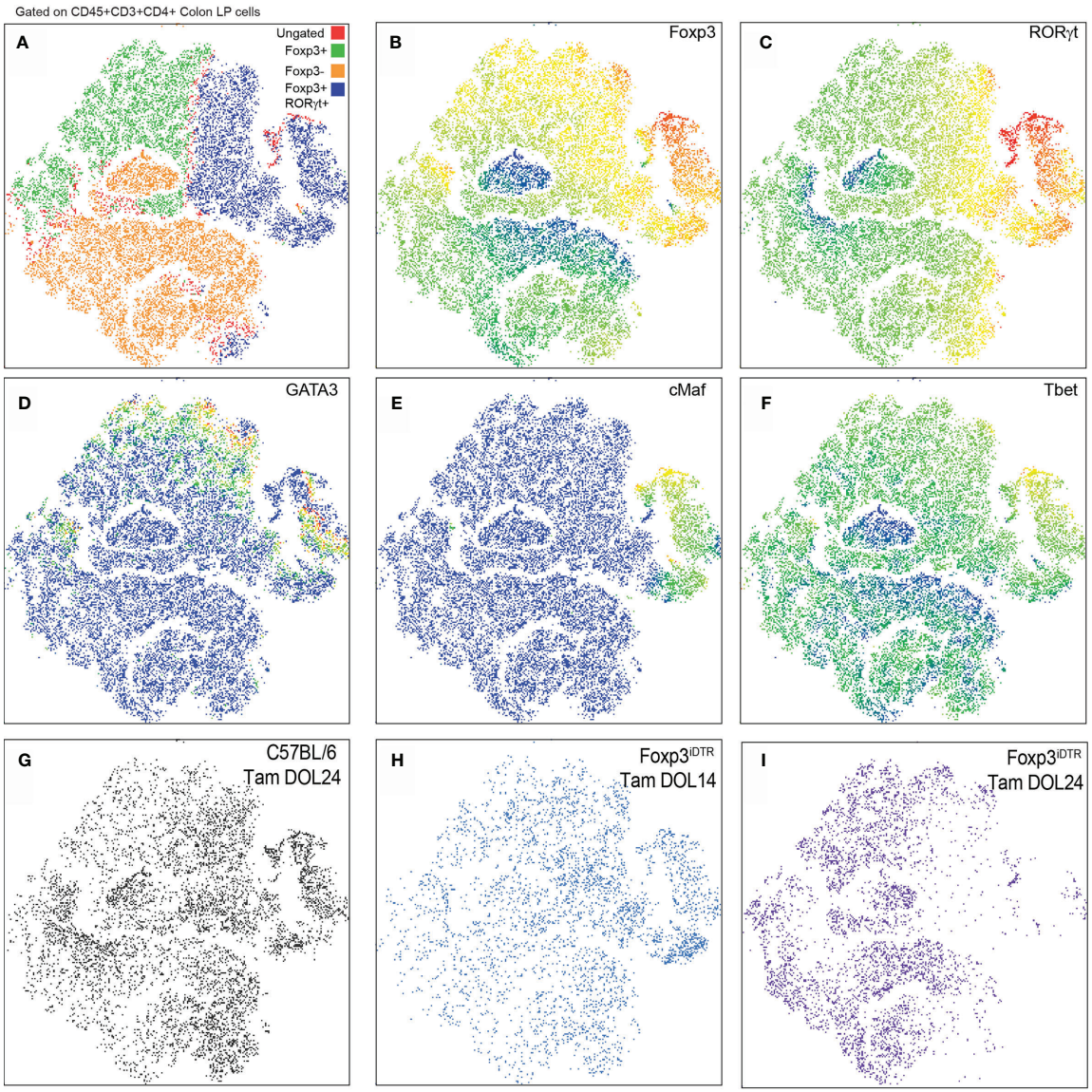


FIGURE 3 | RORγt+FoxP3+ peri-weaning Tregs include a heterogeneous population of Tregs that express GATA3, cMAF, and Tbet. **(A)** viSNE plots of colon CD4 T cell populations from pooled samples denoting Foxp3+ and RORγt+Foxp3+ and Foxp3– clusters. **(B–F)** Plots also show individual expression of Foxp3, RORt, GATA3, cMAF, and Tbet. **(G–I)** Plots of CD4+ T cells in the colon LP from individual experimental conditions. Vsne analysis in **(A–F)** are pooled from n = 3 mice per group, plots in **(G–I)** are representative of n = 3 mice per group.

Following depletion of peri-weaning Tregs, IgE steadily increased suggesting IgE-producing plasma cell populations either expand or increase secretion. The role of IgE in food allergy is well described, and we previously showed an association of decreased RORγt+ pTregs to increased allergen-specific IgE (17, 32). However, here it remains to be seen if increased total IgE may contribute to future allergic sensitization (33) or just indicate a skewing of the immune system to favor allergic responses. Similarly, increased serum IL13 suggests expansion or increased activity of Th2 effector cells or innate cells such as eosinophils and basophils. IL13 has been shown to not only drive goblet cell hyperplasia, but also increase allergen delivery across the intestinal epithelium by the formation of GAPs and secretory cell associated passages (SAPs) (34), and been implemented in promoting oral allergen sensitization (35) and food allergy pathogenesis (33). Thus increased systemic IL13 has the potential to increase delivery of food allergens into the lamina propria and sensitize innate cells triggering the pathogenesis of allergic responses. How peri-weaning Tregs restrain IL13 production and secretion through either direct cell contact or indirectly is of great interest. While more data is necessary to understand how RORγt+ pTregs uniquely prevent allergic reactions, it remains clear their development during early life may be imperative to maintaining tolerance throughout life.

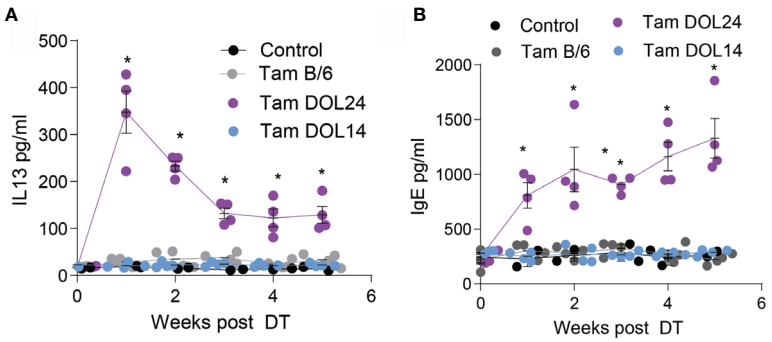


FIGURE 4 | Systemic IL13 and IgE is increased following depletion of peri-weaning Tregs. Serum concentrations of **(A)** IL13 or **(B)** total IgE for 5 weeks following deletion of Tregs. n = 4 mice per group in panels **(A**, **B)**, analyzed by two-way ANOVA with Dunnett's test for multiple comparisons. * denotes statistical significance < 0.05.

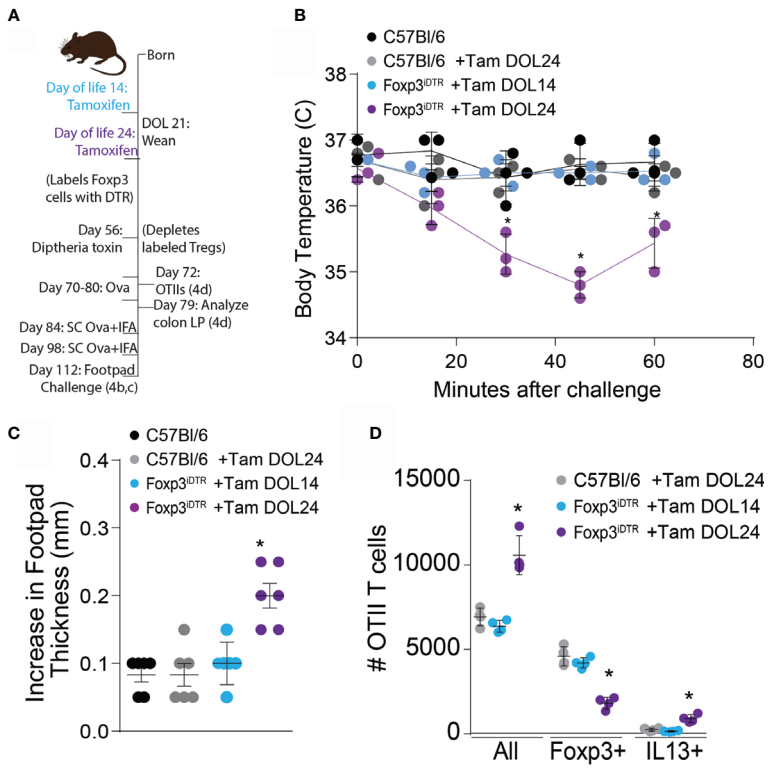


FIGURE 5 | Development of tolerance to new oral antigens is abrogated following depletion of peri-weaning Tregs. **(A)** Experimental schematic describing labeling of Tregs with DTR using tamoxifen followed by deletion of labeled cells on DOL 56, and DTH response (left, **B** and **C**) or transfer of OTII cells (right, **D**) FoxP3iDTR mice were injected with tamoxifen on DOL14 (light blue) or DOL24 (purple). C57Bl6 mice were used as controls and injected with a vehicle (black) or tamoxifen on DOL24 (gray). All groups were injected with diphtheria toxin on DOL56 for deletion of labeled cells on DOL 56. **(B)** Increase in footpad thickness following 7 days of Ova in drinking water, immunization, and footpad challenge with Ova, analyzed by one-way ANOVA with Dunnett's test for multiple comparison. **(C)** Change in temperature following footpad challenge, analyzed by two-way ANOVA with Tukey's test for multiple comparisons. **(D)** Number of Ova-specific OTII cells in the colon LP 7 days following adoptive transfer and dietary Ova, analyzed by one-way ANOVA with Dunnett's test for multiple comparison. n = 6 mice per group in panels **(B, C)** from two independent experiments, c displays data from one representative experiment, n = 3 mice in panel **(D)** * denotes statistical significance < 0.05.

While the generation and function of these pTregs is an area of active investigation, multiple features, that may be unique to early life, have been identified to contribute to the development of this RORγt+ pTreg population. There are two key time points for the role of antigen delivery: GAP formation in the colon prior to weaning, and GAP and SAP formation in the small intestine later in life. The former drives RORγt+ pTregs expansion in response to microbial products, while the later has the potential to promote food allergy pathogenesis through increased food allergen delivery (34). Understanding how the antigen delivery process results in two very different outcomes depends on both location (colon as compared to small intestine) and timing (peri-weaning compared to later in life). During the pre-weaning interval in which naïve T cells are stimulated to generate these pTregs, dietary and microbial antigens are delivered to the colon lamina propria *via* GAPs to generate antigen-specific immune responses (17, 32). This process is regulated by maternal ligands present in breastmilk and bacterial components of the maturing microbiota around weaning, most notably epidermal growth factor (17, 32). Maternal antibodies IgA and IgG also present in breastmilk have been shown to promote tolerance, may protect from food allergy pathogenesis in early life, and may help regulate development of RORγt+ pTregs (22, 36, 37). Following weaning, dietary antigens are largely encountered by the immune system in the small intestine, and dietary antigen-specific pTregs developing post-weaning have a limited life span in absence of antigen exposure (38, 39). This mechanism might explain why allergen avoidance in early life does not reduce food allergy. Indeed, complementary diets combined food allergens with breastmilk protect against food allergy later in life (14). These feeding practices could be directing food allergens to the colon during the first year of life to promote long lived tolerance *via* the induction of dietary antigen-specific RORγt+ pTregs.

Another feature supporting the development of this RORγt+ pTreg population is the gut microbiota. The requirement of the microbiota for inducing RORγt+ pTregs has long been appreciated (21, 29). Antibiotic use, particularly in the first year of life, is strongly associated with later life food allergies (40), and would certainly induce microbial dysbiosis (41). Children with food allergies have distinct microbiotas from healthy children. These bacterial communities induced significantly fewer RORγt+ pTregs (23). Additionally Th2 responses have the potential to create feedback loops, depleting the bacterial taxa necessary for Treg development (42). Administration of individual or a consortium of bacteria partly rescued RORγt+ pTregs where such cells were otherwise lacking or decreased (23). However this effect was restricted to specific bacterial taxa, suggesting microbial cues exclusive to the early life microbiota promote the development of this population of pTregs (43, 44). However attempts to manipulate the microbiota as a therapy for allergies have had limited success possibly due to the lack of other unique features present in early life.

Finally, RORγt+ pTregs are likely different depending on the time of life during which the pTregs differentiate. Recently a set point for the RORγt+ pTreg population was described to be defined in early life, shortly after birth (22), and pTregs may have age-dependent fates (45). RORγt+ expression can be driven by bacteria adherent to the intestinal epithelium in adult mice, including in pTregs (46–48), however pTregs induced in this manner are transient and dependent on the continued presence of the microbiota (29). The manner in which adherent bacterial antigens are encountered by the immune system post-weaning differs from how commensal bacterial antigens are encountered pre-weaning (32, 49). Common adherent bacteria have not been observed in the peri-weaning microbiota (50, 51), suggesting other microbial members or luminal factors drive RORγt expression peri-weaning. During differentiation RORγt+ pTregs potentially express Foxp3 first, becoming a Foxp3+ RORγt- intermediate before final differentiation into RORγt +Foxp3+ (52). Intriguingly induction of Foxp3 and RORγt expression may be initiated by differential microbial products (53, 54), potentially sensed directly by the Tregs (23).

Taken together, peri-weaning pTregs including the RORγt+ pTregs subset induced in the colon, are uniquely capable of enduring suppression systemic inflammation later in life. The heterogenous expression of specific transcription factors suggest peri-weaning Tregs can restrain multiple Th-driven inflammatory responses (55–57). One limitation of this study is through the labeling of all Foxp3 expressing cells at a specified time in life, deletion of cells upon diphtheria treatment results in mass deletion of Foxp3 cells present at the specified labeling time point, suggesting the mass deletion of cells from a specified time point may be as important as their specificity or gene expression pattern representative of the Foxp3 population.

It remains unclear how peri-weaning RORγt+ pTregs restrain allergic responses uniquely when compared to other Tregs subsets. Multiple described roles for Tregs in the restraint of allergic responses include producing cytokines that suppress immune responses, modulating antigen presenting cells to prevent antigen presentation, and preventing proliferation of T effector cells (58–60). While our data are consistent with each of these functions and suggest peri-weaning Tregs are continually required to perform their suppressive role, one alternative interpretation is the presence of the RORγt+ peri-weaning pTregs limits or decreases differentiation of future Tregs capable of restraining Th2 responses through active utilization of space and resources in the lamina propria as these are long-lived Tregs (17). Future work should explore if the peri-weaning Treg phenotype can be replicated later in life.

In conclusion, we have shown the continued presence of peri-weaning Tregs is necessary to restrain Th2 responses and supports tolerance to dietary antigens encountered later in life. These results implicate peri-weaning Tregs, which include a substantial population of RORγt+ pTregs, as playing active roles in suppressing potential Th2 responses and maintaining tolerogenic homeostasis. Further these findings underscore the importance of early life immune education for the proper expansion of this population, as Tregs developing post-weaning do not substitute for the peri-weaning Tregs. These data suggest the peri-weaning RORγt+ pTregs have unique capacities and are potentially not replaced without intervention. Thus risk factors for food allergy such as antibiotic use and allergen avoidance may

contribute to food sensitization by disrupting this population of peri-weaning pTregs.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The animal study was reviewed and approved by Institutional Animal Care and Use Committee at Washington University School of Medicine.

AUTHOR CONTRIBUTIONS

KK performed sample collection, ELISAs, flow cytometry, tSNE analysis, and data analysis. KK, and KM performed animal breeding, genotyping, and injections. KK, C-SH, PT, and RN designed the experiments, analyzed and interpreted the data, and wrote the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

Supported by grants: DK052574-PIT and RDN, DK097317-RDN, AI131342-RDN, AI1407551-RDN and CSH, AI136515-RDN and CSH, AI112626–SPH and RDN, AI131349-CSH, DK109006-KAK, and DK122187-KAK.

REFERENCES

1. Branum AM, Lukacs SL. Food allergy among U.S. children: trends in prevalence and hospitalizations. *NCHS Data Brief* (2008) 10:1–8.
2. Jackson KD, Howie LD, Akinbami LJ. Trends in allergic conditions among children: United States, 1997-2011. *NCHS Data Brief* (2013) 121:1–8.
3. Gupta RS, Warren CM, Smith BM, Blumenstock JA, Jiang J, Davis MM, et al. The Public Health Impact of Parent-Reported Childhood Food Allergies in the United States. *Pediatrics* (2018) 142:e20181235. doi: 10.1542/peds.2018-1235
4. Poole JA, Barriga K, Leung DY, Hoffman M, Eisenbarth GS, Rewers M, et al. Timing of initial exposure to cereal grains and the risk of wheat allergy. *Pediatrics* (2006) 117:2175–82. doi: 10.1542/peds.2005-1803
5. Nwaru BI, Erkkola M, Ahonen S, Kaila M, Haapala AM, Kronberg-Kippila C, et al. Age at the introduction of solid foods during the first year and allergic sensitization at age 5 years. *Pediatrics* (2010) 125:50–9. doi: 10.1542/peds.2009-0813
6. Koplin JJ, Osborne NJ, Wake M, Martin PE, Gurrin LC, Robinson MN, et al. Can early introduction of egg prevent egg allergy in infants? A population-based study. *J Allergy Clin Immunol* (2010) 126:807–13. doi: 10.1016/j.jaci.2010.07.028
7. Du Toit G, Roberts G, Sayre PH, Bahnson HT, Radulovic S, Santos AF, et al. Randomized Trial of Peanut Consumption in Infants at Risk for Peanut Allergy. *New Engl J Med* (2015) 372:803–13. doi: 10.1056/NEJMoa1414850
8. Metsala J, Lundqvist A, Virta LJ, Kaila M, Gissler M, Virtanen SM. Mother's and offspring's use of antibiotics and infant allergy to cow's milk. *Epidemiology* (2013) 24:303–9. doi: 10.1097/EDE.0b013e31827f520f
9. Love BL, Mann JR, Hardin JW, Lu ZK, Cox C, Amrol DJ. Antibiotic prescription and food allergy in young children. *Allergy Asthma Clin Immunol Off J Can Soc Allergy Clin Immunol* (2016) 12:41. doi: 10.1186/s13223-016-0148-7
10. Ni J, Friedman H, Boyd BC, Mcgurn A, Babinski P, Markossian T, et al. Early antibiotic exposure and development of asthma and allergic rhinitis in childhood. *BMC Pediatr* (2019) 19:225. doi: 10.1186/s12887-019-1594-4
11. Anvari S, Miller J, Yeh CY, Davis CM. IgE-Mediated Food Allergy. *Clin Rev Allergy Immunol* (2019) 57:244–60. doi: 10.1007/s12016-018-8710-3
12. De Vries JE. The role of IL-13 and its receptor in allergy and inflammatory responses. *J Allergy Clin Immunol* (1998) 102:165–9. doi: 10.1016/S0091-6749(98)70080-6
13. Koplin JJ, Dharmage SC, Ponsonby AL, Tang ML, Lowe AJ, Gurrin LC, et al. Environmental and demographic risk factors for egg allergy in a population-based study of infants. *Allergy* (2012) 67:1415–22. doi: 10.1111/all.12015
14. Du Toit G, Sayre PH, Roberts G, Sever ML, Lawson K, Bahnson HT, et al. Effect of Avoidance on Peanut Allergy after Early Peanut Consumption. *N Engl J Med* (2016) 374:1435–43. doi: 10.1056/NEJMoa1514209
15. Du Toit G, Katz Y, Sasieni P, Mesher D, Maleki SJ, Fisher HR, et al. Early consumption of peanuts in infancy is associated with a low prevalence of peanut allergy. *J Allergy Clin Immunol* (2008) 122:984–91. doi: 10.1016/j.jaci.2008.08.039
16. Pabst O, Mowat AM. Oral tolerance to food protein. *Mucosal Immunol* (2012) 5:232. doi: 10.1038/mi.2012.4
17. Knoop KA, Mcdonald KG, Coughlin PE, Kulkarni DH, Gustafsson JK, Rusconi B, et al. Synchronization of mothers and offspring promotes tolerance and limits allergy. *JCI Insight* (2020) 5. doi: 10.1172/jci.insight.137943
18. Chehade M, Mayer L. Oral tolerance and its relation to food hypersensitivities. *J Allergy Clin Immunol* (2005) 115:3–12. doi: 10.1016/j.jaci.2004.11.008
19. Wawrzyniak M, O'mahony L, Akdis M. Role of Regulatory Cells in Oral Tolerance. *Allergy Asthma Immunol Res* (2017) 9:107–15. doi: 10.4168/aair.2017.9.2.107
20. Tordesillas L, Berin MC. Mechanisms of Oral Tolerance. *Clin Rev Allergy Immunol* (2018) 55:107–17. doi: 10.1007/s12016-018-8680-5
21. Ohnmacht C, Park J-H, Cording S, Wing JB, Atarashi K, Obata Y, et al. The microbiota regulates type 2 immunity through RORγt+ T cells. *Science* (2015) 349:989–93. doi: 10.1126/science.aac4263
22. Ramanan D, Sefik E, Galván-Peña S, Wu M, Yang L, Yang Z, et al. An Immunologic Mode of Multigenerational Transmission Governs a Gut Treg Setpoint. *Cell* (2020) 181:1276–90.e1213. doi: 10.1016/j.cell.2020.04.030
23. Abdel-Gadir A, Stephen-Victor E, Gerber GK, Noval Rivas M, Wang S, Harb H, et al. Microbiota therapy acts via a regulatory T cell MyD88/RORγt pathway to suppress food allergy. *Nat Med* (2019) 25:1164–74. doi: 10.1038/s41591-019-0461-z
24. Al Nabhani Z, Dulauroy S, Marques R, Cousu C, Al Bounny S, Déjardin F, et al. A Weaning Reaction to Microbiota Is Required for Resistance to Immunopathologies in the Adult. *Immunity* (2019) 50:1276–88.e1275. doi: 10.1016/j.immuni.2019.02.014
25. Barnden MJ, Allison J, Heath WR, Carbone FR. Defective TCR expression in transgenic mice constructed using cDNA-based alpha- and beta-chain genes under the control of heterologous regulatory elements. *Immunol Cell Biol* (1998) 76:34–40. doi: 10.1046/j.1440-1711.1998.00709.x
26. Rubtsov YP, Niec RE, Josefowicz S, Li L, Darce J, Mathis D, et al. Stability of the regulatory T cell lineage in vivo. *Science* (2010) 329:1667–71. doi: 10.1126/science.1191996
27. Buch T, Heppner FL, Tertilt C, Heinen T, Kremer M, Wunderlich FT, et al. A Cre-inducible diphtheria toxin receptor mediates cell lineage ablation after toxin administration. *Nat Methods* (2005) 2:419. doi: 10.1038/nmeth762
28. Mcdonald KG, Leach MR, Brooke KW, Wang C, Wheeler LW, Hanly EK, et al. Epithelial expression of the cytosolic retinoid chaperone cellular retinol binding protein II is essential for in vivo imprinting of local gut dendritic cells by lumenal retinoids. *Am J Pathol* (2012) 180:984–97. doi: 10.1016/j.ajpath.2011.11.009
29. Sefik E, Geva-Zatorsky N, Oh S, Konnikova L, Zemmour D, Mcguire AM, et al. Individual intestinal symbionts induce a distinct population of RORγ+ regulatory T cells. *Science* (2015) 349:993–7. doi: 10.1126/science.aaa9420

30. Yang S, Fujikado N, Kolodin D, Benoist C, Mathis D. Immune tolerance. Regulatory T cells generated early in life play a distinct role in maintaining self-tolerance. *Science* (2015) 348:589–94. doi: 10.1126/science.aaa7017
31. Josefowicz SZ, Niec RE, Kim HY, Treuting P, Chinen T, Zheng Y, et al. Extrathymically generated regulatory T cells control mucosal TH2 inflammation. *Nature* (2012) 482:395–9. doi: 10.1038/nature10772
32. Knoop KA, Gustafsson JK, Mcdonald KG, Kulkarni DH, Coughlin PE, Mccrate S, et al. Microbial antigen encounter during a preweaning interval is critical for tolerance to gut bacteria. *Sci Immunol* (2017) 2. doi: 10.1126/sciimmunol.aao1314
33. Wang M, Takeda K, Shiraishi Y, Okamoto M, Dakhama A, Joetham A, et al. Peanut-induced intestinal allergy is mediated through a mast cell-IgE-FcepsilonRI-IL-13 pathway. *J Allergy Clin Immunol* (2010) 126:306–316, 316.e301-312. doi: 10.1016/j.jaci.2010.05.017
34. Noah TK, Knoop KA, Mcdonald KG, Gustafsson JK, Waggoner L, Vanoni S, et al. IL-13-induced intestinal secretory epithelial cell antigen passages are required for IgE-mediated food-induced anaphylaxis. *J Allergy Clin Immunol* (2019) 144:1058–73.e1053. doi: 10.1016/j.jaci.2019.04.030
35. Brandt EB, Munitz A, Orekov T, Mingler MK, Mcbride M, Finkelman FD, et al. Targeting IL-4/IL-13 signaling to alleviate oral allergen-induced diarrhea. *J Allergy Clin Immunol* (2009) 123:53–8. doi: 10.1016/j.jaci.2008.10.001
36. Mosconi E, Rekima A, Seitz-Polski B, Kanda A, Fleury S, Tissandie E, et al. Breast milk immune complexes are potent inducers of oral tolerance in neonates and prevent asthma development. *Mucosal Immunol* (2010) 3:461–74. doi: 10.1038/mi.2010.23
37. Ohsaki A, Venturelli N, Buccigrosso TM, Osganian SK, Lee J, Blumberg RS, et al. Maternal IgG immune complexes induce food allergen-specific tolerance in offspring. *J Exp Med* (2018) 215:91–113. doi: 10.1084/jem.20171163
38. Kim KS, Hong S-W, Han D, Yi J, Jung J, Yang B-G, et al. Dietary antigens limit mucosal immunity by inducing regulatory T cells in the small intestine. *Science* (2016) 351:858–63. doi: 10.1126/science.aac5560
39. Kulkarni DH, Gustafsson JK, Knoop KA, Mcdonald KG, Bidani SS, Davis JE, et al. Goblet cell associated antigen passages support the induction and maintenance of oral tolerance. *Mucosal Immunol* (2020) 13:271–82. doi: 10.1038/s41385-019-0240-7
40. Netea SA, Messina NL, Curtis N. Early-life antibiotic exposure and childhood food allergy: A systematic review. *J Allergy Clin Immunol* (2019) 144:1445–8. doi: 10.1016/j.jaci.2019.08.001
41. Shu SA, Yuen AWT, Woo E, Chu KH, Kwan HS, Yang GX, et al. Microbiota and Food Allergy. *Clin Rev Allergy Immunol* (2019) 57:83–97. doi: 10.1007/s12016-018-8723-y
42. Campbell C, Dikiy S, Bhattarai SK, Chinen T, Matheis F, Calafiore M, et al. Extrathymically Generated Regulatory T Cells Establish a Niche for Intestinal Border-Dwelling Bacteria and Affect Physiologic Metabolite Balance. *Immunity* (2018) 48:1245–57.e1249. doi: 10.1016/j.immuni.2018.04.013
43. Stefka AT, Feehley T, Tripathi P, Qiu J, Mccoy K, Mazmanian SK, et al. Commensal bacteria protect against food allergen sensitization. *Proc Natl Acad Sci U.S.A.* (2014) 111:13145–50. doi: 10.1073/pnas.1412008111
44. Feehley T, Plunkett CH, Bao R, Choi Hong SM, Culleen E, Belda-Ferre P, et al. Healthy infants harbor intestinal bacteria that protect against food allergy. *Nat Med* (2019) 25:448–53. doi: 10.1038/s41591-018-0324-z
45. Pratama A, Schnell A, Mathis D, Benoist C. Developmental and cellular age direct conversion of CD4+ T cells into RORγ+ or Helios+ colon Treg cells. *J Exp Med* (2019) 217. doi: 10.1084/jem.20190428
46. Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, et al. Induction of Intestinal Th17 Cells by Segmented Filamentous Bacteria. *Cell* (2009) 139:485–98. doi: 10.1016/j.cell.2009.09.033
47. Lathrop SK, Bloom SM, Rao SM, Nutsch K, Lio CW, Santacruz N, et al. Peripheral education of the immune system by colonic commensal microbiota. *Nature* (2011) 478:250–4. doi: 10.1038/nature10434
48. Chai JN, Peng Y, Rengarajan S, Solomon BD, Ai TL, Shen Z, et al. Helicobacter species are potent drivers of colonic T cell responses in homeostasis and inflammation. *Sci Immunol* (2017) 2. doi: 10.1126/sciimmunol.aal5068
49. Ladinsky MS, Araujo LP, Zhang X, Veltri J, Galan-Diez M, Soualhi S, et al. Endocytosis of commensal antigens by intestinal epithelial cells regulates mucosal T cell homeostasis. *Science* (2019) 363:eaat4042. doi: 10.1126/science.aat4042
50. Ivanov II, Frutos RDL, Manel N, Yoshinaga K, Rifkin DB, Sartor RB, et al. Specific Microbiota Direct the Differentiation of IL-17-Producing T-Helper Cells in the Mucosa of the Small Intestine. *Cell Host Microbe* (2008) 4:337–49. doi: 10.1016/j.chom.2008.09.009
51. Nutsch K, Chai JN, Ai TL, Russler-Germain E, Feehley T, Nagler CR, et al. Rapid and Efficient Generation of Regulatory T Cells to Commensal Antigens in the Periphery. *Cell Rep* (2016) 17:206–20. doi: 10.1016/j.celrep.2016.08.092
52. Solomon BD, Hsieh C-S. Antigen-Specific Development of Mucosal Foxp3 +RORγt+ T Cells from Regulatory T Cell Precursors. *J Immunol (Baltimore Md 1950)* (2016) 197:3512–9. doi: 10.4049/jimmunol.1601217
53. Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y, et al. Induction of colonic regulatory T cells by indigenous Clostridium species. *Science* (2011) 331:337–41. doi: 10.1126/science.1198469
54. Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, et al. T induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature* (2013) 500:232–6. doi: 10.1038/nature12331
55. Wang Y, Su MA, Wan YY. An essential role of the transcription factor GATA-3 for the function of regulatory T cells. *Immunity* (2011) 35:337–48. doi: 10.1016/j.immuni.2011.08.012
56. Wohlfert EA, Grainger JR, Bouladoux N, Konkel JE, Oldenhove G, Ribeiro CH, et al. GATA3 controls Foxp3$^+$ regulatory T cell fate during inflammation in mice. *J Clin Invest* (2011) 121:4503–15. doi: 10.1172/JCI57456
57. Xu M, Pokrovskii M, Ding Y, Yi R, Au C, Harrison OJ, et al. c-MAF-dependent regulatory T cells mediate immunological tolerance to a gut pathobiont. *Nature* (2018) 554:373–7. doi: 10.1038/nature25500
58. Noval Rivas M, Chatila TA. Regulatory T cells in allergic diseases. *J Allergy Clin Immunol* (2016) 138:639–52. doi: 10.1016/j.jaci.2016.06.003
59. Satitsuksanoa P, Jansen K, Głobińska A, Van De Veen W, Akdis M. Regulatory Immune Mechanisms in Tolerance to Food Allergy. *Front Immunol* (2018) 9:2939. doi: 10.3389/fimmu.2018.02939
60. Calzada D, Baos S, Cremades-Jimeno L, Cárdaba B. Immunological Mechanisms in Allergic Diseases and Allergen Tolerance: The Role of Treg Cells. *J Immunol Res* (2018) 2018:6012053–6012053. doi: 10.1155/2018/6012053

Conflict of Interest: RN, KK, and KM are inventors on U.S. Nonprovisional Application Serial No. 15/880,658 Compositions and Methods for Modulation of Dietary and Microbial Exposure. PT discloses a financial conflict of interest with MediBeacon Inc (member of their Scientific Advisory Board, consultant, and equity holder), is a consultant to Kallyope Inc., and is a potential recipient of royalties from a patent to test human gut permeability noninvasively.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

MINI REVIEW
published: 17 February 2021
doi: 10.3389/fimmu.2020.623071

Immune-Mediated Mechanisms in Cofactor-Dependent Food Allergy and Anaphylaxis: Effect of Cofactors in Basophils and Mast Cells

Rosa Muñoz-Cano[1,2,3,4], Clara San Bartolome[2,3,5], Rocío Casas-Saucedo[1,2,3], Giovanna Araujo[1,3], Sonia Gelis[1,3], Maria Ruano-Zaragoza[1,2,3], Jordi Roca-Ferrer[3,4,6], Francis Palomares[2,7], Margarita Martin[2,3,8], Joan Bartra[1,2,3,4†] and Mariona Pascal[2,3,5†]*

[1] Allergy Section, Pneumology Department, Institut Clinic Respiratori (ICR), Hospital Clinic, Barcelona, Spain, [2] Asma, Reacciones Adversas y Alergia (ARADyAL), Instituto de Salud Carlos III, Madrid, Spain, [3] Immunoalergia Respiratoria y Experimental - Institut d'Investigacions Biomediques August Pi i Sunyer (IRCE-IDIBAPS), Barcelona, Spain, [4] Universitat de Barcelona, Barcelona, Spain, [5] Immunology Department, Centre de Diagnostic Biomedic (CDB), Hospital Clínic, Barcelona, Spain, [6] Centro de Investigaciones Biomédicas en Red de Enfermedades Respiratorias (CIBERES), Instituto de Salud Carlos III, Madrid, Spain, [7] Allergy Research Group, Instituto de Investigación Biomédica de Málaga-IBIMA, Málaga, Spain, [8] Biochemistry Unit, University of Barcelona Faculty of Medicine and Health Sciences, Barcelona, Spain

Cofactors may explain why in some cases food ingestion leads to anaphylaxis while in others elicits a milder reaction or tolerance. With cofactors, reactions become more severe and/or have a lower allergen threshold. Cofactors are present in up to 58% of food anaphylaxis (FAn). Exercise, NSAIDs, and alcohol are the most frequently described, although the underlying mechanisms are poorly known. Several hypotheses have suggested the influence of these cofactors on basophils and mast cells (MCs). Exercise has been suggested to enhance MC activation by increasing plasma osmolarity, redistributing blood flow, and activating adenosine and eicosanoid metabolism. NSAIDs' cofactor effect has been related with cyclooxygenase inhibition and therefore, prostaglandin E_2 (PGE_2) production. Indeed, overexpression of adenosine receptor 3 (A_3) gene has been described in NSAID-dependent FAn; A_3 activation potentiates FcεRI-induced MC degranulation. Finally, alcohol has been related with an increase of histamine levels by inhibition of diamino oxidase (DAO) and also with and increase of extracellular adenosine by inhibition of its uptake. However, most of these mechanisms have limited evidence, and further studies are urgently needed. In conclusion, the study of the immune-related mechanisms involved in food allergic reactions enhanced by cofactors is of the utmost interest. This knowledge will help to design both tailored treatments and prophylactic strategies that, nowadays, are non-existent.

Keywords: mast cell, basophil, adenosin, NSAID, cofactor, exercise, prostaglandin, food allergy

OPEN ACCESS

Edited by:
Simon Patrick Hogan, University of Michigan, United States

Reviewed by:
Glenn Cruse, North Carolina State University, United States
Joana Vitte, Aix-Marseille Université, France

****Correspondence:***
Rosa Muñoz-Cano
rmunoz@clinic.cat

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology

Received: *29 October 2020*
Accepted: *31 December 2020*
Published: *17 February 2021*

Citation:
Muñoz-Cano R, San Bartolome C, Casas-Saucedo R, Araujo G, Gelis S, Ruano-Zaragoza M, Roca-Ferrer J, Palomares F, Martin M, Bartra J and Pascal M (2021) Immune-Mediated Mechanisms in Cofactor-Dependent Food Allergy and Anaphylaxis: Effect of Cofactors in Basophils and Mast Cells. Front. Immunol. 11:623071. doi: 10.3389/fimmu.2020.623071

INTRODUCTION

Food allergy is the main cause of anaphylaxis in children and in some series, also in adults (1). However, only some food allergic reactions end up being an anaphylaxis, ranging from very low percentages (0.4%) up to 40% of the reactions in some series (2). This disparity may be related with differences in age, food trigger, and geographic area. Interestingly, food allergy in adults usually debuts in the second-third decade of life and, in up to 50% of the cases, as an anaphylaxis (1).

The severity of an allergic reaction is unpredictable. The same individual may present reactions with different severity, even with the same food. The presence of cofactors, such as NSAIDs, exercise or alcohol, may explain this heterogeneity. Patients with cofactor-dependent reactions may have no or mild symptoms with the food alone and a more severe reaction (generalized urticaria or anaphylaxis) when associating a cofactor. Indeed, the same patient may have reactions with different cofactors or even need more than one cofactor to develop the severe reaction. Cofactors may increase the severity of the reaction or decreasing the reactivity threshold, meaning that lower doses of allergen are able to induce a more severe reaction (from two to six times depending on the series) (2–6). Cofactors are identified in up to 58% of food anaphylaxis (FAn) in some series and related with greater severity (3, 4, 7, 8), but the underlying mechanisms are poorly understood.

Their role in anaphylaxis has been more widely studied in adult patients, being not yet optimally studied in children. The high frequency of cofactor-related reactions highlights the clinical impact of recognizing and including cofactors into the routine diagnostic workup. Indeed, the understanding of the underlying mechanisms will help in developing tailored prophylactic treatments and identifying risk biomarkers. Hereby we report the main evidence reported regarding the major cofactors involved in food allergy.

EXERCISE

Exercise is described in up to 10% of anaphylactic reactions (3, 4). Exercise-induced anaphylaxis is a syndrome that may occur in food allergic patients but also independently of food ingestion (9). Considering the number of published studies, for the purpose of this review we will focus on food-dependent exercise-induced anaphylaxis (FDEIA). Several mechanisms have been postulated, although the evidence supporting each of them is scarce and limited by the low number of patients evaluated and the limited quality of the studies, as stated by a recent position paper from the European Academy of Allergy and Clinical Immunology (EAACI) (9).

The increase of both gastrointestinal permeability and tissue transglutaminase activity, two of the proposed mechanisms in the gut mucosa, is splendidly reviewed elsewhere (9). Other suggested mechanisms are related with the direct effect of exercise on basophils and mast cells (MCs) by modifying the cell count and histamine release (HR), redistributing the blood flow and increasing plasma osmolarity.

Exercise and Basophil Reactivity and Count

Acute exercise is related with the transient increase of blood circulating neutrophils, monocytes, dendritic and NK cells, although it remains unknown whether these changes may be also related with an altered immune function (10). Some *in vivo* studies have shown an increase of basophils count and HR after exercise, being more important in atopic individuals (11). In others, only an increase in HR has been demonstrated with no modification of basophil count (12). Interestingly, some authors have not found differences in HR when comparing allergic patients with controls, regardless of a significant basophil count increase in the atopic group (11). Indeed, increase in HR after *in vitro* IgE activation was only observed in highly trained athletes compared with non-trained ones, although both groups were non-atopic (13). Thus, these observations suggest that the atopic status together with the training level might be conditioning factors of HR and therefore, of exercise-induced basophil- and MC-activation. However, the interpretation and comparison of these findings are often complicated due to differences in experimental design of the studies (*i.e.*, measurement times and methods, samples types and exercise intensity/duration).

Exercise and Blood Flow Redistribution

It is well known that during exercise, blood flow is redistributed, being diverted from the viscera to the skeletal muscle, heart, and skin (14). Mounting evidence supports that HR during exercise is part of the physiological mechanism of recovery (15). Histamine acts as a vasodilator and is involved in post-exercise hypotension and hyperemia (15, 16). Therefore, one hypothesis that may need further exploration is whether this exercise recovery system in FDEIA patients is somehow damaged and, therefore, exercise is inducing "excessive" basophil/MC activation.

Some authors (17) have hypothesized that as a consequence of the blood flow redistribution, food allergens are transported away from the gut mucosa where resident MCs tolerate them to other tissues as skin or skeletal muscles, where MCs with a different phenotype react. For this reason, FDEIA patients tolerate the food at rest but have an allergic reaction when doing exercise. This is an interesting hypothesis and biologically plausible, although there is no experimental evidence yet.

Exercise and Plasma Osmolarity

Another effect of exercise is plasma osmolarity increase, which magnitude depends on exercise intensity and the resulting dehydration (9). Several *in vitro* studies have demonstrated that hyperosmolar environments induce MC and basophil activation. Torres-Atencio et al. (18) showed that mannitol, as a hyperosmolar stimulus, induced significant activation in MC from LAD2 cell line and healthy individuals (MC derived from CD34+ peripheral blood cells and primary lung MC). Other authors, in three patients (one FDEIA and two food allergic) and three healthy controls, showed that changes in osmolarity increase basophil activation only in FDEIA, but not in controls or food allergic patients (19). However, the *in vitro* osmolarity

achieved in that study (340 and 450 mOsm) seems difficult to be reached during exercise or dehydration in physiological conditions (293–305 mOsm) (9, 20).

Exercise and Adenosine Metabolism

Adenosine is produced under conditions of increased energy consumption such as hypoxia or stress, rapidly increasing its circulating levels (21). Adenosine induces opposite effects in MC activation depending on the binding receptor (22). Whereas A_{2A} ligation results in an increase of cAMP, and therefore, the inhibition of MC mediator release, A_{2B} enhances MC activation through PLC. Finally, A_3 seems to be involved in the potentiation of IgE-mediated MC activation in mouse and human models (22). Like the observations in PGE_2–EP axis, the expression profile of adenosine receptors in the cell's surface may condition the final effect of adenosine. Gomez et al. (23) demonstrated that adenosine enhanced IgE-mediated degranulation *via* A_3 in human lung derived MC but not in skin MC. Interestingly, lung MCs were shown to express three-fold more A_3 mRNA than the skin ones.

Adenosine is released into the venous efflux from skeletal muscle fibers in response to muscle contraction during exercise. Accumulated evidence shows that it is partially responsible for muscle hyperemia at submaximal and maximal workloads due to its effect on A_{2A} that results in vasodilation (24). Indeed, one of the most important factors regulating exercise capacity is the vasodilatation of the exercising muscle (25). *In vivo* studies performed in chronic heart failure patients have shown that adenosine release is impaired, partially explaining the reduced exercise capacity observed in these patients (25). Interestingly, some studies have shown that trained athletes have higher adenosine baseline plasma levels when compared with recreational ones (26). A previous publication of Muñoz-Cano et al. (27) showed that cofactor-related FAn patients (NSAIDs and exercise) overexpressed A_3 gene (ADORA3) and others related with adenosine metabolism. Interestingly, although A_3 activation has been linked to anti-inflammatory effects in several models of inflammation (28), it has also been related with the enhancement of IgE-mediated degranulation in human MC and, thus contributing to allergic inflammation (29, 30). Therefore, we hypothesized that the adenosine released during exercise in FDEIA patients would preferably bind A_3 with no deleterious effect in the absence of allergen. However, in the presence of allergen, adenosine would have a synergistic effect on MC activation, favoring the allergic reaction. However, further studies need to be conducted to confirm this theory.

Exercise and Eicosanoid Metabolism

Finally, another potential underlying mechanism in FDEIA may be related with the eicosanoid metabolism. Exercise is related with an increase in serum of products from the eicosanoid metabolism, as well as, cyclooxygenase (COX)-1 and 2-derived prostanoids (TXB_2, PGE_2, PGD_2,…) and lipoxygenase (5-LOX, 12-LOX, 15-LOX) as a physiological response (31).

Different models have demonstrated that PGE_2 abrogates IgE-mediated MC activation (32–34). Particularly misoprostol, a PGE_1 analog, has shown to suppress symptoms in wheat-dependent exercise-induced anaphylaxis and IgE-mediated histamine release in both allergic rhinitis and healthy individuals (35–37). Rastogi et al. (38) have shown that patients with hymenoptera anaphylaxis had lower baseline PGE_2 serum levels, suggesting that PGE_2 may protect from anaphylaxis. Conversely, a very recent publication of Muñoz-Cano et al. (39) did not find any differences in plasma PGE_2 at baseline in a series of FAn patients. Differences in the sample type and cause of anaphylaxis may account for this discrepancy.

Although there is no data regarding PGE_2 levels at baseline or otherwise in FDEIA, we could suggest, as a hypothesis, that these patients may have a deficient production of PGE_2 during exercise that would be predisposing to anaphylaxis in the presence of the allergen.

NSAIDS

Non-steroidal anti-inflammatory drugs (NSAIDs) constitute a heterogenous group of widely used drugs with analgesic, anti-pyretic, and anti-inflammatory properties. Their main mechanism of action, despite the differences in their chemical structure, depends on prostanoid (prostaglandins and thromboxane) inhibition by blocking COX activity (40). However, some NSAIDs have COX-independent effects, such as the ability to modulate several transcription factors that control the expression of genes involved in inflammation (*f.i.* nuclear factor-kappa B) or signaling pathways (MAPK or PI3k/Akt) (41).

NSAIDs, as a cofactor, are involved in up to 25% of food-induced anaphylaxis and are considered a risk factor with an odds ratio >11 (42). Several studies have shown that NSAIDs can also induce anaphylaxis in FDEIA patients despite that NSAIDs were not originally involved in previous reactions (43, 44). The underlying mechanisms of this synergistic effect are not completely understood, and two main theories have been suggested. One is related with the alteration of intestinal permeability by NSAIDs leading to an increase of allergen absorption (45) and the other suggesting a direct effect of NSAIDs on basophils and MC.

NSAIDs and Eicosanoid Metabolism

NSAIDs have shown to induce MC activation in certain human and animal models. In NSAID exacerbated respiratory disease (N-ERD) patients, Steinke et al. showed that aspirin induced MC activation by measuring calcium influx and PGD_2 release (46). Interestingly, it has also been demonstrated that N-ERD patients have a decreased expression of PGE_2 receptor 2 (EP2) that may contribute to reducing PGE_2 capacity to mediate anti-proliferative and anti-inflammatory effects (47). Indeed, N-ERD has also decreased production of PGE_2 (48).

Matsuo et al. (49) showed that aspirin did not induce HR by itself but enhanced IgE-mediated basophil activation. Interestingly, the authors suggested that this effect was not related with a COX-dependent mechanism but with Syk phosphorylation. On the contrary, Pascal et al. (50) showed that the ability of NSAID to enhance the IgE-mediated reactions

in FAn patients may be COX1-dependent. Using a model of basophil activation test, these authors demonstrated that the activation with the allergen (peach lipid transfer protein) was enhanced by aspirin. However, this effect was not observed when co-stimulating with valdecoxib (selective COX-2 inhibitor). In the same line, Wojnar et al. showed that several chemically unrelated NSAIDs (non-selective COX inhibitors) enhanced HR induced by ragweed (51). Indeed, Matsukura (43) and Aihara (52) demonstrated a potentiation of the allergic reaction with aspirin but not with nimesulide and etodolac, both preferential COX-2 inhibitors, in FDEIA patients.

Finally, several authors have demonstrated that PGE_2 prevents MC degranulation when acting through EP_2 and induces a pro-inflammatory response when signaling through EP_3 (18, 32, 53). Very recently, Rastogi et al. (38) have shown that anaphylaxis in mice can be prevented by blocking PGE_2 degradation. They also showed that MC IgE-mediated degranulation is suppressed by PGE_2 through EP_4 in mouse MC and through both EP_2 and EP_4 ligation in human skin MC. It has been suggested that the ratio of EP receptors expressed on cell's surface may be determinant in the final effect of PGE_2. EP_3 is considered to mediate pro-inflammatory effects, and EP_2 and EP_4 have anti-inflammatory activity (32). Also very recently, Muñoz-Cano et al. (39) showed that PGE_2 reduced IgE-mediated basophil activation in patients with FAn. Furthermore, these authors showed a decreased expression of EP_4 (anti-inflammatory) and increased expression of EP_3 (pro-inflammatory) receptors in basophils. However, they did not find differences among EP pattern expression when comparing FDNIA and FAn, and all patients had a ratio $EP_3/EP_4{+}EP_2$ favoring a pro-inflammatory activation upon PGE_2 ligation.

All this suggests that eicosanoid metabolism may be involved in the development of anaphylaxis in general, and therefore, anything blocking PGE_2 production, such as NSAIDs, may facilitate the development of a severe reaction. However, if that seems to be a universal mechanism in anaphylaxis, we must wonder why not all food allergic patients require a cofactor in order to have an anaphylaxis. In this line, Pascal et al. (50) showed that the synergistic effect of NSAID was present in both NSAID-dependent (FDNIA) and -independent FAn patients, and the main difference between them was the basophil sensitivity. Thus, FAn (NSAID-independent) patients had higher (about 148-fold) basophil sensitivity, requiring further less allergen concentration to elicit 50% of basophil maximal response compared to FDNIA patients. This suggests that enough allergen concentration could elicit an anaphylaxis in the absence of a cofactor in FDNIA. A similar observation was made in FDEIA patients *in vivo*, where the increase of the amount of allergen was enough to reproduce the anaphylaxis in the absence of exercise (43, 52, 54). Some other evidence of the differences in the pathogenic mechanism in cofactor-dependent and -independent anaphylaxis was provided by Muñoz-Cano et al. (27), who reported differences at transcriptome level. Thus, altered B-cell pathways, increased markers of neutrophil activation and reactive oxygen species levels were exclusively observed in FAn patients. However, adenosine metabolism related genes were differentially expressed only in FDNIA.

Altogether, these findings suggest that (1) eicosanoid metabolism may play a role in the development of any anaphylaxis; (2) NSAID may have a universal synergistic effect in any food allergic patient; (3) the right amount of allergen may induce an anaphylaxis in FDNIA even in the absence of a cofactor; and (4) there are other yet to be confirmed mechanisms that explain the differences between NSAID-dependent and -**in**dependent FAn.

NSAIDs and Adenosine Metabolism

Adenosine metabolism has been linked to some NSAID-exacerbated cutaneous and respiratory diseases. ADORA3 polymorphism has been identified in NSAID-exacerbated urticaria patients (55) and ADORA1 and ADORA2A in N-ERD patients (56). Cronstein et al. (57–59) showed in a series of studies with animal and human models that NSAIDs at pharmacologic concentrations increase the release of adenosine into the extracellular milieu by uncoupling oxidative phosphorylation and, therefore, increasing ATP catabolism. These authors have suggested that the anti-inflammatory effects of NSAIDs are partly COX-independent and mediated by adenosine. However, considering that the receptor expression profile in the cell's surface may condition the final effect of adenosine, as in the PGE_2–EP axis, the anti-inflammatory effect of adenosine in these models may be related with a particular expression pattern of the cells/mouse strains studied.

Muñoz-Cano et al. (27) observed that FDNIA patients had a unique transcriptome signature related with adenosine metabolism genes, particularly an overexpression of ADORA3 that may be having a dual effect in these patients. A_3 agonists have also shown anti-inflammatory effects in several mouse models due to inhibition of IFN-γ (60, 61). Interestingly, these authors also showed that FDNIA patients had a repressed IFN-γ production and IFN-γ-regulated genes. Considering that FDNIA patients usually have no or mild reaction when exposed to the allergen alone, A3R may be exerting its protective (anti-inflammatory) effect in this scenario through IFN-γ repression. However, when the patient is exposed to the food allergen plus NSAID, the adenosine released by NSAIDs, *via* A_3, would enhance the IgE-mediated reaction, resulting in a systemic reaction. Nevertheless, further studies are still needed to completely understand the specific role of adenosine metabolism in FAn.

Finally, Pouliot et al. (62) showed that adenosine up-regulates COX-2 expression, with a consequent increase of PGE_2 production through A_{2A}. These findings suggest that the inhibitory effect of A_{2A} receptor depends on COX2-PGE_2–EP axis. The potential connection between adenosine and PGE_2 metabolism, both apparently involved in the development of FDNIA, opens an exciting research field that must still be developed.

ALCOHOL

Alcohol is one of the classic cofactors in FAn, present in up to 15% of cases in some series (2), although the evidence supporting

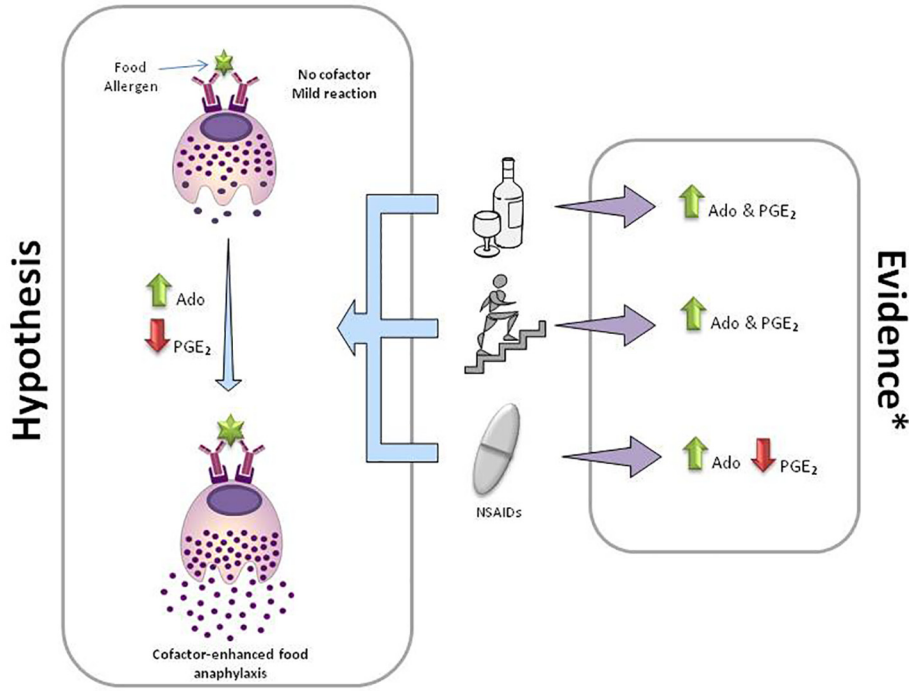


FIGURE 1 | Mechanisms in cofactor-enhanced food anaphylaxis. Adenosine and eicosanoid metabolism hypothesis. The panel depicts the most frequent cofactors involved in food anaphylaxis (from top to bottom): alcohol, exercise, and non-steroidal anti-inflammatory drugs (NSAIDs). Mild reactions in the absence of a cofactor may end up in an anaphylaxis with the same amount of allergen together with the presence of a cofactor. Ado, adenosine; green arrows, increase; red arrows, decrease. *Evidence based on references (23, 26, 28, 29, 38, 39, 41, 50, 57–59, 66, 67).

the underlying mechanism of its effects is scarce. We propose some hypothesis based on evidence of alcohol effect on immune cells that we briefly review hereby.

Some authors have shown that alcohol modifies intestinal permeability due to local activation of MC and modification of the expression of tight junction-associated proteins by acetaldehyde, one of its metabolites (63, 64). Actually, acetaldehyde-induced MC activation is one of the suggested mechanisms involved in alcohol-induced asthma in Japanese patients. It is well known that this population has a defective alcohol catabolism (aldehyde dehydrogenase 2 decreased activity) that facilitates acetaldehyde accumulation (64). Alcohol has also been shown to increase histamine levels by inhibiting diamino oxidase (DAO), an enzyme that catabolizes histamine (65). It has also been described that alcohol induces pro-inflammatory mediator (such IL-6, IL-10, and IFN-γ) release and eicosanoid metabolite production, such as PGE_2 (66). Similar to the hypothesis in FDEIA, food-dependent alcohol-induced anaphylaxis (FDAIA) patients could have a deficient production of PGE_2. Further studies evaluating the productions of eicosanoid metabolites in these patients would shed light upon the underlying mechanism.

Finally, adenosine metabolism again, may be involved in FDAIA. Alcohol inhibits adenosine uptake, increasing its extracellular levels (67). However, this effect is only observed in acute consumption, and chronic intake does not modify adenosine transport (67). So, as suggested in FDNIA, adenosine released upon alcohol consumption may enhance the IgE-mediated reaction induced by food allergen. Conversely to the observation in FDNIA patients, no data regarding expression profile of adenosine receptors in FDAIA exists.

DISCUSSION

The limited knowledge about the mechanisms involved in cofactor-enhanced FAn (CEFA) makes exceedingly difficult the development of prophylactic strategies. Apparently, avoiding strategies in CEFA patients may seem straightforward. However, in those allergic to ubiquitous allergens (*f.i.* nuts) or panallergens such as lipid transfer proteins, avoiding strategies are quite complicated considering that cofactors are everyday common situations (*f.i.* physical activity).

Although the evidence in CEFA is limited, and we are currently working mostly based on hypothesis, the high complexity of the underlying mechanism seems evident. In the light of this data and other existent evidence not reviewed in this manuscript, several pathogenic mechanisms may be intertwined. The "cofactor effect" seems to be a universal phenomenon as demonstrated in *in vivo* and *in vitro* experiments not only in FAn patients but also in healthy individuals (43, 49, 50, 54). That means that (1) a personal

predisposition may be required to develop a CEFA (or any anaphylaxis), and (2) most cofactors are interchangeable and capable of reproduce an anaphylaxis. Considering the unrelated nature of the cofactors, this observation may suggest that all of them may share, somehow, some common pathogenic mechanisms. One may suspect that all these cofactors are interfering with a compensatory system that is blocking (totally or partially) the allergic reaction induced by the food alone. The adenosine and eicosanoid metabolisms (**Figure 1**) and/or the disruption of intestinal permeability (not reviewed here) may be some of these mechanisms. In conclusion, and at risk of sounding cliché, further studies are needed to understand this "cofactor effect" and to identify risk biomarkers and prophylactic treatments.

AUTHOR CONTRIBUTIONS

RM-C has coordinated the different authors and written part of the manuscript. CS, RC-S, GA, MR-Z, JR-F, FP, and MM have written part of the manuscript. JB and MP have supervised the review and written part of the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

RC-S is a recipient of a Rio Hortega fellowship (Carlos III Health Institute CM19/00046).

REFERENCES

1. Kamdar TA, Peterson S, Lau CH, Saltoun CA, Gupta RS, Bryce PJ. Prevalence and characteristics of adult-onset food allergy. *J Allergy Clin Immunol Pract* (2015) 3:114–15.e1. doi: 10.1016/j.jaip.2014.07.007
2. Muraro A, Roberts G, Worm M, Bilò MB, Brockow K, Fernández Rivas M, et al. Anaphylaxis: guidelines from the European Academy of Allergy and Clinical Immunology. *Allergy* (2014) 69:1026–45. doi: 10.1111/all.12437
3. Wölbing F, Fischer J, Köberle M, Kaesler S, Biedermann T. About the role and underlying mechanisms of cofactors in anaphylaxis. *Allergy Eur J Allergy Clin Immunol* (2013) 68:1085–92. doi: 10.1111/all.12193
4. Worm M, Francuzik W, Renaudin JM, Bilo MB, Cardona V, Scherer Hofmeier K, et al. Factors increasing the risk for a severe reaction in anaphylaxis: An analysis of data from The European Anaphylaxis Registry. *Allergy* (2018) 73:1322–30. doi: 10.1111/all.13380
5. Worm M, Moneret-Vautrin A, Scherer K, Lang R, Fernandez-Rivas M, Cardona V, et al. First European data from the network of severe allergic reactions (NORA). *Allergy* (2014) 69:1397–404. doi: 10.1111/all.12475
6. Panesar SS, Javad S, de Silva D, Nwaru BI, Hickstein L, Muraro A, et al. The epidemiology of anaphylaxis in Europe: A systematic review. *Allergy* (2013) 68(11):1353–61. doi: 10.1111/all.12272
7. Cardona V, Luengo O, Garriga T, Labrador-Horrillo M, Sala-Cunill A, Izquierdo A, et al. Co-factor-enhanced food allergy. *Allergy* (2012) 67:1316–8. doi: 10.1111/j.1398-9995.2012.02877.x.
8. Oropeza AR, Bindslev-Jensen C, Broesby-Olsen S, Kristensen T, Møller MB, Vestergaard H, et al. Patterns of anaphylaxis after diagnostic workup: A follow-up study of 226 patients with suspected anaphylaxis. *Allergy* (2017) 72:1944–52. doi: 10.1111/all.13207
9. Ansley L, Bonini M, Delgado L, Del Giacco S, Du Toit G, Khaitov M, et al. Pathophysiological mechanisms of exercise-induced anaphylaxis: an EAACI position statement. *Allergy* (2015) 70:1212–21. doi: 10.1111/all.12677
10. Nieman DC. Exercise immunology: practical applications. *Int J Sports Med* (1997) 18(Suppl 1):S91–100. doi: 10.1055/s-2007-972705
11. Morgan D, Moodley I, Phillips M, Thorax R–. 1983 U. Plasma histamine in asthmatic and control subjects following exercise: influence of circulating basophils and different assay techniques. *Thorax* (1983) 38:771–7. doi: 10.1136/thx.38.10.771
12. Harries MG, Burgue PS, O'Brien I, Cromwell O, Pepys J. Blood histamine levels after exercise testing. *Clin Exp Allergy* (1979) 9:437–41. doi: 10.1111/j.1365-2222.1979.tb02506.x
13. Mucci P, Anselme-Poujol F, Caillaud C, Couret I, Rossi M, Préfaut C. Basophil releasability in young highly trained and older athletes. *Med Sci Sports Exerc* (1999) 31:507–13. doi: 10.1097/00005768-199904000-00003
14. Sheel AW, Boushel R, Dempsey JA. Competition for blood flow distribution between respiratory and locomotor muscles: implications for muscle fatigue. *J Appl Physiol* (2018) 125:820–31. doi: 10.1152/japplphysiol.00189.2018
15. Luttrell MJ, Halliwill JR. The Intriguing Role of Histamine in Exercise Responses. *Exerc Sport Sci Rev* (2017) 45:16–23. doi: 10.1249/JES.0000000000000093
16. Halliwill JR, Buck TM, Lacewell AN, Romero SA. Postexercise hypotension and sustained postexercise vasodilatation: what happens after we exercise? *Exp Physiol* (2013) 98:7–18. doi: 10.1113/expphysiol.2011.058065
17. Robson-Ansley P, Du TG. Pathophysiology, diagnosis and management of exercise-induced anaphylaxis. *Curr Opin Allergy Clin Immunol* (2010) 10:312–7. doi: 10.1097/ACI.0b013e32833b9bb0
18. Torres-Atencio I, Ainsua-Enrich E, de Mora F, Picado C, Martín M. Prostaglandin E2 prevents hyperosmolar-induced human mast cell activation through prostanoid receptors EP2 and EP4. *PloS One* (2014) 9:e110870. doi: 10.1371/journal.pone.0110870
19. Barg W, Wolanczyk-Medrala A Obojski A, Wytrychowski K, B Panaszek WM. Food-dependent exercise-induced anaphylaxis: possible impact of increased basophil histamine releasability in hyperosmolar conditions. *J Investig Allergol Clin Immunol* (2008) 18:312–5.
20. Popowski LA, Oppliger RA, Patrick Lambert G, Johnson RF, Kim Johnson A, Gisolf CV. Blood and urinary measures of hydration status during progressive acute dehydration. *Med Sci Sports Exerc* (2001) 33:747–53. doi: 10.1097/00005768-200105000-00011
21. Bours MJL, Swennen ELR, Di Virgilio F, Cronstein BN, Dagnelie PC. Adenosine 5'-triphosphate and adenosine as endogenous signaling molecules in immunity and inflammation. *Pharmacol Ther* (2006) 112:358–404. doi: 10.1016/j.pharmthera.2005.04.013
22. Rudich N, Ravid K, Sagi-Eisenberg R. Mast cell adenosine receptors function: a focus on the a3 adenosine receptor and inflammation. *Front Immunol* (2012) 3:134. doi: 10.3389/fimmu.2012.00134
23. Gomez G, Zhao W, Schwartz LB. Disparity in FcεRI-induced degranulation of primary human lung and skin mast cells exposed to adenosine. *J Clin Immunol* (2011) 31:479–87. doi: 10.1007/s10875-011-9517-7
24. Marshall JM. The roles of adenosine and related substances in exercise hyperaemia. In: Journal of Physiology. *J Physiol* (2007) 583(Pt 3):835–45. doi: 10.1113/jphysiol.2007.136416
25. Matsui S, Tamura N, Takekoshi N, Murakami E. Plasma adenosine concentration during and after dynamic exercise in patients with chronic heart failure. *Int J Angiol* (1995) 4:188–92. doi: 10.1007/BF02042918
26. Pospieszna B, Kusy K, Słomińska EM, Dudzinska W, Ciekot-Sołtysiak M, Zieliński J. The effect of training on erythrocyte energy status and plasma purine metabolites in athletes. *Metabolites* (2020) 10(1):5. doi: 10.3390/metabo10010005
27. Muñoz-Cano R, Pascal M, Bartra J, Picado C, Valero A, Kim D-K, et al. Distinct transcriptome profiles differentiate nonsteroidal anti-inflammatory drug-dependent from nonsteroidal anti-inflammatory drug-independent food-induced anaphylaxis. *J Allergy Clin Immunol* (2016) 137(1):137–46. doi: 10.1016/j.jaci.2015.05.042
28. Fishman P, Cohen S, Itzhak I, Amer J, Salhab A, Barer F, et al. The A3 adenosine receptor agonist, namodenoson, ameliorates non-alcoholic steatohepatitis in mice. *Int J Mol Med* (2019) 44:2256–64. doi: 10.3892/ijmm.2019.4364
29. Leung CT, Li A, Banerjee J, Gao ZG, Kambayashi T, Jacobson KA, et al. The role of activated adenosine receptors in degranulation of human LAD2 mast cells. *Purinergic Signal* (2014) 10:465–75. doi: 10.1007/s11302-014-9409-4

30. Jin X, Shepherd RK, Duling BR, Linden J. Inosine binds to A3 adenosine receptors and stimulates mast cell degranulation. *J Clin Invest* (1997) 100:2849–57. doi: 10.1172/JCI119833
31. Markworth JF, Vella L, Lingard BS, Tull DL, Rupasinghe TW, Sinclair AJ, et al. Human inflammatory and resolving lipid mediator responses to resistance exercise and ibuprofen treatment. *Am J Physiol Regul Integr Comp Physiol* (2013) 305(11):R1281–96. doi: 10.1152/ajpregu.00128.2013
32. Serra-Pages M, Olivera A, Torres R, Picado C, de Mora F, Rivera J. E-prostanoid 2 receptors dampen mast cell degranulation via cAMP/PKA-mediated suppression of IgE-dependent signaling. *J Leukoc Biol* (2012) 92:1155–65. doi: 10.1189/jlb.0212109
33. Gauvreau GM, Watson RM, O'Byrne PM. Protective effects of inhaled PGE2 on allergen-induced airway responses and airway inflammation. *Am J Respir Crit Care Med* (1999) 159:31–6. doi: 10.1164/ajrccm.159.1.9804030
34. Torres R, Picado C, de Mora F. The PGE2-EP2-mast cell axis: An antiasthma mechanism. *Mol Immunol* (2015) 1:61–8. doi: 10.1016/j.molimm.2014.03.007
35. Inoue Y, Adachi A, Ueno M, Fukumoto T, Nishitani N, Fujiwara N, et al. The inhibition effect of a synthetic analogue of prostaglandin E1 to the provocation by aspirin in the patients of WDEIA. *Arerugi* (2009) 58:1418–25.
36. Babakhin AA, Nolte H, Dubuske LM. Effect of misoprostol on the secretion of histamine from basophils of whole blood. *Ann Allergy Asthma Immunol* (2000) 84:361–5. doi: 10.1016/S1081-1206(10)62787-1
37. Takahashi A, Nakajima K, Ikeda M SS. Pre-treatment with misoprostol prevents food-dependent exercise-induced anaphylaxis (FDEIA). *Int J Dermatol* (2011) 50:233–40. doi: 10.1111/j.1365-4632.2010.04314.x
38. Rastogi S, Willmes DM, Nassiri M, Babina M, Worm M. PGE2 deficiency predisposes to anaphylaxis by causing mast cell hyper-responsiveness. *J Allergy Clin Immunol* (2020) 11:S0091–6. doi: 10.1016/j.jaci.2020.03.046
39. Muñoz-Cano R, Casas-Saucedo R, Araujo G, Roca-Ferrer J, de la Cruz C, Martin M, et al. Prostaglandin E2 Decreases Basophil Activation In Patients With Food-Induced Anaphylaxis. *Allergy* (2020). doi: 10.1111/all.14615
40. Bacchi S, Palumbo P, Sponta A, Coppolino MF. Clinical Pharmacology of Non-Steroidal Anti-Inflammatory Drugs: A Review. *Antiinflamm Antiallergy Agents Med Chem* (2012) 11:52–64. doi: 10.2174/18715231280 3476255
41. Tegeder I, Pfeilschifter J, Geisslinger G. Cyclooxygenase-independent actions of cyclooxygenase inhibitors. *FASEB J* (2001) 15:2057–72. doi: 10.1096/fj.01-0390rev
42. Moneret-Vautrin DA. Drugs as risk factors of food anaphylaxis in adults. *Medecine/Sciences* (2010) 26:719–23. doi: 10.1051/medsci/2010268-9719
43. Matsukura S, Aihara M, Sugawara M, Kunimi Y, Matsuki M, Inoue Y, et al. Two cases of wheat-dependent anaphylaxis induced by aspirin administration but not by exercise. *Clin Exp Dermatol* (2010) 35:233–7. doi: 10.1111/j.1365-2230.2009.03709.x
44. Harada S, Horikawa T, Ashida M, Kamo T, Nishioka E, Ichihashi M. Aspirin enhances the induction of type I allergic symptoms when combined with food and exercise in patients with food-dependent exercise-induced anaphylaxis. *Br J Dermatol* (2001) 145:336–9. doi: 10.1046/j.1365-2133.2001.04329.x
45. Matsuo H, Kaneko S, Tsujino Y, Honda S, Kohno K, Takahashi H, et al. Effects of non-steroidal anti-inflammatory drugs (NSAIDs) on serum allergen levels after wheat ingestion. *J Dermatol Sci* (2009) 53:241–3. doi: 10.1016/j.jdermsci.2008.09.004
46. Steinke JW, Negri J, Liu L, Payne SC, Borish L. Aspirin activation of eosinophils and mast cells: implications in the pathogenesis of aspirin-exacerbated respiratory disease. *J Immunol* (2014) 193:41–7. doi: 10.4049/jimmunol.1301753
47. Machado-Carvalho L, Torres R, Perez-Gonzalez M, Alobid I, Mullol J, Pujols L, et al. Altered expression and signalling of EP2 receptor in nasal polyps of AERD patients: role in inflammation and remodelling. *Rhinology* (2016) 54:254–65. doi: 10.4193/Rhin15.207
48. Roca-Ferrer J, Pérez-Gonzalez M, Garcia-Garcia FJ, Pereda J, Pujols L, Alobid I, et al. Low prostaglandin E2 and cyclooxygenase expression in nasal mucosa fibroblasts of aspirin-intolerant asthmatics. *Respirology* (2013) 18:711–7. doi: 10.1111/resp.12076
49. Matsuo H, Yokooji T, Morita H, Ooi M, Urata K, Ishii K, et al. Aspirin Augments IgE-Mediated Histamine Release from Human Peripheral Basophils via Syk Kinase Activation. *Allergol Int* (2013) 62:503–11. doi: 10.2332/allergolint.13-OA-0536
50. Pascal M, Muñoz-Cano R, Milà J, Sanz ML, Diaz-Perales A, Sánchez-López J, et al. Nonsteroidal anti-inflammatory drugs enhance IgE-mediated activation of human basophils in patients with food anaphylaxis dependent on and independent of nonsteroidal anti-inflammatory drugs. *Clin Exp Allergy* (2016) 46:1111–9. doi: 10.1111/cea.12735
51. Wojnar RJ, Hearn T, Starkweather S. Augmentation of allergic histamine release from human leukocytes by nonsteroidal anti-inflammatory-analgesic agents. *J Allergy Clin Immunol* (1980) 66:37–45. doi: 10.1016/0091-6749(80)90136-0
52. Aihara M, Miyazawa M, Osuna H, Tsubaki K, Ikebe T, Aihara Y, et al. Food-dependent exercise-induced anaphylaxis: influence of concurrent aspirin administration on skin testing and provocation. *Br J Dermatol* (2002) 146:466–72. doi: 10.1046/j.1365-2133.2002.04601.x
53. Feng C, Beller EM, Bagga S, Boyce JA. Human mast cells express multiple EP receptors for prostaglandin E 2 that differentially modulate activation responses. *Blood* (2006) 107:3243–50. doi: 10.1182/blood-2005-07-2772
54. Christensen MJ, Eller E, Mortz CG, Brockow K, Bindslev-Jensen C. Exercise Lowers Threshold and Increases Severity, but Wheat-Dependent, Exercise-Induced Anaphylaxis Can Be Elicited at Rest. *J Allergy Clin Immunol Pract* (2018) 6:514–20. doi: 10.1016/j.jaip.2017.12.023
55. Kim SH, Nam EJ, Kim YK, Ye YM, Park HS. Functional variability of the adenosine A3 receptor (ADORA3) gene polymorphism in aspirin-induced urticaria. *Br J Dermatol* (2010) 163:977–85. doi: 10.1111/j.1365-2133.2010.09983.x
56. Kim S-H, Kim Y-K, Park H-W, Kim S-H, Kim S-H, Ye Y-M, et al. Adenosine deaminase and adenosine receptor polymorphisms in aspirin-intolerant asthma. *Respir Med* (2009) 103:356–63. doi: 10.1016/j.rmed.2008.10.008
57. Cronstein BN, Montesinos MC, Weissmann G. Salicylates and sulfasalazine, but not glucocorticoids, inhibit leukocyte accumulation by an adenosine-dependent mechanism that is independent of inhibition of prostaglandin synthesis and p105 of NFkappaB. *Proc Natl Acad Sci USA* (1999) 96:6377–81. doi: 10.1073/pnas.96.11.6377
58. Cronstein BN, Vandestouwe M, Druska L, Levin RI, Weissmann G. Nonsteroidal Antiinflammatory Agents Inhibit Stimulated Neutrophil Adhesion to Endothelium - Adenosine-Dependent and Independent Mechanisms. *Inflammation* (1994) 18:323–35. doi: 10.1007/BF01534273
59. Cronstein BN, Montesinos MC, Weissmann G. Sites of action for future therapy: an adenosine-dependent mechanism by which aspirin retains its antiinflammatory activity in cyclooxygenase-2 and NFkappaB knockout mice. *Osteoarthr Cartil* (1999) 7:361–3. doi: 10.1053/joca.1998.0236
60. Fishman P, Bar-Yehuda S, Liang BT, Jacobson KA. Pharmacological and therapeutic effects of A3 adenosine receptor agonists. *Drug Discovery Today* (2012) 17:359–66. doi: 10.1016/j.drudis.2011.10.007
61. Cohen S, Barer F, Bar-Yehuda S, AP IJ, Jacobson KA, Fishman P. A(3) adenosine receptor allosteric modulator induces an anti-inflammatory effect: in vivo studies and molecular mechanism of action. *Mediat Inflammation* (2014) 2014:708746. doi: 10.1155/2014/708746
62. Pouliot M, Fiset M-E, Masse M, Naccache PH, Borgeat P. Adenosine Up-Regulates Cyclooxygenase-2 in Human Granulocytes: Impact on the Balance of Eicosanoid Generation. *J Immunol* (2002) 169:5279–86. doi: 10.4049/jimmunol.169.9.5279
63. Ferrier L, Bérard F, Debrauwer L, Chabo C, Langella P, Buéno L, et al. Impairment of the intestinal barrier by ethanol involves enteric microflora and mast cell activation in rodents. *Am J Pathol* (2006) 168:1148–54. doi: 10.2353/ajpath.2006.050617
64. Kawano T, Matsuse H, Kondo Y, Machida I, Saeki S, Tomari S, et al. Acetaldehyde induces histamine release from human airway mast cells to cause bronchoconstriction. *Int Arch Allergy Immunol* (2004) 134:233–9. doi: 10.1159/000078771
65. Zimatkin SM, Anichtchik OV. Alcohol-histamine interactions. *Alcohol Alcohol* (1999) 34:141–7. doi: 10.1093/alcalc/34.2.141
66. van de Loo AJAE, Mackus M, Kwon O, Krishnakumar IM, Garssen J, Kraneveld AD, et al. The Inflammatory Response to Alcohol Consumption and Its Role in the Pathology of Alcohol Hangover. *J Clin Med* (2020) 9:2081. doi: 10.3390/jcm9072081

67. Nagy LE, Diamond I, Casso DJ, Franklin C, Gordon AS. Ethanol increases extracellular adenosine by inhibiting adenosine uptake via the nucleoside transporter. *J Biol Chem* (1990) 265:1946–51. doi: 10.1016/S0021-9258(19)39923-5

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 19 February 2021
doi: 10.3389/fimmu.2021.595762

Eosinophilic Esophagitis and Microbiota: State of the Art

Maurizio Mennini[1], Renato Tambucci[2], Carla Riccardi[1], Francesca Rea[2], Paola De Angelis[2], Alessandro Fiocchi[1] and Amal Assa'ad[3]*

[1] Division of Allergy, Bambino Gesù Children's Hospital-Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS), Rome, Italy, [2] Digestive Endoscopy and Surgery Unit, Bambino Gesù Children's Hospital-IRCCS, Rome, Italy, [3] Division of Allergy and Immunology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States

OPEN ACCESS

Edited by:
Simon Patrick Hogan, University of Michigan, United States

Reviewed by:
Carine Blanchard, Nestlé Research Center, Switzerland
David Katzka, Mayo Clinic, United States
Brooke Polk, Washington University in St. Louis, United States

***Correspondence:**
Maurizio Mennini
Maurizio.mennini@opbg.net

Specialty section:
This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology

Received: *17 August 2020*
Accepted: *04 January 2021*
Published: *19 February 2021*

Citation:
Mennini M, Tambucci R, Riccardi C, Rea F, De Angelis P, Fiocchi A and Assa'ad A (2021) Eosinophilic Esophagitis and Microbiota: State of the Art. Front. Immunol. 12:595762. doi: 10.3389/fimmu.2021.595762

Eosinophilic esophagitis (EoE) is a chronic, food-triggered, immune-mediated disease of the oesophagus, clinically characterized by symptoms referred to oesophagal dysfunction, and histologically defined by an eosinophil productive inflammation of the oesophagal mucosa, among other cell types. The involvement of an adaptive Th2-type response to food antigens in EoE was known since 2000; several cytokines and chemokines promote food-specific responses, during which local production of IgE, but also IgG4 derived from plasma cells in lamina propria of oesophagal mucosa might play an important role. Evidence pointing towards a possible role for the innate immunity in EoE has arisen recently. Together, this evidence gives rise to a potential role that the innate immune system in general, and also the microbial pattern recognition receptors (PRRs) might play in EoE pathogenesis. Among PRRs, Toll-like receptors (TLRs) are type-I transmembrane receptors expressed both on epithelial and lamina propria cells with the capacity to distinguish between pathogen and commensal microbes. As TLRs in the different intestinal epithelia represent the primary mechanism of epithelial recognition of bacteria, this evidence underlines that oesophagal TLR-dependent signaling pathways in EoE support the potential implication of microbiota and the innate immune system in the pathogenesis of this disease. The oesophagal mucosa hosts a resident microbiota, although in a smaller population as compared with other districts of the gastrointestinal tract. Few studies have focused on the composition of the microbiota of the normal oesophagus alone. Still, additional information has come from studies investigating the oesophagal microbiota in disease and including healthy patients as controls. Our review aims to describe all the evidence on the oesophagal and intestinal microbiota in patients with EoE to identify the specific features of dysbiosis in this condition.

Keywords: microbiota, eosinophilic esophagitis, probiotics, dysbiosis, toll-like receptor

MICROBIOTA IN EOSINOPHILIC ESOPHAGITIS: A REVISION OF HYGIENE HYPOTHESIS

Eosinophilic esophagitis (EoE) is a chronic immune-mediated disease of the oesophagus, characterized by oesophageal dysfunction and by a productive eosinophil inflammation of the oesophageal mucosa (1–3). The incidence of EoE has increased in recent years (4).

The involvement of an adaptive Th2-type response to food antigens in EoE is well demonstrated (5, 6); several cytokines and chemokines promote food-specific responses (7, 8), during which local production of IgE (9), but also IgG4 in lamina propria of oesophageal mucosa (10) may play an important role. Pro-fibrogenic factors released by inflammatory cells can provoke fibrous remodeling of the oesophageal mucosa (11, 12). Avoiding specific food triggers is, in some contexts, the first line of therapy for EoE (13, 14).

Rather than the specific adaptive immunity, the innate immune system recognizes and reacts to ecological insults and microbes without the need for an immunoglobulin-driven antigen-specific reaction. Proof pointing towards a possible role for the innate immunity in EoE has emerged. Oesophageal epithelial cells appear to be critical effectors provoking the inflammatory phenomena in EoE, not directly through eotaxin-3 release and other chemoattractants for eosinophils (15), but also by the recruitment of invariant natural killer T (iNKT) cells toward the oesophageal epithelium (16), which constitutes a crucial cytokine source. A pivotal role for mast cells (MCs) has also been recognized in the pathophysiology and symptoms of EoE, which reverse after effective dietary treatment (17). This evidence gives rise to a possible role that the innate immune system and raises some possible questions regarding the role of the microbial pattern recognition receptors (PRRs) in EoE pathogenesis.

Among PRRs, Toll-like receptors (TLRs) are type-I transmembrane receptors expressed both on epithelial and lamina propria cells with the ability to recognize microorganism and commensal organisms (18). In humans, there is an aggregate of 11 distinctive TLR, each having various specificities which, when activated, promote intracellular sign transduction pathways intervened by MAP kinases and NF-κB, at last setting off a supportive of inflammatory reaction. TLRs initiation is mindful, among different capacities, for setting off provocative reactions by going about as a connection among adaptive and innate immunity (19–21). Enactment and development of antigen-presenting cells and regulatory T cells (Tregs) rely in part upon TLR-intervened flagging, featuring their job on mucosal resistant homeostasis. A few investigations have assessed the association between hypersensitivity and TLR activation (19, 22, 23). TLR activity in oesophageal epithelial samples has been described (24).

Arias et al. shown that bacterial load and TLR1, TLR2, TLR4, and TLR9 were overexpressed on oesophageal biopsies with EoE compared to controls. Muc1 and Muc5B genes were downregulated while Muc4 was overexpressed. Upregulation of MyD88 and NFκB was discovered along with IL-1β, IL-6, IL-8, and IL-10 and PER-1, iNOS, and GRZA effectors. NG-K2D (KLRK1, IL-15, MICB) were likewise upregulated. In all cases, changes in EoE were neutralized after six food elimination diet (SFED) and mucosal healing.

As TLRs in the different intestinal epithelia represent the essential instrument of epithelial recognition of microbes (25), this proof underlines that oesophageal TLR-subordinate flagging pathways in EoE support the implication of microbiota and the innate immune system in the advancement of this condition.

Sterility in infant mice can prompt a move in the IgE-basophil axis, an unevenness in Th1/Th2 activation, just as inappropriate implication of Tregs. Human examinations to assess these components are not many (26, 27). These varieties may be suggestive of a connection between commensal microscopic organisms and hereditary demeanor to atopic illness. These bacterial ligands additionally could represent therapeutic targets in atopic disease.

Therefore, it appears potentially useful to understand the function of microbial flora in healthy human beings and the specific alterations in atopic diseases.

The White House Office of Science and Technology declared that the Fact Sheet for the National Microbiome Initiative (NMI) was intended to satisfy three explicit objectives: (1) to address central inquiries concerning the microbiome in different biological systems; (2) to create stage innovations for upgrading information sharing on microbiomes; and (3) to grow the microbiome workforce.

Since then, the interest in microbiome composition in different allergic conditions has grown (28–32).

The examination concerning how the microbiome can change, even in healthy individuals, is critical to improve comprehension of how the microbiome unthinkingly causes infection.

OESOPHAGEAL MICROBIOTA IN HEALTH

Introduction Comments

Once thought composed of few microbes, the oesophageal mucosa showed a composition of around 300 bacteria species. New culture-independent techniques have allowed scientists to identify the microbial composition of the oesophagus.

The oesophageal mucosa hosts a resident microbiota, although in a smaller population as compared with other districts of the gut. The human microbiota of the digestive tract exhibits considerable qualitative and quantitative differences, with communities starting from 10 cells per g/mL of sampled material within the oesophagus and stomach to 10^{12} per g/mL of tested material in the large intestine (33, 34).

The Main Findings

It was initially not clear whether the oesophagus was characterized by a defined microbiota. The first studies on the oesophageal microbiota and based on cultivation methods demonstrated that the oesophagus did not merely contain a

transient microbial population originating from the oral cavity by swallowing or from the stomach by gastroesophageal reflux (GER) (35–37). It was later observed that bacteria were associated with the oesophageal mucosal surface, confirming the presence of a resident microbiota at this site (38). Knowledge regarding the composition of the microbiota of healthy individuals has been expanded using investigations based on metagenomics approaches (39–41).

In general, the distal oesophageal microbiota was described as simply like that of the oropharynx, yet not indistinguishable (38, 42, 43).

Scarcely any examinations have concentrated on the organization of the microbiota of healthy oesophagus, (44–46) but extra data has originated from considers researching the oesophageal microbiota in disease compared to controls (47–56).

Influencers and Limitations

The accessible data on the microbiota are biased by various methodologies, contrasts in the tested parts of the oesophagus, and the heterogeneity of consideration/avoidance rules utilized in the different investigations don't permit comparisons and make it hard to agree on the general microbiota synthesis of the healthy oesophagus.

The presence of Streptococcus spp. was described by all studies: along these lines, members from this genus, seem to be a dominant taxon in the microbiota of the healthy oesophagus. Other bacterial genera frequently identified in association with streptococci, albeit in lower extents, incorporate Fusobacterium, Veillonella and Prevotella. The nearness of different genera (e.g., Neisseria, Haemophilus, Gemella, Granulicatella, Actinomyces, Lactobacillus, Bacteroides, Porphyromonas, and Staphylococcus), was lower.

Outstandingly, the commonness of Fusobacterium, Streptococcus, Prevotella and Veillonella has reliably been accounted in several studies based on either culture-dependent or culture-independent methodologies and on various examples (biopsies, aspirates, brushes), subsequently giving a reliable sign of their commitment to the piece of the microbiota that colonizes the healthy oesophageal mucosa. The strength of streptococci and the continuous nearness of other taxa regular of the oropharyngeal microbiota have been identified with the piece of the microbial networks of the oropharyngeal cavity, where a high commonness of streptococci is discovered, along with Gemella, Fusobacterium, Veillonella, Rothia and Granulicatella, (57, 58) have bolstered the idea that the oesophageal microbiota is basically of oral inception.

In any case, not every single oral bacterium can colonize the oesophageal mucosa, while a few individuals from the oesophageal microbiota appear to be underrepresented in the oral cavity, highlighting an alternate microbiota variation in the two-body destinations (38, 45, 46).

OESOPHAGEAL MICROBIOME AND DIET

There is no sufficient data on the effect of the diet on the oesophageal microbiota. A study of 47 patients showed that a diet higher in fiber was associated with a decrease of Proteobacteria and an increase of the Firmicutes phylum in the oesophagus (59). Additional studies correlating oesophageal microbiome and nutrition are needed. Studies that further define the stability of the oesophageal microbiome over time as well as other factors that determine inter-individual microbiome composition will aid in our understanding of the role of the diet.

OESOPHAGEAL MICROBIOTA IN EOE

The Main Findings

In 2015, Benitez et al. described the bacterial composition of the oral and oesophageal mucosa through 16S rRNA assessment of buccal swabs and oesophageal biopsies from 33 pediatric EoE subjects compared to 35 non-EoE healthy controls. They applied a longitudinal model before and after defined dietary changes.

In this study, Firmicutes were more abundant in oesophageal compared to oral samples, and oesophageal microbiota was more abundant of Proteobacteria in controls than EoE. The authors detected a significant difference between activated EoE and controls biopsies. The targeted dietary intervention did not produce substantial differences in either oesophageal or oral microbiota; the reintroduction of allergens led to enrichment in Campylobacter and Ganulicatella genera in the oesophagus (55).

Corynebacterium was enriched in the EoE samples, as was Neisseria genus, as previously described in different inflammatory conditions (60, 61). The Atopobium and Streptococcus genera were consistently enriched in non-EoE control samples.

The oesophageal microbiome of non-EoE control subjects showed a prevalence of Gram (+) bacteria of the Streptococcus genus, in agreement with previous findings in adult (62) and pediatric individuals (45) without oesophageal inflammation. Most of Benitez et al. study subjects were pediatric males with nearly 100% documented and concurrent proton-pump inhibitors (PPI) use, providing a further indication of the resilience of the Streptococcus-dominated microbiome in the healthy oesophagus in all genders and age groups.

They did not detect differences in the oral microbiome between inactive EoE, active EoE or non-EoE control samples, suggesting that in pediatric EoE, bacterial communities are stable and might not be altered by dietary modification. Data do not support the use of oral samples for EoE surveillance instead of biopsies.

In the same year, Harris et al. performed a prospective evaluation of secretions from EoE adult and children oesophageal biopsies, Gastro-oesophageal Reflux Disease (GERD) and healthy mucosa through Esophageal String Test (EST). Bacterial load was determined by quantitative PCR. Bacterial communities, determined by 16S rRNA and 454 pyrosequencing, were compared between disease and health. The bacterial amount was increased in both GERD and EoE and compared to healthy subjects. In EoE individuals, the amount was increased regardless of treatment status or level of mucosal eosinophilia. Haemophilus was significantly increased in untreated EoE individuals as compared with healthy subjects. Streptococcus was diminished in GERD subjects in PPI therapy as compared with healthy subjects. These data affirmed that diseases related to mucosal

eosinophilia are characterized by a different microbiome from that found in the ordinary mucosa (56).

The EoE activity did not seem to directly affect a load of bacteria in EoE. However, eosinophils possess extracellular DNA traps and numerous anti-microbial properties with the release of defensins (63–65). The microbiota of untreated EoE subjects showed a shift from a mostly Gram (+) population to an increase in Gram (−) bacteria similar to what has been described in GERD (49). The implication of Gram (−) bacterial involvement in reflux esophagitis (66) is consistent with the observed increase in Haemophilus and Proteobacteria in EoE. These data suggest that treatment could affect the microbiota.

Norder Grusell et al. enrolled 17 subjects with GERD and 10 with EoE. All patients performed endoscopic brush sampling and biopsies from the upper and lower oesophagus and the oral cavity. Bacterial growth was identified to the species or genus level. The major part of bacterial groups or species was found in specimens from the lower oesophagus in EoE subjects compared to GERD subjects. *Streptococcus viridans* was the most common bacteria in both groups. GERD individuals had significantly inferior bacterial diversity in both oesophageal and oral samples. This discrepancy could depend on the protective mucosal biofilm by the acid content in GERD patients. The authors endorsed cultural method and speculated that bacteria identified by 16SrDNA/RNA techniques might not be alive, and there might be amplification bias following the processing steps. Nevertheless, they acknowledge that it is impossible to cultivate some bacterial species, and essential bacteria might, therefore, be overlooked (67).

Table 1 summarizes the main findings about microbiota in EoE.

HELICOBACTER PYLORI: A CONTROVERSIAL ROLE

Helicobacter pylori infection often occurs in early childhood and it seems to enhance immune-tolerance driving immune-mediated diseases in a susceptible host (69–71).

In this context, previous or current infection with Helicobacter pylori (exposure) has been reported to protect against EoE, perhaps owing to H. pylori-induced immunomodulation. In 2019 a meta-analysis evaluated 11 observational studies comprising data on 377,795 individuals worldwide. H. pylori exposure vs non-exposure was associated with a 37% reduction in odds of EoE (odds ratio, 0.63; 95% CI, 0.51–0.78) and a 38% reduction in odds of esophageal eosinophilia (odds ratio, 0.62; 95% CI, 0.52–0.76). Fewer prospective studies found a significant association between H. pylori exposure and EoE (p= .06) than retrospective studies. Effect estimates were not affected by study location, whether the studies were performed in pediatric or adult populations, time period, or prevalence of H. pylori in the study

TABLE 1 | Main findings about oesophageal Microbiota in EoE.

Author, year	Study population	Method	Microbial differences
Benitez AJ. et al, 2015 (55)	Non-EoE pediatric controls and pediatric EoE subjects before and after defined dietary changes.	Bacterial composition through 16S rRNA gene sequencing of the oral and esophageal microenvironments using oral swabs and esophageal biopsies	1) Enrichment of Proteobacteria (Neisseria and Corynebacterium) in the EoE cohort, and predominance of the Firmicutes in non-EoE control subjects. 2) Targeted dietary intervention did not lead to significant differences in either oral or esophageal microbiota, reintroduction of highly allergenic foods led to enrichment in Ganulicatella and Campylobacter genera in the esophagus.
Harris JK. et al., 2015 (56)	EoE adult and children, Gastro-oesophageal Reflux Disease and healthy mucosa.	Bacterial composition of secretions and biopsies through 16S rRNA gene amplification from samples obtained with Esophageal String Test (EST). Bacterial load was determined by quantitative PCR.	1) In EoE, bacterial load was increased regardless of treatment status or degree of mucosal eosinophilia compared with normal. 2) Haemophilus was significantly increased in untreated EoE subjects
Norder Grussell E. et al., 2018 (67)	Subjects diagnosed with GERD and with EoE	Brush sampling and biopsies from the oral cavity, upper and lower esophagus. The samples were cultivated on agar plates, and bacterial growth was identified to the genus or species level and semi-quantified.	1) Significantly higher numbers of bacterial groups or species were found in specimens from the lower esophagus in subjects with EoE compared to subjects with GERD. 2) Streptococci were present in all of the EoE-subjects but only in approximately 75% in lower esophagus of the GERD-subjects, regardless of the sampling method.
Hiremath G. et al., 2019 (68)	Non-EoE pediatric controls and pediatric EoE.	The salivary microbiome was determined through 16S rRNA gene sequencing.	1) A trend toward lower microbial richness and alpha diversity was noted in children with EoE. 2) Specific taxa such as Streptococcus tended to be abundant in children with active EoE compared with non-EoE controls. 3) Haemophilus was significantly abundant in children with active EoE compared with inactive EoE and increased with disease activity.

population (72). The role of H. pylori would therefore deserve more evidence.

POSSIBLE ALTERNATIVES TO THE OESOPHAGEAL BIOPSIES FOR MICROBIOTA EVALUATION

In 2012 Fillon et al. described the oesophageal microbiome in healthy children through the Enterotest™ (EST), a minimally invasive string technology. EST samples and mucosal biopsies were collected from healthy children (n=15) and their microbiome composition determined by 16S rRNA gene sequencing. Microbiota from oesophageal biopsies and ESTs produced nearly identical profiles of bacterial genera and were different from the bacterial contents of oral and nasal cavity samples. They concluded that the EST could be a useful device for the study of the oesophageal microbiome (45).

In 2019 Hiremath et al. collected saliva samples from 19 non-EoE controls and 26 children with EoE. The salivary microbiome was determined through 16S rRNA gene sequencing, and disease activity was assessed through the Eosinophilic Esophagitis Histologic Scoring System (EoEHSS) and the Eosinophilic Esophagitis Endoscopic Reference Score.

A trend toward lower microbial richness was recognized in EoE children. The salivary microbiome was similar between children with and without EoE.

Streptococcus tended to be more abundant in children with active EoE compared with non-EoE controls. Haemophilus was significantly plentiful in active EoE compared with inactive EoE and increased with the increasing Eosinophilic Esophagitis Histology Scoring System and EoEHSS. Besides, four broad salivary microbial communities correlated with the EoEHSS.

The authors concluded that the composition of the salivary microbiome community structure could be different in children with EoE. The disease activity positively correlates with the relative abundance of Haemophilus. Perturbations in the salivary microbiome may play a role in EoE pathobiology and could be a noninvasive marker of disease activity. The disease activity in the oesophagus does not seem to affect a load of bacteria in EoE directly (68).

ATTEMPTS AT THE MODULATION OF EOE WITH PROBIOTIC

Recently, it was demonstrated that there are huge contrasts in gut microbial community structure, microbial abundance, and uniformity in patients with EoE.

Faecal microbiota was evaluated through 16SrRNA amplification from 12 EoE patients and 12 controls. Patients with EoE showed inferior gut microbiota alpha diversity. The authors observed at the phylum level an important increase in Bacteroidetes and a decrease in Firmicutes and a significant reduction in Clostridiales and Clostridia at the order and family level in patients with EoE.

The authors speculated that Clostridia based interventions could be tested as adjuncts to current therapeutic strategies in EoE (73).

The probiotic Lactococcus lactis NCC 2287 has previously been shown to decrease clinical scores in a food allergy model based on co-administration of cholera toxin and ovalbumin (74). Also, NCC 2287 is a potent inhibitor of the eosinophil survival cytokine IL-5 and an inducer of the immune-modulatory cytokine IL-10 and in Th2-skewed cultures of peripheral blood mononuclear cells (PBMC) (75). The probiotic Bifidobacterium lactis NCC 2818 is also known for its immunomodulatory properties in allergy (76).

Holvoet et al. in 2016 tested L. lactis NCC 2287 and B. lactis NCC 2818, for their capacity to decrease oesophageal inflammation in EoE murine model (77).

To test whether probiotics could decrease oesophageal eosinophilia in an EoE animal model, the strain NCC 2287 was added to the drinking water as prevention (day 0 to day 28; n = 8), as a treatment (day 28 to day 38; n = 10) or as continuous exposure (day 0 to day 38; n = 10). The maximum eosinophil count was significantly greater in the oesophagus of sensitized mice challenged with Af extract than in the oesophagus of non-sensitized mice. Interestingly, sensitized mice receiving NCC 2287 from day 28 to 38 had significantly less oesophageal eosinophilia than the non-supplemented sensitized group. However, there was no significant effect on oesophageal eosinophilia when the NCC 2287 strain was administered as a preventive measure or when it was given continuously, throughout the study.

This study demonstrates that the time frame of supplementation is fundamental: the beneficial effect of L. lactis NCC 2287 was only observed when it was administered as a treatment. Altogether, the data suggest that L. lactis NCC 2287 may be an exciting candidate for reducing EoE inflammation.

B. lactis NCC 2818 and L. lactis NCC 2287 both induce an increase of IL-10 in a Th2-skewed PBMC model, suggesting a possible immunoregulatory effect of these strains (76–78). However, in the same model, L. lactis NCC 2287 is more potent at reducing IL-5 levels and a stronger inducer of IFN-c than B. lactis NCC 2818 (36). It seems debatable the evaluation of the predictive value of these assays in human disease.

Probiotics seem to stabilize IL10 mRNA expression and to dysregulate microRNAs in human monocyte (78). These results suggest that other epigenetic mechanisms could explain the effect observed in the oesophagus and that the understanding of probiotic impact is at the beginning. Akei et al. have demonstrated that oesophageal eosinophilia is IL-5-dependent (79).

The balancing impact of NCC 2287 on IL-5 expression seen in Th2-slanted PBMCs (75) and an ovalbumin/cholera toxin-induced food hypersensitivity model (74) may somewhat clarify the abatement in oesophageal eosinophilia seen with NCC 2287 treatment in this study.

These results suggest that L. lactis NCC 2287 may lead to a steady decrease in gastrointestinal Th2 inflammation independently of the level of antigen sensitization in experimental allergy models.

Previous studies have highlighted the noticeable link between oesophageal and lung inflammation (45). Furthermore, 14–70% of

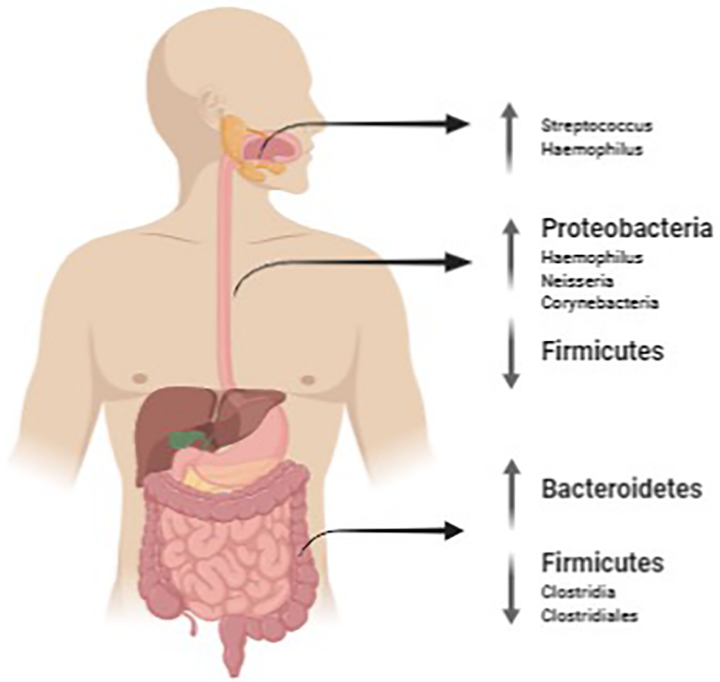


FIGURE 1 | Salivary, oesophageal and gut microbiota mutations in EoE. The image was created with Biorender.com.

EoE patients also presented with asthma (1, 46). The effect of L. lactis NCC 2287 on lung eosinophilia and oesophageal eosinophilia could be referred to a common consequence of the probiotic on eosinophil recruitment. Nevertheless, oral supplementation with B. lactis NCC 2818 had no effect on EoE, although it significantly reduced bronchoalveolar eosinophilia.

Their data suggest that a decrease in eosinophils in the lung is not concomitant with a significant decline in oesophageal eosinophilia. These outcomes are to be expected as various preclinical examinations have indicated that lung eosinophilia can diminish with probiotic supplementation (80, 81) or auxiliary to changes in the gut microbiota (82, 83).

Probiotics could vary in their ability to prevent or deal with the allergic response.

The proof in the animal model recommends that specific probiotics might be gainful in lessening oesophageal eosinophilic inflammation.

Instead, probiotics seem to permit a more comprehensive approach which may restore and maintain homeostasis in humans.

Figure 1 summarizes the main findings of microbiota found in EoE at oral, esophageal and intestinal level.

CONCLUSION

In conclusion, the oesophageal mucosa hosts a resident microbiota and there is evidence that it may change in the presence of EoE with an increase of bacterial load (56, 67) and with Streptococcus as recurrent taxa (67, 68) and with Haemophilus as possible marker of disease activity in different studies (56, 68).

It is not yet clear what is the cause-effect link that regulates these changes.

It is therefore not yet possible to imagine possible rational interventions for modulating the esophageal microbiota.

It therefore appears essential to carry out immunological studies that clarify this phenomenon and that allow to hypothesize also possible alternative therapies for EoE.

AUTHOR CONTRIBUTIONS

MM, AA, and AF conceived the review and research method of bibliographic sources. MM and RT performed the research, the analysis and the selection of the sources. MM and AA wrote the first draft of the manuscript. CR, FR, and PA performed the critical analysis of the sources and the final revision of the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

The Scientific Direction of Bambino Gesù Children's Hospital approved and contributed to the payment of the publication fee of this scientific contribution.

REFERENCES

1. Lucendo AJ, Molina-Infante J, Arias A, von Arnim U, Bredenoord AJ, Bussmann C, et al. Guidelines on eosinophilic esophagitis: evidence-based statements and recommendations for diagnosis and management in children and adults. *United Eur Gastroenterol J* (2017) 5(3):335–58. doi: 10.1177/2050640616689525
2. Molina-Infante J, Bredenoord AJ, Cheng E, Dellon ES, Furuta GT, Gupta SK, et al. Proton pump inhibitor-responsive oesophageal eosinophilia: an entity challenging current diagnostic criteria for eosinophilic oesophagitis. *Gut* (2016) 65:521–31. doi: 10.1136/gutjnl-2015-310991
3. Lucendo AJ, Navarro M, Comas C, Pascual JM, Burgos E, Santamaría L, et al. Immunophenotypic characterization and quantification of the epithelial inflammatory infiltrate in eosinophilic esophagitis through stereology: an analysis of the cellular mechanisms of the disease and the immunologic capacity of the esophagus. *Am J Surg Pathol* (2007) 31:598–606. doi: 10.1097/01.pas.0000213392.49698.8c
4. Arias Á, Pérez-Martínez I, Tenías JM, Lucendo AJ. Systematic review with meta-analysis: The incidence and prevalence of eosinophilic oesophagitis in children and adults in populationbased studies. *Aliment Pharmacol Ther* (2016) 43:3–15. doi: 10.1111/apt.13441
5. Straumann A, Bauer M, Fischer B, Blaser K. Simon HU Idiopathic eosinophilic esophagitis is associated with a T (H)2-type allergic inflammatory response. *J Allergy Clin Immunol* (2001) 108:954–61. doi: 10.1067/mai.2001.119917
6. Bullock JZ, Villanueva JM, Blanchard C, Filipovich AH, Putnam PE, Collins MH, et al. Interplay of adaptive th2 immunity with eotaxin-3/c-C chemokine receptor 3 in eosinophilic esophagitis. *J Pediatr Gastroenterol Nutr* (2007) 45:22–31. doi: 10.1097/MPG.0b013e318043c097
7. Lucendo AJ, Lucendo B. An update on the immunopathogenesis of eosinophilic esophagitis. *Expert Rev Gastroenterol Hepatol* (2010) 4:141–8. doi: 10.1586/egh.10.9
8. Lucendo AJ. Cellular and molecular immunological mechanisms in eosinophilic esophagitis: an updated overview of their clinical implications. *Expert Rev Gastroenterol Hepatol* (2014) 8:669–85. doi: 10.1586/17474124.2014.909727
9. Vicario M, Blanchard C, Stringer KF, Collins MH, Mingler MK, Ahrens A, et al. Local B cells and IgE production in the oesophageal mucosa in eosinophilic oesophagitis. *Gut* (2009) 51:12–20. doi: 10.1136/gut.2009.178020
10. Clayton F, Fang JC, Gleich GJ, Lucendo AJ, Olalla JM, Vinson LA, et al. Eosinophilic esophagitis in adults is associated with IgG4 and not mediated by IgE. *Gastroenterology* (2014) 147:602–9. doi: 10.1053/j.gastro.2014.05.036
11. Aceves SS, Newbury RO, Dohil R, Bastian JF, Broide DH. Esophageal remodeling in pediatric eosinophilic esophagitis. *J Allergy Clin Immunol* (2007) 119:206–12. doi: 10.1016/j.jaci.2006.10.016
12. Lucendo AJ, Arias A, De Rezende LC, Yagüe-Compadre JL, Mota-Huertas T, González-Castillo S, et al. Subepithelial collagen deposition, profibrogenic cytokine gene expression, and changes after prolonged fluticasone propionate treatment in adult eosinophilic esophagitis: a prospective study. *J Allergy Clin Immunol* (2011) 128:1037–46. doi: 10.1016/j.jaci.2011.08.007
13. Arias A, González-Cervera J, Tenias JM, Lucendo AJ. Efficacy of dietary interventions for inducing histologic remission in patients with eosinophilic esophagitis: a systematic review and metaanalysis. *Gastroenterology* (2014) 146:1639–48. doi: 10.1053/j.gastro.2014.02.006
14. Molina-Infante J, Arias Á, Alcedo J, Garcia-Romero R, Casabona-Frances S, Prieto-Garcia A, et al. Step-up empiric elimination diet for pediatric and adult eosinophilic esophagitis: the 2-4-6 Study. *J Allergy Clin Immunol* (2017) 141(4):1365–72. doi: 10.1016/j.jaci.2017.08.038
15. Blanchard C, Mingler MK, Vicario M, Abonia JP, Wu YY, Lu, et al. IL-13 involvement in eosinophilic esophagitis: transcriptome analysis and reversibility with glucocorticoids. *J Allergy Clin Immunol* (2007) 120:1292–300. doi: 10.1016/j.jaci.2007.10.024
16. Lexmond WS, Neves JF, Nurko S, Olszak T, Exley MA, Blumberg RS, et al. Involvement of the iNKT cell pathway is associated with early-onset eosinophilic esophagitis and response to allergen avoidance therapy. *Am J Gastroenterol* (2014) 109:646–57. doi: 10.1038/ajg.2014.12
17. Arias Á, Lucendo AJ, Martínez-Fernández P, González-Castro AM, Fortea M, González-Cervera J, et al. Dietary treatment modulates mast cell phenotype, density, and activity in adult eosinophilic oesophagitis. *Clin Exp Allergy* (2016) 46:78–91. doi: 10.1111/cea.12504
18. Jiménez-Dalmaroni MJ, Gerswhin ME, Adamopoulos IE. The critical role of toll-like receptors–from microbial recognition to autoimmunity: a comprehensive review. *Autoimmun Rev* (2016) 15:1–8. doi: 10.1016/j.autrev.2015.08.009
19. Satoh T, Akira S. Toll-like receptor signaling and its inducible proteins. *Microbiol Spectr* (2016) 4(6). doi: 10.1128/microbiolspec.MCHD-0040-2016
20. Kumagai Y, Akira S. Identification and functions of pattern-recognition receptors. *J Allergy Clin Immunol* (2016) 125:985–92. doi: 10.1016/j.jaci.2010.01.058
21. van Egmond M, Vidarsson G, Bakema JE. Cross-talk between pathogen recognizing Toll-like receptors and immunoglobulin Fc receptors in immunity. *Immunol Rev* (2015) 268:311–27. doi: 10.1111/imr.12333
22. Tunis MC, Marshall JS. Toll-like receptor 2 as a regulator of oral tolerance in the gastrointestinal tract. *Mediators Inflammation* (2014) 2014:606383. doi: 10.1155/2014/606383
23. Tsybikov NN, Petrisheva I, Fefelova EV, Kuznik BII, Magen E. Expression of TLR2 and TLR4 on peripheral blood monocytes during exacerbation of atopic dermatitis. *Allergy Asthma Proc* (2015) 36(6):e140–5. doi: 10.2500/aap.2015.36.3901
24. Mulder DJ, Lobo D, Mak N, Justinich CJ. Expression of toll-like receptors 2 and 3 on esophageal epithelial cell lines and on eosinophils during esophagitis. *Dig Dis Sci* (2012) 57(3):630–42. doi: 10.1007/s10620-011-1907-4
25. Burgueño JF, Abreu MT. Epithelial Toll-like receptors and their role in gut homeostasis and disease. *Nat Rev Gastroenterol Hepatol* (2020) 17(5):263–78. doi: 10.1038/s41575-019-0261-4
26. Woo JG, Assa'ad A, Heizer AB, Bernstein JA, Hershey GK. The -159 C–>T polymorphism of CD14 is associated with nonatopic asthma and food allergy. *J Allergy Clin Immunol* (2003) 112(2):438–44. doi: 10.1067/mai.2003.1634
27. Liu J, Rädler D, Illi S, Klucker E, Turan E, von Mutius E, et al. TLR2 polymorphisms influence neonatal regulatory T cells depending on maternal atopy. *Allergy* (2011) 66(8):1020–9. doi: 10.1111/j.1398-9995.2011.02573.x
28. Bunyavanich S, Berin MC. Food allergy and the microbiome: Current understandings and future directions. *J Allergy Clin Immunol* (2019) 144(6):1468–77. doi: 10.1016/j.jaci.2019.10.019
29. Tanabe H, Sakurai K, Kato T, Kawasaki Y, Nakano T, Yamaide F, et al. Association of the maternal microbiome in Japanese pregnant women with the cumulative prevalence of dermatitis in early infancy: A pilot study from the Chiba study of Mother and Child Health birth cohort. *World Allergy Organ J* (2019) 12(10):100065. doi: 10.1016/j.waojou.2019.100065
30. Guo J, Lv Q, Ariff A, Zhang X, Peacock CS, Song Y, et al. Western oropharyngeal and gut microbial profiles are associated with allergic conditions in Chinese immigrant children. *World Allergy Organ J* (2019) 12(8):100051. doi: 10.1016/j.waojou.2019.100051
31. Chiu CY, Chan YL, Tsai MH, Wang CJ, Chiang MH, Chiu CC. Gut microbial dysbiosis is associated with allergen-specific IgE responses in young children with airway allergies. *World Allergy Organ J* (2019) 12(3):100021. doi: 10.1016/j.waojou.2019.100021
32. Gilbert JA, Quinn RA, Debelius J, Xu ZZ, Morton J, Garg N, et al. Microbiome-wide association studies link dynamic microbial consortia to disease. *Nature* (2016) 535(7610):94–103. doi: 10.1038/nature18850
33. O'Hara AM, Shanahan F. The gut flora as a forgotten organ. *EMBO Rep* (2006) 7(7):688–93. doi: 10.1038/sj.embor.7400731
34. Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Nageshwar Reddy D. Role of the normal gut microbiota. *World J Gastroenterol* (2015) 21(29):8787–803. doi: 10.3748/wjg.v21.i29.8787
35. Lau WF, Wong J, Lam KH, Ong GB. Oesophageal microbial flora in carcinoma of the oesophagus. *Aust New Z J Surg* (1981) 51(1):52–5. doi: 10.1111/j.1445-2197.1981.tb05905.x
36. Finlay IG, Wright PA, Menzies T, McArdle CS. Microbial flora in carcinoma of oesophagus. *Thorax* (1982) 37(3):181–4. doi: 10.1136/thx.37.3.181
37. Mannell A, Plant M, Frolich J. The microflora of the oesophagus. *Ann R Coll Surg Engl* (1983) 65(3):152–4.
38. Pei Z, Bini EJ, Yang L, Zhou M, Francois F, Blaser MJ. Bacterial biota in the human distal esophagus. *Proc Natl Acad Sci United States America* (2004) 101(12):4250–5. doi: 10.1073/pnas.0306398101
39. Kuczynski J, Lauber CL, Walters WA, Parfrey LW, Clemente JC, Gevers D, et al. Experimental and analytical tools for studying the human microbiome. *Nat Rev Genet* (2011) 13(1):47–58. doi: 10.1038/nrg3129

40. Morgan XC, Huttenhower C. Meta'omic analytic techniques for studying the intestinal microbiome. *Gastroenterology* (2014) 146(6):1437–48.e1. doi: 10.1053/j.gastro.2014.01.049
41. Walker AW, Duncan SH, Louis P, Flint HJ. Phylogeny, culturing, and metagenomics of the human gut microbiota. *Trends Microbiol* (2014) 22:267–74. doi: 10.1016/j.tim.2014.03.001
42. Baghdadi J, Chaudhary N, Pei Z, Yang L. Microbiome, innate immunity, and esophageal adenocarcinoma. *Clin Lab Med* (2014) 34:721–32. doi: 10.1016/j.cll.2014.08.001
43. May M, Abrams JA. Emerging Insights into the Esophageal Microbiome. *Curr Treat Options Gastroenterol* (2018) 16:72–85. doi: 10.1007/s11938-018-0171-5
44. Gagliardi D, Makihara S, Corsi PR, Viana Ade T, Wiczer MV, Nakakubo S, et al. Microbial flora of the normal esophagus. *Dis Esophagus* (1998) 11:248–50. doi: 10.1093/dote/11.4.248
45. Fillon SA, Harris JK, Wagner BD, et al. Novel device to sample the esophageal microbiome–the esophageal string test. *PloS One* (2012) 7:e42938. doi: 10.1371/journal.pone.0042938
46. Norder Grusell E, Dahlén G, Ruth M, Ny L, Quiding-Järbrink M, Bergquist H, et al. Bacterial flora of the human oral cavity, and the upper and lower esophagus. *Dis Esophagus* (2013) 26:84–90. doi: 10.1111/j.1442-2050.2012.01328.x
47. Pei Z, Yang L, Peek RM C.OMMAJ.R.X.X.X, Levine SM, Pride DT, Blaser MJ. Bacterial biota in reflux esophagitis and Barrett's esophagus. *World J Gastroenterol* (2005) 11:7277–83. doi: 10.3748/wjg.v11.i46.7277
48. Macfarlane S, Furrie E, Macfarlane GT, Dillon JF. Microbial colonization of the upper gastrointestinal tract in patients with Barrett's esophagus. *Clin Infect Dis* (2007) 45:29–38. doi: 10.1086/518578
49. Yang L, Lu X, Nossa CW, Francois F, Peek RM, Pei Z. Inflammation and intestinal metaplasia of the distal esophagus are associated with alterations in the microbiome. *Gastroenterology* (2009) 137:588–97. doi: 10.1053/j.gastro.2009.04.046
50. Liu N, Ando T, Ishiguro K, Maeda O, Watanabe O, Funasaka K, et al. Characterization of bacterial biota in the distal esophagus of Japanese patients with reflux esophagitis and Barrett's esophagus. *BMC Infect Dis* (2013) 13:130. doi: 10.1186/1471-2334-13-130
51. Blackett KL, Siddhi SS, Cleary S, Steed H, Miller MH, Macfarlane S, et al. Oesophageal bacterial biofilm changes in gastro-oesophageal reflux disease, Barrett's and oesophageal carcinoma: association or causality? *Aliment Pharmacol Ther* (2013) 37:1084–92. doi: 10.1111/apt.12317
52. Amir I, Konikoff FM, Oppenheim M, Gophna U, Half EE. Gastric microbiota is altered in oesophagitis and Barrett's oesophagus and further modified by proton pump inhibitors. *Environ Microbiol* (2014) 16:2905–14. doi: 10.1111/1462-2920.12285
53. Zaidi AH, Kelly LA, Kreft RE, Barlek M, Omstead AN, Matsui D, et al. Associations of microbiota and toll-like receptor signaling pathway in esophageal adenocarcinoma. *BMC Cancer* (2016) 16:52. doi: 10.1186/s12885-016-2093-8
54. Pajecki D, Zilberstein B, dos Santos MA, Ubriaco JA, Quintanilha AG, Cecconello I, et al. Megaesophagus microbiota: a qualitative and quantitative analysis. *J Gastrointest Surg* (2002) 6:723–9. doi: 10.1016/s1091-255x(02)00028-8
55. Benitez AJ, Hoffmann C, Muir AB, Dods KK, Spergel JM, Bushman FD, et al. Inflammation-associated microbiota in pediatric eosinophilic esophagitis. *Microbiome* (2015) 3:23. doi: 10.1186/s40168-015-0085-6
56. Harris JK, Fang R, Wagner BD, Choe HN, Kelly CJ, Schroeder S, et al. Esophageal microbiome in eosinophilic esophagitis. *PloS One* (2015) 10: e0128346. doi: 10.1371/journal.pone.0128346
57. Wade WG. The oral microbiome in health and disease. *Pharmacol Res* (2013) 69:137–43. doi: 10.1016/j.phrs.2012.11.006
58. Dewhirst FE, Chen T, Izard J, Paster BJ, Tanner AC, Yu WH, et al. The human oral microbiome. *J Bacteriol* (2010) 192:5002–17. doi: 10.1128/JB.00542-10
59. Nobel YR, Snider EJ, Compres G, Freedberg DE, Khiabanian H, Lightdale CJ, et al. Increasing Dietary Fiber Intake Is Associated with a Distinct Esophageal Microbiome. *Clin Transl Gastroenterol* (2018) 9:199. doi: 10.1038/s41424-018-0067-7
60. Mukhopadhya I, Hansen R, El-Omar EM, Hold GL. IBD-what role do Proteobacteria play? *Nat Rev Gastroenterol Hepatol* (2012) 9:219–30. doi: 10.1038/nrgastro.2012.14
61. Larsen JM, Musavian HS, Butt TM, Ingvorsen C, Thysen AH, Brix S. Chronic obstructive pulmonary disease and asthma-associated Proteobacteria, but not commensal Prevotella spp., promote Toll-like receptor 2-independent lung inflammation and pathology. *Immunology* (2015) 144:333–42. doi: 10.1111/imm.12376
62. Yang L, Chaudhary N, Baghdadi J, Pei Z. Microbiome in reflux disorders and esophageal adenocarcinoma. *Cancer J* (2014) 20:207–10. doi: 10.1097/PPO.0000000000000044
63. Lehrer RI, Szklarek D, Barton A, Ganz T, Hamann KJ, Gleich GJ. Antibacterial properties of eosinophil major basic protein and eosinophil cationic protein. *J Immunol* (1989) 142:4428–34.
64. Driss V, Legrand F, Hermann E, Loiseau S, Guerardel Y, Kremer L, et al. TLR2-dependent eosinophil interactions with mycobacteria: role of alpha-defensins. *Blood* (2009) 113:3235–44. doi: 10.1182/blood-2008-07-166595
65. Yousefi S, Gold JA, Andina N, Lee JJ, Kelly AM, Kozlowski E, et al. Catapult-like release of mitochondrial DNA by eosinophils contributes to antibacterial defense. *Nat Med* (2008) 14:949–53. doi: 10.1038/nm.1855
66. Yang L, Francois F, Pei Z. Molecular pathways: pathogenesis and clinical implications of microbiome alteration in esophagitis and Barrett esophagus. *Clin Cancer Res* (2012) 18:2138–44. doi: 10.1158/1078-0432.CCR-11-0934
67. Norder Grusell E, Dahlén G, Ruth M, Bergquist H, Bove M. The cultivable bacterial flora of the esophagus in subjects with esophagitis. *Scand J Gastroenterol* (2018) 53:650–6. doi: 10.1080/00365521.2018.1457712
68. Hiremath G, Shilts MH, Boone HH, Correa H, Acra S, Tovchigrechko A, et al. The Salivary Microbiome Is Altered in Children With Eosinophilic Esophagitis and Correlates With Disease Activity. *Clin Transl Gastroenterol* (2019) 10:e00039. doi: 10.14309/ctg.0000000000000039
69. Arnold IC, Hitzler I, Müller A. The immunomodulatory properties of Helicobacter pylori confer protection against allergic and chronic inflammatory disorders. *Front Cell Infect Microbiol* (2012) 2:10. doi: 10.3389/fcimb.2012.00010
70. Kyburz A, Urban S, Altobelli A, Floess S, Huehn J, Cover TL, et al. Helicobacter pylori and its secreted immunomodulator VacA protect against anaphylaxis in experimental models of food allergy. *Clin Exp Allergy J Br Soc Allergy Clin Immunol* (2017) 47(10):1331–41. doi: 10.1111/cea.12996
71. Wu YZ, Tan G, Wu F, Zhi FC. H. pylori attenuates TNBS-induced colitis via increasing mucosal Th2 cells in mice. *Oncotarget* (2017) 8(43):73810–6. doi: 10.18632/oncotarget.17962
72. Shah SC, Tepler A, Peek R. M. C.OMMAJ.R.X.X.X, Colombel JF, Hirano I, Narula N. Association Between Helicobacter pylori Exposure and Decreased Odds of Eosinophilic Esophagitis-A Systematic Review and Meta-analysis. *Clin Gastroenterol Hepatol Off Clin Pract J Am Gastroenterol Assoc* (2019) 17 (11):2185–98.e3. doi: 10.1016/j.cgh.2019.01.013
73. Kashyap PC, Johnson S, Geno DM, Lekatz HR, Lavey C, Alexander JA, et al. A decreased abundance of clostridia characterizes the gut microbiota in eosinophilic esophagitis. *Physiol Rep* (2019) 7:e14261. doi: 10.14814/phy2.14261
74. Zuercher AW, Weiss M, Holvoet S, Moser M, Moussu H, van Overtvelt L, et al. Lactococcus lactis NCC 2287 alleviates food allergic manifestations in sensitized mice by reducing IL-13 expression specifically in the ileum. *Clin Dev Immunol* (2012) 2012:485750. doi: 10.1155/2012/485750
75. Holvoet S, Zuercher AW, Julien-Javaux F, Perrot M, Mercenier A. Characterization of candidate anti-allergic probiotic strains in a model of th2-skewed human peripheral blood mononuclear cells. *Int Arch Allergy Immunol* (2013) 161:142–54. doi: 10.1159/000343703
76. Singh A, Hacini-Rachinel F, Gosoniu ML, Bourdeau T, Holvoet S, Doucet-Ladeveze R, et al. Immune-modulatory effect of probiotic Bifidobacterium lactis NCC2818 in individuals suffering from seasonal allergic rhinitis to grass pollen: an exploratory, randomized, placebo-controlled clinical trial. *Eur J Clin Nutr* (2013) 67:161–7. doi: 10.1038/ejcn.2012.197
77. Holvoet S, Doucet-Ladevèze R, Perrot M, Barretto C, Nutten S, Blanchard C. Beneficial effect of Lactococcus lactis NCC 2287 in a murine model of eosinophilic esophagitis. *Allergy* (2016) 71(12):1753–61. doi: 10.1111/all.12951
78. Demont A, Hacini-Rachinel F, Doucet-Ladevèze R, Ngom-Bru C, Mercenier A, Prioult G, et al. Live and heat-treated probiotics differently modulate IL10 mRNA stabilization and microRNA expression. *J Allergy Clin Immunol* (2016) 137:1264–1267.e10. doi: 10.1016/j.jaci.2015.08.033

79. Akei HS, Mishra A, Blanchard C, Rothenberg ME. Epicutaneous antigen exposure primes for experimental eosinophilic esophagitis in mice. *Gastroenterology* (2005) 129:985–94. doi: 10.1053/j.gastro.2005.06.027
80. Pellaton C, Nutten S, Thierry AC, Boudousquié C, Barbier N, Blanchard C, et al. Intragastric and Intranasal Administration of Lactobacillus paracasei NCC2461 Modulates Allergic Airway Inflammation in Mice. *Int J Inflam* (2012) 2012:686739. doi: 10.1155/2012/686739
81. Feleszko W, Jaworska J, Rha RD, Steinhausen S, Avagyan A, Jaudszus A, et al. Probiotic-induced suppression of allergic sensitization and airway inflammation is associated with an increase of T regulatory-dependent mechanisms in a murine model of asthma. *Clin Exp Allergy* (2007) 37:498–505. doi: 10.1111/j.1365-2222.2006.02629.x
82. Trompette A, Gollwitzer ES, Yadava K, Sichelstiel AK, Sprenger N, Ngom-Bru C, et al. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat Med* (2014) 20:159–66. doi: 10.1038/nm.3444
83. Hogenkamp A, Thijssen S, van Vlies N, Garssen J. Supplementing pregnant mice with a specific mixture of nondigestible oligosaccharides reduces symptoms of allergic asthma in male offspring. *J Nutr* (2015) 145:640–6. doi: 10.3945/jn.114.197707

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

ORIGINAL RESEARCH
published: 25 February 2021
doi: 10.3389/fimmu.2021.599296

Mucosal Nanoemulsion Allergy Vaccine Suppresses Alarmin Expression and Induces Bystander Suppression of Reactivity to Multiple Food Allergens

*Mohammad Farazuddin[1], Jeffrey J. Landers[1], Katarzyna W. Janczak[1], Hayley K. Lindsey[1], Fred D. Finkelman[2], James R. Baker Jr[1] and Jessica J. O'Konek[1]**

[1] Mary H. Weiser Food Allergy Center, University of Michigan, Ann Arbor, MI, United States, [2] Division of Allergy, Immunology and Rheumatology, University of Cincinnati College of Medicine, Cincinnati, OH, United States

OPEN ACCESS

Edited by:
Karen Laky,
National Institutes of Health (NIH), United States

Reviewed by:
Ya-Jen Chang,
Academia Sinica, Taiwan
Giorgio Walter Canonica,
University of Genoa, Italy

***Correspondence:**
Jessica J. O'Konek
jjoz@umich.edu

Specialty section:
This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology

Received: 26 August 2020
Accepted: 06 January 2021
Published: 25 February 2021

Citation:
Farazuddin M, Landers JJ, Janczak KW, Lindsey HK, Finkelman FD, Baker JR and O'Konek JJ (2021) Mucosal Nanoemulsion Allergy Vaccine Suppresses Alarmin Expression and Induces Bystander Suppression of Reactivity to Multiple Food Allergens. Front. Immunol. 12:599296. doi: 10.3389/fimmu.2021.599296

We have demonstrated that intranasal immunotherapy with allergens formulated in a nanoemulsion (NE) mucosal adjuvant suppresses Th2/IgE-mediated allergic responses and protects from allergen challenge in murine food allergy models. Protection conferred by this therapy is associated with strong suppression of allergen specific Th2 cellular immunity and increased Th1 cytokines. Here we extend these studies to examine the effect of NE-allergen immunization in mice sensitized to multiple foods. Mice were sensitized to both egg and peanut and then received NE vaccine formulated with either one or both of these allergens. The animals were then subjected to oral challenges with either egg or peanut to assess reactivity. Immunization with NE formulations containing both egg and peanut markedly reduced reactivity after oral allergen challenge with either allergen. Interestingly, mice that received the vaccine containing only peanut also had reduced reactivity to challenge with egg. Protection from oral allergen challenge was achieved despite the persistence of allergen-specific IgE and was associated with strong suppression of both Th2-polarized immune responses, alarmins and type 2 innate lymphoid cells (ILC2). NE-induced bystander suppression of reactivity required IFN-γ and the presence of an allergen in the NE vaccine. These results demonstrate that anaphylactic reactions to food allergens can be suppressed using allergen-specific immunotherapy without having to eliminate allergen-specific IgE and suggests that modulation of Th2 immunity towards one allergen may induce bystander effects that suppress reactivity to other allergens through the induction of IFN-γ and suppression of alarmins in the intestine. In addition, these data suggest that a NE vaccine for a single food allergen may lead to a global suppression of allergic responses to multiple foods.

Keywords: adjuvant, alarmins, allergy treatment, food allergy, immunotherapy vaccines and mechanisms, vaccine

INTRODUCTION

Food allergy is an emerging epidemic that now affects up to 15 million people in the US, including 8% of children. The economic burden of food allergy in the US alone exceeds $24.8 billion (1). Allergen-specific immunotherapy for food allergy involves the progressive administration of increasing amounts of a specific allergen by one of several routes and has been the primary approach to suppress allergic reactivity. This approach, however, does not provide long-term protection following cessation of therapy and requires prolonged treatment protocols burdening to patients and their families. Specifically, subcutaneous immunotherapy to food allergens showed promise for protection against IgE-mediated food allergies, however significant adverse reactions limited successful implementation (2–4). Sublingual, oral (OIT) and epicutaneous immunotherapy have demonstrated efficacy in animal models and human trials, however these approaches desensitize only a portion of patients and the protection achieved is rapidly lost after cessation of the therapy (5–11). Thus, there is a need to understand immune mechanisms that modulate Th2-biased immune responses to food and could lead to long-lasting protection from allergic reactions.

The primary immunologic mechanism of allergic hypersensitivity is the induction of Th2-polarized cellular immune responses leading to the production of allergen-specific IgE antibodies critical for mast cell activation. Th2 cytokines also are critical mediators of local allergic inflammation, including IL-4– and IL-13–dependent mucus production and IL-5–mediated eosinophil recruitment (12). Oral or subcutaneous allergen immunotherapy (AIT) appears to achieve desensitization to the allergen by temporarily reducing Th2-biased immunity and allergen-specific IgE. While OIT has been proven clinically useful for treating food allergy, it has not induced a long term redirection of allergen-specific immunity away from a Th2 phenotype (13). Thus, interest has been directed toward new strategies that are able to permanently suppress Th2 cellular immune responses or redirect these cellular Th2 responses towards a Th1 phenotype (14, 15).

We have developed a novel nasal vaccine-based immunotherapy system employing a nanoscale oil-in-water emulsion (nanoemulsion, NE) adjuvant. When administered intranasally (i.n.) with viral and bacterial antigens, this formulation induces robust systemic and mucosal immunity, and cell-mediated immune responses are polarized towards Th1 and Th17 (16–24). We have previously reported that therapeutic immunization with NE and antigen/allergen can suppress established Th2-polarized immunity and protect from allergen challenge in murine models of food allergy (25–28). NE-based allergy vaccines induced sustained unresponsiveness lasting at least 16 weeks, and protection was associated with increased IL-10 and regulatory T cells. Here, we extended our previous work to determine the ability of NE-based allergy vaccines to broadly suppress allergic reactions in mice sensitized to more than one food.

MATERIALS AND METHODS

Antigen and Adjuvants

Nanoemulsion adjuvant (NE) was produced by a high speed emulsification of ultra-pure soybean oil with cetyl pyridinium chloride, Tween 80 and ethanol in water, with resultant NE droplets with average 350–400 nm diameter (17, 29). Aluminum hydroxide (alum, alhydrogel) was purchased from InvivoGen. Peanut extract (Greer) was used for all intraperitoneal (i.p.) and intranasal (i.n.) immunizations. For oral/intragastric (i.g.) challenges, peanut flour (12% fat, light roast, Byrd Mill) was solubilized in PBS. Endotoxin-free ovalbumin (OVA) was purchased from Hyglos. Endotoxin content of all vaccine components was determined by a limulus amebocyte lysate (LAL) assay (Pierce).

Mice and Immunizations

Specific pathogen-free BALB/c mice (females 3 weeks old) were purchased from Jackson Laboratory. Mice were 4 weeks of age at the onset of the experiment. The experimental design is shown in **Figure 1**. In all experiments, allergic sensitization was induced with i.p. immunizations of 20 μg OVA and 20 μg peanut extract

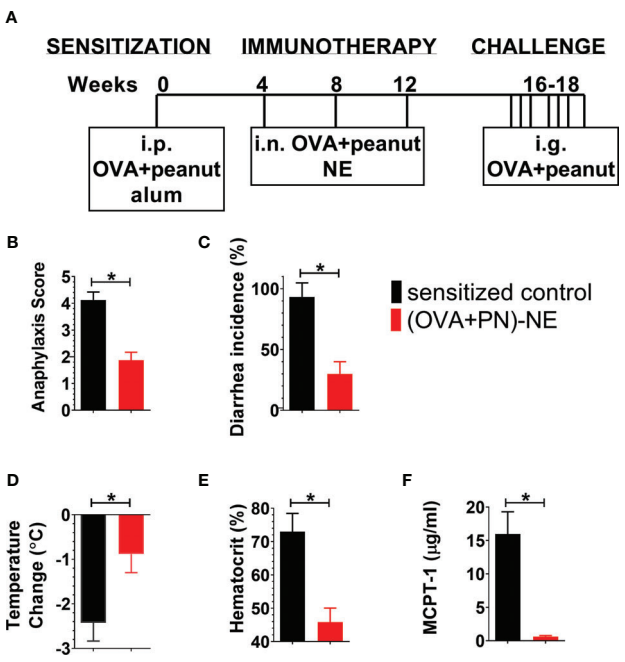


FIGURE 1 | NE immunotherapy protects against allergic reactivity in polysensitized mice. **(A)** Mice were sensitized with OVA and peanut-alum and treated i.n. with 3 administrations of PBS (sensitized control) or OVA and peanut-NE (OVA+PN-NE) Mice were challenged orally with OVA and peanut and **(B)** symptoms of anaphylaxis, **(C)** diarrhea and **(D)** temperature change were monitored. **(E)** Hemoconcentration was determined by hematocrit. **(F)** Levels of MCPT-1 in the serum 60 min after challenge were determined by ELISA. Statistically significant differences ($p < 0.05$) are indicated by *.

(PN) adsorbed on 1 mg alum at week 0. The experimental design for each experiment is as follows.

Assessment of Combination Vaccine Containing OVA and Peanut

Mice received i.n. immunizations of 12 µl (6 µl/nare) of a formulation containing 20 µg of OVA and 20 µg PN at weeks 4, 8 and 12. Sensitized control mice received i.n. PBS. Beginning 4 weeks after the final i.n. vaccine dose, anaphylaxis was induced by repeated oral challenge with allergen. Mice were challenged orally every other day for a total of 7 gavages (30). For each challenge, mice were fasted for 5–6 h to ensure gastric emptying and then were challenged by oral gavage (i.g.) with 0.2 ml containing of 10 mg OVA and 10 mg peanut. Reactivity was measured as described below.

Assessment of Bystander Protection

Mice received i.n. immunizations of a formulation containing either 20 µg of OVA or 20 µg PN mixed with 20% NE at weeks 4, 8 and 12. Mice were orally challenged with either 10 mg OVA or 10 mg peanut protein and reactivity was determined as described below.

Assessment of Requirement of Specific Antigen for Protection

Following the same schedule as described above, sensitized mice received i.n. immunizations of 20% NE alone (no antigen) or 20 µg of OVA in 20% NE. Sensitized control mice received i.n. PBS. Mice were challenged orally with OVA and reactivity was determined as described above. In a separate experiment following the same schedule, mice were immunized with 20 µg hepatitis B surface antigen (HBsAg) mixed with 20% NE.

IFN-γ Depletion Experiments

Mice were sensitized to OVA and peanut at week 0 and received i.n. PN-NE vaccine at weeks 4, 8 and 12. Mice were subjected to our repeated OVA challenge protocol beginning at week 16. Mice were injected i.p. with 0.5 mg anti-IFN-γ (XMG-6) or isotype control rat IgG1 (GL113) [both produced in house (31)] the day before starting oral challenges and every 4 days until the final challenge. All animal procedures were performed according to the National Institutes of Health guide for the care and use of laboratory animals and approved by the University of Michigan Institutional Animal Care and Use Committee (IACUC).

Assessment of Hypersensitivity Reactions

Anaphylactic symptoms were evaluated for one hour following the final (7^{th}) challenge with OVA using the following scoring system [modified from (32, 33)]: 0, no symptoms; 0.5, transient rubbing and scratching; 1, prolonged rubbing and scratching around the nose, eyes or head; 2, puffiness around the eyes or mouth, diarrhea, piloerection, and/or decreased activity with increased respiratory rate; 3, labored respiration, wheezing, stridor, and/or cyanosis around the mouth and tail; 4, tremor, convulsion, no activity after prodding and/or moribund; 5, death. Rectal temperature was monitored prior to and every 15 min for 60 min following challenge, and the maximum temperature change from baseline was reported. Mice were bled 60 min following challenge, and serum mouse mast cell protease-1 (MCPT-1) was determined by ELISA (eBioscience). To determine hemoconcentration, blood was drawn 60 min following challenge into heparinized capillary tubes and centrifuged for 5 min at 10,000 rpm. Hematocrit values were calculated as the length of packed RBCs as a percentage of the total length of serum and red cells in the capillary tube.

Measurement of Serum IgE

Sera were obtained by cardiac puncture post-euthanasia one day after the final (7^{th}) allergen challenge. Serum was separated from whole blood by centrifugation at 1,500×g for 5 min after allowing coagulation for 30–60 min at room temperature. Serum samples were stored at −20°C until analyzed. OVA-specific IgE antibody levels were determined by ELISA. Serially diluted serum samples were incubated on microtiter plates coated with 20 µg/ml OVA. IgE antibodies were detected with alkaline phosphatase conjugated anti-mouse IgE (Rockland) and Sigma Fast™ p-nitrophenyl phosphate substrate and quantified by measuring the optical density (OD) at 405 nm. The antibody concentrations are presented as endpoint titers defined as the reciprocal of the highest serum dilution producing an OD above background of naïve sera. The cutoff value is determined as the OD (mean+2 standard deviations) of the corresponding dilution of naive sera (34, 35).

Analysis of Cytokine Production

Mice were sacrificed one day after the final (7^{th}) oral challenge, and mesenteric lymph nodes were harvested. The cellular recall response was evaluated in lymphocytes isolated from mesenteric lymph nodes. Single cell lymphocyte suspensions were cultured *ex vivo* ± OVA (20 µg/ml) at 37°C. After 72 h, cytokine secretion was measured in cell culture supernatants using Luminex Multiplex detection system (Millipore). For real-time PCR analysis, RNA was isolated from duodenum homogenates with an RNeasy mini kit (Qiagen), and cDNA was generated with a Superscript II reverse transcription kit (Invitrogen). qPCR was performed with SYBR green master mix and commercially available primer sets (Bio-Rad). Values were normalized to GAPDH and displayed as fold induction over control samples.

Lamina Propria Mononuclear Cells Isolation

Mice were sacrificed one day after the final (7^{th}) oral challenge. Small intestine (SI, 15 cm) was dissected from the mouse and Peyer's patches were trimmed off. SI was cut longitudinally and washed with PBS thoroughly to remove ingested food. SI was incubated in a petri dish with 10 ml PBS with EDTA (5 mM) for 10 min on ice. Intestines were washed with PBS (no EDTA) by vortexing vigorously to remove the epithelial cells. These two steps were repeated 3–4 times until the tissue became clear. Tissue was minced finely and transferred to 8 ml digestion buffer (16 mg collagenase A (Roche) and 1.6mg DNase I (Roche) in RPMI (10% FBS) and incubated at 37°C for 30 min. After incubation, digested tissue was passed through a 10 ml syringe with 18G needle a few times. Liberated cells were filtered through 70 µm filter. The cell suspension was washed by adding 20 ml of RPMI with 10% FBS. The cell pellet was suspended in 44% Percoll (4 ml) and loaded on 67% Percoll (3 ml) for

centrifugation. A mononuclear cell gradient was created by spinning the cells down at 1,800 rpm for 20 min at room temperature with centrifuge acceleration set at 5 and deceleration set to 0. The middle interphase of mononuclear cells was collected from the interface and washed again with RPMI (10% FBS). The obtained cells were counted and used for subsequent analysis.

Antibodies

All the antibodies used for flow cytometry were purchased from eBioscience, Biolegend and BD biosciences. For cell surface staining, a lineage cocktail consisting anti-mouse CD3 (clone 145-2C11), anti-mouse Ly-6G/Ly-6C (clone RB6-8C5), anti-mouse CD11b (clone M1/70), anti-mouse CD45R/B220 (clone RA3-6B2), and anti-mouse TER-119/Erythroid cells (clone Ter-119) was used. FITC-streptavidin was used to stain biotin labelled primary antibody cocktail. Other antibodies used were rat anti-mouse CD45, anti-mouse CD127 (clone A7R34), anti-mouse CD90.2 (clone 53-2.1), anti-mouse KLRG1 (clone 2F1), and anti-mouse GATA3 (clone TWAJ). Foxp3 fixation and permeabilization kit (eBioscience) was used for intracellular staining.

Flow Cytometry

Cells were stained with lineage antibody cocktail on ice for 20 min followed by washing with flow staining buffer (PBS with 0.1% BSA) two times. Cells were then incubated with FITC-streptavidin antibody on ice for another 20 min. Cells were washed two times with flow staining buffer. Cells were then stained with live-dead ef450, CD45, CD127, CD90.2, and KLRG1 on ice for 20 min followed by washing two times with flow staining buffer. Intracellular staining of GATA3 was done using Foxp3 fixation and permeabilization kit as per the manufacturer's protocol (eBiosciences). Samples were acquired on Novocyte 3000 (Acea biosciences) and data were analyzed using FlowJo v10.1. The gating strategy for identifying ILC2s is shown in **Supplementary Figure 1**.

Statistics

Results presented here are the representatives of at least two independent experiments. Each experiment contained 8–10 mice per group. Statistical comparisons were assessed by the Mann-Whitney test using GraphPad Prism version 8 (GraphPad Software). The p value < 0.05 was considered as significant.

RESULTS

Intranasal Immunization With Allergens in NE Adjuvant Suppresses Allergic Reactions in Polysensitized Mice

BALB/c mice were sensitized to egg and peanut and immunized with OVA and peanut formulated in NE to determine if suppression of reactivity to 2 allergens could be achieved simultaneously. Sensitized control mice had profound physiological reactions to challenge as indicated by severe symptoms of anaphylactic shock, including diarrhea, labored respiration, wheezing, lack of activity when prodded, core body temperature loss of greater than 2°C, hemoconcentration and increased mast cell degranulation (MCPT-1) (**Figure 1**). The NE vaccine markedly suppressed these responses to allergen challenge. Anaphylaxis symptoms were markedly reduced to mild symptoms such as pruritus or reduced activity (**Figure 1B**), and the incidence of diarrhea was reduced from 100–40% (**Figure 1C**). Mice treated with the NE vaccine also were protected from hypovolemic shock and experienced minimal body temperature loss while hemoconcentration also was prevented (**Figures 1D, E**). MCPT-1 measured in serum following challenge was used to assess mast cell degranulation. Consistent with the clinical symptoms of allergic reaction, immunized mice had significant reductions in MCPT-1, with average levels of 0.6 μg/ml compared with 16 μg/ml in sensitized control mice (**Figure 1F**; p=0.0079).

Immunization of Polysensitized Mice With NE Adjuvant Induces Bystander Suppression of Allergic Reactivity

Next, we wanted to assess the effects of immunotherapy with NE and only one allergen on protection in polysensitized animals. Mice were again sensitized to both egg and peanut, and then were nasally treated with either OVA or peanut or both allergens formulated in NE. In general, mice were protected from allergic reaction to whatever allergen was contained in the vaccines, and protection for each allergen was similar if the mice were treated with the vaccine containing either a single allergen or both allergens (**Figure 2**). Surprisingly, mice that were treated with the OVA-NE vaccine were also protected from reactivity to challenge with peanut and mice treated with the PN-NE vaccine were protected from challenge with OVA. While there was a trend that this "bystander protection" was not as complete as protection induced by immunotherapy with NE and both allergens, these differences were not significant, and mice immunized with only one allergen in NE had significantly less severe allergic reactions compared with sensitized control mice that did not receive the i.n. vaccine. Bystander suppression of reactivity persisted for at least 8 weeks after the final vaccine dose (**Supplementary Figure 2**).

We next aimed to determine if the observed bystander suppression of allergic reactivity required immunotherapy with NE and at least one allergen to which the mice were sensitized. As shown in **Figures 3A–C**, in mice that were sensitized to OVA and peanut, i.n. instillation of NE alone (no allergen) did not induce any suppression of allergic reactivity to oral OVA challenge, as reactivity in these mice was equivalent to sensitized control mice and significantly more severe than mice that received the OVA-NE vaccines. Similarly, NE formulated with an unrelated antigen [hepatitis B surface antigen (HBsAg)] did not confer protection from challenge with OVA (**Figures 3D–F**).

Intranasal Immunization With NE Adjuvant Suppresses Allergy Associated Th2 Responses and Alarmin Expression

While food allergic reactions are dependent upon the presence of allergen-specific IgE, many patients with allergen-specific IgE to

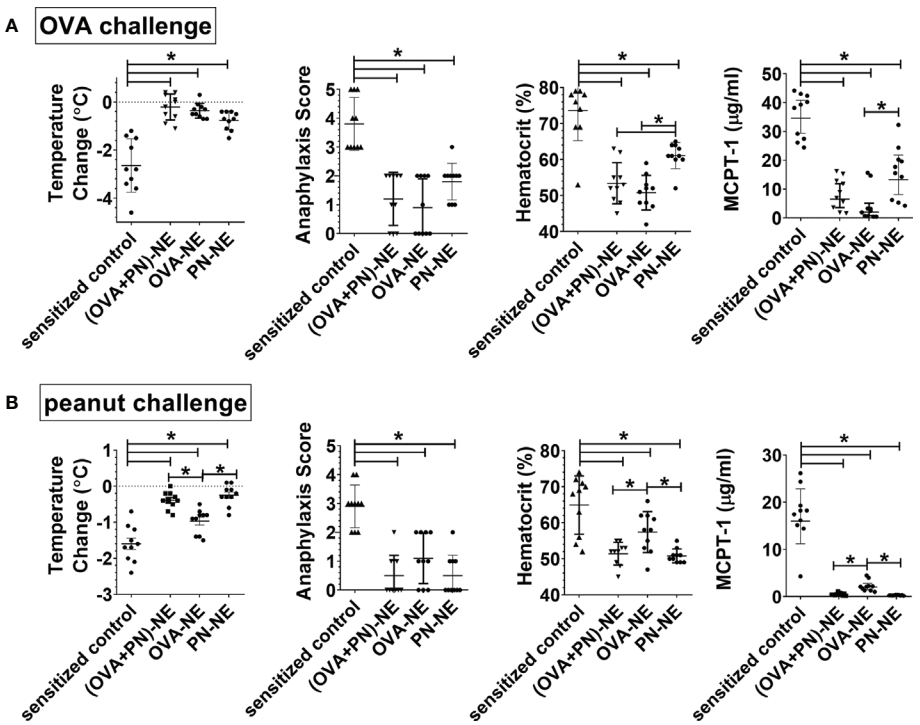


FIGURE 2 | Immunization of polysensitized mice with NE and one allergen provides protection against reactivity to another allergen. As described in Figure 1A, mice were sensitized with OVA and peanut-alum and treated i.n. with 3 administrations of PBS (sensitized control), ova and peanut-NE (OVA+PN-NE), OVA-NE or peanut-NE (PN-NE). Mice were challenged orally with **(A)** OVA or **(B)** peanut and temperature change and symptoms of anaphylaxis were monitored. Hemoconcentration was determined by hematocrit. Levels of MCPT-1 in the serum 60 min after challenge were determined by ELISA. Statistically significant differences ($p < 0.05$) are indicated by *.

foods do not clinically react to those foods. In the present study, immunization with PN-NE suppressed allergic reactivity to both peanut and OVA without significantly reducing OVA-specific IgE (**Figure 4A**). This apparent disconnect between the presence of allergen-specific IgE and reactivity to an allergen suggests that other immune changes are behind the suppression of allergic reactions observed here.

Allergen-specific cytokine secretion was measured to characterize changes to the Th2-biased cellular immune responses associated with allergic disease. Upon stimulation with OVA, cells from (OVA and peanut)-alum sensitized mice produced significant levels of Th2-type cytokines IL-4 and IL-13 but not IFN-γ (**Figure 4B**). Immunotherapy with either OVA or peanut in NE reduced OVA-specific IL-4 and IL-13 and increased IFN-γ. While OVA-specific IL-17 and IL-10 were increased in mice that received the OVA-NE vaccine, the PN-NE vaccine did not affect OVA-specific production of these cytokines. Because of the interplay between Th2 immunity and expression of alarmins, expression of the genes for the alarmins IL-25, IL-33, and TSLP was also evaluated in the small intestine (36–40). Immunization with the NE vaccines significantly reduced the expression of *Il25*, *Il33* and *Tslp* such that the fold change over expression in naïve mice was approximately 1 (**Figure 4C**). These data indicate that immunization with NE prevents increased alarmin expression normally observed in allergen sensitized mice.

Intranasal Immunization With NE Adjuvant Suppresses ILC2 Populations

We observed a significant decrease in alarmin expression, in animals that received the NE-allergen vaccines compared to sensitized control animals. It has been previously established that IL-25 and IL-33 acts as activation cytokines and regulate ILC2 populations in allergic inflammation (41–43). ILC2s were quantified in the small intestine lamina propria to determine if reduction in reactivity was associated with reduce ILC2 accumulation in the tissue. ILC2s were increased in the intestine of sensitized mice compared to naïve. Conversely, ILC2s were significantly reduced in OVA-NE-and PN-NE immunized animals compared to sensitized controls (**Figure 4D**). These data suggest that immunization with either OVA-NE-or PN-NE modulates epithelial alarmin production, which in turn prevents the accumulation of ILC2s in the tissues.

Bystander Protection Induced by NE Allergy Vaccines Requires IFN-γ

Because NE immunization increased IFN-γ, which has been shown to suppress Th2 immunity and alarmins, we hypothesized that bystander protection associated with NE was IFN-γ dependent. Mice were sensitized to OVA and peanut and then treated with PN-NE. During the allergen challenge phase in which mice were treated with OVA, IFN-γ was depleted.

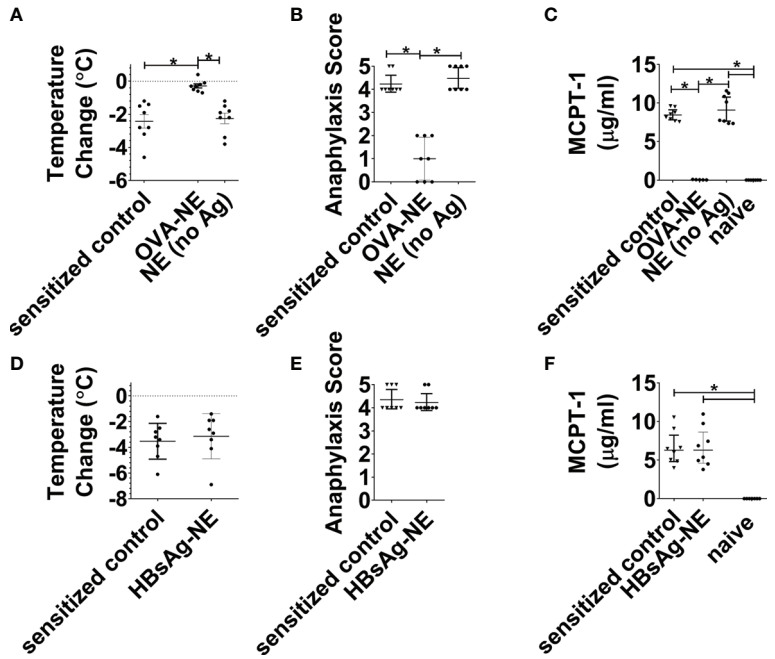


FIGURE 3 | Intranasal administration of NE without allergen does not suppress the allergic response. **(A–C)** Mice were sensitized with OVA and peanut-alum and treated i.n. with 3 administrations of PBS (sensitized control), OVA-NE (OVA-NE) or NE only (no antigen) or Mice were challenged orally with OVA and temperature change and symptoms of anaphylaxis were monitored. Serum MCPT-1 levels were determined by ELISA. **(D–F)** In a separate experiment, mice were similarly sensitized with OVA and peanut with alum and treated i.n. with 3 administrations of PBS (sensitized control) or hepatitis B surface antigen-NE (HBsAg-NE). Statistically significant differences ($p < 0.05$) are indicated by *.

Depletion of IFN-γ during the challenge phase completely abrogated the protection induced by the PN-NE vaccine, as mice treated with anti-IFN-γ antibody had severe reactions to challenge, including decreases in core body temperature and increased clinical symptoms, diarrhea, hematocrit and MCPT-1 similar to animals not treated with NE (**Figure 5**). Suppression of alarmin expression by the PN-NE vaccine was also reversed following IFN-γ depletion, as mice that were depleted of IFN-γ had similar expression of alarmins in the small intestine as sensitize mice that did not receive the vaccine (**Figure 6**).

DISCUSSION

Thirty to forty percent of patients with food allergies are sensitized to multiple foods (44, 45). While allergen-specific immunotherapy has the potential to relieve the burden of fear of reactivity to specific foods, this approach is more difficult for polysensitized individuals. Approved, allergen-specific immunotherapy for food allergy involves a single food, and regulatory issues may preclude the development of therapies containing multiple foods. While some studies have demonstrated the ability to desensitize patients with OIT for up to 5 foods simultaneously (multi-OIT) (46, 47), the amount of each food required to be consumed daily is a burden for some children as the food required for multi-OIT can be a significant proportion of the daily caloric intake for a child. Given this, there is interest in the development of therapies for food allergy that work more broadly and are not specific to one allergen.

The present studies demonstrate that NE can be formulated with multiple allergens and lead to reduction of allergic reactivity to all allergens included in the vaccine. NE was formulated with two allergens and maintained therapeutic efficacy for both foods. Based on the protein loading capacity of NE, formulation with more than 2 allergens would also be possible and the therapy only has to be administered three to four times to achieve sustained unresponsiveness of at least 4 months (25–27). Given that long-term protection can be achieved with only a few doses administered at monthly intervals, this approach would significantly reduce the burden on patients with multiple food allergies over daily, allergen-specific immunotherapies.

Importantly, these studies also demonstrate a "bystander" effect in that NE-induced reduction of allergic reactivity with one allergen also reducing reactions to unrelated allergens not included in the vaccine. The bystander protection did require immunization with an allergen to which the animal was previously sensitized, suggesting the non-specific reduction of food allergic reactions required prior sensitization and immune recognition of the vaccine. This bystander suppression of reactivity was maintained for at least 8 weeks, supporting our previous work that demonstrated the long-term sustained unresponsiveness induced by this approach (27). The mechanism of this effect appeared to be redirection of the underlying immune polarization from a Th2 to a Th1

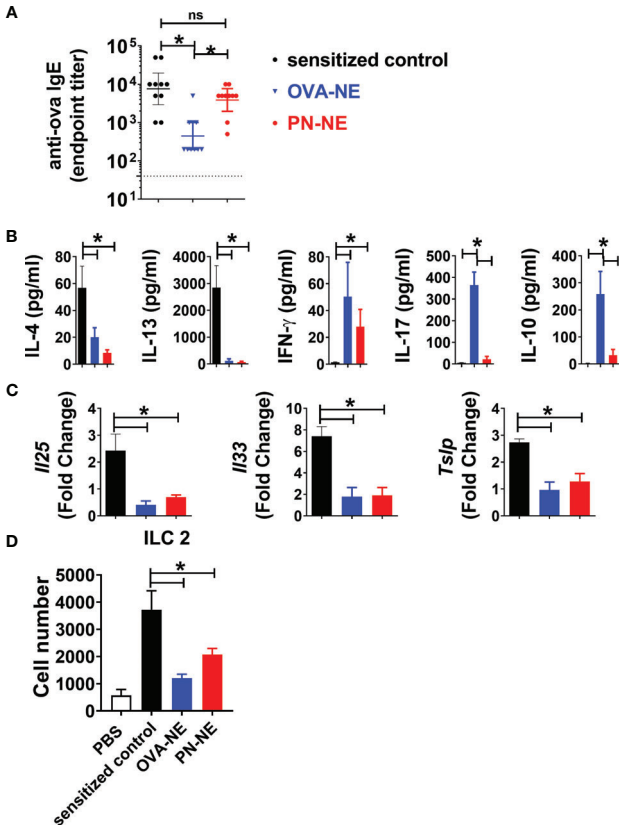


FIGURE 4 | NE immunotherapy induces antigen-specific and bystander suppression of Th2 cytokines and alarmins. As described in **Figure 1A**, mice were sensitized with OVA and peanut-alum and treated i.n. with 3 administrations of PBS (sensitized control), OVA-NE or peanut-NE (PN-NE). Mice were challenged orally with OVA, and **(A)** OVA-specific IgE was measured in serum obtained after the final challenge and **(B)** OVA-specific cytokine secretion was determined in cultures of mLN lymphocytes. **(C)** Duodenum samples were homogenized and mRNA extracted to determine relative gene expression compared to GAPDH. **(D)** Total number of ILC2 (Lin- CD45+ CD127+ CD90.2+ KLRG1+ GATA3+) from SI. Statistically significant differences ($p < 0.05$) are indicated by *.

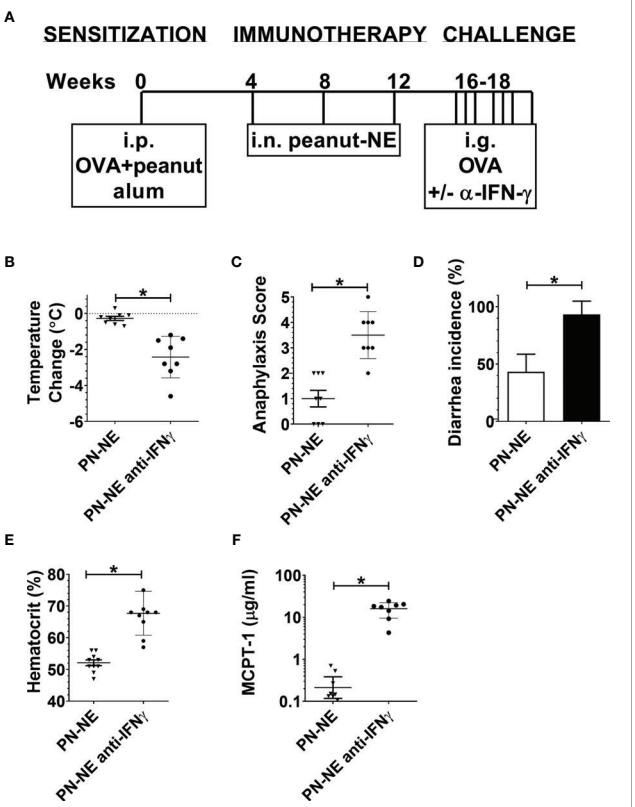


FIGURE 5 | Bystander protection induced by NE allergy vaccines requires IFN-γ **(A)** Mice were sensitized with OVA and peanut-alum and treated i.n. with 3 administrations of PBS (sensitized control) or peanut-NE (PN-NE). Mice were challenged orally with OVA and IFN- γ was depleted during the challenge phase. **(B)** Temperature change, **(C)** symptoms of anaphylaxis and **(D)** diarrhea were monitored. **(E)** Hemoconcentration was determined by hematocrit. **(F)** Levels of MCPT-1 in the serum 60 min after challenge were determined by ELISA. Statistically significant differences ($p < 0.05$) are indicated by *.

phenotype, especially since no suppressive activity was demonstrated with treatment using either the adjuvant alone or with an immunogen for which there was no pre-existing allergy (HBsAg). This demonstrates that while NE allergy vaccines provide bystander protection, it requires the recognition and induction of an antigen-specific immune response.

There are very few reports of bystander activation of antigen non-specific immune responses, but there is some evidence these do exist. Immunization with aluminum adjuvants (alum) has been shown to induce bystander polarization of Th2 immune responses to unrelated antigens. Specifically, if mice were immunized with alum and "antigen 1", and then later exposed to antigens 1 and 2 together in the absence of alum, mice developed Th2-polarized immune responses to antigen 2 (48, 49). These studies demonstrated the need for co-administration of antigens to drive the bystander effects. Eisenbarth et al. described this phenomenon as "collateral priming" and demonstrated that it was based on an adaptive T cell-derived, cytokine dependent mechanism that did not require innate toll-like receptor 4 signaling (49).

Other studies also support the concept of protection to proteins not included in the immunotherapy. For example, after sensitization with whole peanut extract, immunotherapy with individual peanut components such as Ara h 1 or Ara h 2 conferred protection against challenge with the whole allergen, despite the fact that mice were sensitized to multiple protein components of the allergen that the immunotherapy did not target (50–52). It also has been reported that epicutaneous immunotherapy with peanut in mice generates a Treg population that can prevent the subsequent sensitization to either peanut or house dust mite (53, 54). These Tregs were broadly effective at suppressing inflammation, including T cell-mediated intestinal inflammation in a mouse model of colitis (55). Along with our data, these reports suggest that antigen-specific T cell responses to newly introduced immunogens can be

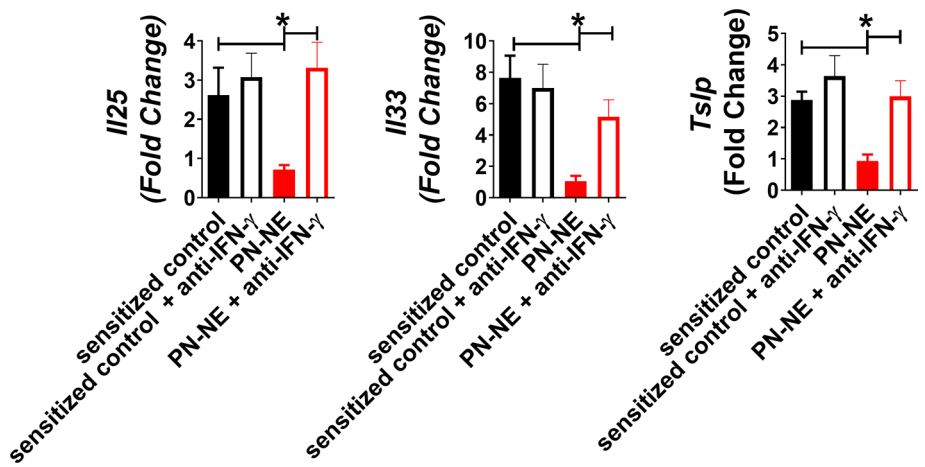


FIGURE 6 | IFN-γ is required for suppression of alarmins by NE allergy vaccines. Mice were sensitized with OVA and peanut-alum and treated i.n. with 3 administrations of PBS (sensitized control) or peanut-NE (PN-NE). Mice were challenged orally with OVA and IFN- γ was depleted during the challenge phase. Duodenum samples were homogenized and mRNA was extracted to determine relative gene expression compared to GAPDH. Statistically significant differences ($p < 0.05$) are indicated by *.

influenced by non-specific elements (mainly regulatory cells and cytokines) from ongoing immune responses to other antigens.

While our previous work has shown that induction of Tregs and IL-10 were important to NE mediated immune modulation (25, 28), we now include the production of IFN-γ as critical for allergic protection. Interestingly, while the NE vaccines induced antigen-specific production of IL-17 and IL-10, there was no bystander modulation of these cytokines, as immunization with PN-NE did not induce production of OVA-specific IL-17 or IL-10. The significant increase in OVA-specific IFN-γ production in PN-NE-immunized mice suggested a key role of IFN-γ in driving the bystander modulation of allergen-specific immune responses and reduction of allergic reactivity. The induction of Th1 cytokines, including IFN-γ, has been associated with resolution of food allergy and favorable outcomes for immunotherapy in humans (56). It has been suggested that IFN-γ can reduce allergic disease through suppression of Th2 cells as well as effects on the innate cells and alarmins which are required for both the induction and maintenance of allergic disease. Innate cytokines including the alarmins IL-25, IL-33, and TSLP are produced by epithelial cells and are key mediators of allergic disease (57). The NE vaccines in our studies suppressed alarmin expression in an IFN-γ dependent mechanism, and this correlated with reduction of allergic reactivity to bystander allergens. This suggests local NE-induced IFN-γ modulates the small intestine environment to suppress the allergic response. Since these components of the innate immune system function in an antigen-independent manner, this may be responsible for the non-antigen specific bystander effects that confer protection against allergens not included in the NE vaccine. Additional studies are required to determine if modulation of a specific alarmin is required for suppression of reactivity, and further dissection of the mechanism of IFN-γ induction on suppression of reactivity is currently in progress.

A role for IFN-γ–mediated suppression of ILC2s in the small intestine cannot be ruled out, as mice that received the NE vaccines also had reduced ILC2s in the small intestine. Previous work has demonstrated that in the lung, IFN-γ prevents the accumulation of these lymphoid cells in mucosal tissues by limiting the recruitment and maintenance of these cells (58–60). The reduction in ILC2s in the mice that received the NE vaccines may also be a critical factor for the observed reduction in allergic reactivity, as IL-4 and IL-13 producing ILC2s have also been shown to promote experimental food allergy (36, 61).

Overall, these results show that modulation of Th2 immunity towards one food can induce bystander effects that suppress allergic reactivity to unrelated foods. This may lead to a global reduction of allergic reactivity to multiple foods. This allergen-non-specific protection may also be induced by other therapies that increase IFN-γ and decreased expression of alarmins in the gut mucosa. Taken together, these results suggest new targets for the suppression of allergic disease.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal studies were reviewed by the University of Michigan Institutional Animal Care and Use Committee.

AUTHOR CONTRIBUTIONS

MF, FF, JB, and JO designed the studies and prepared the manuscript. MF, JL, KJ, HL, and JO performed experiments and analyzed data. All authors contributed to the article and approved the submitted version.

FUNDING

This project has been funded by a Food Allergy Research and Education New Investigator Award, the National Institute for Allergy and Infectious Disease, National Institutes of Health under Grant R01AI145991, the Michigan Food Allergy Research Accelerator (M-FARA), and a generous gift from Robert and Caren Vondell.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2021.599296/full#supplementary-material

REFERENCES

1. Gupta R, Holdford D, Bilaver L, Dyer A, Holl JL, Meltzer D. The economic impact of childhood food allergy in the United States. *JAMA Pediatr* (2013) 167:1026–31. doi: 10.1001/jamapediatrics.2013.2376
2. Shamji MH, Durham SR. Mechanisms of immunotherapy to aeroallergens. *Clin Exp Allergy* (2011) 41:1235–46. doi: 10.1111/j.1365-2222.2011.03804.x
3. Oppenheimer JJ, Nelson HS, Bock SA, Christensen F, Leung DY. Treatment of peanut allergy with rush immunotherapy. *J Allergy Clin Immunol* (1992) 90:256–62. doi: 10.1016/0091-6749(92)90080-L
4. Nelson HS, Lahr J, Rule R, Bock A, Leung D. Treatment of anaphylactic sensitivity to peanuts by immunotherapy with injections of aqueous peanut extract. *J Allergy Clin Immunol* (1997) 99:744–51. doi: 10.1016/S0091-6749(97)80006-1
5. Palisade Group of Clinical Investigators, Vickery BP, Vereda A, Casale TB, Beyer K, du Toit G, et al. AR101 Oral Immunotherapy for Peanut Allergy. *N Engl J Med* (2018) 379:1991–2001. doi: 10.1056/NEJMoa1812856
6. Jones SM, Sicherer SH, Burks AW, Leung DY, Lindblad RW, Dawson P, et al. Epicutaneous immunotherapy for the treatment of peanut allergy in children and young adults. *J Allergy Clin Immunol* (2017) 139:1242–52.e9. doi: 10.1016/j.jaci.2016.08.017
7. Mondoulet L, Dioszeghy V, Vanoirbeek JA, Nemery B, Dupont C, Benhamou PH. Epicutaneous immunotherapy using a new epicutaneous delivery system in mice sensitized to peanuts. *Int Arch Allergy Immunol* (2011) 154:299–309. doi: 10.1159/000321822
8. Jones SM, Burks AW, Dupont C. State of the art on food allergen immunotherapy: oral, sublingual, and epicutaneous. *J Allergy Clin Immunol* (2014) 133:318–23. doi: 10.1016/j.jaci.2013.12.1040
9. Keet CA, Frischmeyer-Guerrerio PA, Thyagarajan A, Schroeder JT, Hamilton RG, Boden S, et al. The safety and efficacy of sublingual and oral immunotherapy for milk allergy. *J Allergy Clin Immunol* (2012) 129:448–55.e1-5. doi: 10.1016/j.jaci.2011.10.023
10. Burks AW, Jones SM, Wood RA, Fleischer DM, Sicherer SH, Lindblad RW, et al. Oral immunotherapy for treatment of egg allergy in children. *N Engl J Med* (2012) 367:233–43. doi: 10.1056/NEJMoa1200435
11. Bauer RN, Manohar M, Singh AM, Jay DC, Nadeau KC. The future of biologics: applications for food allergy. *J Allergy Clin Immunol* (2015) 135:312–23. doi: 10.1016/j.jaci.2014.12.1908
12. Galli SJ, Tsai M. IgE and mast cells in allergic disease. *Nat Med* (2012) 18:693–704. doi: 10.1038/nm.2755
13. Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy: multiple suppressor factors at work in immune tolerance to allergens. *J Allergy Clin Immunol* (2014) 133:621–31. doi: 10.1016/j.jaci.2013.12.1088
14. Broide DH. Immunomodulation of Allergic Disease. *Annu Rev Med* (2009) 60:279–91. doi: 10.1146/annurev.med.60.041807.123524
15. Casale T, Stokes J. Immunomodulators for allergic respiratory disorders. *J Allergy Clin Immunol* (2008) 121:288 – 96. doi: 10.1016/j.jaci.2007.11.040
16. Bielinska AU, Janczak KW, Landers JJ, Makidon P, Sower LE, Peterson JW, et al. Mucosal immunization with a novel nanoemulsion-based recombinant anthrax protective antigen vaccine protects against Bacillus anthracis spore challenge. *Infect Immun* (2007) 75:4020–9. doi: 10.1128/IAI.00070-07
17. Makidon PE, Bielinska AU, Nigavekar SS, Janczak KW, Knowlton J, Scott AJ, et al. Pre-clinical evaluation of a novel nanoemulsion-based hepatitis B mucosal vaccine. *PloS One* (2008) 3:e2954. doi: 10.1371/journal.pone.0002954
18. Passmore C, Makidon PE, O'Konek JJ, Zahn JA, Pannu J, Hamouda T, et al. Intranasal immunization with W 5EC adjuvanted recombinant RSV rF-ptn enhances clearance of respiratory syncytial virus in a mouse model. *Hum Vaccin Immunother* (2013) 10:615–22. doi: 10.4161/hv.27383
19. Lindell DM, Morris SB, White MP, Kallal LE, Lundy PK, Hamouda T, et al. A novel inactivated intranasal respiratory syncytial virus vaccine promotes viral clearance without Th2 associated vaccine-enhanced disease. *PloS One* (2011) 6:e21823. doi: 10.1371/journal.pone.0021823
20. Hamouda T, Chepurnov A, Mank N, Knowlton J, Chepurnova T, Myc A, et al. Efficacy, immunogenicity and stability of a novel intranasal nanoemulsion-adjuvanted influenza vaccine in a murine model. *Hum Vaccin* (2010) 6:585–94. doi: 10.4161/hv.6.7.11818
21. Bielinska AU, Chepurnov AA, Landers JJ, Janczak KW, Chepurnova TS, Luker GD, et al. A novel, killed-virus nasal vaccinia virus vaccine. *Clin Vaccine Immunol* (2008) 15:348–58. doi: 10.1128/CVI.00440-07
22. Bielinska AU, Gerber M, Blanco LP, Makidon PE, Janczak KW, Beer M, et al. Induction of Th17 cellular immunity with a novel nanoemulsion adjuvant. *Crit Rev Immunol* (2010) 30:189–99. doi: 10.1615/CritRevImmunol.v30.i2.60
23. Bielinska AU, Makidon PE, Janczak KW, Blanco LP, Swanson B, Smith DM, et al. Distinct pathways of humoral and cellular immunity induced with the mucosal administration of a nanoemulsion adjuvant. *J Immunol* (2014) 192:2722–33. doi: 10.4049/jimmunol.1301424
24. Makidon PE, Belyakov IM, Blanco LP, Janczak KW, Landers J, Bielinska AU, et al. Nanoemulsion mucosal adjuvant uniquely activates cytokine production by nasal ciliated epithelium and induces dendritic cell trafficking. *Eur J Immunol* (2012) 42:2073–86. doi: 10.1002/eji.201142346
25. O'Konek JJ, Landers JJ, Janczak KW, Goel RR, Mondrusov AM, Wong PT, et al. Nanoemulsion adjuvant-driven redirection of TH2 immunity inhibits allergic reactions in murine models of peanut allergy. *J Allergy Clin Immunol* (2018) 141:2121–31. doi: 10.1016/j.jaci.2018.01.042
26. O'Konek JJ, Baker JR Jr. Treatment of allergic disease with nanoemulsion adjuvant vaccines. *Allergy* (2019) 175:246–9. doi: 10.1111/all.13977
27. O'Konek JJ, Landers JJ, Janczak KW, Lindsey HK, Mondrusov AM, Totten TD, et al. Intranasal nanoemulsion vaccine confers long-lasting immunomodulation and sustained unresponsiveness in a murine model of milk allergy. *Allergy* (2020) 75:872–81. doi: 10.1111/all.14064
28. Bielinska AU, O'Konek JJ, Janczak KW, Baker JR Jr. Immunomodulation of TH2 biased immunity with mucosal administration of nanoemulsion adjuvant. *Vaccine* (2016) 34:4017–24. doi: 10.1016/j.vaccine.2016.06.043
29. Myc A, Kukowska-Latallo JF, Bielinska AU, Cao P, Myc PP, Janczak K, et al. Development of immune response that protects mice from viral pneumonitis after a single intranasal immunization with influenza A virus and nanoemulsion. *Vaccine* (2003) 21:3801–14. doi: 10.1016/S0264-410X(03)00381-5
30. Ahrens R, Osterfeld H, Wu D, Chen CY, Arumugam M, Groschwitz K, et al. Intestinal mast cell levels control severity of oral antigen-induced anaphylaxis in mice. *Am J Pathol* (2012) 180:1535–46. doi: 10.1016/j.ajpath.2011.12.036
31. Finkelman FD, Katona IM, Mosmann TR, Coffman RL. IFN-gamma regulates the isotypes of Ig secreted during in vivo humoral immune responses. *J Immunol* (1988) 140:1022–7.

32. Li XM, Serebrisky D, Lee SY, Huang CK, Bardina L, Schofield BH, et al. A murine model of peanut anaphylaxis: T- and B-cell responses to a major peanut allergen mimic human responses. *J Allergy Clin Immunol* (2000) 106:150–8. doi: 10.1067/mai.2000.107395
33. Rodriguez B, Prioult G, Hacini-Rachinel F, Moine D, Bruttin A, Ngom-Bru C, et al. Infant gut microbiota is protective against cow's milk allergy in mice despite immature ileal T-cell response. *FEMS Microbiol Ecol* (2012) 79:192–202. doi: 10.1111/j.1574-6941.2011.01207.x
34. Classen DC, Morningstar JM, Shanley JD. Detection of antibody to murine cytomegalovirus by enzyme-linked immunosorbent and indirect immunofluorescence assays. *J Clin Microbiol* (1987) 25:600–4. doi: 10.1128/JCM.25.4.600-604.1987
35. Frey A, Di Canzio J, Zurakowski D. A statistically defined endpoint titer determination method for immunoassays. *J Immunol Methods* (1998) 221:35–41. doi: 10.1016/S0022-1759(98)00170-7
36. Lee JB, Chen CY, Liu B, Mugge L, Angkasekwinai P, Facchinetti V, et al. IL-25 and CD4(+) TH2 cells enhance type 2 innate lymphoid cell-derived IL-13 production, which promotes IgE-mediated experimental food allergy. *J Allergy Clin Immunol* (2016) 137:1216–1225 e5. doi: 10.1016/j.jaci.2015.09.019
37. Oyoshi MK, Larson RP, Ziegler SF, Geha RS. Mechanical injury polarizes skin dendritic cells to elicit a T(H)2 response by inducing cutaneous thymic stromal lymphopoietin expression. *J Allergy Clin Immunol* (2010) 126:976–84, 984 e1-5. doi: 10.1016/j.jaci.2010.08.041
38. Allinne J, Scott G, Lim WK, Birchard D, Erjefalt JS, Sanden C, et al. IL-33 blockade affects mediators of persistence and exacerbation in a model of chronic airway inflammation. *J Allergy Clin Immunol* (2019) 144:1624–1637 e10. doi: 10.1016/j.jaci.2019.08.039
39. Muto T, Fukuoka A, Kabashima K, Ziegler SF, Nakanishi K, Matsushita K, et al. The role of basophils and proallergic cytokines, TSLP and IL-33, in cutaneously sensitized food allergy. *Int Immunol* (2014) 26:539–49. doi: 10.1093/intimm/dxu058
40. Hussain M, Borcard L, Walsh KP, Pena Rodriguez M, Mueller C, Kim BS, et al. Basophil-derived IL-4 promotes epicutaneous antigen sensitization concomitant with the development of food allergy. *J Allergy Clin Immunol* (2018) 141:223–234 e5. doi: 10.1016/j.jaci.2017.02.035
41. Stier MT, Zhang J, Goleniewska K, Cephus JY, Rusznak M, Wu L, et al. IL-33 promotes the egress of group 2 innate lymphoid cells from the bone marrow. *J Exp Med* (2018) 215:263–81. doi: 10.1084/jem.20170449
42. von Moltke J, Ji M, Liang HE, Locksley RM. Tuft-cell-derived IL-25 regulates an intestinal ILC2-epithelial response circuit. *Nature* (2016) 529:221–5. doi: 10.1038/nature16161
43. Huang Y, Guo L, Qiu J, Chen X, Hu-Li J, Siebenlist U, et al. IL-25-responsive, lineage-negative KLRG1(hi) cells are multipotential 'inflammatory' type 2 innate lymphoid cells. *Nat Immunol* (2015) 16:161–9. doi: 10.1038/ni.3078
44. Gupta RS, Springston EE, Warrier MR, Smith B, Kumar R, Pongracic J, et al. The prevalence, severity, and distribution of childhood food allergy in the United States. *Pediatrics* (2011) 128:e9–17. doi: 10.1542/peds.2011-0204
45. Gupta RS, Warren CM, Smith BM, Jiang J, Blumenstock JA, Davis MM, et al. Prevalence and Severity of Food Allergies Among US Adults. *JAMA Netw Open* (2019) 2:e185630. doi: 10.1001/jamanetworkopen.2018.5630
46. Begin P, Winterroth LC, Dominguez T, Wilson SP, Bacal L, Mehrotra A, et al. Safety and feasibility of oral immunotherapy to multiple allergens for food allergy. *Allergy Asthma Clin Immunol* (2014) 10:1. doi: 10.1186/1710-1492-10-1
47. Andorf S, Manohar M, Dominguez T, Block W, Tupa D, Kshirsagar RA, et al. Feasibility of sustained response through long-term dosing in food allergy immunotherapy. *Allergy Asthma Clin Immunol* (2017) 13:52. doi: 10.1186/s13223-017-0224-7
48. Wang LF, Chen JS, Hsu CJ, Liu CY, Yu JS, Miaw SC. Antigen-driven bystander effect accelerates epicutaneous sensitization with a new protein allergen. *J BioMed Sci* (2009) 16:28. doi: 10.1186/1423-0127-16-28
49. Eisenbarth SC, Zhadkevich A, Ranney P, Herrick CA, Bottomly K. IL-4-dependent Th2 collateral priming to inhaled antigens independent of Toll-like receptor 4 and myeloid differentiation factor 88. *J Immunol* (2004) 172:4527–34. doi: 10.4049/jimmunol.172.7.4527
50. Gregory JA, Shepley-McTaggart A, Umpierrez M, Hurlburt BK, Maleki SJ, Sampson HA, et al. Immunotherapy using algal-produced Ara h 1 core domain suppresses peanut allergy in mice. *Plant Biotechnol J* (2016) 14:1541–50. doi: 10.1111/pbi.12515
51. Storni F, Zeltins A, Balke I, Heath MD, Kramer MF, Skinner MA, et al. Vaccine against peanut allergy based on engineered virus-like particles displaying single major peanut allergens. *J Allergy Clin Immunol* (2020) 145:1240–53. doi: 10.1016/j.jaci.2019.12.007
52. Bannon GA, Cockrell G, Connaughton C, West CM, Helm R, Stanley JS, et al. Engineering, characterization and in vitro efficacy of the major peanut allergens for use in immunotherapy. *Int Arch Allergy Immunol* (2001) 124:70–2. doi: 10.1159/000053672
53. Mondoulet L, Dioszeghy V, Puteaux E, Ligouis M, Dhelft V, Plaquet C, et al. Specific epicutaneous immunotherapy prevents sensitization to new allergens in a murine model. *J Allergy Clin Immunol* (2015) 135:1546–57.e4. doi: 10.1016/j.jaci.2014.11.028
54. Mondoulet L, Dioszeghy V, Busato F, Plaquet C, Dhelft V, Bethune K, et al. Gata3 hypermethylation and Foxp3 hypomethylation are associated with sustained protection and bystander effect following epicutaneous immunotherapy in peanut-sensitized mice. *Allergy* (2019) 74:152–64. doi: 10.1111/all.13479
55. Dunkin D, Berin MC, Mondoulet L, Tobar S, Yeretssian G, Tordesillas L, et al. Epicutaneous Tolerance Induction to a Bystander Antigen Abrogates Colitis and Ileitis in Mice. *Inflammation Bowel Dis* (2017) 23:1972–82. doi: 10.1097/MIB.0000000000001273
56. Turcanu V, Maleki SJ, Lack G. Characterization of lymphocyte responses to peanuts in normal children, peanut-allergic children, and allergic children who acquired tolerance to peanuts. *J Clin Invest* (2003) 111:1065–72. doi: 10.1172/JCI200316142
57. Hammad H, Lambrecht BN. Barrier Epithelial Cells and the Control of Type 2 Immunity. *Immunity* (2015) 43:29–40. doi: 10.1016/j.immuni.2015.07.007
58. Molofsky AB, Van Gool F, Liang HE, Van Dyken SJ, Nussbaum JC, Lee J, et al. Interleukin-33 and Interferon-gamma Counter-Regulate Group 2 Innate Lymphoid Cell Activation during Immune Perturbation. *Immunity* (2015) 43:161–74. doi: 10.1016/j.immuni.2015.05.019
59. Li BWS, de Bruijn MJW, Lukkes M, van Nimwegen M, Bergen IM, KleinJan A, et al. T cells and ILC2s are major effector cells in influenza-induced exacerbation of allergic airway inflammation in mice. *Eur J Immunol* (2019) 49:144–56. doi: 10.1002/eji.201747421
60. Starkey MR, McKenzie AN, Belz GT, Hansbro PM. Pulmonary group 2 innate lymphoid cells: surprises and challenges. *Mucosal Immunol* (2019) 12:299–311. doi: 10.1038/s41385-018-0130-4
61. Noval Rivas M, Burton OT, Oettgen HC, Chatila T. IL-4 production by group 2 innate lymphoid cells promotes food allergy by blocking regulatory T-cell function. *J Allergy Clin Immunol* (2016) 138:801–11.e9. doi: 10.1016/j.jaci.2016.02.030

Conflict of Interest: JB holds stock in Blue Willow Biologics, a company that has licensed the adjuvant technology from the University of Michigan. JB and JO'K are inventors of the adjuvant technology involved in this research and patent applications have been submitted for this technology.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

ORIGINAL RESEARCH
published: 09 March 2021
doi: 10.3389/fimmu.2021.604192

Stem Cell Factor Neutralization Protects From Severe Anaphylaxis in a Murine Model of Food Allergy

Catherine Ptaschinski[1,2]*, *Andrew J. Rasky*[1], *Wendy Fonseca*[1] *and Nicholas W. Lukacs*[1,2]

[1] Department of Pathology, Ann Arbor, MI, United States, [2] Mary H. Weiser Food Allergy Center, University of Michigan, Ann Arbor, MI, United States

Food allergy is a growing public health problem with ∼15 million people affected in the United States. In allergic food disease, IgE on mast cells bind to ingested antigens leading to the activation and degranulation of mast cells. Stem cell factor (SCF) is mast cell growth and activation factor that is required for peripheral tissue mast cells. We targeted a specific isoform of SCF, the larger 248 amino acid form, that drives peripheral tissue mast cell differentiation using a specific monoclonal antibody in a model of food allergy. Ovalbumin sensitized and intragastrically challenged mice were monitored for symptoms of anaphylaxis including respiratory distress, diarrhea, and a reduction in body temperature. During the second week of challenges, allergic mice were injected with an antibody to block SCF248 or given IgG control. Mice treated with α-SCF248 had a decreased incidence of diarrhea and no reduction in body temperature suggesting a reduction in anaphylaxis compared to IgG control treated animals. Re-stimulated mesenteric lymph nodes indicated that α-SCF248 treated mice had decreased OVA-specific Th2 cytokine production compared to IgG control treated allergic animals. The reduction of food induced anaphylaxis was accompanied by a significant reduction in gut leak. The mesenteric lymph node cells were analyzed by flow cytometry and showed a decrease in the number of type 2 innate lymphoid cells in mice injected with α-SCF248. Morphometric enumeration of esterase+ mast cells demonstrated a significant reduction throughout the small intestine. Using a more chronic model of persistent food-induced anaphylaxis, short term therapeutic treatment with α-SCF248 during established disease effectively blocked food induced anaphylaxis. Together, these data suggest that therapeutically blocking SCF248 in food allergic animals can reduce the severity of food allergy by reducing mast cell mediated disease activation.

Keywords: food allergy, stem cell factor, mast cell, anaphylaxis, innate lymphoid cell

OPEN ACCESS

Edited by:
Pamela Guerrerio, National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH), United States

Reviewed by:
Philipp Starkl, Medical University of Vienna, Austria
Hans Oettgen, Boston Children's Hospital and Harvard Medical School, United States

***Correspondence:**
Catherine Ptaschinski
cptaschi@med.umich.edu

Specialty section:
This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology

Received: *08 September 2020*
Accepted: *16 February 2021*
Published: *09 March 2021*

Citation:
Ptaschinski C, Rasky AJ, Fonseca W and Lukacs NW (2021) Stem Cell Factor Neutralization Protects From Severe Anaphylaxis in a Murine Model of Food Allergy. Front. Immunol. 12:604192. doi: 10.3389/fimmu.2021.604192

INTRODUCTION

The incidence and severity of food allergy early in life has been growing considerably over the past two decades. Presently, it is estimated that one in 13 children have food allergic responses that predispose them to anaphylaxis (1, 2). Diagnostic assessment of children with potential food anaphylaxis include elevated food specific serum IgE and severe skin challenge reactivity (3).

Unfortunately, these latter parameters are not predictive of whether a child will fail a food challenge in the clinic (4). Furthermore, it is unclear whether a negative food challenge is predictive of future potential reactivity to accidental challenge later in life. Importantly, we do know that the mechanisms that drive an anaphylactic response begins with a rapid and systemic activation of mast cells that cause the release of mediators that initiate the vascular response (5, 6). Several strategies have been studied and utilized in the clinics with some specifically blocking mast cell activation, especially targeting IgE (7–9). Recent use of biologics primarily targeting type 2 immune responses have been suggested or are beginning in initial trials, including α-IL-4/13R, α-IL-5, and α-IL-33 (10–14). These latter therapeutic targets are focused on the type 2 immune response that inhibit immune environments but do not alter the effector responses of anaphylaxis directly. Few strategies have pursued reduction of mast cell numbers as a means for inhibiting adverse allergic responses. The presence of increased mast cell numbers in mucosal gastrointestinal (GI) tract tissue may be critical for driving the severity of anaphylactic responses in patients with increased food specific IgE.

A key molecule that has a central role in mast cell development, survival and activation is stem cell factor (SCF also knowns as kit ligand) (15, 16). In both humans and mice, endogenous SCF occurs in two isoforms, "membrane" (220 amino acids) and "soluble" (248 amino acids) forms (17, 18). They differ by the inclusion of Exon 6 in the SCF248 form, both are membrane expressed, and can induce a c-kit receptor-dependent signal to cells by enhanced cross-linking. SCF248 is known as the "soluble" form because it is enzyme cleavable within exon 6 that allows the SCF extracellular domain fragment to be more easily released from the surface of the cell. This is the source of the vast majority of serum SCF248, which is monomeric and therefore cannot activate c-kit$^+$ cells due to an inability to cross-link c-kit (19). Differences in the isoforms' biology come from studies with Sl/Sld mice that are runted, anemic (due to SCF220's role in erythropoiesis), and have altered inflammatory responses. Sl/Sld mutant mice were transfected with either SCF248 or SCF220 and examined to determine whether the specific forms could differentially reconstitute defective biology. Induced SCF220 expression corrected the runting and anemia with little effect on the inflammatory responses in the Sl/Sld mice, whereas SCF248 expression did not correct the runting or anemia but increased myeloid cell populations including mast cells (20). A separate study that utilized mice that express only SCF220 showed that animals developed normally with no runting or anemia, yet did not have peripheral mast cells (21). Together, these genetic studies define that these SCF isoforms have different biologic functions with SCF220 associated with homeostatic and SCF248 associated with peripheral immune responses, especially mast cells. We have recently published that SCF248 is the dominate form expressed in peripheral tissues during chronic disease. Furthermore, our studies indicate that α-SCF248 specific mAb can inhibit chronic asthma and severe remodeling diseases by reducing pathogenesis, cytokine production and critical c-kit$^+$ innate cells, including mast cells, eosinophils and ILC2s (22, 23).

In the present set of studies we have identified that specifically blocking SCF248 during and after food allergen induced disease can block anaphylaxis and is associated with diminished numbers of mast cells throughout the small intestine. Furthermore, inhibition of SCF248 reduced the overall type 2 immune response and reduced serum IgE suggesting that the systemic immune environment may be affected using this strategy. Importantly, the blockade of SCF248 led to reduced gut leak that suggests a more stable gut barrier that is necessary for controlling systemic allergen distribution. Our studies highlight that blocking a specific isoform of SCF (SCF248) associated with development and activation of mast cells diminished the anaphylactic phenotype in food allergic mice that may be developed for future therapeutic application in food-induced anaphylaxis.

MATERIALS AND METHODS

Animals

Female BALB/c mice were purchased from Jackson Laboratories and used at 6–7 weeks of age. All experiments were approved by the University of Michigan Institutional Animal Care and Use Committee.

Allergic Model and Monoclonal Antibody Production

Mice were treated as in **Figure 1A** or **Figure 5A**. Mice were sensitized intraperitoneally on day 0 with 100 μg of endotoxin-free ovalbumin (OVA, Invivogen, San Diego, CA, USA) adsorbed to 1mg of alum (Imject® Alum, ThermoFisher Scientific, Waltham, MA, USA), then were challenged with 5 mg of OVA protein (Sigma Cat#A5503, St. Louis, MO, USA) in 200 μl or with 200 μl of PBS on days 14, 16, 18, 21, 23, 25, and 28. Mice were fasted for 5–6 h prior to each challenge. To neutralize SCF248, mice were injected with 20 mg/kg of α-SCF248 or control IgG on days 21, 23, and 25, or on days 28, 31, and 35 for the delayed response model. The specific SCF248 monoclonal antibody was made as previously described (22). A similarly produced control IgG1 antibody was used to a non-mammalian target (Genscript).

After each challenge, animals were monitored for 60 min, and rectal temperatures were recorded at 0, 15, 30, and 60 min following oral gavage with PBS or OVA. A score of 0–5 was assigned to each mouse based on anaphylaxis symptoms, as previously described (24). The scale is as follows: (0) no symptoms; (1) scratching and rubbing around the nose and head; (2) puffiness around the eyes and mouth, diarrhea, pilar erecti, reduced activity, and/or decreased activity with increased respiratory rate; (3) wheezing, labored respiration, and cyanosis around the mouth and the tail; (4) no activity after prodding or tremor and convulsion; (5) death.

Histopathology

The small intestine was removed and flushed with cold PBS. Tissue was divided into duodenum, jejunum, and ileum. Each section was opened longitudinally and coiled onto a wooden stick

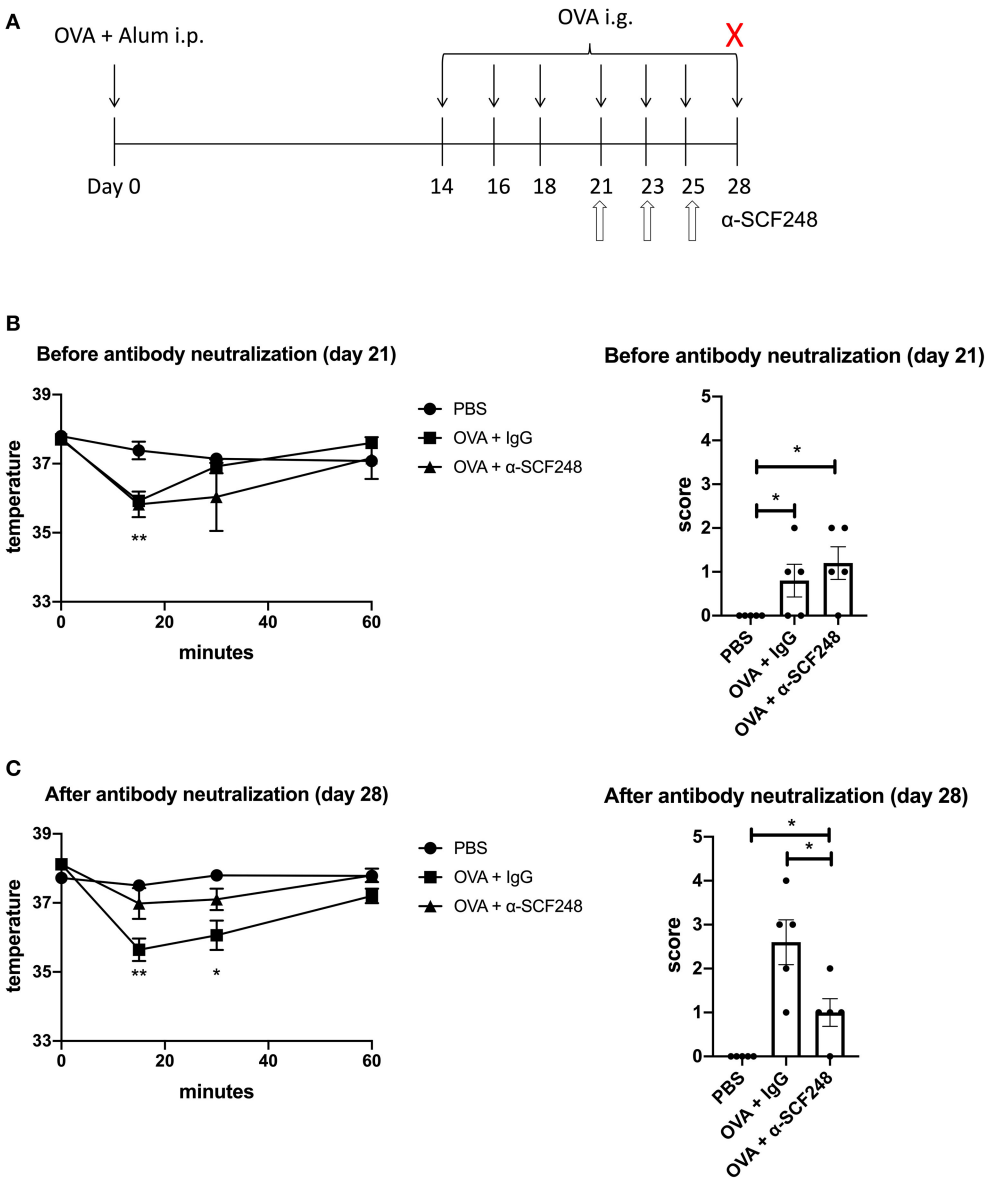


FIGURE 1 | Neutralizing SCF248 reduces anaphylactic symptoms in allergic mice. **(A)** Model to induce anaphylaxis. Mice were sensitized with OVA and alum on day 0, then challenged with OVA by oral gavage on days 14, 16, 18, 21, 23, 25, and 28. SCF248 was neutralized by intraperitoneal injection on days 21, 23, and 25. Animals were euthanized 60 min after final challenge. **(B)** Prior to SCF248 neutralization (day 21, challenge four), temperatures were recorded at 15, 30, and 60 min following oral challenge. Symptoms were continuously monitored for 60 min, and mice were assigned as score on a scale of one to five based on symptom presentation. $^{*}p < 0.05$, $^{**}p < 0.01$ for OVA + IgG and OVA + αSCF248 compared to PBS. **(C)** Following SCF248 neutralization (day 28, challenge seven), temperatures were monitored for 60 min, and animals were euthanized. Mice were assigned a clinical score based on symptoms. Results are from five mice per group. Date show mean ± SEM, and are from one of three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$ for OVA + IgG compared to PBS and OVA + αSCF248.

to create a roll. Tissue was fixed in 10% formalin, followed by 70% ethanol, then embedded in paraffin and 5 μm sections were cut and mounted onto slides. Mast cells were visualized using chloroacetate esterase staining as previously described, as this staining protocol is known to detect mucosal mast cells (25, 26). At least five high powered fields (HPF) were counted on each section of the intestine per animal.

Flow Cytometry

Mesenteric lymph nodes were removed and single cells were isolated by enzymatic digestion with 1 mg/ml collagenase A (Roche, Indianapolis, IN, USA) and 20 U/ml DNaseI (Sigma, St. Louis, MO, USA) in RPMI containing 10% FCS. Lymphocytes from the lamina propria were isolated as previously described (27). Briefly, the small intestine was opened longitudinally and

mucus was removed by washing the tissue with PBS + 2% FCS + 5mM DTT at 37°C for 20 min. The intestinal epithelial cell lining was removed by three washes with PBS + 2% FCS% 5 mM EDTA, each for 10 min at 37°C. The tissue was then minced into small pieces, and at digested in HBSS supplemented with 10mM HEPES, 37.5 U/ml Liberase TM (Roche Applied Science, Indianapolis, IN, USA), and 300 U/ml DNase I for 30 min at 37°C. The resulting cell suspension was filtered through a 70 μm sieve, counted, and plated for flow cytometry at 2×10^6 per well. Cells were washed and resuspended in PBS and live cells were identified using LIVE/DEAD Fixable Yellow Dead Cell Stain kit (ThermoFisher Scientific, Waltham, MA, USA), then were washed and resuspended in PBS with 1% FCS and Fc receptors were blocked with purified α-CD16/32 (clone 93; BioLegend, San Diego, CA, USA). Surface markers were identified using antibodies against the following antigens, all from BioLegend: CD3 (17A2), CD11b (M1/70), CD45R/B220 (RA3-6B2), Ter119 (TER-119), Gr-1 (RB6-8C5), CD45 (30-F11), SiglecF (E50-2440), c-kit/CD117 (2B8), CD90 (5E10), IL7Rα/CD127 (A019D5), ST2/IL33Rα (DIH4), and FcεRIα (MAR-1). Intracellular staining for GATA3 (clone 16E10A23 was performed using the Transcription Factor Staining Buffer Set (eBioscience) according to the manufacturer's protocol. Eosinophils were identified as $CD45^+SiglecF^+SSC^{hi}$. Mast cells were identified as $CD45^+c\text{-}kit^+FcεRIα^+$. ILC2s were identified as $lin^-CD45^+c\text{-}kit^+CD90^+IL7Rα^+ST2^+Gata3^+$. Lineage negative cells were defined as $CD3^-CD11b^-CD45R/B220^-Ter119^-Gr1^-$.

Lymph Node Restimulation

Mesenteric lymph nodes (MLN) were removed and single cells were isolated by enzymatic digestion, as described above. 5×10^5 cells were plated in 200 μl of complete medium (RPMI 1640 supplemented with 10% FCS, L-glutamine, penicillin/streptomycin, non-essential amino acids, sodium pyruvate, 2-mercaptoethanol) and were restimulated with 50 μg/ml of ovalbumin for 48 h. Unstimulated cells were used as baseline controls. Supernatants were collected and levels of the cytokines IL-4, IL-5, and IL-13 were measured by Bioplex assay (Bio-Rad, Hercules, CA, USA).

IgE and mMCP1 Enzyme Linked Immunosorbant Assay

OVA-specific antibody isotypes were measured in the serum of treated mice. Immunosorbant 96-well plates (ThermoFisher Scientific) were coated with OVA overnight at 4°C, then incubated with blocking buffer (1% dry non-fat milk in PBS) for 1 h at 37°C. Serial dilutions of serum were made in 1% BSA and were incubated on the plates overnight at 4°C. Plates were washed PBS + 0.05% Tween 20. Alkaline phosphatase-labeled secondary antibodies (Jackson ImmunoResearch, West Grove, PA, USA) were incubated at 37°C for 1.5 h. Plates were washed and incubated at room temperature with p-nitrophenyl phosphate (Sigma-Aldrich, St. Louis, MO, USA) and color was developed for 1 h. Plates were read on a SpectraMax iD3 (Molecular Devices, San Jose, CA, USA) at 405 nm, and results are presented as optical density. Optical density in allergic mice was calculated as $OD_{allergic}-(OD_{control} + 2^*SD_{control})$. mMCP1 was measured in the serum using a mouse MCPT-1 kit (Invitrogen) according to the manufacturer's instructions.

Epithelial Barrier Permeability Assay

Mice were fasted for 4 h, then given 150 μl of 4 kDa FITC-dextran by oral gavage at a concentration of 80 mg/ml. Blood was collected after 4 h and centrifuged to separate plasma. Plasma was then diluted 1:10 in PBS at and read on a SpectraMax iD3 at 530 nm with excitation at 485 nm. Serial dilutions of FITC-dextran were made to create a standard curve.

Western Blot of JAM-A

Protein was extracted from a section of the jejunum by homogenization in RIPA buffer (Cell Signaling Technology, Danvers, MA, USA) supplemented with 1mM PMSF (ThermoFisher Scientific). Protein was clarified by centrifugation, and concentration was determined by Bradford assay as previously described (28). Fifteen μg of protein was loaded onto Bis-Tris polyacrylamide gels (Invitrogen), and protein was transferred onto a nitrocellulose membrane using an iBlot 2 dry blotting system (ThermoFisher Scientific). Western blot was performed using antibodies again JAM-A (R&D Systems, Minneapolis, MN, USA) and b-actin (Cell Signaling Technology), followed by horseradish peroxidase-conjugated secondary antibodies. JAM-A was detected, followed by stripping of the membrane with Restore™ Western Blot Stripping Buffer (ThermoFisher Scientific), then detection of b-actin. Detection was done using enhanced chemiluminescence (GE Healthcare, Pittsburgh, PA, USA). Band densities were measured using the rectangle measurement tool on ImageLab software (BioRad, Hercules, CA, USA), and JAM-A densities were normalized to b-actin.

Statistical Analysis

Results are expressed as mean ± standard error. Statistical significance was measured by one-way or two-way ANOVA followed by *post-hoc* Student's *t*-test as appropriate. A $p \leq 0.05$ was considered significant.

RESULTS

Neutralization of SCF248 Protects Mice From Food-Induced Anaphylaxis With Reduction in Intestinal Mast Cells

The induction of food allergy in mice has been established using several methodologies. In these studies we utilized systemic immunization with alum and ovalbumin to establish a strong allergic response followed by seven intragastric allergen challenges (**Figure 1A**), as previously described (29). In order to examine the potential role of SCF248 in the development and elicitation of food allergic responses we administered α-SCF248 mAb or control IgG by IP injection after the 4th intragastric challenge with ovalbumin (**Figure 1A**). Prior to injection the animals were assessed for clinical signs of food allergen responses by temperature drop and a standard anaphylaxis scale (0–5) as previously described (24). The data in **Figure 1B** indicate that mice in each of the groups, IgG

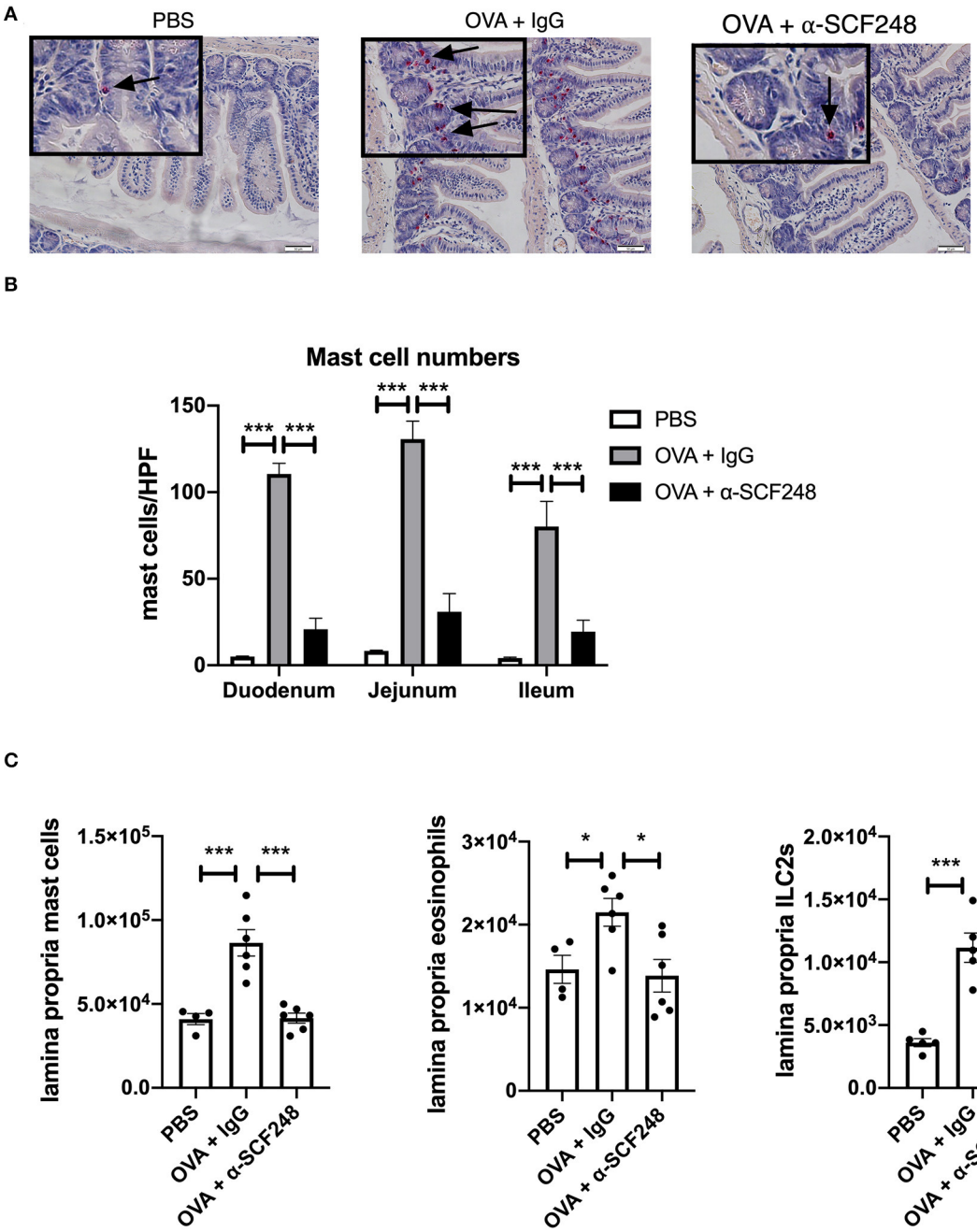


FIGURE 2 | Anti-SCF248 treatment reduces mast cells, eosinophils, and ILC2s in the intestines of allergic mice. **(A)** Representative sections of the small intestine stained with chloroacetate esterase to visualize mast cells. Details are shown in the inset boxes. Scale bar = 50 μm. Mast cells are indicated by arrows. **(B)** Mast cells were counted in the duodenum, jejunum, and ileum. Numbers represent the mean of five high-powered fields per section. **(C)** Mast cells, eosinophils, and ILC2s in the small intestine lamina propria were quantified by flow cytometry. Results are from four to six mice per group and represent one of three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

and α-SCF248, had similar anaphylactic characteristics and based upon temperature loss and anaphylactic scale prior to therapeutic administration of antibody. The animals were then subjected to three additional challenges in the following week while being treated with α-SCF248 or control antibody. After the 7th challenge mice were assessed by temperature and anaphylactic scores for the severity of their responses. Those mice treated with α-SCF248 displayed a minimal loss

of temperature and a minimal anaphylactic score overall, while those treated with control IgG showed a significant decrease in body temperature and a substantial anaphylactic response (**Figure 1C**).

To further examine whether this effect was associated with local mast cell numbers in the GI tract, histopathologic staining was utilized with non-specific esterase for identification of mast cell numbers (**Figure 2**). The histology shown in **Figure 2A** represent that while PBS treated, non-allergic mice have virtually no mast cells evident, ovalbumin sensitized and challenged mice have significant increases in esterase positive mast cells in the small intestine. Those animals treated with α-SCF248 appear to have a significant decrease in mast cell staining. Enumeration of mast cell numbers in stained slides demonstrate that there were significant increases in the control treated food allergic mice that were significantly reduced throughout the small intestine, the duodenum, jejunum, and ileum (**Figure 2B**). Mast cells in the lamina propria were also analyzed by flow cytometry following enzymatic digestion, and these results confirmed a decrease in the number of mast cells in mice treated with α-SCF248 (**Figure 2C**). Representative flow cytometry plots with gating strategies are shown in **Supplementary Figure 1**. We also noted a decrease in the number of eosinophils and type 2 innate lymphoid cells (ILC2), indicating an overall decrease in the T-helper type 2 (Th2) environment in the small intestine in the absence of SCF248 signaling.

Neutralization of SCF248 Alters Th2 Cytokine Profiles

In order to better understand whether blocking SCF248 alters the ongoing allergic responses and can change immune phenotypes mesenteric lymph nodes were isolated and restimulated with ovalbumin. The data presented in **Figure 3A** illustrate that inhibition of SCF248 during the challenge period in food allergic animals reduced type 2 cytokines, IL-4, IL-5, and IL-13, although there were no differences in the overall number of cells from the mesenteric lymph nodes. Our studies have previously identified SCF248 as an inducer of ILC2, as these are c-kit^{+} cell populations (22). When ILC2 numbers were examined in the mesenteric lymph nodes by flow cytometry, we also observed decreases in these cells that likely contribute to the type 2 allergic environment (**Figure 3B**), while there were no differences in Th2 cytokines in unstimulated cells from these animals (**Supplementary Figure 2**).

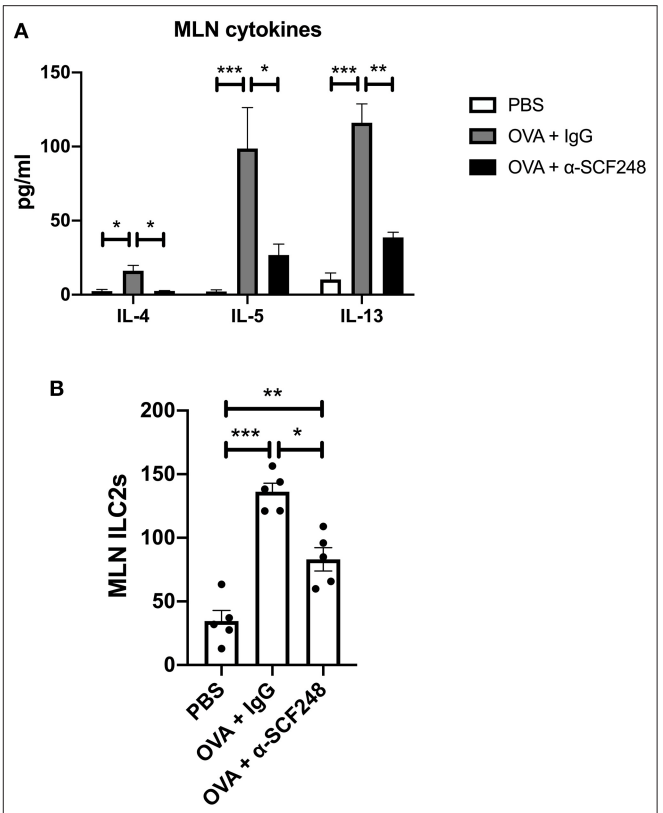


FIGURE 3 | The Th2 response is decreased in the lymph nodes of mice treated with α-SCF248. **(A)** Mesenteric lymph nodes were removed and single cell suspensions were restimulated with OVA. Th2 cytokines were measured in the supernatant by Bioplex assay after 48 h of culture. **(B)** The number of ILC2s in the mesenteric lymph nodes were measured by flow cytometry. Five mice were used in each group, and data represent one of three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Decreased Mast Cell Activity Following SCF248 Neutralization

When mast cells degranulate, one of the inflammatory mediators that is released is the specific protease murine mast cell protease-1 (mMCP1). We measured mMCP1 levels in the serum 30 min after challenge and found a significant reduction in the levels of this protease in the serum of α-SCF248 treated mice (**Figure 4A**). The activation of mast cells can affect the barrier function of the intestinal epithelium. To test this, mice were gavaged with 4 kD FITC-Dextran, and plasma was collected to measure the concentration of this molecule through the epithelial barrier. We found that mice treated with OVA had increased FITC-Dextran in the plasma, but this was inhibited in mice treated with α-SCF248 (**Figure 4B**), indicating that the decreased number and activation of mast cells protects barrier function. To support this, we performed a Western blot for JAM-A in the jejunum. JAM-A is part of the tight junction between epithelial cells in the small intestine, and contributes to barrier function (30). We found that JAM-A expression in the small intestine was decreased in OVA-treated mice, but that this was rescued by treating mice with α-SCF248 (**Figure 4C**, full blots shown in **Supplementary Figure 3**). Therefore, the increased number of mast cells correlates with decreased JAM-A in the GI tract, and an increase in intestinal permeability. In IgE-mediated food allergy, mast cells degranulation occurs when IgE bound to the mast cell encounters its antigen. We therefore measured OVA-specific IgE in the serum of allergic mice. We did not observe any difference in mice treated with control IgG or α-SCF248, indicating that there was no difference in sensitization to OVA (**Figure 4D**). There was no effect on other immunoglobulins, including IgG1, IgG2a, and IgG2b. Thus, the neutralization of SCF248 significantly reduced the anaphylactic responses and was

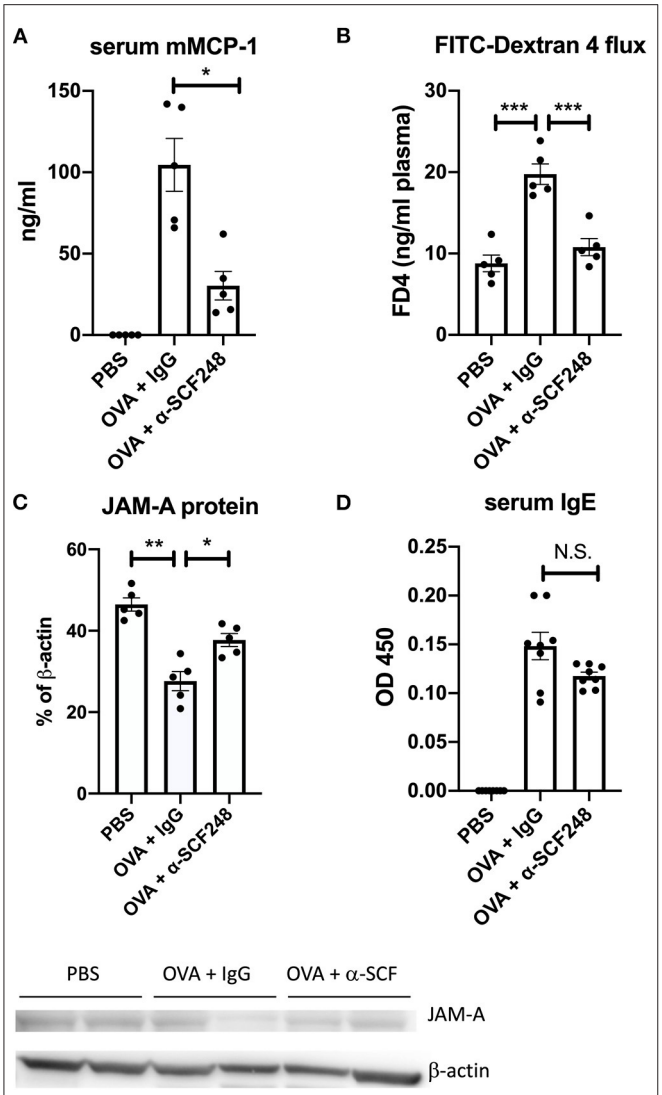


FIGURE 4 | Mast cell activation and intestinal permeability are decreased in mice treated with α-SCF248. **(A)** Mast cell activation was assessed by measuring murine mast cell protease concentration in the serum 30 min after final challenge. Protease concentration was measured by ELISA. **(B)** Intestinal permeability was analyzed by delivering 4 kDa FITC-dextran by oral gavage, then measuring FITC in the plasma by fluorescence 4 h later. **(C)** Western blot of JAM-A was performed from the small intestine, with b-actin as a control. Densitometry analysis of JAM-A results are shown as a percentage of b-actin control. Each lane represents a different tissue sample, and one of two representative blots is shown. **(D)** OVA-specific IgE was measured in the serum of allergic mice by ELISA. Results represent one of three independent experiments with four to five mice per group. ELISA samples were run in duplicate, in addition to the biological replicates. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

correlated to the number and activation of mast cells in the gastrointestinal tract of allergic mice.

Inhibition of Food Induced Anaphylaxis in Established Food Allergy Responses

The previous studies indicated that blocking SCF248 during ongoing food challenges reduces the severity of the food allergic responses. In order to better define whether SCF248 neutralization could inhibit the re-occurrence of an established response a second model was established. In this model (**Figure 5A**) the antibody was administered during a one week "rest" period where no food challenge was administered after the original model was established followed by a food challenge to the sensitized food allergic mice. The data presented in **Figure 5** demonstrates that inhibition of SCF248 abrogates the effects of food induced anaphylaxis as indicated by no decrease in temperature in α-SCF248 treated animals in this more chronic model of established food allergy (**Figure 5B**). Similarly, the overall clinical parameters were reduced as indicated by the anaphylactic index (**Figure 5C**) that includes diarrhea and itching.

We enumerated the mast cells in the small intestine by staining sections with chloroacetate esterase. As previously, we found that blocking SCF248 in an established model of food allergy resulted in decreased mast cell numbers in the tissue (**Figures 6A,B**). As a measure of the extent of mast cell degranulation, the serum mMCP-1 enzyme levels were significantly reduced in the α-SCF248 treated animals (**Figure 6C**). Finally, we measured the Th2 environment in these animals by restimulating mesenteric lymph node cells with OVA. We found that treating mice with α-SCF248 decreased IL-4 and IL-5 production, although there were no differences in IL-13 in allergic mice treated with α-SCF248 or control IgG (**Figure 6D**). Additionally, there were no differences in IFN-g or IL-17, indicating that only Th2 cytokines are altered by the neutralization of SCF248. Thus, inhibition of this pathway that is known to be involved in the expansion and activation of mast cells reduces the severity of food induced anaphylaxis.

DISCUSSION

The elicitation of severe allergic food responses has increased significantly over the past two decades and has caused changes in social and personal behavior in those households where it is present. The critical cellular mediator during a food anaphylactic response is the mast cell. A number of studies have suggested that the number of mast cells in the GI tract may correspond to the generation of anaphylaxis (31, 32). This is also likely accompanied by the ability of food to move through a normally tight epithelial barrier in the GI tract, which is modified by the local activation of mast cell mediated responses (33). Therefore, a central premise would be to control mast cell numbers in tissue that can provide the initial response to limit amplification in subsequent exposures due to the breakdown in barrier from the previous episode. The clinical observations regarding IgE levels and the ability to predict an anaphylactic food challenge at any given time is likely complicated by these factors, numbers of mast cells and barrier maintenance. The present studies demonstrate that by blocking SCF248 levels using a therapeutic antibody approach that a reduction in mast cells throughout the small intestine was achieved. This correlates well with genetic studies in mice that only express the SCF220 splice variant and not SCF248 that lack peripheral mast cells, even though myeloid bone marrow progenitor cell populations were not affected

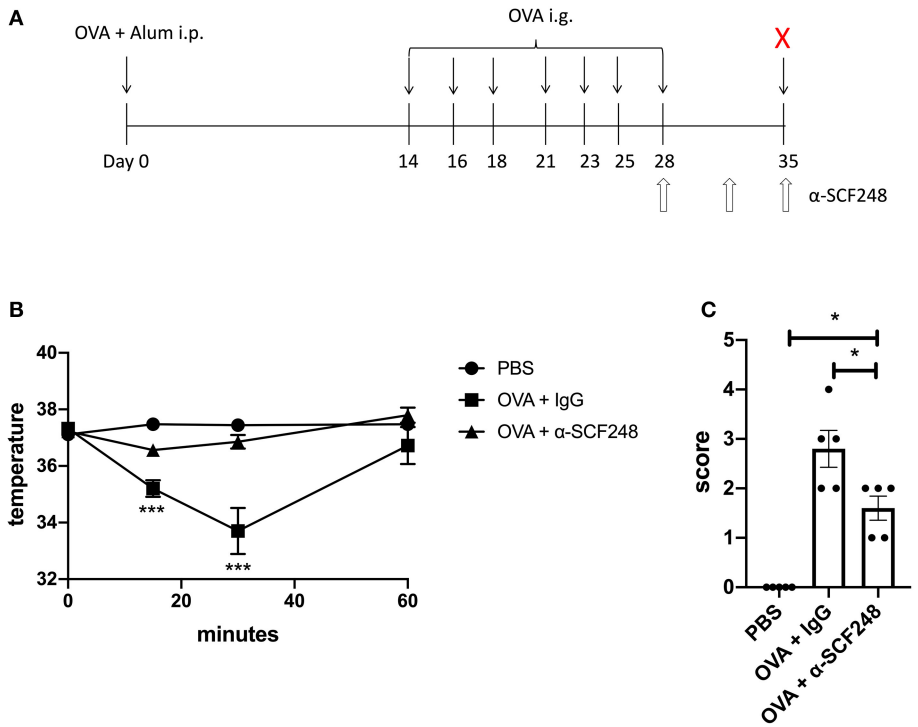


FIGURE 5 | Anti-SCF248 treatment protects mice in a model of established food allergy. **(A)** Food allergy was induced by sensitizing mice with OVA and alum, followed by seven challenges with OVA, ending on day 28. SCF248 was blocked by injection with a monoclonal antibody on days 28, 31, and 35. Mice were given one additional challenge with OVA on day 35, following SCF248 neutralization. **(B)** Rectal temperatures were recorded for at 15, 30, and 60 min following challenge at day 35. **(C)** Mice were monitored for 60 min following final challenge, and assigned a score based on clinical symptoms. Results are from five mice per group and represent one of three independent experiments. $^{*}p < 0.05$, $^{***}p < 0.001$.

(21, 32). The reduction in clinical disease parameters in the food allergy challenges and the reduction of mast cells and type 2 cytokines also contributed to the protective phenotype after a therapeutic challenge in these studies. Thus, a reasonable strategy for reducing the incidence of food anaphylaxis would be to reduce mast cell numbers and therefore the anaphylactic environment would be mitigated. While we have not examined the effect of a-SCF248 antibody on steady state mast cell numbers, the genetic evidence suggests that tissue mast cells are dependent upon SCF248 and not SCF220 (23). Furthermore, the antibody used in this study does not recognized the cleaved domain of SCF248, and is therefore unlikely to interact with mast cells. Instead, the antibody targets the SCF248 isoform on fibroblasts, leading to a reduction in the amount of the cleaved domain available to bind to mast cells (23). However, the long-term effects of a-SCF248 should still be a consideration for future studies, particularly with regards to the possibility of using this antibody in a clinical setting.

One outcome in these studies was the reduction in mast cell numbers and activation, combined with reduced gut leak. These results suggest an important interaction between the systemic anaphylactic responses and the ability of allergen to pass through the gut epithelium and activate the immune cells in the lamina propria. In conjunction with these observations was the reduced numbers of Type 2-associated immune cells locally in the tissue as well as a reduced recall response from draining lymph node cultures. Similar studies using a peanut allergy model of anaphylaxis in mice in which mast cells were targeted found similar changes in the local Th2 response from the draining lymph nodes (34). Thus, these data suggest that reducing local gut mast numbers and local activation, mediated by IgE, may reduce the barrier leak of food antigens into the periphery and alter future responses, including protection from anaphylactic responses. This central premise may help to explain differences in how patients with systemic responses to food do or do not respond to a food challenge.

Epithelial barriers are established with multiple protein interactions that include claudins, occludins, junctional adhesion molecules and others that are differentially regulated to control movement of soluble antigens into the tissue and systemically (35–37). The critical role of these molecules has been established in models of inflammatory bowel diseases, but little is known about how their maintenance is associated with antigen sensitization (38). While more is known about barrier function and tolerance in food allergy, as well as the role of increased gastric acid production, the role of immune cell mediators in barrier function is not at well understood (39). Mast cells are the critical mediators of food allergy, but in order to trigger

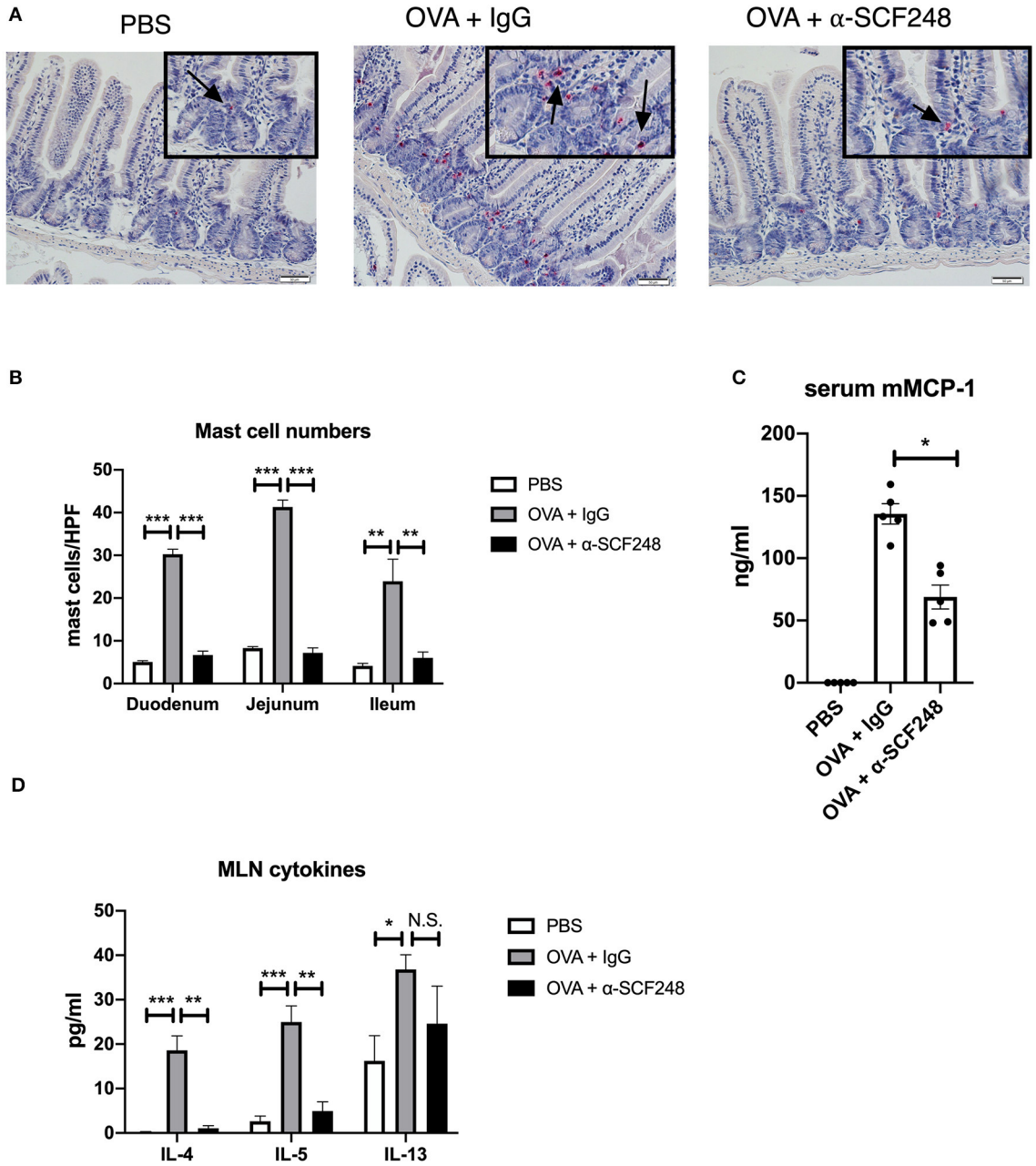


FIGURE 6 | Anti-SCF248 treatment decreases intestinal mast cells and Th2 inflammation in model of established food allergy. **(A)** Mast cells were enumerated following staining of sections with chloroacetate esterase to visualize cells. Details are shown in the inset boxes. Scale bar = 50 μm. Arrows point to mast cells. **(B)** Mast cell numbers per high powered field were determined by counting five sections each in the duodenum, jejunum, and ileum. **(C)** Murine mast cell protease was measured by ELISA to determine the extent of mast cell activation. **(D)** Mesenteric lymph nodes were dissociated and single cell suspensions restimulated with OVA. After 48 h of culture, Th2 cytokines were measured in the supernatants by Bioplex assay. Five mice were used in each group, and data represent one of three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

a reaction, antigen must bind to IgE on the mast cell in the lamina propria, which rapidly release a number of preformed mediators. Thus, food antigens must pass through the epithelial barrier. Mast cell tryptase, one of these mediators, has been shown to directly impact the epithelial barrier by decreasing junction adhesion molecule-A (JAM-A) (40). JAM-A is a critical protein in the tight junction, which is part of the mechanism that determines epithelial permeability. Previous studies have shown that mice that lack JAM-A in the intestinal epithelium have a leaky barrier, as determined by passage of 4 kDa FITC-dextran into the blood following oral gavage (30). Other studies have found that mast cell activation impacts epithelial barrier

function in the small intestine. These include *in vitro* studies have shown that mast cell chymase can affect the distribution of the tight junction proteins ZO-1 and occludin, and can reduce the expression of the claudin five protein (41, 42). A number of cytokines produced by mast cells also affect barrier integrity, including TNF-a, IL-1b, and IL-9 (31, 43–45). In this study, we show that increased mast cell activation results in greater passage of FITC-dextran across the barrier in allergic mice, suggesting that increased permeability to OVA would result in more severe anaphylactic symptoms. However, mast cell activation, as measured by murine mast cell protease-1, is decreased in mice treated with SCF248 neutralizing antibody, although it must be noted that we did not directly visualize mast cell degranulation in the lamina propria. Nevertheless, the decrease in local mast cell numbers and systemic detection mast cell protease-1 correlates with increased barrier function and decreased disease.

In a food allergic reaction, and individual is sensitized to food antigen, resulting in an established Th2 immune response. This is sustained not only by Th2 cells, but also by type 2 innate lymphoid cells in the lamina propria of the small intestine, as well as in the local lymph nodes (46–49). As ILC2s express c-kit, the receptor for SCF, neutralizing SCF248 has the potential to decrease the Th2 environment prior to allergic challenge. Indeed, we found that the number of ILC2s in the lamina propria as well as in the mesenteric lymph nodes were lower in mice treated with α-SCF248 compared to allergic mice treated with control IgG. Previous studies have found that increased mast cell numbers drive ILC2 expansion (50). Here, we find that both ILC2 numbers and mast cell numbers are decreased in mice treated with α-SCF248, however whether the decrease in ILC2 numbers is due to the lack of c-kit signaling on the cell or is linked to mast cell number is currently unknown. In addition, we found that the overall Th2 response was decreased, including the antigen specific T cell reaction in the lymph nodes, indicating that SCF248 is important for maintaining the Th2 environment in an allergic individual. This is further supported by the decreased number of eosinophils in the lamina propria of mice treated with α-SCF248 antibody.

In this study, we examined the role of SCF248 in sensitized animals. In some experiments, we began challenging the mice, and when they began to show symptoms, we neutralized SCF248. In this model, each subsequent challenge results in increased severity of anaphylactic symptoms, as mast cells continue to accumulate with each challenge. Therefore, we are able to halt the increase in mast cells following the initial challenges. Because all animals were sensitized equally, mice treated with α-SCF248 maintain similar levels of OVA-specific IgE compared to mice treated with control IgG. However, with fewer mast cells to bind to, this IgE is unable to activate a strong anaphylactic response. These results were maintained even in a delayed challenge model, in which SCF248 was neutralized following a full allergic response. Together, our findings suggest that SCF248 is an attractive target to minimize robust anaphylaxis in established food allergy.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by University of Michigan Institutional Animal Care and Use Committee.

AUTHOR CONTRIBUTIONS

CP and NWL conceived of the project and wrote the manuscript. CP, AJR, and WF performed the experiments and analyzed the data. All authors contributed to the article and approved the submitted version.

FUNDING

This study was funded by NIH grants R01HL138013 and R35HL150682, and a Michigan Food Allergy Research Accelerator (M-FARA) Grant from the Mary H. Weiser Food Allergy Center at the University of Michigan.

ACKNOWLEDGMENTS

We would like to thank Lisa Johnson, Diana Zhu, and Airy Garcia for technical assistance.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2021.604192/full#supplementary-material

Supplementary Figure 1 | Representative flow plots for cellular analysis. **(A)** Gating strategy for ILC2s. **(B)** Gating for mast cells, gated on $CD45^+$ cells. **(C)** Gating for eosinophils.

Supplementary Figure 2 | Baseline Th2 cytokines from lymph nodes are unchanged in OVA-treated mice compared to control. Mesenteric lymph nodes were dissociated and single cells suspensions were cultured without restimulation. Bioplex analysis was performed after 48 h.

Supplementary Figure 3 | JAM-A Western blot. Detection of JAM-A antibody, followed by stripping of the blot and reprobing with b-actin.

REFERENCES

1. Savage J, Johns CB. Food allergy: epidemiology and natural history. *Immunol Allergy Clin North Am*. (2015) 35:45–59. doi: 10.1016/j.iac.2014.09.004
2. Kattan J. The Prevalence and Natural History of Food Allergy. *Curr Allergy Asthma Rep*. (2016) 16:47. doi: 10.1007/s11882-016-0627-4
3. Sicherer SH, Sampson HA. Food allergy: A review and update on epidemiology, pathogenesis, diagnosis, prevention, and management. *J Allergy Clin Immunol*. (2018) 141:41–58. doi: 10.1016/j.jaci.2017.11.003

4. Gupta RS, Walkner MM, Greenhawt M, Lau CH, Caruso D, Wang X, et al. Food Allergy Sensitization and Presentation in Siblings of Food Allergic Children. *J Allergy Clin Immunol Pract*. (2016) 4:956–62. doi: 10.1016/j.jaip.2016.04.009
5. Hagel AF, Derossi T, Zopf Y, Konturek P, Dauth W, Kressel J, et al. Mast cell tryptase levels in gut mucosa in patients with gastrointestinal symptoms caused by food allergy. *Int Arch Allergy Immunol*. (2013) 160:350–5. doi: 10.1159/000341634
6. Valenta R, Hochwallner H, Linhart B, Pahr S. Food allergies: the basics. *Gastroenterology*. (2015) 148:1120–1131.e1124. doi: 10.1053/j.gastro.2015.02.006
7. Shek LP, Soderstrom L, Ahlstedt S, Beyer K, Sampson HA. Determination of food specific IgE levels over time can predict the development of tolerance in cow's milk and hen's egg allergy. *J Allergy Clin Immunol*. (2004) 114:387–91. doi: 10.1016/j.jaci.2004.04.032
8. Elizur A, Rajuan N, Goldberg MR, Leshno M, Cohen A, Katz Y. Natural course and risk factors for persistence of IgE-mediated cow's milk allergy. *J Pediatr*. (2012) 161:482–487.e481. doi: 10.1016/j.jpeds.2012.02.028
9. Sicherer SH, Wood RA, Vickery BP, Jones SM, Liu AH, Fleischer DM, et al. The natural history of egg allergy in an observational cohort. *J Allergy Clin Immunol*. (2014) 133:492–9. doi: 10.1016/j.jaci.2013.12.1041
10. Stone KD, Prussin C. Immunomodulatory therapy of eosinophil-associated gastrointestinal diseases. *Clin Exp Allergy*. (2008) 38:1858–65. doi: 10.1111/j.1365-2222.2008.03122.x
11. Straumann A, Conus S, Grzonka P, Kita H, Kephart G, Bussmann C, et al. Anti-interleukin-5 antibody treatment. (mepolizumab) in active eosinophilic oesophagitis: a randomised, placebo-controlled, double-blind trial. *Gut*. (2010) 59:21–30. doi: 10.1136/gut.2009.178558
12. Chinthrajah S, Cao S, Liu C, Lyu SC, Sindher SB, Long A, et al. Phase 2a randomized, placebo-controlled study of anti-IL-33 in peanut allergy. *JCI Insight*. (2019) 4. doi: 10.1172/jci.insight.131347
13. Rial MJ, Barroso B, Sastre J. Dupilumab for treatment of food allergy. *J Allergy Clin Immunol Pract*. (2019) 7:673–4. doi: 10.1016/j.jaip.2018.07.027
14. Albuhairi S, Rachid R. Novel Therapies for Treatment of Food Allergy. *Immunol Allergy Clin North Am*. (2020) 40:175–86. doi: 10.1016/j.iac.2019.09.007
15. Galli SJ, Tsai M, Wershil BK. The c-kit receptor, stem cell factor, and mast cells. *What each is teaching us about the others Am J Pathol*. (1993) 142:965–74.
16. Mcniece IK, Briddell RA. Stem cell factor. *J Leukoc Biol*. (1995) 58:14–22. doi: 10.1002/jlb.58.1.14
17. Williams, D. A., and Majumdar, M. K. (1994). Analysis of steel factor. (stem cell factor) isoforms in the hematopoietic microenvironment. *Stem Cells 12 Suppl 1, 67-74; discussion* 75–67.
18. Smith, K. A., and Zsebo, K. M. (2001). Measurement of human and murine stem cell factor. (c-kit ligand). *Curr Protoc Immunol Chapter* 6, Unit 6.17.
19. Hsu YR, Wu GM, Mendiaz EA, Syed R, Wypych J, Toso R, et al. The majority of stem cell factor exists as monomer under physiological conditions. *Implications for dimerization mediating biological activity J Biol Chem*. (1997) 272:6406–15. doi: 10.1074/jbc.272.10.6406
20. Kapur R, Majumdar M, Xiao X, Mcandrews-Hill M, Schindler K, Williams DA. Signaling through the interaction of membrane-restricted stem cell factor and c-kit receptor tyrosine kinase: genetic evidence for a differential role in erythropoiesis. *Blood*. (1998) 91:879–89. doi: 10.1182/blood.V91.3.879
21. Tajima Y, Moore MA, Soares V, Ono M, Kissel H, Besmer P. Consequences of exclusive expression in vivo of Kit-ligand lacking the major proteolytic cleavage site. *Proc Natl Acad Sci U S A*. (1998) 95:11903–8. doi: 10.1073/pnas.95.20.11903
22. Fonseca W, Rasky AJ, Ptaschinski C, Morris SH, Best SKK, Phillips M, et al. Group 2 innate lymphoid cells. (ILC2) are regulated by stem cell factor during chronic asthmatic disease. *Mucosal Immunol*. (2019) 12:445–56. doi: 10.1038/s41385-018-0117-1
23. Rasky A, Habiel DM, Morris S, Schaller M, Moore BB, Phan S, et al. Inhibition of the stem cell factor 248 isoform attenuates the development of pulmonary remodeling disease. *Am J Physiol Lung Cell Mol Physiol*. (2020) 318:L200–l211. doi: 10.1152/ajplung.00114.2019
24. Li XM, Serebrisky D, Lee SY, Huang CK, Bardina L, Schofield BH, et al. A murine model of peanut anaphylaxis: T- and B-cell responses to a major peanut allergen mimic human responses. *J Allergy Clin Immunol*. (2000) 106:150–8. doi: 10.1067/mai.2000.107395
25. Friend DS, Ghildyal N, Austen KF, Gurish MF, Matsumoto R, Stevens RL. Mast cells that reside at different locations in the jejunum of mice infected with Trichinella spiralis exhibit sequential changes in their granule ultrastructure and chymase phenotype. *J Cell Biol*. (1996) 135:279–90. doi: 10.1083/jcb.135.1.279
26. Benedé, S., Cody, E., Agashe, C., and Berin, M. C. (2018). Immune Characterization of Bone Marrow-Derived Models of Mucosal and Connective Tissue Mast Cells. *Allergy Asthma Immunol Res* 10, 268–277. doi: 10.4168/aair.2018.10.3.268
27. Azcutia V, Kelm M, Luissint AC, Boerner K, Flemming S, Quiros M, et al. *Neutrophil expressed CD47 regulates CD11b/CD18-dependent neutrophil transepithelial migration in the intestine in vivo*. *Mucosal Immunol*. (2020). doi: 10.1038/s41385-020-0316-4
28. Harlow E, Lane D. Bradford assay. *CSH Protoc*. (2006) 2006. doi: 10.1101/pdb.prot4644
29. Kucuk ZY, Strait R, Khodoun MV, Mahler A, Hogan S, Finkelman FD. Induction and suppression of allergic diarrhea and systemic anaphylaxis in a murine model of food allergy. *J Allergy Clin Immunol*. (2012) 129:1343–8. doi: 10.1016/j.jaci.2012.03.004
30. Laukoetter MG, Nava P, Lee WY, Severson EA, Capaldo CT, Babbin BA, et al. JAM-A regulates permeability and inflammation in the intestine in vivo. *J Exp Med*. (2007) 204:3067–76. doi: 10.1084/jem.20071416
31. Forbes EE, Groschwitz K, Abonia JP, Brandt EB, Cohen E, Blanchard C, et al. IL-9- and mast cell-mediated intestinal permeability predisposes to oral antigen hypersensitivity. *J Exp Med*. (2008) 205:897–913. doi: 10.1084/jem.20071046
32. Ahrens R, Osterfeld H, Wu D, Chen CY, Arumugam M, Groschwitz K, et al. Intestinal mast cell levels control severity of oral antigen-induced anaphylaxis in mice. *Am J Pathol*. (2012) 180:1535–46. doi: 10.1016/j.ajpath.2011.12.036
33. Albert-Bayo M, Paracuellos I, González-Castro AM, Rodríguez-Urrutia A, Rodríguez-Lagunas MJ, Alonso-Cotoner C, et al. Intestinal Mucosal Mast *Cells*: Key Modulators of Barrier Function and Homeostasis. *Cells*. (2019) 8. doi: 10.3390/cells8020135
34. Burton OT, Noval Rivas M, Zhou JS, Logsdon SL, Darling AR, Koleoglou KJ, et al. Immunoglobulin E signal inhibition during allergen ingestion leads to reversal of established food allergy and induction of regulatory T cells. *Immunity*. (2014) 41:141–51. doi: 10.1016/j.immuni.2014.05.017
35. Stevenson BR, Siliciano JD, Mooseker MS, Goodenough DA. Identification of ZO-1: a high molecular weight polypeptide associated with the tight junction. (zonula occludens) in a variety of epithelia. *J Cell Biol*. (1986) 103:755–66. doi: 10.1083/jcb.103.3.755
36. Furuse M, Hirase T, Itoh M, Nagafuchi A, Yonemura S, Tsukita S, et al. Occludin: a novel integral membrane protein localizing at tight junctions. *J Cell Biol*. (1993) 123:1777–88. doi: 10.1083/jcb.123.6.1777
37. Martìn-Padura I, Lostaglio S, Schneemann M, Williams L, Romano M, Fruscella P, et al. Junctional adhesion molecule, a novel member of the immunoglobulin superfamily that distributes at intercellular junctions and modulates monocyte transmigration. *J Cell Biol*. (1998) 142:117–27. doi: 10.1083/jcb.142.1.117
38. Munkholm P, Langholz E, Hollander D, Thornberg K, Orholm M, Katz KD, et al. Intestinal permeability in patients with Crohn's disease and ulcerative colitis and their first degree relatives. *Gut*. (1994) 35:68–72. doi: 10.1136/gut.35.1.68
39. Samadi N, Klems M, Untersmayr E. The role of gastrointestinal permeability in food allergy. *Ann Allergy Asthma Immunol*. (2018) 121:168–73. doi: 10.1016/j.anai.2018.05.010
40. Wilcz-Villega, E. M., Mcclean, S., and O'sullivan, M. A. (2013). Mast cell tryptase reduces junctional adhesion molecule-A. (JAM-A) expression in intestinal epithelial cells: implications for the mechanisms of barrier dysfunction in irritable bowel syndrome. *Am J Gastroenterol* 108, 1140–1151. doi: 10.1038/ajg.2013.92
41. Scudamore CL, Jepson MA, Hirst BH, Miller HR. The rat mucosal mast cell chymase, RMCP-II, alters epithelial cell monolayer permeability in association with altered distribution of the tight junction proteins ZO-1 and occludin. *Eur J Cell Biol*. (1998) 75:321–30. doi: 10.1016/S0171-9335(98)80065-4

42. Groschwitz KR, Wu D, Osterfeld H, Ahrens R, Hogan SP. Chymase-mediated intestinal epithelial permeability is regulated by a protease-activating receptor/matrix metalloproteinase-2-dependent mechanism. *Am J Physiol Gastrointest Liver Physiol*. (2013) 304:G479–489. doi: 10.1152/ajpgi.00186.2012
43. Mankertz J, Tavalali S, Schmitz H, Mankertz A, Riecken EO, Fromm M, et al. Expression from the human occludin promoter is affected by tumor necrosis factor alpha and interferon gamma. *J Cell Sci*. (2000) 113:2085–90.
44. Zolotarevsky Y, Hecht G, Koutsouris A, Gonzalez DE, Quan C, Tom J, et al. A membrane-permeant peptide that inhibits MLC kinase restores barrier function in in vitro models of intestinal disease. *Gastroenterology*. (2002) 123:163–72. doi: 10.1053/gast.2002.34235
45. Al-Sadi R, Ye D, Said HM, Ma TY. IL-1beta-induced increase in intestinal epithelial tight junction permeability is mediated by MEKK-1 activation of canonical NF-kappaB pathway. *Am J Pathol*. (2010) 177:2310–22. doi: 10.2353/ajpath.2010.100371
46. Amoli MM, Hand S, Hajeer AH, Jones KP, Rolf S, Sting C, et al. Polymorphism in the STAT6 gene encodes risk for nut allergy. *Genes Immun*. (2002) 3:220–4. doi: 10.1038/sj.gene.6363872
47. Sicherer SH, Wood RA, Stablein D, Burks AW, Liu AH, Jones SM, et al. Immunologic features of infants with milk or egg allergy enrolled in an observational study. (Consortium of Food Allergy Research) of food allergy. *J Allergy Clin Immunol*. (2010) 125:1077–1083.e1078. doi: 10.1016/j.jaci.2010.02.038
48. Lee JB, Chen CY, Liu B, Mugge L, Angkasekwinai P, Facchinetti V, et al. IL-25 and CD4(+) TH2 cells enhance type 2 innatelymphoid cell-derived IL-13 production, which promotes IgE-mediated experimental food allergy. *J Allergy Clin Immunol*. (2016) 137:1216–1225.e1215. doi: 10.1016/j.jaci.2015.09.019
49. Noval Rivas M, Burton OT, Oettgen HC, Chatila T. IL-4 production by group 2 innate lymphoid cells promotes food allergy by blocking regulatory T-cell function. *J Allergy Clin Immunol*. (2016) 138:801–811.e809. doi: 10.1016/j.jaci.2016.02.030
50. Burton OT, Medina Tamayo J, Stranks AJ, Miller S, Koleoglou KJ, Weinberg EO, et al. IgE promotes type 2 innate lymphoid cells in murine food allergy. *Clin Exp Allergy*. (2018) 48:288–96. doi: 10.1111/cea.13075

Conflict of Interest: NL was a co-founder of Opsidio, LLC, which is developing anti-SCF248 for commercial use.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

ORIGINAL RESEARCH
published: 25 March 2021
doi: 10.3389/fimmu.2021.636198

Thermoneutrality Alters Gastrointestinal Antigen Passage Patterning and Predisposes to Oral Antigen Sensitization in Mice

*Taeko K. Noah[1,2], Jee-Boong Lee[3], Christopher A. Brown[4], Amnah Yamani[1,2], Sunil Tomar[1,2], Varsha Ganesan[1,2], Rodney D. Newberry[5], Gary B. Huffnagle[2,6], Senad Divanovic[7,8,9] and Simon P. Hogan[1,2]**

[1] Department of Pathology, University of Michigan, Ann Arbor, MI, United States, [2] Mary H Weiser Food Allergy Center, University of Michigan, Ann Arbor, MI, United States, [3] Division of Allergy and Immunology, Cincinnati Children's Medical Center, Cincinnati, OH, United States, [4] Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, University of Michigan, Ann Arbor, MI, United States, [5] Department of Medicine, Division of Gastroenterology, Washington University School of Medicine, St. Louis, MO, United States, [6] Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI, United States, [7] Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH, United States, [8] Division of Immunobiology, Cincinnati Children's Medical Center, Cincinnati, OH, United States, [9] Center for Inflammation and Tolerance, Cincinnati Children's Medical Center, Cincinnati, OH, United States

OPEN ACCESS

Edited by:
Karen Laky,
National Institute of Allergy and Infectious Diseases (NIH), United States

Reviewed by:
Michael D. Kulis,
University of North Carolina at Chapel Hill, United States
Clinton Mathias,
Western New England University, United States

***Correspondence:**
Simon P. Hogan
sihogan@med.umich.edu

Specialty section:
This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology

Received: 01 December 2020
Accepted: 05 March 2021
Published: 25 March 2021

Citation:
Noah TK, Lee J-B, Brown CA, Yamani A, Tomar S, Ganesan V, Newberry RD, Huffnagle GB, Divanovic S and Hogan SP (2021) Thermoneutrality Alters Gastrointestinal Antigen Passage Patterning and Predisposes to Oral Antigen Sensitization in Mice. Front. Immunol. 12:636198. doi: 10.3389/fimmu.2021.636198

Food allergy is an emerging epidemic, and the underlying mechanisms are not well defined partly due to the lack of robust adjuvant free experimental models of dietary antigen sensitization. As housing mice at thermoneutrality (Tn) - the temperature of metabolic homeostasis (26–30°C) – has been shown to improve modeling various human diseases involved in inflammation, we tested the impact of Tn housing on an experimental model of food sensitization. Here we demonstrate that WT BALB/c mice housed under standard temperature (18–20°C, Ts) conditions translocated the luminal antigens in the small intestine (SI) across the epithelium *via* goblet cell antigen passages (GAPs). In contrast, food allergy sensitive *Il4ra*^{F709} mice housed under standard temperature conditions translocated the luminal antigens in the SI across the epithelium *via* secretory antigen passages (SAPs). Activation of SI antigen passages and oral challenge of *Il4ra*^{F709} mice with egg allergens at standard temperature predisposed *Il4ra*^{F709} mice to develop an anaphylactic reaction. Housing *Il4ra*^{F709} mice at Tn altered systemic type 2 cytokine, IL-4, and the landscape of SI antigen passage patterning (villus and crypt involvement). Activation of SI antigen passages and oral challenge of *Il4ra*^{F709} mice with egg antigen under Tn conditions led to the robust induction of egg-specific IgE and development of food-induced mast cell activation and hypovolemic shock. Similarly, Tn housing of WT BALB/c mice altered the cellular patterning of SI antigen passage (GAPs to SAPs). Activation of SI antigen passages and the oral challenge of WT BALB/c mice with egg antigen led to systemic reactivity to egg and mast cell activation. Together these

data demonstrate that Tn housing alters antigen passage cellular patterning and landscape, and concurrent oral exposure of egg antigens and SAP activation is sufficient to induce oral antigen sensitization.

Keywords: food sensitization, mouse models, antigen passage, thermoneutral conditions, food allergy

INTRODUCTION

Food allergy is an emerging epidemic (1) that is estimated to affect 32 million people in the United States (2, 3). Clinical and experimental studies have advanced our understanding of food allergy pathogenesis by revealing that food sensitization, characterized by the presence of allergen-specific IgE and $CD4^+$ Th2 cells, is pathognomonic to disease (4). Although food sensitization can occur at various mucosal surfaces, including the skin and gastrointestinal tract (5, 6), the underlying mechanisms that drive food sensitization in humans have been elusive. This is in part due to mice resistance to becoming sensitized to allergens (environmental, aeroallergen, and foods) naturally, and the requirement of adjuvants such as aluminum hydroxide, cholera toxin, and staphylococcus enterotoxin B to break oral tolerance and induce sensitization to dietary antigens (7). Recent studies have demonstrated that oral exposure of food allergens in the absence of adjuvant to mice with enhanced IL4Rα signaling by a gain of function mutation (*Il4ra*^{F709} mice) is sufficient to promote allergen-specific Th2 and IgE responses (8, 9). Subsequent studies revealed that heightened IL-4 signaling promoted reprogramming of regulatory T cells to Th2 (10), enhanced the number and function of ILC2 cells (11), and activated mast cells that perpetuated the IgE-mast cell response following food allergen exposure (12). Collectively, these studies revealed a role for the hematopoietic compartment in oral food sensitization in under heightened IL-4ra signaling. However, the contribution of the non-hematopoietic compartment to food sensitization and reactivity was largely unexplored (9).

Goblet cell antigen passages (GAP) are a mechanism by which the intestinal epithelium passages luminal antigens to underlying immune cells (antigen presenting cells) to mount a tolerizing response (13). The formation of GAPs in the gastrointestinal tract facilitates the tolerenergic environment by maintaining regulatory T cells, modulating dendritic cell function, and inducing IL-10 production by macrophages (13). Blockade of GAPs impairs oral tolerance as such that oral allergen exposure failed to prevent allergen specific T cell responses driven by the subcutaneous immunization (13). Accordingly, various signals including the microbiome, pathogens, epidermal growth factor (EGF), and carbachol (CCh) have been demonstrated to modulate antigen passage cellular patterning (intestinal epithelial cell population involvement) and landscape of antigen passages (villus to crypt involvement) to ensure the proper tolerizing response to the innocuous antigens (14–16). We recently demonstrated that under food allergic conditions the small intestine (SI) antigen passage cellular patterning and landscape were altered. In a food allergic state, antigen passages formed through goblet, enteroendocrine, and Paneth cells (termed SAPs) and were present in both the villus and crypt epithelium following dietary allergen exposure (17). Blockade of SAP formation inhibited a food-induced anaphylactic response suggesting the deviation in antigen passage cellular patterning and landscape is associated with allergic reactions toward dietary antigens. Currently, the contribution of altered antigen passage cellular patterning and landscape in the SI in driving food sensitization remains unexplored.

Recent investigations demonstrate that housing temperature robustly influences mice physiology (18). Vivariums in research facilities throughout the world maintain laboratory mice at 18-20°C (standard housing temperature, Ts). Under Ts conditions, mice are under chronic cold stress, exhibiting elevated stress hormones, including corticosterone (rodent stress hormone) and catecholamines (neurotransmitters to induce fight or flight responses through the sympathetic nervous system) (19, 20). Consequently, Ts housed mice exhibit elevated heart rate and oxygen consumption and dampened immune cell functions as an adaptation to the cold housing temperature (18). The ambient temperature for mice to maintain thermoneutrality is 26-30°C (Tn 30°C). At this temperature, mice do not activate thermogenic pathways to sustain core body temperature (18). Notably, at this ambient temperature Tn, the heightened stress hormone response, immune suppression, and the heightened metabolic rates observed in Ts housed mice are significantly reduced (18).

One of the limitations of studying human immunological disease processes in mice is that mice do not necessarily respond to immunological challenges similarly to humans. For example, mice are resistant to developing human disease conditions such as obesity (21), atherosclerosis (22), and severe non-alcoholic fatty liver disease (NAFLD) (23). Recent studies have revealed that the observed differences in physiological outcomes in mice are a consequence of the housing temperature at which the mice are maintained. Given the historical and recent reports of the inhibitory effects of the stress hormones on the innate and adaptive immune functions (24, 25), we examined the impact of altering the housing temperature on the intestinal immune environment and test the effects of Tn housing temperature on oral food sensitization in mice.

Herein, we show that antigen passage cellular patterning was altered in *IL4ra*^{F709} mice at Ts conditions compared to WT BALB/c mice. Activation of antigen passage formation predisposed *IL4ra*^{F709} mice to oral food sensitization at Ts conditions. Tn housing of *IL4ra*^{F709} mice altered the antigen passage landscape and increased penetrance of food sensitization and reactivity. Tn housing of WT BALB/c mice altered the SI

antigen passage cellular patterning (GAP to SAP) and landscape promoting passage formation from the villus to the crypt region. Activation of antigen passages and oral exposure of Tn conditioned housed *IL4ra*^{F709} and WT BALB/c mice to egg antigen significantly exacerbated allergen-specific IgE and IgG1 responses and led to hypovolemic shock that coincided with the activation of mucosal mast cells following systemic and oral allergen challenge.

MATERIALS AND METHODS

Animals

BALB/c WT and *Il4ra*^{F709} mice (provided from Fred D. Finkelman at Cincinnati Children's Hospital Medical Center, CCHMC) were maintained and bred under standard housing conditions (T_S temperature, 18-20°C). Mice were transferred to the thermoneutral housing conditions (Tn temperature, 30-33°C) after weaning and acclimated for at least two weeks prior to experimentation. We have previously demonstrated that two weeks is sufficient to decrease serum stress hormones in WT mice (23). The Tn conditions are provided by the University of Michigan Unit for Laboratory Animal Medicine within the specific pathogen-free facility as part of IACUC-approved animal protocol. The thermostat of a small room (~ 72 ft^2) within the facility was set at 30°C (86°F), with a 12-hour light/dark cycle. The room is equipped with a laminar flow workstation and a Rodent Cage system to maintain the mice in the room at all times. Mouse cages are maintained in a Rodent Cage system that delivers HEPA-filtered ventilated air and is equipped with an automated water supply to individual cages providing air and water at ambient temperature. The room temperature is monitored daily to ensure the temperature is maintained between 27.7-31.1 °C (82-88 °F). Six to 10-week old mice were used for all the experiments described in the study. All animals were maintained and used as approved by the Animal Care and Use Committee at CCHMC and the University of Michigan.

Reagents

Food allergen, egg white powder from Jay Robb Enterprises (North Palm Beach, FL) was stored at -20°C until the time of use. Reagents used are as follows: Imaging antigen, lysine fixable dextran tetramethylrhodamine in 10,000 MW (Invitrogen), CCh, and paraformaldehyde (Sigma-Aldrich). Antibodies used are as follows: wheat germ agglutinin (WGA) conjugated to Alexa 488 (Invitrogen), rat anti-mouse IgE (BD Bioscience), anti rat IgG conjugated to biotin (Vector lab), avidin conjugated to HRP (BD Bioscience), or anti-mouse IgG1 (Abcam) conjugated to HRP.

Oral Sensitization and Measurement of Food Sensitization Parameters

Il4ra^{F709} mice were orally exposed to egg 23 mg in 400 µl water twice a week for two weeks. The mice were fasted for 5 hours and treated subcutaneously with either saline or CCh (3 µg/mouse in 100 µl saline) 15 minutes before oral food allergen exposure. The serum was harvested on day 14. On day 16, mice were challenged intravenously with 100 µg of egg allergen, and body temperature and hemoconcentration were measured as previously described (26). For oral food reactivity, *Il4ra*^{F709} mice were exposed to the food allergens over 8 weeks with twice a week of oral allergen exposure following either saline or CCh treatment. Mice were orally challenged with egg allergen (23 mg) in 400 µl water every 2 weeks after 4 weeks of oral allergen exposure, and serum was harvested following each oral allergen challenge. WT BALB/c mice were exposed to egg 23 mg in 400 µl water twice a week for four weeks. Each oral allergen exposure was performed as described earlier. The serum was harvested on day 28. On day 30, mice were challenged intravenously with 100 µg of egg allergen, and blood was harvested, hemoconcentration were measured as previously described (26).

Flow Cytometry and Single-Cell Preparation

Mononuclear cells in the lamina propria (LP) from the small intestine (SI) were isolated as previously described (27). The isolated cells were stained with anti CD3 conjugated to Brilliant Violet 605, anti CD4 conjugated to Horizon V500, anti IL-25R conjugated to allophycocyanin (APC), anti GITR conjugated to FITC, anti OX40 conjugated to Pacific Blue, anti CD8 conjugated to APC Cy7, anti c-kit conjugated to Brilliant Violet 711 and anti FcεR conjugated to phycoerythrin (PE)-Cy7. Anti γδTCR conjugated to PE, followed by counterstain with lineage markers (CD11b, CD11c, GR1, B220) conjugated with PerCP-Cy5.5. Separately the isolated cells were stained with anti B220 conjugated with APC, anti CD3 conjugated with PerCP-CY5.5, anti CD64 conjugated to Pacific Blue, anti CD11c conjugated to PE, anti CD11b conjugated to FITC, anti MHCII conjugated to APC-Cy7, and anti CD103 conjugated to PE-Cy7. Stained cells were analyzed with FACSCanto I (B.D. Bioscience) or Novocyte (ACEA Bioscience).

RNA Extraction and Quantitative PCR Analysis

The intestinal epithelium isolated from the mononuclear cell preparation of the lamina propria were used to extract RNA as previously described (28). 1 µg of total RNA was reverse transcribed and analyzed using SYBR green based real-time PCR with the following primer sets; Il25, ACAGGGACTTGA ATCGGGTC and TGGTAAAGTGGGACGGAGTTG.

Enzyme-Linked Immunosorbent Assay (ELISA)

Serum MCPT-1 was analyzed with a kit (eBioscience) as described by the manufacture. Allergen specific IgE and IgG1 were captured with allergens coating of the plate, and the levels were detected with rat anti-mouse IgE along with biotinylated anti rat IgG or biotinylated IgG1, detection antibodies in the presence of TMB substrate solution (B.D. Bioscience). The *in vitro* cytokine capture assay (IVCCA) for IL-4 was performed as previously described (29). Serum corticosterone was measured by a kit as described by the manufacture (Arbor Assays)

Histology, Immunofluorescence, and Microscopy

Harvested tissues were fixed and processed as previously described (17). Immunofluorescence images were acquired with a Zeiss Apotome, and bright-field images were captured with an Olympus BX51. Antigen passage was assessed and quantified per villus or crypts as previously described (17).

Microbiome Analysis

Stools were collected from mice housed at the standard temperature. The mice were transferred to the thermoneutral temperature to collect stools after the acclimation period. DNA was isolated from the stools, as previously described (30). DNA library was prepared and sequenced with a MiSeq instrument from Illumina at the University of Michigan. Sequence data were processed and analyzed as previously described (30). Briefly, the data was processed with mothur (v.1.42.3) and put through standardization methods for community ecology using the vegan package (version 2.5.6) in R. Operational taxonomic units (OTUs) were binned at 97% similarity, and Bacteria_unclassified and any OTUs which did not cross an abundance threshold of 0.05% in any sample were excluded from the data. PERMANOVA was used for the statistical analysis.

Statistical Analysis

Student t-test or one-way ANOVA was performed to determine statistical significance using GraphPad Prism 8 unless otherwise noted. $P<0.05$ is considered statistical significance unless otherwise noted.

RESULTS

Antigen Passage Cellular Patterning Is Altered in *Il4ra*^{F709} Mice Under Standard Housing Conditions

Previous studies have reported that oral antigen exposure of *Il4ra*^{F709} and not WT BALB/C mice induce food sensitization due to the effects of heightened IL-4rα signaling on the immune compartment (31). To determine whether the differential immunological responses to dietary antigen exposure were related to differences in antigen passage formation, we evaluated steady state antigen passages in WT BALB/c and *Il4ra*^{F709} mice. We show that the frequency of antigen passages in both saline-treated *Il4ra*^{F709} and WT BALB/c mice was comparable, ~ 0.5 antigen passages per villus (**Figures 1A–D**). The antigen passages in the saline-treated WT BALB/c mice were restricted to wheat germ agglutinin $(WGA)^+$ cells indicating goblet cell-restricted antigen passages (GAPs). In contrast, we observed WGA^+ and WGA^- antigen passages in the saline-treated *Il4ra*^{F709} mice, indicating the presence of SAPs (**Figures 1B** and **D**). Consistent with previous reports, treatment of WT BALB/c mice with carbachol (CCh) enhanced the frequency of GAPs in the villus (**Figures 1A** and **C**, green arrow). CCh treatment of *Il4ra*^{F709} mice induced a significant increase in the frequency of WGA^+ and WGA^- antigen passages (**Figures 1B, D**, red arrows). The presence of SAPs was confirmed by the demonstration that WGA^- antigen passages in *Il4ra*^{F709} mice were enteroendocrine cells (17) (data not shown). Collectively, these data indicate that genetic manipulation of IL4Rα is sufficient to alter SI antigen passage cellular patterning at a steady state.

To gain insight into the impact of hyperactivation of IL4-pathway on gastrointestinal immunity in *Il4ra*^{F709} mice housed

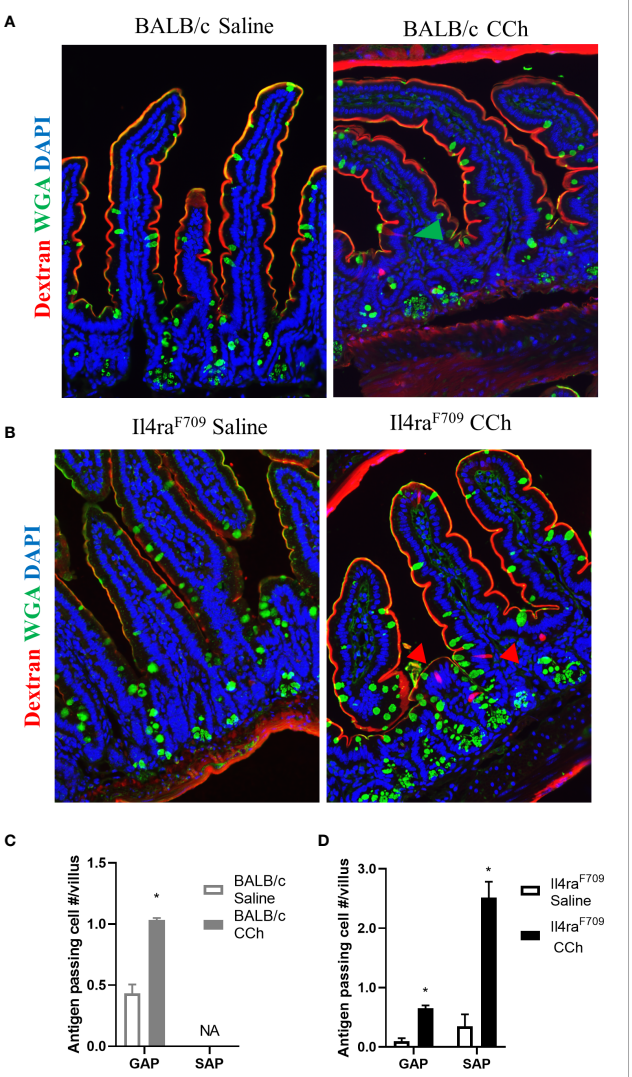


FIGURE 1 | Antigen passage formation is altered in *Il4ra*^{F709}. Immunofluorescence analysis for WGA (green) to identify goblet cells and dextran (red) with the small intestine of **(A)** wild type BALB/c mice and **(B)** *Il4ra*^{F709} mice treated with saline or CCh. The nucleus is visualized with DAPI (blue). The green arrow points to GAP, and the red arrows point to non-GAPs. Quantification of antigen passage formation in **(C)** wild type BALB/c and **(D)** *Il4ra*^{F709} mice treated with saline or CCh. n=3 per group. *denotes statistical significance, $p<0.05$. NA indicates 0 value. SAP includes antigen passages across goblet and non-goblet cells.

under Ts conditions, we examined the intestinal immune tissues of Ts housed WT BALB/c and *Il4ra*^{F709} mice at a steady state. Flow cytometry analysis revealed that Th2, ILC2, Treg, γδ T cells, dendritic cells, and mast cell frequency in the SI were unaltered in *Il4ra*^{F709} mice (**Table 1**). However, total CD4$^+$ and CD8$^+$ T cells were significantly increased in the SI of *Il4ra*^{F709} mice compared to the WT BALB/c mice (**Table 1**). Consistent with previous reports, these results indicate that the intestinal immune compartment of *Il4ra*^{F709} housed at Ts condition is not biased toward Th2 at a steady state (10).

Activation of SAPs Is Associated With Increased Oral Food Sensitization Incidence in *Il4ra*^{F709} Mice Under Standard Housing Conditions

Given *Il4ra*^{F709} mice are susceptible to IgE-mediated food allergy (9), and we have previously demonstrated an association between SAP formation and IgE-mediated food allergic reactions, we examined the potential role of SAPs in oral food sensitization in *Il4ra*^{F709} mice. *Il4ra*^{F709} mice received repeated oral exposure of egg followed by either vehicle (saline) or CCh treatment to induce antigen passage formation (**Figure 2A**). 16 days following the first oral exposure, mice received systemic antigen challenge to assess for sensitization. Notably, systemic antigen challenge of CCh-treated *Il4ra*^{F709} mice lead to a shock response (decrease in core body temperature) in 55% of the mice compared to 0% of the saline treated *Il4ra*^{F709} mice (**Figure 2B**). The incidence of shock following systemic allergen challenge was significantly higher in the CCh-treated *Il4ra*^{F709} mice (**Figure 2B**), suggesting that oral antigen exposure and SAP activation predisposed to sensitization in *Il4ra*^{F709} mice. This was supported by the demonstration of a strong negative correlation between antigen-specific IgE and shock response (**Figure 2C**) and a positive correlation between antigen-specific IgE and hemoconcentration (evidence of hypovolemic shock) (**Figure 2D**). Importantly, we observed a positive correlation between serum MCPT-1 (mast cell activation) and antigen-specific IgE in *Il4ra*^{F709} mice demonstrating IgE-mast cell activation. Collectively, these results demonstrate that activation of SAPs in *Il4ra*^{F709} mice was associated with antigen sensitization and IgE-mast cell-mediated systemic reactions.

TABLE 1 | List of the immune cells analyzed in the LP SI of wild type BALB/c and *Il4ra*^{F709} mice.

Cell Population (absolute cell #)	WT BALB/C Ts	Y709F Ts
CD4$^+$ T cells (Lin$^-$ CD3$^+$CD4$^+$)	3.60 ± 0.27 x 10^5	5.40 ± 0.71 x 10^{5*}
CD8+ T cells (Lin$^-$CD4$^-$CD8$^+$)	1.17 ± 0.14 x 10^5	2.24 ± 0.41 x 10^{5*}
CD4$^+$ Th2 T cells (Lin$^-$ CD3$^+$CD4$^+$ IL17RB$^+$)	0.76 ± 0.11x 10^4	1.12 ± 0.20 x 10^4
CD4$^+$ Treg cells (Lin$^-$ CD3$^+$CD4$^+$ GITR$^+$ OX40$^+$)	3.87 ± 0.30 x 10^4	4.70 ± 0.53 x 10^4
γδ T cells (Lin$^-$ CD4$^-$ CD8$^-$ γδ TCR$^+$)	4.87 ± 0.82 x 10^4	6.29 ± 1.1x 10^4
ILC2 (Lin$^-$ CD3$^-$CD4$^-$ ckit$^-$ IL17RB$^+$)	1.55 ± 0.17 x 10^5	1.45 ± 0.11 x 10^5
MC (Lin$^-$CD4$^-$IL17RB$^-$ckit$^+$FcεR$^+$)	3.68 ± 0.61 x 10^4	4.25 ± 0.73 x 10^4
B cells (CD3$^-$CD64$^-$B220$^+$CD11c$^-$)	7.0 ± 1.1 x 10^5	3.25 ± 1.0 x 10^{5*}
Plasmatoid DCs (CD3$^-$CD64$^-$ B220$^+$CD11c$^+$)	1.02 ± 0.09 x 10^5	0.93 ± 0.09 x 10^5
CD103+CD11b- DCs (CD3$^-$CD64$^-$B220$^-$ CD11c$^+$MHCII$^+$CD11b$^-$CD103$^+$)	1.9 ± 0.20 x 10^4	1.7 ± 0.25 x 10^4
CD103+CD11b+ DCs(CD3$^-$CD64$^-$B220$^-$ CD11c$^+$MHCII$^+$CD11b$^+$CD103$^+$)	5.2 ± 0.90 x 10^3	5.7 ± 0.73 x 10^3
CD103- DCs (CD3$^-$CD64$^-$B220$^-$ CD11c$^+$MHCII$^+$CD103$^-$)	1.2 ± 0.09 x 10^4	1.86 ± 0.30 x 10^4

**denotes statistical significance. Lin, lineage; MC, mast cells; DC, dendritic cells.*

Thermoneutral Housing Has Minimum Effects on the Gastrointestinal Immune Compartment in *Il4ra*^{F709} Mice

We have previously reported that Tn housing decreases serum stress hormones in WT C57BL/6 mice (23). To confirm the Tn housing effect across the different mouse strains, we examined the systemic stress hormone level in the WT BALB/c mice housed under Ts and Tn conditions. Systemic corticosterone level was significantly decreased in the Tn housed WT BALB/c mice (Ts 897.2 ± 74.2 ng/ml and Tn 125.6 ± 29.6 ng/ml serum; mean ± SEM, n = 7 – 9 mice per group; $p < 0.0001$), indicating that Tn housing reduces systemic corticosterone levels in different strains of mice. Examination of the effect of Tn housing on systemic Type-2 cytokine levels, revealed 1.2-fold increase in IL-4 (n = 7 - 8 $p < 0.05$) and 2-fold increase in IL-13 (n = 7 – 8, $p < 0.01$) levels in WT C57BL/6 mice. Consistent with this, serum IL-4 levels were significantly increased in Tn- vs Ts-housed *Il4ra*^{F709} mice (**Figure 3A**). Given the impact of Tn on corticosterone and IL-4 levels and their respective role in immune function (19, 32), we examined the impact of Tn housing on the intestinal immune cells within the LP SI of *Il4ra*^{F709} mice. We revealed that LP SI CD4$^+$ Th2 (CD3$^+$ CD4$^+$ IL-17RB$^+$), ILC2 (Lin$^-$ CD4$^-$ CD8$^-$ c-kit$^-$ IL17RB$^+$), or the regulatory T (Tregs) (Lin$^-$ CD3$^+$ CD4$^+$ GITR$^+$ OX40$^+$) cells were not significantly different between Ts- and Tn-housed *Il4ra*^{F709} mice (**Figures 3B** and **C**). Furthermore, colonic LP and mesenteric lymph node immune cells were comparable between Ts and Tn housed *Il4ra*^{F709} mice (data not shown and **Supplementary Figure 1**). Consistent with these observations, SI epithelial *Il25* mRNA levels were similar between Ts and Tn housed *Il4ra*^{F709} mice suggesting that housing temperature has very little impact on pro type 2 immune phenotype at a steady state (data not shown). Taken together, the data suggest that Tn housing did not alter the gastrointestinal immune compartment in *Il4ra*^{F709} mice.

Thermoneutral Housing Has Minimal Effects on the Intestinal Microbiome of Il4ra^{F709} Mice

Given the previously described effect on Tn housing on the microbiome (23) and that intestinal dysbiosis has been associated with the experimental and clinical food sensitization (33–36), we analyzed the intestinal microbiome of *Il4ra*^{F709} mice housed at Ts and Tn conditions. Analysis of 16 S rRNA gene content high-throughput sequencing of V4 amplicons from fecal samples revealed no significant separation between the microbiome of Ts and Tn housed *Il4ra*^{F709} mice (**Supplementary Figure 2**, $p = 0.075$). To further dissect the potential impact of the housing conditions on the microbial

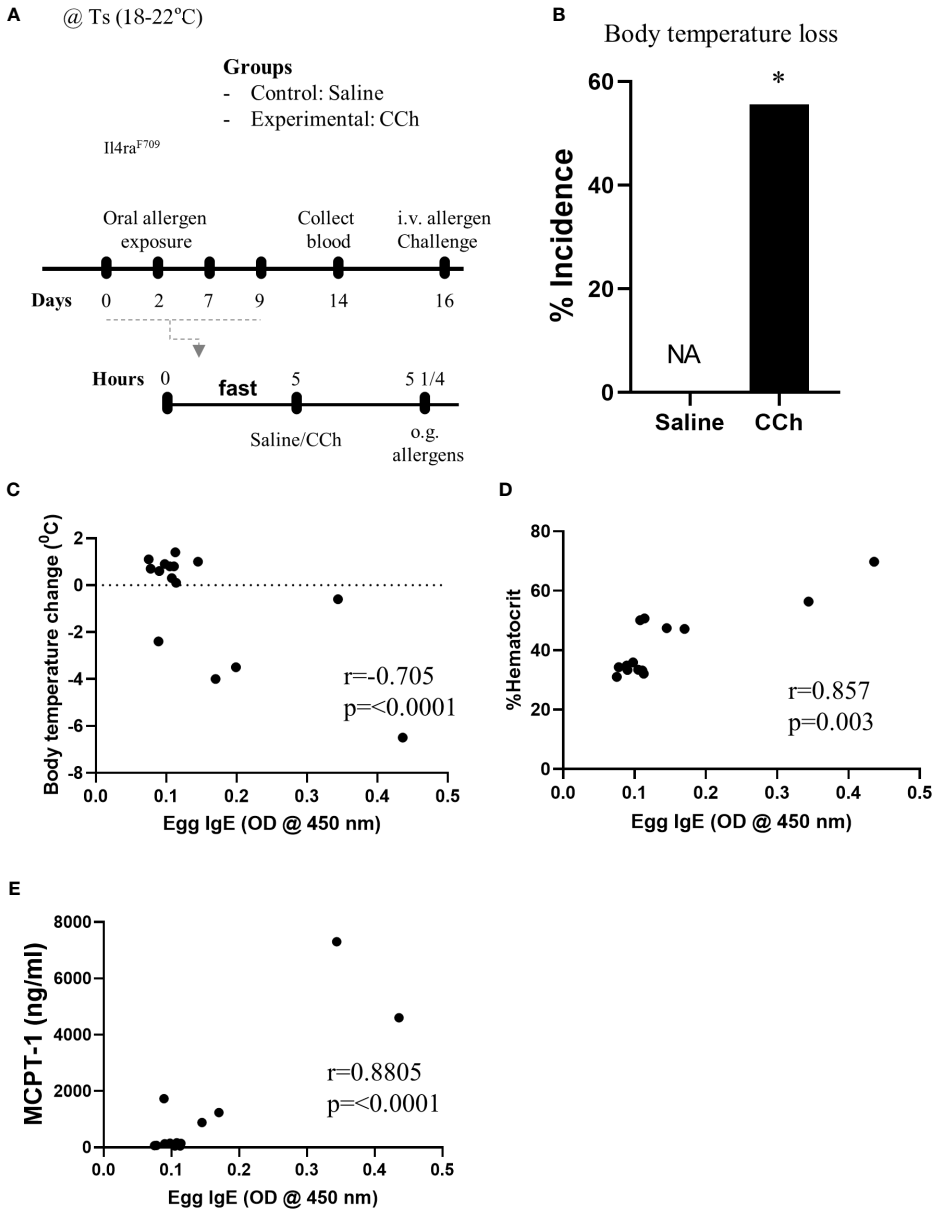


FIGURE 2 | Antigen passage activation predisposes *Il4ra*^{F709} mice to food sensitization at the standard temperature. **(A)** Experimental scheme of the adjuvant free oral sensitization with Il4ra^{F709} mice. **(B)** % incidence of clinical reactivity following the systemic allergen challenge on day 16. NA indicates 0 value. Fisher's exact test was used for the statistical analysis of the odds ratio. **(C-E)** Correlation analysis for egg IgE and **(C)** body temperature change, **(D)** % hemoconcentration, and **(E)** MCPT-1 following the systemic allergen challenge on day 16. r indicates the Pearson correlation coefficient. CCh, carbachol; i.v., intravenous; o.g., oral gavage; Ts, standard housing temperature. n=6-8 per group. * denotes statistical significance.

community of *Il4ra*^{F709} mice, we performed additional analysis on the microbial sequence data. First, we identified the top 50 OTUs abundant in the *Il4ra*^{F709} mice housed at Ts conditions, then compared their % relative abundance against Tn housed *Il4ra*^{F709} mice. All but one OTUs among them, including OTU0004 (Prevotella) and OTU0008 (Bacteroides) were similarly represented between Ts and Tn housed *Il4ra*^{F709} mice supporting the principal component analysis (**Supplementary Figure 2B**). Interestingly, % relative abundance of OTU0116 (Clostridium_XIVa) was significantly reduced in the Tn housed compared to Ts housed *Il4ra*^{F709} mice (**Supplementary Figure 2B**, p = 0.046). These data indicate that Tn housing has a minimum impact on the composition of the intestinal microbiome in *Il4ra*^{F709} mice.

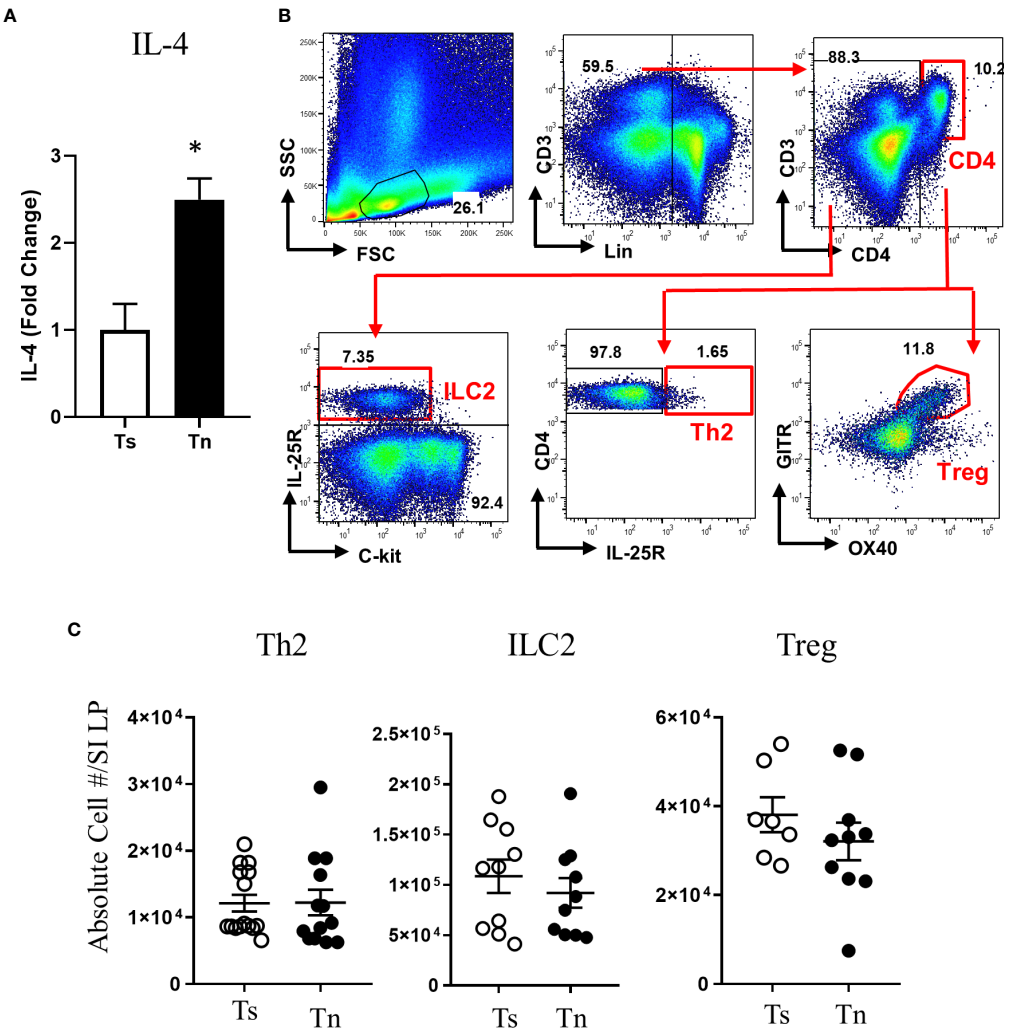


FIGURE 3 | Thermoneutral housing has minimum effects on the gastrointestinal immune cells in *Il4ra*^{F709} mice. **(A)** serum IL-4 was assessed by *in vitro* cytokine capture assay (IVCCA) in Ts and Tn housed Il4ra^{F709} mice. **(B)** Gating strategies for flow cytometry analysis of the small intestinal lamina propria for ILC2, Th2 and Treg. **(C)** Flow cytometry analysis of small intestinal lamina propria for CD4+ Th2 (Lin$^-$ CD3$^+$CD4$^+$ IL17RB$^+$), ILC2 (Lin-CD4-CD8-ckit-IL17RB+), and Treg (Lin$^-$ CD3$^+$CD4$^+$ GITR$^+$ OX40$^+$) from Ts and Tn housed Il4ra^{F709} mice. These immune cells were identified with the panel of markers described in **Table 1** and presented as the absolute cell number in the lamina propria (LP) of the small intestine (SI). n=5 per group for **(A)**. n=7-10 per group for **(B)**. * denotes statistical significance.

The Thermoneutral Housing Altered Antigen Passage Landscape in *Il4ra*^{F709} Mice

With the observed increase in systemic IL-4 levels in Tn-housed *Il4ra*^{F709} mice and knowledge that cellular patterning of antigen passages are sensitive to IL-4rα signaling (17), we examined the impact of housing temperature on antigen passage formation in *Il4ra*^{F709} mice. Similar to the Ts housing condition, saline treated *Il4ra*^{F709} mice exhibited SAPs (~ 0.2 per villi) in the villus of the SI epithelium (**Figures 4A** and **C**). CCh stimulation lead to a significant increase in SAP frequency in the villus of *Il4ra*^{F709} mice under Tn conditions (**Figure 4C**). Notably, we also observed a significant induction of crypt SAPs (~10 fold increase) in CCh-treated *Il4ra*^{F709} mice under Tn conditions (**Figures 4B, C**). The frequency of total SAP formation across the crypt-villus unit of the Tn housed *Il4ra*^{F709} mice (**Figure 4C**), ~2.0 SAPs/villus + crypt unit) was similar to that observed in the Ts-housed *Il4ra*^{F709} mice (**Figure 1D**, ~2.0 SAPs/villus). These studies suggest that Tn conditions do not alter antigen passage frequency or cellular patterning but altered the antigen passage landscape in *Il4ra*^{F709} mice.

Thermoneutral Housing and SAP Activation Promotes Oral Food Reactivity to Egg Antigen in *Il4ra*^{F709} Mice

To test the impact of increased systemic IL-4 levels and altered SI antigen passage landscape on oral antigen sensitization, Tn-housed *Il4ra*^{F709} mice were orally exposed to egg following

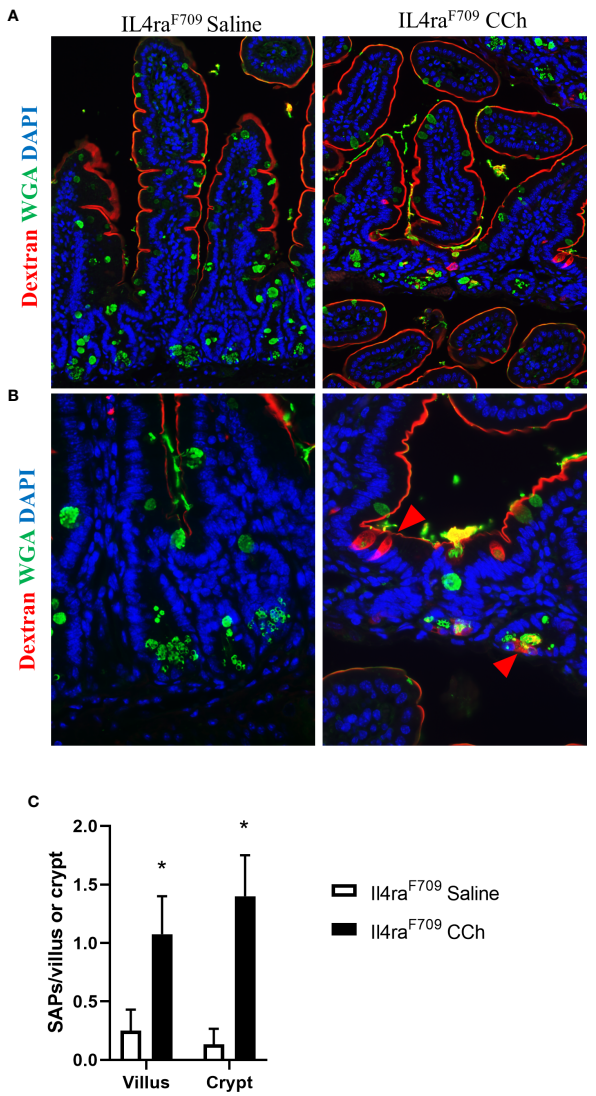


FIGURE 4 | Thermoneutral housing temperature alters structural patterning of antigen passage formation in *Il4ra*^{F709} mice. (A&B) Immunofluorescence analysis for WGA (green) and dextran (red) with the small intestine of Il4ra^{F709} mice treated with saline or CCh that are housed at the thermoneutral temperature. **(A)** shows a lower magnification image and **(B)** shows a higher magnification image. The nucleus is visualized with DAPI (blue). **(C)** Quantification of antigen passage formation for villi and crypts. n=2-3 per group. * denotes statistical significance.

either saline- or CCh-treatment (**Figure 2A**). CCh-treated *Il4ra*^{F709} mice housed under Tn conditions exhibited significantly higher levels of egg-specific IgE and IgG_1 compared with saline-treated *Il4ra*^{F709} mice housed under Tn-conditions (**Figures 5A, B**). Systemic antigen challenge of these mice induced a shock response as evidenced by a significant increase in hemoconcentration (**Figure 5C**). Importantly, the shock response was associated with a significant increase in serum MCPT-1 (**Figure 5D**), indicating activation of mucosal mast cells. Greater than 80% of CCh-treated *Il4ra*^{F709} mice housed under Tn conditions exhibited shock (**Figure 5E**), suggesting that Tn housing increased the penetrance of oral antigen sensitization in *Il4ra*^{F709} mice (**Figures 2B** and **5E**). Longitudinal analyses (**Figure 6A**) of saline- and CCh-treated *Il4ra*^{F709} mice housed under Tn conditions revealed reactivity in CCh- and not saline-treated *Il4ra*^{F709} mice following oral antigen challenge. Moreover, CCh-treated *Il4ra*^{F709} mice housed under Tn conditions 8-weeks following sensitization began to show evidence of increased SI mast cell frequency and mucosal mast cell activation following oral antigen challenge (**Figures 6B, C**). Collectively, oral antigen exposure and SAP activation in *Il4ra*^{F709} mice housed under Tn conditions induce oral antigen sensitization and oral antigen reactivity.

Thermoneutral Housing Alters Cellular Antigen Passage Patterning, and Activation Promotes Oral Food Reactivity to Egg Antigen in WT BALB/c Mice

We next examined the impact of Tn conditions on antigen passage cellular patterning and oral food reactivity in WT BALB/c mice. Intriguingly, WT BALB/c mice housed under Tn conditions exhibited the presence of SI WGA^+ and WGA^- antigen passages under steady state conditions suggesting that Ts to Tn conditions altered antigen passage cellular patterning and induced SAP formation (**Figures 7A, B**). Furthermore, we observed the presence of both villus and crypt antigen passages suggesting that Tn conditions also altered the antigen passage landscape (**Figures 7A, B**). To determine whether the altered SI antigen passages observed in WT BALB/c mice housed under Tn conditions predisposed to oral antigen sensitization, WT BALB/c mice under Tn conditions were orally exposed to egg allergen following either saline- or CCh-treatment and subsequently received repeated oral antigen challenge (**Figure 7C**). Repeated oral egg antigen exposure of Tn-conditioned housed WT BALB/c mice that were orally exposed to egg with CCh treatment exhibited significantly higher egg-specific IgG1 and IgE compared to saline-treated mice (**Figure 7D**). Consistent with this, repeated oral antigen challenge lead to a significant increase in hemoconcentration of CCh-treated BALB/c mice compared to the saline-treated BALB/c mice (**Figure 7E**), which coincided with the significant increase in serum MCPT-1 (**Figure 7F**) following the systemic allergen challenge. These data indicate that Tn conditions induced a shift in the antigen passage cellular patterning and landscape and that activation of antigen passage is sufficient to induce oral food sensitization and reactivity in WT BALB/c mice.

DISCUSSION

Herein, we demonstrate that increased IL-4 signaling is sufficient to alter cellular patterning of antigen passage in the SI. We show that activation of antigen passages in the presence of egg antigen is sufficient to promote food sensitization and reactivity. Furthermore, we show that Tn housing alters antigen cellular patterning and landscape in the SI and that activation of SAPs

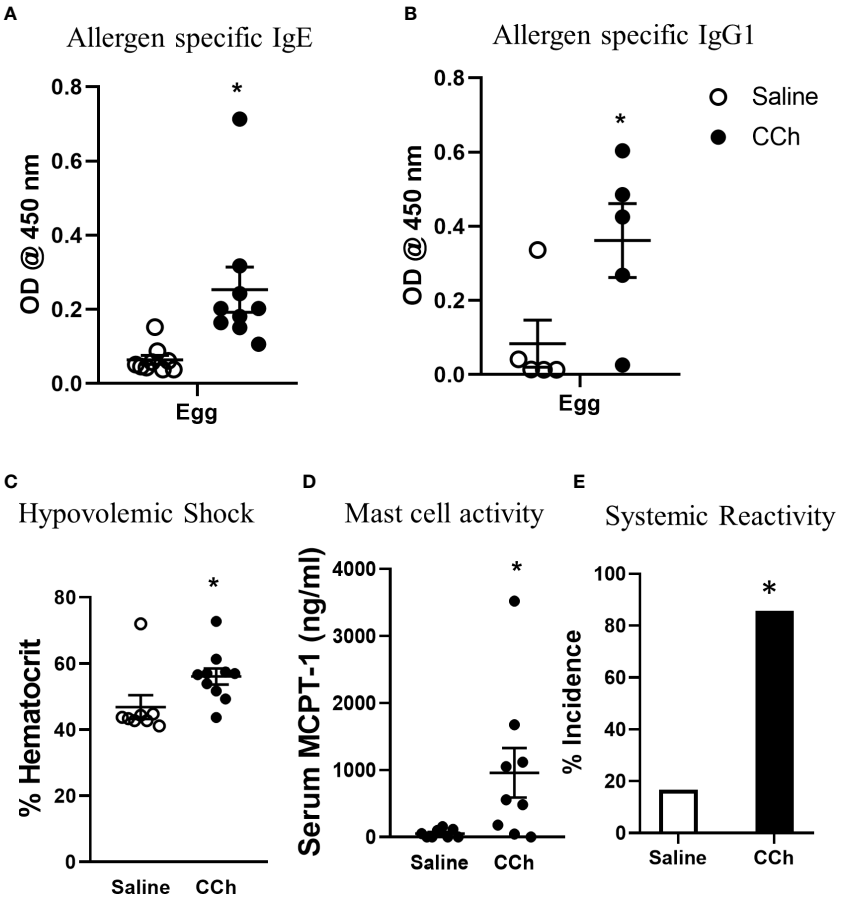


FIGURE 5 | Housing *Il4ra*^{F709} mice at thermoneutral temperature allow robust adjuvant free food sensitization upon activation of antigen passage formation. **(A)** allergen specific IgE and **(B)** IgG1 in the serum of saline or CCh treated Il4ra^{F709} mice housed at the thermoneutral housing temperature. The graph **(B)** shows a representative experiment and has been repeated at least 3 times. Clinical reactivity was assessed by **(C)** % hematocrit and **(D)** serum MCPT-1 level following the systemic allergen challenge. The disease penetrance **(E)** was assessed by the % incidence of clinical reactivity. Fisher's exact test was performed for the statistical analysis. n=5-6 per group per experiment. *denotes statistical significance.

promotes oral antigen sensitization in the WT BALB/c mice. These studies suggest that SAPs are a mechanism by which the intestinal epithelium promotes oral food sensitization.

Goblet cell antigen passage formation and translocation of luminal antigens across GAPs have been associated with oral tolerance to microbial and dietary antigens (13, 15). In the absence of GAPs, either by the deletion of goblet cells, genetic inhibition of GAP formation, or the intraluminal administration of a GAP inhibitor, oral tolerance toward dietary antigens was disrupted, and dietary antigen specific T cell responses were observed (13). Consistent with the previous studies, our current study suggests that a shift in cellular patterning of antigen passage from GAPs to SAPs under the heightened IL-4Rα signaling conditions can promote oral sensitization. Given that Tn housing induced the alteration in cellular patterning of antigen passage in WT BALB/c mice from GAP to SAP, these results further support the notion that Tn housing promotes oral food sensitization by altering the tolerizing signals such as GAP formation. In line with this, the frequency of antigen passage formation seems to play a minimal role in oral sensitization enhanced by the Tn conditions. Similarly, the shift in landscape of antigen passage formation to the crypts seems to play a minimum role in oral food sensitization promoted by Tn housing since it was not enhanced in Tn housed BALB/c mice treated with CCh.

Type 2 cytokines IL-4 and IL-13 are known to regulate differentiation of particular intestinal epithelial cell populations, including goblet cells (37). Functionally, we previously showed that IL-13 reprogramed the cellular patterning of antigen passages formation through goblet, enteroendocrine, and Paneth cells (SAPs) in the SI (17). In the current study, antigen passage also formed through non-goblet cells of the intestinal epithelium under the heightened IL-4rα signaling condition, demonstrating the functional impact of type 2 cytokines on the intestinal epithelium. Yet, precise mechanisms by which antigen passage formation is activated by type 2 cytokines in intestinal epithelial secretory cells

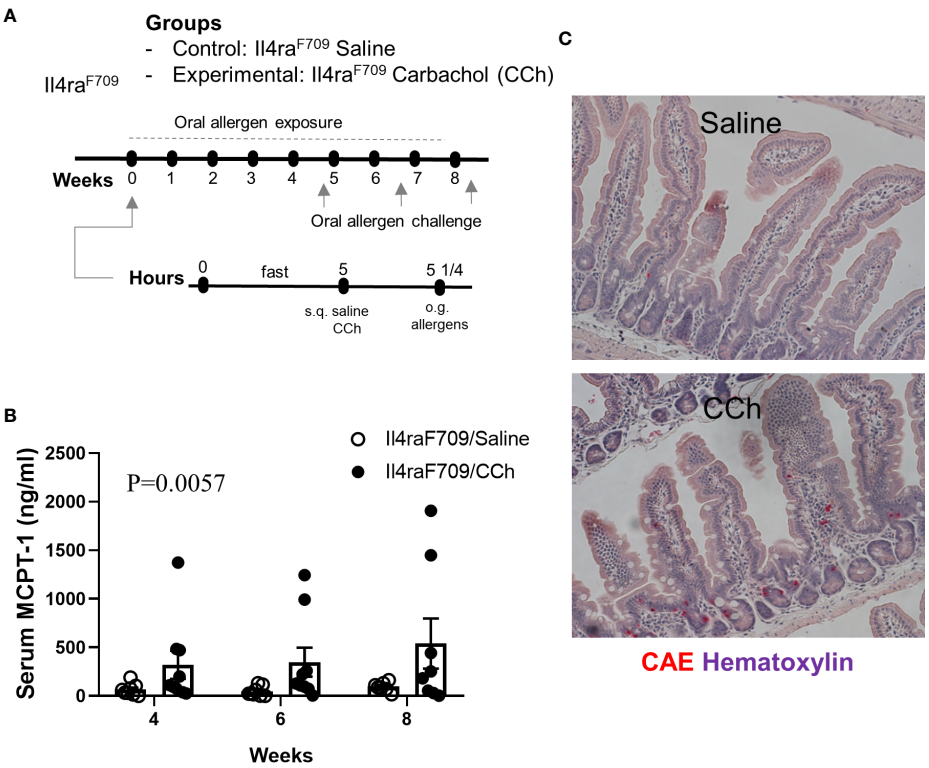


FIGURE 6 | *Il4ra*^{F709} mice housed at thermoneutral temperature develop food reactivity when antigen passage is activated. **(A)** experimental scheme to test the oral reactivity of Il4ra^{F709} mice at the thermoneutral housing temperature. Clinical reactivity was assessed with **(B)** the serum MCPT-1 level following oral allergen challenge. Intestinal mast cell number was examined by **(C)** CAE staining. n=8-9 per group.

are still unclear. Given that intestinal epithelial of secretory cells contribute to immune responses in other pathogenic conditions such as inflammatory bowel disease (38) and infection (39), it is tempting to speculate that the type 2 cytokines may have similar effects in these disease processes. It will be important to uncover the mechanisms by which secretory antigen passages are induced by the heightened IL-4rα signaling since it will provide potential therapeutic targets to prevent induction of SAP formation.

Thermoneutral housing decreases the level of stress hormones and catecholamines which leads to a systemic increase in IL-4 levels. Stress hormones such as corticosterone are known to suppress the immune system, including ILC2 (32), decreasing type 2 cytokine production in response to cytokine activation. Also, experimental studies have recently identified intimate interactions between the sympathetic nervous system and ILC2. In these studies, it was indicated that β2 adrenergic receptor agonists suppress ILC2 mediated Th2 responses in the lung and intestine by suppressing cell proliferation and effector functions of ILC2 (40), including the production of type 2 cytokines. These studies, in aggregate, suggest that the stress hormones and catecholamines may be responsible for the systemic IL-4 increase observed in thermoneutral housing conditions. Moreover, since the catecholamines inhibit IL-4 synthesis in peripheral blood mononuclear cells *in vitro* (41), it would be important to identify the cellular source of IL-4 within peripheral blood mononuclear cells affected by catecholamines. Mast cells, basophils, and CD4^{+} T cells, which are the key immune cells involved in allergic inflammation and the cellular source of IL-4, are also under neuronal regulation (42), suggesting they may also be the cellular sources of IL-4 induced by the Tn condition.

Although it has been reported that Tn housing alters the intestinal microbiome of WT C57BL/6 mice (23), it had a minimum effect on the intestinal microbiome of *Il4ra*^{F709} mice, indicating that enhancement of food sensitization by Tn housing is independent of the intestinal microbiome. Although Clostridium_XIVa was significantly reduced in the Tn housed *Il4ra*^{F709} mice, the contribution of this microbial community change in the enhancement of food sensitization driven by the thermoneutral housing condition is unclear. Since saline treated *Il4ra*^{F709} mice were not sensitized at the thermoneutral conditions indicating that this change in the microbial community was not sufficient to promote sensitization. Since Clostridium_XIVa influences intestinal epithelial cell gene expression and function (35), it may contribute to enhanced food sensitization driven by Tn housing conditions through the intestinal epithelium. Thus, it would be interesting to assess the impact of Clostridium_XIVa on antigen passage formation and patterning in the future studies.

The impact of ambient housing temperature on laboratory rodents has been known for a while (43), yet the recommended

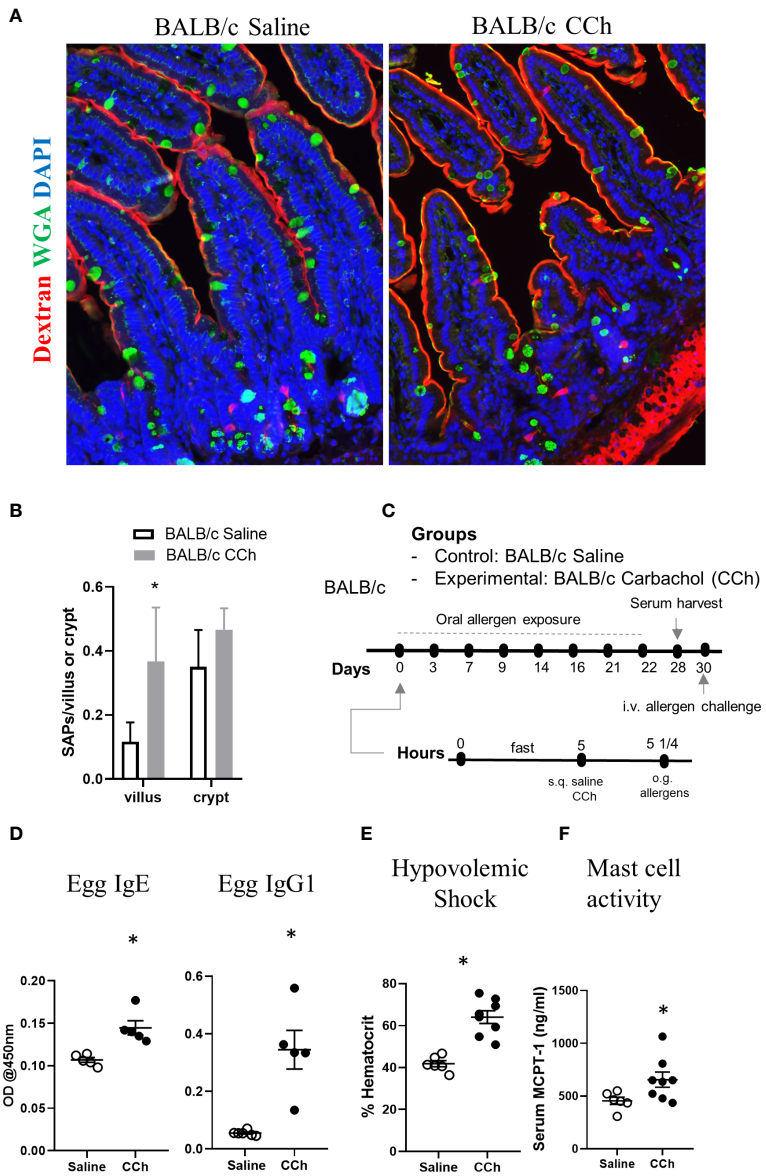


FIGURE 7 | Thermoneutral housing temperature alters cellular and structural patterning of antigen passage formation and allows adjuvant free food sensitization upon activation of antigen passage formation in BALB/c mice. **(A)** Immunofluorescence analysis for WGA (green) and dextran (red) with the small intestine of BALB/c mice treated with saline or CCh that are housed at the thermoneutral temperature. **(B)** Quantification of antigen passage formation for villi and crypts. n=3 per group. **(C)** Experimental scheme of oral sensitization with BALB/c mice. **(D)** Egg specific IgE and IgG1 in the serum of saline or CCh treated BALB/c mice housed at the thermoneutral housing temperature at day 28. The graphs show representative results, and the experiment was repeated once. **(E)** % hematocrit and **(F)** serum MCPT-1 level following the systemic allergen challenge at day 30. *denotes statistical significance.

housing temperature of mice has been set well below their thermoneutral temperature, likely to meet human comfort (44). As the impact of Tn housing been focused on modeling tumor immunotherapy, metabolic diseases, and infection in mice (18, 45), the current study illuminates the utility of Tn housing in modeling food allergy for the first time and offers an improved model of food allergy that does not rely on the adjuvants or genetic manipulation to drive sensitization, which more closely mimics the pathogenesis of human food allergy. As various environmental factors such as diet, microbiome, and vitamin D deficiency (46) have been proposed as risk factors for food allergy, the current experimental model provides a system to evaluate the impact of these risk factors and supports uncovering mechanisms by which these factors are driving food allergy in human.

Our current study emphasizes the relevance of the proper cellular and structural patterning of antigen passage in the

homeostatic immune regulation toward dietary antigens in the SI. As a deviation of antigen passage patterning was associated with food sensitization, it would be interesting to examine the effect of risk factors associated with food allergy on antigen passage patterning to identify a mechanistic link with the development of food allergy.

DATA AVAILABILITY STATEMENT

The microbial sequence datasets for this study can be found in the NCBI BioProject as (PRJNA682183) at https://www.ncbi.nlm.nih.gov/bioproject/PRJNA682183.

ETHICS STATEMENT

The animal study was reviewed and approved by University of Michigan and Cincinnati Children's Medical Center.

AUTHOR CONTRIBUTIONS

TN, J-BL, VG, AY, and ST performed experiments. TN and SH analyzed the data, and wrote the manuscript. CB and GH processed stool samples and analyzed the microbiome. SD, RN, and GH assisted in study design and discussion. SH supervised and acquired funding. All authors contributed to the article and approved the submitted version.

FUNDING

Funding was provided by National Institutes of Health grants; AI138177 (to SH), and AI112626R01(to SH), AI138348 (to GH); DK099222 (to SD); the Mary H. Weiser Food Allergy Center (MHWFAC) (to GH, SH, and TN), and the Nina and Jerry D. Luptak Endowment of the MHWFAC (to GH) and the Askwith Endowment of the MHWFAC (to SH).

ACKNOWLEDGMENTS

We would like to thank Dr. Fred Finkelman for kindly providing animals and reagents necessary for this study. We also would like to thank Mary H. Weiser Food Allergy Center at the University of Michigan Medicine for providing generous support for the current study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2021.636198/full#supplementary-material

REFERENCES

1. Keet CA, Savage JH, Seopaul S, Peng RD, Wood RA, Matsui EC. Temporal trends and racial/ethnic disparity in self-reported pediatric food allergy in the United States. *Ann Allergy Asthma Immunol* (2014) 112:222–9 e3. doi: 10.1016/j.anai.2013.12.007
2. Gupta RS, Warren CM, Smith BM, Jiang J, Blumenstock JA, Davis MM, et al. Prevalence and Severity of Food Allergies Among US Adults. *JAMA Netw Open* (2019) 2:e185630. doi: 10.1001/jamanetworkopen.2018.5630
3. Gupta RS, Warren CM, Smith BM, Blumenstock JA, Jiang J, Davis MM, et al. The Public Health Impact of Parent-Reported Childhood Food Allergies in the United States. *Pediatrics* (2018) 142(6):e20181235. Pediatrics 143 (2019). doi: 10.1542/peds.2018-3835
4. Lopes JP, Sicherer S. Food allergy: epidemiology, pathogenesis, diagnosis, prevention, and treatment. *Curr Opin Immunol* (2020) 66:57–64. doi: 10.1016/j.coi.2020.03.014
5. Samadi N, Klems M, Untersmayr E. The role of gastrointestinal permeability in food allergy. *Ann Allergy Asthma Immunol* (2018) 121:168–73. doi: 10.1016/j.anai.2018.05.010
6. Brough HA, Nadeau KC, Sindher SB, Alkotob SS, Chan S, Bahnson HT, et al. Epicutaneous sensitization in the development of food allergy: What is the evidence and how can this be prevented? *Allergy* (2020) 75:2185–205. doi: 10.1111/all.14304
7. Schülke S, Albrecht M. Mouse Models for Food Allergies: Where Do We Stand? *Cells* (2019) **8**(6). doi: 10.3390/cells8060546
8. Hershey GK, Friedrich MF, Esswein LA, Thomas ML, Chatila TA. The association of atopy with a gain-of-function mutation in the alpha subunit of the interleukin-4 receptor. *N Engl J Med* (1997) 337:1720–5. doi: 10.1056/NEJM199712113372403
9. Mathias CB, Hobson SA, Garcia-Lloret M, Lawson G, Poddighe D, Freyschmidt EJ, et al. IgE-mediated systemic anaphylaxis and impaired tolerance to food antigens in mice with enhanced IL-4 receptor signaling. *J Allergy Clin Immunol* (2011) 127:795–805 e1-6. doi: 10.1016/j.jaci.2010.11.009
10. Noval Rivas M, Burton OT, Wise P, Charbonnier LM, Georgiev P, Oettgen HC, et al. Regulatory T cell reprogramming toward a Th2-cell-like lineage impairs oral tolerance and promotes food allergy. *Immunity* (2015) 42:512–23. doi: 10.1016/j.immuni.2015.02.004
11. Noval Rivas M, Burton OT, Oettgen HC, Chatila T. IL-4 production by group 2 innate lymphoid cells promotes food allergy by blocking regulatory T-cell function. *J Allergy Clin Immunol* (2016) 138:801–11 e9. doi: 10.1016/j.jaci.2016.02.030
12. Burton OT, Noval Rivas M, Zhou JS, Logsdon SL, Darling AR, Koleoglou KJ, et al. Immunoglobulin E signal inhibition during allergen ingestion leads to reversal of established food allergy and induction of regulatory T cells. *Immunity* (2014) 41:141–51. doi: 10.1016/j.immuni.2014.05.017
13. Kulkarni DH, Gustafsson JK, Knoop KA, McDonald KG, Bidani SS, Davis JE, et al. Goblet cell associated antigen passages support the induction and maintenance of oral tolerance. *Mucosal Immunol* (2020) 13:271–82. doi: 10.1038/s41385-019-0240-7
14. Kulkarni DH, McDonald KG, Knoop KA, Gustafsson JK, Kozlowski KM, Hunstad DA, et al. Goblet cell associated antigen passages are inhibited during Salmonella typhimurium infection to prevent pathogen dissemination and limit responses to dietary antigens. *Mucosal Immunol* (2018) 11:1103–13. doi: 10.1038/s41385-018-0007-6
15. Knoop KA, Gustafsson JK, McDonald KG, Kulkarni DH, Coughlin PE, McCrate S, et al. Microbial antigen encounter during a preweaning interval is critical for tolerance to gut bacteria. *Sci Immunol* (2017) 2(18). doi: 10.1126/sciimmunol.aao1314
16. McDonald KG, Leach MR, Brooke KWM, Wang C, Wheeler LW, Hanly EK, et al. Epithelial expression of the cytosolic retinoid chaperone cellular retinol binding protein II is essential for in vivo imprinting of local gut dendritic cells by lumenal retinoids. *Am J Pathol* (2012) 180:984–97. doi: 10.1016/j.ajpath.2011.11.009

17. Noah TK, Knoop KA, McDonald KG, Gustafsson JK, Waggoner L, Vanoni S, et al. IL-13-induced intestinal secretory epithelial cell antigen passages are required for IgE-mediated food-induced anaphylaxis. *J Allergy Clin Immunol* (2019) 144:1058–1073 e3. doi: 10.1016/j.jaci.2019.04.030
18. Ganeshan K, Chawla A. Warming the mouse to model human diseases. *Nat Rev Endocrinol* (2017) 13:458–65. doi: 10.1038/nrendo.2017.48
19. Bowers SL, Bilbo SD, Dhabhar FS, Nelson RJ. Stressor-specific alterations in corticosterone and immune responses in mice. *Brain Behav Immun* (2008) 22:105–13. doi: 10.1016/j.bbi.2007.07.012
20. Tian XY, Ganeshan K, Hong C, Nguyen KD, Qiu Y, Kim J, et al. Thermoneutral Housing Accelerates Metabolic Inflammation to Potentiate Atherosclerosis but Not Insulin Resistance. *Cell Metab* (2016) 23:165–78. doi: 10.1016/j.cmet.2015.10.003
21. Osborn O, Olefsky JM. The cellular and signaling networks linking the immune system and metabolism in disease. *Nat Med* (2012) 18:363–74. doi: 10.1038/nm.2627
22. Giles DA, Ramkhelawon B, Donelan EM, Stankiewicz TE, Hutchison SB, Mukherjee R, et al. Modulation of ambient temperature promotes inflammation and initiates atherosclerosis in wild type C57BL/6 mice. *Mol Metab* (2016) 5:1121–30. doi: 10.1016/j.molmet.2016.09.008
23. Giles DA, Moreno-Fernandez ME, Stankiewicz TE, Graspeuntner S, Cappelletti M, Wu D, et al. Thermoneutral housing exacerbates nonalcoholic fatty liver disease in mice and allows for sex-independent disease modeling. *Nat Med* (2017) 23:829–38. doi: 10.1038/nm.4346
24. Quatrini L, Vivier E, Ugolini S. Neuroendocrine regulation of innate lymphoid cells. *Immunol Rev* (2018) 286:120–36. doi: 10.1111/imr.12707
25. Strehl C, Ehlers L, Gaber T, Buttgereit F. Glucocorticoids-All-Rounders Tackling the Versatile Players of the Immune System. *Front Immunol* (2019) 10:1744. doi: 10.3389/fimmu.2019.01744
26. Yamani A, Wu D, Waggoner L, Noah T, Koleske AJ, Finkelman F, et al. The vascular endothelial specific IL-4 receptor alpha-ABL1 kinase signaling axis regulates the severity of IgE-mediated anaphylactic reactions. *J Allergy Clin Immunol* (2018) 142:1159–1172 e5. doi: 10.1016/j.jaci.2017.08.046
27. Chen CY, Lee JB, Liu B, Ohta S, Wang PY, Kartashov AV, et al. Induction of Interleukin-9-Producing Mucosal Mast Cells Promotes Susceptibility to IgE-Mediated Experimental Food Allergy. *Immunity* (2015) 43:788–802. doi: 10.1016/j.immuni.2015.08.020
28. Noah TK, Kazanjian A, Whitsett J, Shroyer NF. SAM pointed domain ETS factor (SPDEF) regulates terminal differentiation and maturation of intestinal goblet cells. *Exp Cell Res* (2010) 316:452–65. doi: 10.1016/j.yexcr.2009.09.020
29. Finkelman F, Morris S, Orekhova T, Sehy D. The in vivo cytokine capture assay for measurement of cytokine production in the mouse. *Curr Protoc Immunol Chapter* (2003) 6:Unit 6 28. doi: 10.1002/0471142735.im0628s54
30. Ashley SL, Sjoding MW, Popova AP, Cui TX, Hoostal MJ, Schmidt TM, et al. Lung and gut microbiota are altered by hyperoxia and contribute to oxygen-induced lung injury in mice. *Sci Transl Med* (2020) 12(556). doi: 10.1126/scitranslmed.aau9959
31. Tachdjian R, Al Khatib S, Schwinglshackl A, Kim HS, Chen A, Blasioli J, et al. In vivo regulation of the allergic response by the IL-4 receptor alpha chain immunoreceptor tyrosine-based inhibitory motif. *J Allergy Clin Immunol* (2010) 125:1128–36 e8. doi: 10.1016/j.jaci.2010.01.054
32. Yu QN, Guo YB, Li X, Li CL, Tan WP, Fan XL, et al. ILC2 frequency and activity are inhibited by glucocorticoid treatment via STAT pathway in patients with asthma. *Allergy* (2018) 73:1860–70. doi: 10.1111/all.13438
33. Diesner SC, Bergmayr C, Pfitzner B, Assmann V, Krishnamurthy D, Starkl P, et al. A distinct microbiota composition is associated with protection from food allergy in an oral mouse immunization model. *Clin Immunol* (2016) 173:10–8. doi: 10.1016/j.clim.2016.10.009
34. Noval Rivas M, Burton OT, Wise P, Zhang YQ, Hobson SA, Garcia Lloret M, et al. A microbiota signature associated with experimental food allergy promotes allergic sensitization and anaphylaxis. *J Allergy Clin Immunol* (2013) 131:201–12. doi: 10.1016/j.jaci.2012.10.026
35. Stefka AT, Feehley T, Tripathi P, Qiu J, McCoy K, Mazmanian SK, et al. Commensal bacteria protect against food allergen sensitization. *Proc Natl Acad Sci U S A* (2014) 111:13145–50. doi: 10.1073/pnas.1412008111
36. Azad MB, Konya T, Guttman DS, Field CJ, Sears MR, HayGlass KT, et al. Infant gut microbiota and food sensitization: associations in the first year of life. *Clin Exp Allergy* (2015) 45:632–43. doi: 10.1111/cea.12487
37. Andrews C, McLean MH, Durum SK. Cytokine Tuning of Intestinal Epithelial Function. *Front Immunol* (2018) 9:1270. doi: 10.3389/fimmu.2018.01270
38. Parikh K, Antanaviciute A, Fawkner-Corbett D, Jagielowicz M, Aulicino A, Lagerholm C, et al. Colonic epithelial cell diversity in health and inflammatory bowel disease. *Nature* (2019) 567:49–55. doi: 10.1038/s41586-019-0992-y
39. Haber AL, Biton M, Rogel N, Herbst RH, Shekhar K, Smillie C, et al. A single-cell survey of the small intestinal epithelium. *Nature* (2017) 551:333–9. doi: 10.1038/nature24489
40. Moriyama S, Brestoff JR, Flamar AL, Moeller JB, Klose CSN, Rankin LC, et al. beta2-adrenergic receptor-mediated negative regulation of group 2 innate lymphoid cell responses. *Science* (2018) 359:1056–61. doi: 10.1126/science.aan4829
41. Wahle M, Neumann RP, Moritz F, Krause A, Buttgereit F, Baerwald CG. Beta2-adrenergic receptors mediate the differential effects of catecholamines on cytokine production of PBMC. *J Interferon Cytokine Res* (2005) 25:384–94. doi: 10.1089/jir.2005.25.384
42. Kabata H, Artis D. Neuro-immune crosstalk and allergic inflammation. *J Clin Invest* (2019) 129:1475–82. doi: 10.1172/JCI124609
43. Gordon CJ. Twenty-four hour rhythms of selected ambient temperature in rat and hamster. *Physiol Behav* (1993) 53:257–63. doi: 10.1016/0031-9384(93)90202-Q
44. Karp CL. Unstressing intemperate models: how cold stress undermines mouse modeling. *J Exp Med* (2012) 209:1069–74. doi: 10.1084/jem.20120988
45. Hylander BL, Gordon CJ, Repasky EA. Manipulation of Ambient Housing Temperature To Study the Impact of Chronic Stress on Immunity and Cancer in Mice. *J Immunol* (2019) 202:631–6. doi: 10.4049/jimmunol.1800621
46. Koplin JJ, Allen KJ, Tang MLK. Important risk factors for the development of food allergy and potential options for prevention. *Expert Rev Clin Immunol* (2019) 15:147–52. doi: 10.1080/1744666X.2019.1546577
47. Loh W, Tang MLK. The Epidemiology of Food Allergy in the Global Context. *Int J Environ Res Public Health* (2018) 15(9). doi: 10.3390/ijerph15092043

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership