DIETARY INTERVENTIONS AND NUTRITIONAL FACTORS IN THE PREVENTION OF ALLERGIC DISEASES IN INFANTS

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EDITORIAL
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Editorial: Dietary Interventions and Nutritional Factors in the Prevention of Allergic Diseases in Infants

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Keywords: allergic diseases, diet, atopic march, hydrolyzed formulas, preterm newborns, gut microbiota, biotics, diet diversity

Editorial on the Research Topic

Dietary Interventions and Nutritional Factors in the Prevention of Allergic Diseases in Infants

INTRODUCTION

Since allergic diseases represent a great public health, there is a strong need for a better understanding of modifiable risk factors. The present Research Topic discusses the main topic related to allergic disease prevention and addresses possible intervention strategies, since pregnancy to postnatal period.

Both primary prevention, which prevents the sensitization development, and secondary prevention, aiming to decrease the development of further disease after sensitization, are addressed. Primary prevention may play a role in reducing the burden of allergic disease, especially in high-risk infants, although some preventive measures should be considered as useful preventive strategies for general population.

PRIMARY PREVENTION

Many dietary factors, from prenatal life through infancy, have been proposed to influence the susceptibility to allergic diseases, by modulating the gut microbiota composition and promoting tolerance to allergens (Ferrante et al.).

The concept of allergen avoidance as a preventive measure has been challenged in the wake of recent randomized studies which shed light on the role of early oral exposure in inducing tolerance.

As a result of these recent findings, primary prevention recommendations have recently been updated. Hereby, new recommendations, are reported and critically discussed, as well as the implications for clinical practice (Corica et al.). It remains unknown whether the window of opportunity to induce tolerance varies depending on the food. This Research Topic and other knowledge gaps are addressed.

There is a paucity of studies on the role of early exposure to CM on the development of allergic diseases and the role of early introduction of cow's milk formula is still debated (Mastrorilli et al.); further studies are warranted to understand the prospective for allergy prevention related to early exposure to CMF and the optimal timing of CM introduction. There is no actual recommendation for or against using partially or extensively hydrolysed formula to prevent food allergy in infants

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and young children. However, if exclusive breastfeeding is not possible, many substitutes are available for families to choose from, including partially or extensively hydrolyzed formulas.

Paucity of data exist on preterms; a 3-year follow-up study of a previous triple-blind, placebo-controlled randomized trial, aiming to investigate the prevalence of atopic diseases in preterm, found that extensively hydrolyzed protein formula seems to be ineffective in allergic diseases prevention in this population (Di Mauro et al.). The authors concluded that further adequately powered, randomized controlled trials, evaluating hydrolyzed protein formula administration to prevent allergic diseases in preterm newborns, are needed.

Interest in microbiota manipulating strategies to restore the microbial balance for atopic disease prevention, through prebiotics, probiotics, or synbiotics supplementation, has been increasing. In this Research Topic main findings regarding the effects of biotics in prevention of allergic diseases are summarized (Sestito et al.).

Overall, the use of different strains, period of intervention, duration of supplementation in clinical studies hamper to draw definitive conclusions on the effects of probiotics and/or prebiotics for prevention of allergic diseases in infants.

The latest data addressing prenatal and perinatal nutritional and dietary interventions in the primary prevention of atopic dermatitis are hereby reported (Trikamjee et al.).

Encouraging results on the use of probiotics in at risk infants exist; however, no consistent evidence of a clear benefit of nutritional factors in the alteration of the risk of AD in children is available.

Among nutritional factors, the role of vitamin D in prevention of allergic diseases has been reviewed.

In regard to atopic dermatitis, available data are conflicting (Trikamjee et al.) and not conclusive.

Potential relationship between vitamin D and food allergy development mainly derive from ecologic studies showing an association between lower sunlight exposure and food allergies incidence. However, as well as for AD, the evidence on the role of vitamin D in the development of food allergy is still contrasting (Giannetti et al.).

Infants with severe atopic dermatitis may be sensitized to foods that have not been introduced into their diet, posing a risk for developing an immediate hypersensitivity reaction on the first exposure to the food to which they are sensitized. Thus, broad-spectrum sensitization studies are necessary before introducing complementary diet (Bilbao et al.).

In the last 20 years, a large number of epidemiological studies showed a significant increase of incidence and prevalence of eosinophilic esophagitis especially in children in Western Countries, varying widely across North America and Europe. Evidence suggests that epithelial barrier impairment along with esophageal dysbiosis may play a role in the development of this disease Risk factors that might contribute to the increasing prevalence of EoE, focusing on the possible preventive role of early interventions, are discussed (Votto et al.).

SECONDARY PREVENTION

Along with atopic dermatitis, cow's milk allergy may also represent the first step of the so called "allergic march" (AM), a clinical sequence beginning with AD and culminating with respiratory allergies.

Indeed, the occurrence of allergic sensitization in these infants increases the risk of later developing asthma and allergic oculorhinitis (AR), in particular when sensitization occurs along with atopic dermatitis.

Latest findings on the role of prenatal and perinatal dietary factors in the development of asthma, and whether the modulation of such factors could contribute to the prevention of childhood-onset asthma is discussed (Trambusti et al.).

Respiratory viruses in general and sincitial respiratory virus in particular are recognized as one of the causes of early life wheezing that, in turn, may contribute to the development of childhood asthma.

As innate immunity receptors seem to play a critical role in inflammation and host defense, as well as allergy and nutritional factors, the study by Savino et al. addresses the role of innate immunity key receptors polymorphism.

Interestingly, some evidence suggests that consumption of safe, raw, unpastorized cow's milk might be considered among the preventive strategies to halt the atopic march (Baars et al.).

The modern approach to CMA and food allergy in general is not simply avoiding the allergen, but also the possibility to actively modulate the immune system, in order to reduce disease duration and to protect against the occurrence of other atopic manifestations.

Several non-immune (gut barrier integrity) and immune (cytokines, immune cells) tolerogenic factors are involved in such modulatory action.

Extensively hydrolyzed formulas (eHFs), in which milk proteins have been fragmented (hydrolyzation) to make them less allergenic, are the first choice in infants and children diagnosed with CMA.

In addition to their role in symptoms relief, hydrolysed formula contain peptides that may act on immune system favoring the tolerance mechanisms. These special formulas may be considered an active therapy in infants with cow's milk allergy, that means the possibility to influence the CMA natural history and to limit the occurrence of other atopic manifestations later in the life. Many effects are mediated by epigenetic mechanisms (Carucci et al.).

Although hydrolyzed formulas represent the first choice for CMA treatment, soy formulas have been long used for the treatment of CMA. In the last few decades, soy formulas have been changed over the years to improve digestibility, nutritional values, and protein quality. The actual role of soy formulas in CMA treatment and their potential application also in CMA prevention is discussed and controversial are highlighted (Verduci et al.). Further

studies are warranted to study not only the prevalence of soy allergy in children with CMA and the entire pediatric population but also the preventive effect of soybean on allergic diseases development.

More than considering the properties of a single nutrient on the immune system, there is growing evidence of the effects of diet as a whole on immune function and development. The exposure to a variety of food antigens during early life may increase intake of nutrients and positively affect the gut microbiome composition and the development of immune tolerance.

Recent findings that an increased diversity of food introduced in the 1st year of life protects against allergic diseases are consistent with this hypothesis (D'Auria et al.).

Further studies are warranted to investigate the effects of diet diversity during pregnancy and lactation on the development of allergic diseases in infants and children.

AUTHOR CONTRIBUTIONS

ED'A designed and wrote the article. RB wrote the article. GZ and RB equally designed the article and read and made comments on the manuscript. All authors contributed to the article and approved the submitted version.

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MINI REVIEW
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Early Life Risk Factors in Pediatric EoE: Could We Prevent This Modern Disease?

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Eosinophilic esophagitis (EoE) is a chronic antigen-mediated inflammatory disease that affects the esophagus. In the last 20 years, a large number of epidemiological studies showed a significant increase in the incidence and prevalence of EoE, especially in developed countries. This phenomenon might correlate to the overall increase in pediatric allergic diseases or might be a result of improved medical awareness and knowledge through modern diagnostic instruments. Since 1993, when EoE was first recognized as a distinct clinical entity, several signs of progress in the pathophysiology of EoE were achieved. However, a few studies reported data on early risk factors for pediatric EoE and how these factors may interfere with genes. Currently, the most defined risk factors for EoE are male sex, Caucasian race, and atopic comorbidities. Other putative risk factors may include alterations in epithelial barrier function and fibrous remodeling, esophageal dysbiosis, variation in the nature and timing of oral antigen exposure, and early prescription of proton pump inhibitors and antibiotics. Notably, the timing and nature of food antigen exposure may be fundamental in inducing or reversing immune tolerance, but no studies are reported. This review summarized the current evidence on the risk factors that might contribute to the increasing development of EoE, focusing on the possible preventive role of early interventions.

Keywords: eosinophilic esophagitis, allergy, risk factors, early life exposures, food allergens, microbiome, prevention

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INTRODUCTION

EoE is a chronic, antigen-mediated, inflammatory disease of the esophagus characterized by symptoms due to esophageal inflammation, dysmotility, and fibrosis (1, 2). EoE occurs in children and adults, and symptoms are often non-specific and depending on the age of onset (1, 2). While in toddlers and children EoE presents with inflammatory symptoms mimicking gastroesophageal reflux disease (GERD), in adolescents and adults EoE frequently appears with food impaction, dysphagia, odynophagia, or esophageal strictures, as a consequence of the ongoing fibrosis process (1, 2). EoE is a multifactorial disorder resulting from the combination of genetic predisposition, epithelial barrier dysfunction, environmental risk factors (**Table 1**), and allergen sensitization, leading to a T helper type 2 (Th2) atopic inflammation of the esophagus (2).

Since 1993, when EoE was first recognized as a distinct clinical entity, several signs of progress in the pathophysiology of EoE were achieved; however, few studies reported data on early risk factors and how these factors might interfere with the genes in the disease onset and evolution. EoE is strictly associated with atopic disorders (asthma, atopic dermatitis, IgE mediated food allergy, allergic rhinitis), suggesting that EoE and allergic diseases share the same environmental risk factors and early life exposures.

We reviewed the recent evidence about the well-known risk factors of EoE, also reporting the less-investigated early exposures, to open future ideas of investigation in the limited field of prevention. Finally, we speculate about the possible strategies for EoE prevention.

WHY IS EoE A MODERN DISEASE OF WESTERN COUNTRIES?

Recently, it was estimated that EoE affects 1/2,000 patients in the United States, with higher prevalence rate in adults (43.4/100,000; 95% CI, 22.5–71.2) than in children (29.5/100,000; 95% CI, 17.5–44.7), prevailing in Caucasian patients and male sex (**Table 1**) (1, 3, 19). In the last 20 years, a large number of epidemiological studies showed a significant increase of incidence and prevalence of EoE especially in children in Western Countries, varying widely across North America and Europe (19–21). This interesting phenomenon might be related to (1) an overall increased incidence of allergic and non-allergic diseases, (2) the chronic disease-course of EoE, and (3) the improved medical awareness and knowledge through modern diagnostic instruments (18). Although EoE is associated with some genetic polymorphisms (22, 23), this rapid increase in EoE frequency might indicate a prevalent role of environmental risk factors in disease development.

Hygienic Hypothesis, Dysbiosis, and Esophageal Infection

The hygienic hypothesis postulated for the first time in 1989 by Strachan (24), and recently reviewed (25), has explained the global rise of allergic and autoimmune diseases. Animal and human studies demonstrated that the increased frequency of allergic diseases in developed countries is a consequence of the modern hygienic conditions and fewer bacterial, viral, and parasitic infections during infancy and childhood (26). Although fundamental to reduce infectious diseases, an excessively hygienic environment in early life might induce adverse effects on the host microbiome, altering certain strains of necessary commensal bacteria (dysbiosis). Furthermore, microbial dysbiosis might arise from the modern lifestyle that is characterized by limited physical activity, low intake of fibers, a diet high in saturated fats, and more frequent use of antibiotics. An impaired microbiota might also result from early life events such as cesarean section, premature birth, early antibiotic exposure, and formula feeding (**Table 2**) (27). Patients with EoE showed differences in the esophageal microbiome and an increase of bacterial load compared to patients with GERD and healthy controls (28, 29, 62). Harris et al. have demonstrated that the esophageal microbiome in children with untreated and active EoE is characterized by the predominance of *Haemophilus* strain, compared to patients with disease-remission and healthy controls (29). Also, Benitez et al. characterized the bacterial composition of the oral and esophageal microenvironments from children with EoE and healthy controls, showing that specific bacterial strains (mainly Firmicutes) were more abundant in the esophagus compared to the oral cavity in EoE patients (62). These data suggest that eosinophilic inflammation might specifically alter the esophageal microbiota, and the oral microbiota could not be used as a surrogate for monitoring the disease activity.

Evidence on the role of the microbiome in EoE pathogenesis is still limited to a few studies. However, two possible hypotheses could explain the relationship between the gut microbiome and EoE: (1) early life risk factors might specifically influence the correct development of the esophageal microbiome, predisposing to EoE, (2) eosinophilic inflammation could lead to esophageal dysmotility and decrease the esophageal compliance; thus EoE itself might induce esophageal microbiome alteration (28). Both hypotheses might coexist in a vicious circle, and the first one opens the unexplored field of the early prevention of EoE. Currently, only a single study in a murine model showed the beneficial effect of the probiotic *Lactococcus lactis* NCC 2287 on the esophageal inflammation (63). Although raising evidence explained the pivotal role of the well-balanced gut microbiome in the correct development of the immune system (25), the precise mechanisms whereby hygienic environment and dysbiosis interact with each other and result in allergic and autoimmune disease is still understood (64). Moreover, further studies are needed to clarify the role of dysbiosis in EoE pathogenesis and to identify possible preventive strategies.

Infectious diseases might act as promotive or protective factors for atopic diseases, including the EoE. Studies reported the development of EoE after herpes simplex virus (HSV) infection in immunocompetent adults and children. These data suggest that HSV esophagitis might predispose to EoE, impairing the esophageal barrier, and increasing the epithelial permeability (11, 12).

In Western countries, the overall prevalence of *Helicobacter pylori* infection was decreased in the last decades, probably contributing to the rise of allergic diseases (65). Experiments in murine models demonstrated that the *H. pylori* infection early in life was protective against asthma through the induction of regulatory T cells (T-regs) (66). Furthermore, epidemiological data showed that the *H. pylori* infection was negatively associate with EoE, demonstrating the potential protective role in EoE pathogenesis (67–70). The decrease of H. *pylori* infection in Western countries might also be a consequence of better hygienic conditions; furthermore, its possible protective role might explain the lower prevalence of EoE in developing countries,

Abbreviations: AA, arachidonic acid; ADHD, attention deficit hyperactivity disorder; CAPN14, calpain 14; DHA, docosahexaenoic acid; EA, esophageal atresia; EoE, eosinophilic esophagitis; EPA, eicosapentaenoic acid; GERD, gastroesophageal reflux disease; HSV, herpes simplex virus; NICU, neonatal intensive care unit; OIT, oral immunotherapy; PPI, proton pump inhibitor; PUFA, polinsatured fatty acid; SLIT, sublingual immunotherapy; Th2, T helper type 2; TLSP, thymic stromal lymphopoietin; T-regs, regulatory T cells.

TABLE 1 | Risk factors of eosinophilic esophagitis [adapted from Dellon and Hirano (3)].

Male sex	Gene encoding for thymic stromal lymphopoietin (TLSP), a central mediator of eosinophilic inflammation, is located on a pseudo-autosomal region of the X and Y chromosomes (Xp22.3 and Yp 11.3). A single nucleotide polymorphism of this region predisposes male patients to develop EoE (4)
Family members of patients with EoE	Monozygotic twins had a 44% disease concordance, a 2-fold increase compared with dizygotic twins (5, 6). Also, the relative risk to develop this disease in dizygotic twins might increase more than 10-fold compared to siblings (5)
Genetic *loci*	Studies of Genome-wide association studies (GWAS) identified different genetic *loci* that are likely contributing to the development of EoE and mainly include thymic stromal lymphopoietin (TSLP), calpain 14 (CAPN14), EMSY, LRRC32, STAT6 and ANKRD27 (7). These genetic loci are mainly involved in T-helper 2 type inflammation (allergic inflammation) and epithelial barrier function and integrity
Non-atopic diseases	EoE prevails in patients with connective tissue disorders, coeliac disease, autoimmune diseases, autism, and ADHD (8)
Atopic diseases	EoE may be a late manifestation of the atopic march (9)
OIT for foods and aeroallergens	EoE is a complication of oral immunotherapy (OIT) in 3–5% of cases. EoE is also reported during sublingual immunotherapy (SLIT) for respiratory allergies (10)
Infectious Esophagitis (HSV)	HSV might impair the esophageal barrier and increase the epithelial permeability (11, 12)
GERD	GERD alters the esophageal barrier function, increases the epithelial permeability, and the passage of food allergens that might trigger EoE. Furthermore, GERD might induce the expression of inflammatory molecules and eosinophil chemoattractants (13–15)
Aeroallergens	Environment allergens might increase disease activity and explain the seasonal variation of EoE reactivations and diagnosis (16, 17)
Food allergens	Food allergens directly trigger EoE (1)
Cold climate regions	Higher exposition to aeroallergens (18)

TABLE 2 | Putative early risk factors of eosinophilic esophagitis (EoE).

Microbial gut dysbiosis	Microbial dysbiosis might arise from a modern lifestyle (limited physical activity, low intake of fibers, high saturated fats in the diet, and frequent use of antibiotics) and early life events (cesarean section, premature birth, early antibiotic exposure, and formula feeding) (25, 27–29)
Monogenic diseases	Hyper-IgE syndrome, Ehlers-Danlos syndrome, ERBIN deficiency, Loeys-Dietz syndrome, Netherton's syndrome, PTEN hamartoma tumor syndrome, severe atopy syndrome associated with metabolic wasting syndrome (7)
Esophageal atresia (EA)	EA and EoE might share same risk factors: genes, early life factors (prematurity, NICU admission), early exposure to acid suppressants and antibiotics, GERD and esophageal dysmotility and epithelial injury (30–33)
Esophageal injury in childhood, and fetal chest malformations	Caustic damage and diaphragmatic hernia might allow the development of EoE with mechanisms not well-understood and investigated (34, 35)
Western diet and obesity	A recent study in mice demonstrated that a high fat diet and obesity aggravated the immune histopathological characteristics and increased inflammatory cells in the EoE experimental model (36)
Low level of vitamin D	The supplementation of vitamin D *in utero* and early life seems to reduce the risk of atopy (37–43)
Early life exposures	Cesarean section, preterm birth, NICU admission, formula feeding, early prescription of PPI, and antibiotics might impair the host microbiome and the developing immature immune system (44–49)
Early prescription and long-term therapy with proton pump inhibitors (PPI)	PPIs prevent the digestion of food allergens, increase the gastric permeability, and alter the intestinal microbiome (27, 49–56)
Early prescription of antibiotics	Antibiotics might impair the immature gut microbiome, that is essential for the developing of immune system (27, 49, 57–59)
Formula feeding	Human milk shows potentially anti-allergic immune properties and is fundamental for the correct development of a well-balanced gut microbiome (27, 60, 61)

where the infection is usually acquired in childhood. On the other hand, Molina-Infante et al. recently published the results of a large prospective case-control study conducted in 23 centers, and showed that the prevalence of *H. pylori* infection was not different between EoE cases and controls (37 vs. 40%; $p = 0.3$; OR 0.97; 95% CI 0.73–1.30), neither in children (42 vs. 46%; $p = 0.1$) nor in adults (36 vs. 38%; $p = 0.4$) (71). Therefore, there are already insufficient and conflicting data to support the protective role of *H. pylori* infection, and several issues are still open.

Diseases of Modern Life and Phenotypes of EoE

Recent advances in disease pathogenesis and prognosis have demonstrated that EoE could be classified in different phenotypes based on specific comorbidities. Epidemiological data demonstrated that EoE is so strongly associated with atopic comorbidities (asthma, allergic rhinitis, IgE-mediated food allergy, atopic dermatitis) (3, 9, 72) to follow allergic conditions in the atopic march, as a late manifestation (73).

However, a significant number of EoE patients do not present allergic diseases, suggesting a possible non-atopic phenotype (2). Interestingly, several reports have suggested that EoE may be more frequently associated with some non-allergic disorders, including connective tissue disorders (74), autoimmune diseases (coeliac disease) (8), and contradictorily inflammatory bowel diseases (IBD) (8, 75–77), that are increased in the last decades, especially in Western countries (8). The pathogenetic mechanisms explaining the association between these non-atopic diseases and EoE are poorly understood and investigated. EoE and coeliac disease (CD) are two inflammatory diseases induced by food allergens. Although CD resulted more frequent in EoE patients than controls (5.6% of EoE, 0.9% of non-EoE, $P < 0.0001$) (8), Lucendo et al. did not find a common genetic basis between these two diseases (78). The frequency of the HLA DQ2 and DQ8 alleles predisposing to CD was not observed in adult EoE patients compared to controls (78). Also, type 1 diabetes, cystic fibrosis, adrenal insufficiency, autism, attention deficit hyperactivity disorder (ADHD) (8), and monogenic diseases (7) appear to be significantly associated with a non-atopic phenotype of EoE (2).

An increasing amount of evidence showed that children with esophageal atresia (EA) (30–32) or with diaphragmatic hernia (34) are at higher risk to develop EoE (33, 34, 79). Several risk factors have been associated with the development of EoE in children with EA, such as early life factors, early exposure to acid suppressants and antibiotics, GERD, esophageal dysmotility, and epithelial injury (79). Interestingly, Krishnan et al. demonstrated that children with EoE + EA share the same dysregulated genes (that encode for proteins involved in epithelial barrier functions and Th2 inflammation) compared to patients with EoE and without EA (33).

Although not widely demonstrated, another possible risk factor for EoE might be childhood exposure to caustic ingestion. Homan et al. reported a case of EoE development after caustic damage in a child with allergic comorbidity (35). The authors proposed two possible explanations for this association: (1) the caustic ingestion primarily triggered the eosinophilic inflammation of the esophagus or (2) after caustic damage the esophageal lesion might allow the trigger exposure (mainly food allergens) that might lead to EoE (35). Although fascinating, this report is characterized by some bias (child presented allergic diseases); however, further and extensive studies are required to confirm this data.

The diagnosis of gastroesophageal reflux disease (GERD) was also increased in the last two decades in Western countries (80), in parallel to allergic diseases, and, as a result of cow's milk allergy in the half of infants with refractory GER (81). Some authors reported that GERD might play a role in the pathogenesis of esophageal eosinophilia, more relevant in PPI-responsive cases (82). GERD, esophageal eosinophilia, and EoE are not mutually exclusive and may coexist in the same patient. However, there was no precise data about this association, and four mechanisms were proposed to explain it. (1) GERD causes esophageal eosinophilia in the absence of EoE, (2) GERD and EoE coexist but are unrelated, (3) EoE contributes to or causes GERD, (4) GERD contributes to or causes EoE (82). In patients with GERD, acid reflux alters the epithelial barrier of the esophagus, increasing the permeability and the passage of food allergens that might trigger EoE. Furthermore, acid reflux in GERD may induce the expression of inflammatory molecules and eosinophil chemoattractants (13, 83). On the other hand, eosinophilic inflammation produces different molecules (vasoactive intestinal peptide and interleukine-6) that might impair the esophageal peristalsis and delay the esophageal acid clearance (14). The subepithelial fibrosis, a delayed complication of EoE, might promote esophageal dysmotility (15). Further studies are needed to understand if this possible pathogenetic correlation might early predispose children with GERD to develop the EoE.

Interestingly, the 10–15% of children with EoE presented to the otolaryngologist before to be referred to the gastroenterologist (84), and the 33% of these patients required one or more otolaryngologic surgical interventions (20% bilateral myringotomy, 14% tonsillectomy, 18.5% adenoidectomy, 1.4% sinus irrigation, 3.3% bronchoscopy, and 1.4% laryngotracheoplasty), suggesting that EoE might overlap with otolaryngologic pathology (85).

Western Diet and Lifestyle

Although foods are the primary triggers of EoE, there are limited data about the role of the Western diet in the contribution of the EoE pathogenesis. Higher levels of fatty acids characterize the Western diet and could be related to the increased risk of developing allergic diseases. In a recent study in mice, Silva et al. demonstrated that high-fat diet and obesity aggravated the immune histopathological characteristics and increased inflammatory cells in the EoE experimental model (36). These fascinating data provide new insights about obesity as a possible risk factor, impairing EoE symptoms; however, further prospective studies are needed.

No studies evaluated tobacco exposure in children and adolescents with EoE. Only a recent case-control study of adult patients showed that smoking was inversely associated with EoE compared to controls (86).

Geographic Risk Factors and Vitamin D Levels

As previously reported and already described for other inflammatory gastrointestinal diseases, EoE prevails in specific geographic areas of the world. Prevalence rates of EoE were higher in Western regions of Europe, North America, and Australia than Asia and Africa (3). These geographic differences between Western countries (high prevalence) and Eastern countries (low prevalence) suggest that environmental factors might play a significant role in etiological mechanisms. The effects of people migration on the future development of EoE have not yet been investigated.

A few and conflicting studies evaluated the geographic distribution of EoE, based on the population density. An extensive US survey of Spergel et al. showed that EoE prevalence was higher in urban (0.58) and suburban (0.44) compared with rural settings (0.36, $P < 0.0065$) (87). Lee et al. demonstrated no significant difference in the incidence of EoE between people living in the rural area (50.9%) vs. patients from the urban

ones (49.1%) (88). On the other hand, more recently, Jensen et al. found a strong inverse association between the population density and development of esophageal eosinophilia or EoE, demonstrating that EoE was more common in rural areas, in contrast with the hygienic hypothesis (89). A possible explanation of these results might be the geographic variation of specific environmental allergens.

Eosinophilic esophagitis prevails in cold climate zones, suggesting a possible association with specific aeroallergens (tree or grass pollens) and with low serum vitamin D levels (18). Increasingly significant evidence showed a link between vitamin D deficiency (maternal diet during pregnancy, early childhood diet, lack of exposure to sunlight) and risk of atopy, as described for asthma, allergic rhinitis, food allergy, and atopic dermatitis (37–39). This association is generally strongest in early life; in fact, interventional studies showed that the supplementation of vitamin D *in utero* and early life reduces the risk of recurrent wheeze and asthma (40–43). Although vitamin D enhances antimicrobial pathways, promotes peripheral immunological tolerance, and maintains mucosal barrier integrity, no studies have evaluated its possible preventive role in EoE development or its help in disease remission.

Climate zones might also affect the season of EoE diagnosis. Several single-center studies have evaluated the seasonality of symptoms and new diagnoses of EoE. In pediatric cohort studies, the seasonal exposure to aeroallergens increased the esophageal eosinophilic inflammation in children with EoE and allergic rhinitis (90, 91). However, the association between EoE relapse and season is still unclear, and available results were contradictory (16, 17, 92–97).

EARLY LIFE RISK FACTORS OF EoE: STATE OF ART

Early life is a critical period during the immune system and microbiota mature, becoming susceptible to early environmental exposures. A well-balanced microbiome is fundamental for the correct development of the immune system (98–100), and numerous early life exposures, including prenatal (maternal diseases, mother diet, and lifestyle), intrapartum (cesarean section, maternal fever, and infections, prematurity), and postnatal factors (early antibiotic and acid suppressants use, formula feeding), might impair the gut microbiome, and predispose to allergic diseases (101–109). The association between early impaired microbiota and risk of atopy is widely described for asthma, allergic rhinitis and food allergy (44, 45, 110). A few studies postulated that early life exposures might also predispose to EoE in childhood (**Table 2**). However, few studies focalized on early life exposures and their effects on the future development of EoE (27, 46–49). The available studies reported that formula feeding (27, 60), neonatal intensive care (NICU) admission, prematurity (47, 49), maternal fever (47), antibiotic and acid suppressants use in infancy (27, 49), cesarean delivery (27, 47) were putative early risk factors of EoE. The antibiotic and proton pump inhibitor (PPI) use in infancy showed the most consistent evidence of a positive correlation with the future development of EoE.

Effect of Early-Life Use of PPIs and Antibiotics

Although PPIs are used to treat GERD and esophageal eosinophilia, some studies paradoxically showed that the early PPI use might predispose to the development of autoimmune gastrointestinal diseases (celiac disease) (60), food allergies (13), and EoE (50). Physiologically, digestion of food proteins–and potential food allergens–begins into the stomach through pepsin proteinases, that are activated by the gastric acid *milieu*. PPI therapy might inactive proteinases and facilitate the digestive escape of food allergens, increasing the gastric pH. Also, PPI might increase the gastric mucosal permeability and the passage of allergens through the gastric mucosa, allowing their exposure to immune cells and the activation of atopic inflammation (51–53). Finally, PPI might alter the esophageal microbiota, and the modulation of immune response (54, 55). The risk to develop EoE after PPI therapy later in life has minimally been evaluated and could be higher after a long-term therapy (56, 111, 112). However, these data suggest that the immune system of infants might be more susceptible to PPI exposure, which might trigger the allergen-mediated inflammation of EoE. Since 1989 when the first PPI (Omeprazole) has been introduced into clinical practice, a worldwide escalation of PPI prescriptions was described at any age. Surprisingly, a pediatric study documented an 11-fold increase of new PPI prescriptions under 12 months of age in the last two decades (113).

The use of antibiotics in pregnancy is related to the treatment of several infections, such as bacterial vaginosis and urinary tract infections. Also, *intrapartum* and *peripartum* antibiotic prophylaxis are fundamental to decrease the risk of Group B *Streptococcus* infection in positive mothers and newborns. However, antibiotics might alter the immature gut microbiome of the newborn. Studies in rodents demonstrated that the administration of antibiotics in pregnancy decreased the microbiota diversity and permanently altered the immunity (57, 58). In newborns, the early administration of antibiotics resulted in decreased *Bifidobacterium* and increased enterococci strains (59). The worldwide increase of antibiotics prescriptions, especially in infancy, might partially explain the rise of allergic diseases. Observational studies demonstrated that the early life antibiotic administration was associated with asthma, atopic dermatitis, and allergic rhinitis (114–116). As previously mentioned, Jensen et al. founded a significant association with early antibiotic use and the development of EoE in children (27). Although an exact cause-effect mechanism cannot be deducted, these data suggest that the early exposure to antibiotics potentially might alter the immature microbiome and the developing immune system, allowing the risk of EoE (117).

The worldwide increase of PPI and antibiotic prescriptions in early life, associated with their possible pathogenetic role in

allergic disease and EoE, suggests a conscious and rational use of these drugs, especially in childhood.

Breastfeeding and Timing of Food Introduction in Children

Breastfeeding might be a possible factor that could prevent the development of food allergy through different mechanisms. Human milk shows potentially anti-allergic immune properties; in particular, the presence of maternal antibodies might prevent exposure to food allergens and induce oral immuno-tolerance (118). However, there is limited evidence on the direct correlation between breastfeeding and the development of EoE. In a pediatric case-control study, Jensen et al. identified a strong interaction between the calpain14 (CAPN14) gene variant (rs6736278) and breastfeeding, suggesting the possible protective role of human milk against EoE. CAPN14 is a cysteine protease and plays a fundamental role in the integrity of the esophageal epithelial barrier. Furthermore, its expression is only limited to the esophageal mucosa (119). CAPN14 expression was almost 4-fold increased in EoE patients compared to controls. Higher levels of CAPN14 expression are associated with the downregulation of desmoglein 1, filaggrin, and zonulin, which are pivotal proteins of the epithelial barrier (119). Although the exact mechanism of interaction between breastfeeding and CAPN14 is still unknown, human milk with its immunological properties might protect the esophagus from the epithelial barrier impairment and the development of EoE in patients with specific genotypes (120).

Over the last decade, food allergy research mainly focused on the timing of food introduction and oral tolerance. Murine models well-explained the concept of oral tolerance, and previous works showed how early and regular oral exposure to food allergens induced clinical tolerance and immunological changes. A large amount of evidence demonstrated that an early introduction of allergens might protect against the risk to develop IgE-mediated food allergy (61, 121, 122). In the last years, an increasing scientific interest focused on the diagnosis of non-IgE mediated food allergy, which often presents with a delayed onset of gastrointestinal symptoms. The EAT study evaluated data of non-IgE mediated symptoms (colic, vomiting, regurgitation, diarrhea, and constipation), demonstrating that infants in the early intervention arm reported significantly more non-IgE type symptoms than children in the standard intervention arm. However, rates of non-IgE mediated symptoms were equivalent in both groups at any time point, suggesting that the reporting of these symptoms did not depend on the introduction of the specific food allergen (121, 123). Further research is needed to understand if early food introduction could prevent non-IgE mediated food allergies, including EoE. Although the understanding of the EoE pathogenesis achieved notable progress, there are no published studies about the timing of food introduction in infancy and the future development of EoE.

Genetic Risk Factors

EoE has a strong familiar hereditability pattern. Monozygotic twins had a 44% disease concordance, a 2-fold increase compared with dizygotic twins (5, 6). These data underly a complex interplay between genic loci and environmental exposures, through epigenetic mechanisms that are partially understood (6). Also, the relative risk to develop this disease in dizygotic twins might increase more than 10-fold compared to siblings. The increased rate of EoE development in dizygotic twins could be attributed to the same early-life environmental factors, previously mentioned.

The inheritance mechanism of EoE could be related to the effects of multiple single nucleotide gene polymorphisms (SNPs) that increase disease risk, depending on the environmental exposures and disease risk-modifying factors (119, 124). Several studies, including candidate-gene identification and genome-wide association studies (GWAS), have identified different genetic *loci* that are likely contributing to the development of EoE and mainly include thymic stromal lymphopoietin (TSLP), calpain 14 (CAPN14), EMSY, LRRC32, STAT6, and ANKRD27 (7). These genetic loci are mainly involved in T-helper 2 type inflammation (allergic inflammation) and epithelial barrier function and integrity. Interestingly, EoE is also associated with several monogenic inherited diseases, especially with connective tissue disorders and skin diseases. Connective tissue disorders, such as Marfan and Ehlers Danlos Syndromes, share a common pathogenic mechanism through the dysregulation of the TGF-β signaling. Children with autosomal dominant Hyper-IgE Syndrome (HIES) and Netherton Syndrome have also significantly increased the incidence of EoE (125, 126). Defects in PTEN, dehydrogenase E1, and transketolase domain–containing 1 (DHTKD1) genes are also associated with EoE (127, 128).

HOW COULD WE PREVENT EoE?

The rise of EoE diagnosis, especially in children, is an actual problem, and preventive strategies are needed to limit this phenomenon. Although there are no published studies about the prevention of EoE, we could speculate that possible strategies of primary prevention of EoE might be:

1. Sustaining breastfeeding in the first 6 months of life, especially in preterm babies and newborns from mothers that underwent cesarean section.
2. Limiting the uncontrolled prescriptions of acid suppressants and antibiotics only in specific and right circumstances.
3. Do not delay the introduction of food allergens in infants.
4. Providing adequate levels of vitamin D in infant and children, especially in those from cold climate regions.
5. Encouraging a well-balanced diet and a healthy lifestyle both in pregnant women both in children.

This work has several strengths. Firstly, this is a comprehensive review, summarizing the current knowledge on EoE risk factors, and focusing on the role of early exposures. Also, this review tried to answer to two main clinical issues: (1) the increased prevalence and incidence of EoE in Western countries, especially in children; (2) the lack of knowledge on early risk factors and possible preventive strategies.

There are several limitations. First of all, the lack of extensive and prospective studies evaluating the real burden of

environmental risk factors, particularly the pathogenetic role of early exposures. Secondly, the vast majority of genetic and epidemiological studies were realized in Western Countries and mostly in the US. Finally, a few studies evaluated the gene-environmental interactions and the possible preventive strategies for EoE. Therefore, the lack of prospective and extensive studies from Eastern and developing Countries did not allow to draw reliable conclusions on the role of early risk factors and preventive strategies in EoE.

In conclusion, EoE is an emerging atopic disease that affects people at any age and characterized by symptoms due to esophageal inflammation, dysmotility, and fibrosis. As described for allergic diseases, several environmental risk factors and early-life exposures might interfere with genes, alter tolerance mechanisms, and activate the Th2 inflammation of EoE. Further studies are needed to identify risk factors of EoE, understand the interaction between genes and environment, finally find possible early preventive strategies.

AUTHOR CONTRIBUTIONS

MV and MD reviewed the literature and wrote the manuscript. AL, SC, IB, and GM reviewed the literature and helped with the writing of the manuscript. All authors approved the final version of the manuscript and agreed to be accountable for all aspects of the work. Questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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MINI REVIEW
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Primary Prevention of Allergic Diseases: The Role of Early Exposure to Cow's Milk Formula

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The burden of atopic disorders is continuously worsening worldwide, especially in childhood. Therefore, risk factors and preventive measures have been called into question. The age when infants introduce complementary foods, varies greatly according to traditional habits, clinical practice recommendations, and breastfeeding duration. It is still debated the impact of early exposure to cow's milk on the increase of allergic diseases, mainly food allergy, and atopic dermatitis. Many factors may play a role in this potential link, such as genetic variation, parental atopy, infant feeding regimens. Recent evidences suggest that the early introduction of complementary foods (up to 6 months of age), including cow's milk, could prevent the development of food allergies. So, several countries included this new approach into feeding guidelines. Our review will focus on the influence of early exposure to cow's milk formula on the development of allergic diseases. Some trials found that cow's milk supplementation in the first days of life could even increase the development of IgE sensitization and food allergies. Other trials did not show any efficacy on prevention of allergic disorders. Further studies are needed to understand the prospective for allergy prevention related to optimal timing of cow's milk formula introduction.

Keywords: atopy, allergy prevention, breastfeeding, children, cow's milk allergy, atopic dermatitis, food allergy, asthma

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INTRODUCTION

The burden of atopic disorders is continuously increasing worldwide, especially in the pediatric setting. Therefore, several risk factors and diverse preventive measures have been called into question (1). The age when infants introduce complementary foods varies greatly according to traditional habits, clinical practice recommendations, and breastfeeding duration. Several studies suggest that the timing of introduction of food allergens may be fundamental for the development of allergic diseases, principally food allergy, and atopic dermatitis (2). In particular, the effects of early exposure to cow's milk (CM) are still debated. Many factors may play a role in this potential relation (3–5). In the past, clinical practice guidelines had recommended a delayed exposure to allergenic foods including CM, egg, fish, nuts among children with parental atopy (6, 7) to prevent allergy with poor results (8). The timing of exposure to complementary foods corresponds to the healthy gut colonization, found to be crucial in stimulating allergen tolerance (9). So, it has been hypothesized that allergic disorders can be due to immature immunoregulatory networks and reduced diversity and intensity of microbial exposure (10, 11). Moreover, another suggestion of allergy risk is the dual-barrier hypothesis, theorizing that allergic sensitization is a consequence

of cutaneous exposure, and tolerance results from oral exposure to foods (3). So, avoidance of specific foods (e.g., egg or peanuts) can increase the risk of developing allergy, especially in high risk infants (e.g., barrier defects, such as eczema or filaggrin deficiency). Therefore, preventive strategies shifted from avoidance to controlled exposure, suggesting that allergen avoidance may be even harmful for allergy prevention. This has raised the search of an "optimal window" for introduction of complementary foods to prevent allergic disorders. Data suggest to start weaning not before 3-4 months of age, because gut colonization and immune network are not well-established yet (12–14). Updated guidelines nowadays recommend introducing complementary foods from 4 to 6 months of age irrespective of potential food allergenicity and atopic family history (15–21). However, if these guidelines can be recommended to general population or only in high-risk infants it has still to be elucidated (22). Moreover, no current guideline defines the optimal timing of introduction of cow's milk formula (CMF) other than that it can be comprised along with other foods.

Cow's milk allergy (CMA) represents the most common food allergy in infancy with an estimated prevalence of 2–5% (23). Only in 60% of cases CMA is IgE-mediated with symptoms, such as urticaria, wheezing, anaphylaxis, starting within 15–30 min after exposure to CMP, even in low amounts (24, 25). Other cases include food protein–induced enterocolitis syndrome (FPIES) or a mixed IgE-associated or cell-mediated reaction, such as atopic dermatitis (26) and eosinophilic gastroenteritis. The child becomes sensitized to food allergens *in utero*, via breastmilk and by ingestion, skin contact, and inhalation (27). Even if it is unclear whether breastfed infants are less prone to develop atopic dermatitis or food allergy, breastfeeding keeps on being fundamental in infants' diet and it is advised at least until 6 months of age (28).

The aim of the current review is focused on the influence of early exposure to cow's milk proteins (CMP) in the first days of life on the development of allergic diseases. We perform a literature search in PubMed and Cochrane library for English-language studies published during the period 1985–2019 that assessed whether early administration of CMF in the first 3–4 days of life was associated with development of CMA or atopic diseases. Information was obtained from randomized controlled trials (RCT) published in peer-reviewed journals. Trials that compared CMF only with hydrolyzed formula, rice formula, soy formula, or other mammalian milk and studies in preterm infants were excluded. In addition, we discussed selected papers that may be useful for the purpose of this review. We planned to provide a practical approach for introducing CMP in the infant's diet.

DESCRIPTION OF RCT STUDIES

Five RCT (**Table 1**) met the inclusion criteria (29–35). In 1988, Lindfors and Enocksson (29) and Lindfors et al. (30) randomly assigned a 216 healthy term infants to CMF feeding ($n = 112$) or breastfeeding ($n = 104$) for the first week of life since their earliest meal (at 6 h of age) and studied the incidence of atopic diseases up to 18 months of age. Children were examined by welfare center's pediatricians who participated in the study at 3, 6, 18 months of age and at the age of 4–6 year. Allergic symptoms were recorded as obvious or probable. Moreover, infants with high risk for atopy were assigned to *single* heredity group (one parent or a sibling with a positive history of allergy) or double heredity group (two first-degree relatives with a positive history of allergy). Serum IgE antibodies to inhalants, pediatric food allergen mix and skin prick tests to egg, cow's milk, and inhalants were performed at 4–6 years of age.

Juvonen P et al. randomized 129 infants at birth to human milk (HM), CMF, or highly casein hydrolysate formula during the first 3 days of life (31). Subsequently, infants were exclusively breastfed. Children were clinically examined and serum IgE levels to alpha-lactoglobulin were detected at 2, 4, 8, 12, and 24 months of age. At 1 and 2 years of age, infants underwent skin prick tests to food and inhalant allergens. Food allergy or allergic symptoms were diagnosed by a telephone interview at 3 years of age.

A double-blind RCT exposed 1,533 breastfed newborns to CMF or protein free placebo during the first 3 days of life with the aim of detecting the occurrence of atopic diseases in the first 5 years of life by history, physical examination, and questionnaire (32, 33). Serum specific IgE antibodies to cow's milk, egg and inhalants were measured at 1 and 5 year of age.

Saarinen et al. (34) studied whether supplementary feeding of CMF at maternity hospital would influence the frequency of CMA at about 27 months of age when compared with pasteurized HM among 6,209 unselected full-term newborns. CMA was diagnosed by oral food challenge.

A recent Japanese RCT (35) (Atopy Induced by Breastfeeding or Cow's Milk Formula [ABC] trial) randomized 312 newborns with a family history of atopy to breastfed *plus* aminoacid–based elemental formula group in the first 3 days of life (BF/EF) or breastfed plus cow's milk formula (BF/CMF) from the first day of life to 5 months. Primary outcome of the study was rate of positive IgE antibodies to cow's milk (IgE $>$ 0.35 allergen units [UA]/mL) at 2 years of age. The occurrence of immediate food allergy, including CMA, diagnosed by oral food challenge test, or triggered by food ingestion with positive IgE to the relevant food was also investigated.

COW'S MILK ALLERGY/SENSITIZATION

The development of CMA diagnosed by oral food challenge that is the gold standard for ascertaining food allergy, in subjects that were early fed with CMF was investigated by two of RCTs we reviewed (34, 35). They showed that the administration of CMF in the first 2–4 days of life may play a role for the onset of CMA. In infants with atopic family history (35), the incidence of CMA at age 2 was lower among breastfed newborns at risk for atopy supplemented with aminoacid-based formula compared to those supplemented with CMF (CMA: 1/151 in BF/EF group in the first 3 days of life vs. 10/151 in BF/CMF group; RR 0.10; 95%CI, 0.01–0.77). Moreover, at 2 years of age, infants in the BF/EF group were less frequently sensitized to CM (specific IgE levels $\geq$0.35 UA/ml) compared to infants in the BF/CMF group (16 vs. 32%; relative risk, 0.52; 95%CI, 0.34–0.81). Infants who avoided CMF

TABLE 1 | Characteristics of randomized controlled studies on introduction of cow's milk formula in the first days of life.

Author, Country	Population	Inclusion criteria	N active group/N control group	Cow's milk challenge	Age at outcome assessment	N active group/N control group with cow's milk allergy	N active group/N control group with atopic diseases	N active group/N control group with other food allergy	Feeding after 3 days
Lindfors and Enocksson. (29), Sweden	General population	Healthy term infant, birth weight between −1 SD and −2 SD	109 cow's milk formula/98 breast milk	No	18 months	Not reported	18%/33% ($p < 0.05$)	Not reported	Cow's milk formula or breast milk
Lindfors et al. (30), Sweden	General population	Healthy term infant, birth weight between −1 SD and −2 SD	95 cow's milk formula/88 breast milk	No	4–6 years	Not reported	20%/32%	Not reported	Cow's milk formula or breast milk
Juvonen et al. (31), Sweden	General population	All newborns	39 cow's milk formula/53 breast milk	No	3 years	0/1	7%/7%	5.1%/5.6%	Breast milk
De Jong et al. (32), Netherlands	General population	Healthy full-term newborns	758 cow's milk formula/775 placebo	No	2 years	Not reported	11.9%/12.4%	Not reported	Breast milk Cow's milk formula if required
De Jong et al. (33), Netherlands	General population	Healthy full-term newborns	542 cow's milk formula/566 placebo	No	5 years	Not reported	26.3%/25%	Not reported	Breast milk Cow's milk formula if required
Saarinen et al. (34), Finland	General population	Healthy full-term newborns	1,758 cow's milk formula/1,844 human milk	Yes	mean (± SD) 6.7 ± 2.3 months	2.4%/1.7%	Not reported	Not reported	Breast milk Cow's milk formula if required
Urashima et al. (35), Japan	At least one 1-degree relative with current and/or past atopic disease	Healthy full-term newborns	156 or breastfed plus cow's milk formula /156 breastfed plus amino acid–based elemental formula	Open food challenge or a suggestive reaction	2 years	6.6%/0.7%	Not reported	Egg, 19.9%/11.3% Wheat, 4.6%/0.7%	In breastfed plus amino acid–based group, 115 infants had breast milk + cow's milk formula, 39 had breast milk/Elemental formula

for 3 days could be then fed with CMF according to maternal preferences, and only 39 infants did not receive CMF until 5 months of age. However, a *post-hoc* analysis did not show any difference for food allergy in this subgroup. On the other hand, Saarinen et al. (34) noted a non-significant increase of the risk of CMA in general population who were fed with CMF for an average of 2 days after birth. CMA occurred in 2.4% of infants who received CMF supplementation at maternity hospital vs. 1.7% of those supplemented with pasteurized HM at maternity hospitals [Odd Ratio (95% CI) 0.70 (0.44–1.12)]. The mean follow-up period was 27 months (range, 18–34 months). Juvonen et al. (31) showed that no child who received CMF during the first 3 days of life developed CMA at 3 years of age while one child who received HM developed atopic eczema to cheese. Regarding sensitization to cow's milk, Lindfors et al. (30) found that at 5 years of age, skin prick test results to cow's milk were positive in 3/80 children who were early fed with CMF and in 0/74 children who did not received infant formula. Finally, de Jong et al. (32, 33) found that at 1 and 5 year of age there was no difference in serum specific IgE levels to cow's milk between infants who early received CMF and those who received placebo. Differences in selected populations and study design may explain different findings among studies (36). Several explanations may be offered for the potential enhanced risk for CM sensitization in subjects with allergic family history who in their initial days of life are exposed to CMF. It is possible that an early occasional ingestion of CMF may initiate sensitization and predispose infants to CMA. Accordingly, a retrospective case-control study by Kelly et al. (37) showed that CMA were seven times more frequent in breastfed infants who received CMF in the first 24 h of life than in exclusively breastfed infants. Infants who were exclusively formula fed were not at increased risk for CMA. Along this line, Katz et al. (38) showed that occurrence of CMA was less likely in infants who were regularly exposed to CMF following discontinuation of exclusive or almost exclusive breast-feeding in the first 15 day of life than those who received CMF later in the first year of life. Moreover, a continuous administration of the allergen during allergen specific immunotherapy has been shown to be effective to induce tolerance to the allergen in question (39). Unfortunately, the studies we reviewed did not assessed this issue. Another possibility is that may be due to immature local immune system and bacterial intestinal colonization (13). Indeed, germ free mice rated with antibiotics or empty of any bacteria colonization have exhibited great susceptibility to anaphylaxis and food allergy (10).

ONSET OF ALLERGY/SENSITIZATION TO OTHER FOODS

There is no clear evidence that hypersensitivity to foods other than CM was associated with early administration of CMF in the studies we reviewed. In the ABC trial (35), sensitization rate to egg white or wheat did not increase in infants who had CMF at birth. At 2 years of age, the immediate and anaphylactic types of food allergy independent of food type (i.e., not simply CM but additionally wheat, hen's egg, and others) were more common in the group early exposed to CMF. However, it is unclear what are the results when CMA is excluded and the mechanisms are undetermined. Moreover, Juvonen et al. found that at 3 years of age there was no difference in food allergy rates between CMF group and HM group during the first 3 days of life (3/53 infants in the HM group, 2/39 in the CMF group) (31). The most frequently sensitizing food was hen's egg. Finally, de Jong et al. (32, 33), found no differences in serum specific IgE levels for egg at 1 and 5 year of age between active group and placebo group.

ALLERGIC SYMPTOMS

For atopic diseases, observational studies have reported discordant results on timing of introduction of CMF and their prevention (40–43). Regarding the studies we reviewed, the judgement is unclear and new studies are warranted. A significant association of atopic diseases with early CMF administration has been reported in one case (29, 30) and no link in two cases (31–33). Lindfors et al. (29, 30) showed a significantly increased frequency of allergic symptoms in the formula-fed infants compared to breast-fed infants at 18 months of age, particularly in children with double atopic heredity. At 5 years of age, findings of the study found a significant lower frequency of allergic symptoms among formula-fed infants compared to controls only in the double positive atopic family history group. Juvonen et al. (31) found that infants who avoided CMF were not more likely to develop allergic symptoms at 3 years of age than those who did not avoid. In the trial by de Jong et al. (32, 33) no significant difference was found in the development of atopic diseases among children exposed or not to CMF, at 1 year of life (10 vs. 9.3%), at 2 year of life (9.6 vs. 10.2) and at 5 years of life (26.3 vs. 25%, relative risk 1.05).

INDIVIDUAL ALLERGIC SYMPTOMS

Atopic dermatitis was not associated with early administration of CMF in the studies we reviewed Indeed, a Swedish study showed that both at 18 months of age (9 vs. 18%) (29) and at 5 years of age (17 vs. 25%) (30) there was no significant difference in the frequency of atopic eczema between infants fed with CMF or breastfed in the first week of life. This was the case also in subgroups with family history of atopy. Over the first 3 years of life, Juvonen et al. (31) showed that the incidence of atopic eczema was not statistically different in infants fed in the first 3 days of life with HM in comparison with those fed with CMF (3/53 vs. 3/39). The results of a follow-up analysis of the trial by de Jong et al. (33) showed that brief neonatal exposure to CMP was not associated with atopic eczema at 5 years of age.

Timing of CM introduction very early in life did not prevent the development of wheeze. In infants fed with CMF or breastfed in the first days of life, Lindfors et al. did not find any difference in the incidence of wheeze, at 18 months of age (29) and at 5 years of age (30), even in relation to family history of allergy. The incidence of asthma until 3 years of age was not different in infants who received CMF vs. HM during the first 3 days of life (3/53 infants in the HM group, 0/39 in the CMF group) (31).

At 5 years of age, the frequency of wheeze during the past 12 months was similar in infants who received CMF and in those who received placebo in the first 3 days of life (33). As well, sensitization to inhalant allergens was not dissimilar between intervention groups. Introduction of CMF in the first days of life compared with breastfeeding did not increase the frequency of urticaria and gastrointestinal symptoms at 18 months of age (29) or at 5 years of age (30).

For rhinoconjunctivitis, CMF intake in the first days of life did not increase the risk of developing rhinoconjunctivitis or positive specific IgE antibodies to seasonal or perennial allergens (30, 33). It is difficult to understand why the risk of rhinoconjunctivitis significantly increased among children with double atopic heredity (30).

CONCLUSIONS

There is a long-standing debate on the link between early introduction of CMF and onset of CMA and allergic diseases that led us to review the results of RCT. Our paper extends findings of a previous review (44) by considering more studies (32, 33, 35). Yuan showed that early introduction of CMF did not have any effect on development of asthma, atopic dermatitis and CMA.

Some data in children with positive family history of atopy might suggest that an early exposure to CM may predispose to the onset of CM sensitization. It is unlikely that an early exposure to CMF prevents the development of allergic diseases or hypersensitivity to foods other than CM. However, several items limit conclusions of our review. There is a paucity of studies on the role of early exposure to CM on the prevention of allergy. We search only RCTs in English language so relevant studies might potentially be excluded. Study designs are heterogeneous, and inconsistent. Some trials are small. Findings of trials are divergent and associated to confounders, such as family history of atopy, number of outcomes, duration of breastfeeding, weaning (45), age at analysis, definition. Amount, dose, frequency and composition of formula supplementation may also affect the results. CM challenge test was performed only in two studies (34, 35). A third arm fed with hydrolyzed milk formula has also been introduced in a minority of studies (31, 34). Another issue is that an RCT study may not mirror the real life since the supplementation is often given when a weight loss is observed or lack of breast milk is perceived and it doesn't necessarily continue as milk comes in.

Much more effort is still needed to understand the prospective for allergy prevention related to early exposure to CMF, the optimal timing of CM introduction, continuous intake of CM over time, and the potential consequences of current strategies on breastfeeding.

AUTHOR CONTRIBUTIONS

CM, AS, and CC co-wrote the manuscript and approved the final version. All authors contributed to the article and approved the submitted version.

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Hydrolyzed Protein Formula for Allergy Prevention in Preterm Infants: Follow-Up Analysis of a Randomized, Triple-Blind, Placebo-Controlled Study

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Background: Allergic diseases are a major public health burden worldwide. Evidence suggests that early nutrition might play a key role in the future development of allergies and the use of hydrolyzed protein formulas have been proposed to prevent allergic disease, mainly in term infants with risk factors.

Aim: To evaluate the preventive effect of a hydrolyzed protein formula vs. an intact protein formula on allergy development in preterm infants with or without risk factors.

Methods: We performed a 3-year follow-up study of a previous triple-blind, placebo-controlled randomized trial. Evidence of atopic dermatitis, asthma and IgE-mediated food allergies were evaluated according to a validated parental questionnaire (Comprehensive Early Childhood Allergy Questionnaire). Food sensitization was also investigated by skin prick test at 3 years of chronological age.

Results: Of the 30 subjects in the intact protein formula group and 30 in the extensively hydrolyzed formula group, respectively 18 and 16 completed the 3-year follow-up and entered the final analysis. No group differences in the incidence of atopic dermatitis, asthma, IgE-mediated food allergies, and food sensitization were found.

Conclusion: Despite the small number of cases, extensively hydrolyzed protein formula seems to be ineffective in allergic diseases prevention in preterm neonates. Further adequately powered, randomized controlled trials evaluating hydrolyzed protein formula administration to prevent allergic diseases in preterm neonates are needed.

Keywords: preterm/full term infants, infant formula, hydrolyzed protein formula, hypersensitivity, allergy

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INTRODUCTION

Allergic rhinitis (AR), asthma, eczema, and food allergy are some of the most common pediatric chronic conditions worldwide and have a major impact on children health and quality of life (1).

Allergic diseases are genetically determined but also influenced by several factors such as environmental pollution, smoke, aeroallergens, and early feeding pattern (2). To date, a tailored approach seems to be the best strategy to hamper the so-called "atopic march" (3). In this perspective, standard operative procedures to prevent allergy have become a priority in managing public health and infant feeding is considered the most important modifiable factor that can be targeted (4).

World Health Organization (WHO) states that breast milk is the best source of nutrients for both term and preterm infants, and there is some evidence of its role in decreasing the risk of allergy development (5, 6). Unfortunately human milk is not always available and the challenge for many pediatric societies remains to draw up standardized and definitive guidelines to recommend the most effective infant formula in allergy prevention (7).

More recently, hydrolyzed formulas (HF) have been proposed for prevention of allergy and many studies suggested the use of these formulas in formula-fed infants with a family history of allergic diseases (8).

The main differences between each HF are the degree and method of hydrolysis, with consequent different immunological, clinical, and nutritional effects: extensively hydrolyzed formulas (eHF) contain mostly peptides $\leq$3 kD, while partially hydrolyzed formulas (pHF) $\leq$5 kD (9).

Despite preterm infants could be at higher risk for food allergy because of their increased intestinal permeability and their possible higher food antigen uptake, they do not show higher incidence of allergic diseases when compared to term infants (10, 11).

At the moment, human milk represents the best source of nutrients for preterm infants for its bioactive effect (12). On the contrary, there is limited evidence regarding nutritional preventive action against the future development of allergies in this vulnerable population (13). This paper describes the follow-up results of a previous published triple-blind, controlled, clinical trial, in preterm neonates fed with either intact protein formula or extensively hydrolyzed formula (14, 15).

METHODS

Study design, inclusion and exclusion criteria, randomization and study group allocation, and feeding protocol are thoroughly described in the previous articles (14, 15).

Abbreviations: AD, atopic dermatitis; AR, Allergic rhinitis; CM, Cow's milk; CMA, Cow's milk allergy; eHF-C, extensively hydrolyzed casein formula; eHF, extensively hydrolyzed formula; FGIDs, functional gastrointestinal diseases; HF, hydrolyzed formula; HM, human milk; IPF, intact protein formula; pHF-W, partially hydrolyzed whey formula; pHF, partially hydrolyzed formula; RCT, randomized controlled trials; SPT, skin prick test; VLBW, very low birth weight; WHO, World Health Organization.

In brief, all mothers were encouraged to exclusively breastfeed and to have an unrestricted diet during lactation. At birth all eligible preterm neonates, regardless family history of allergy, were randomized to receive one of two different blinded formulas: either preterm intact protein formula [IPF: marketed Enfamil® Premature, Mead Johnson Nutrition (MJN), Evansville, IN, USA] or infant extensively hydrolyzed protein formula (eHF: marketed Pregestimil®, MJN, Evansville, IN, USA). When breastfeeding was not sufficient, one of the two formulas, according to randomization, was given for 2 weeks. The research protocol was approved by the ethical committee of "Azienda Ospedaliero—Universitaria Consorziale Policlinico" (number 4122—date 17/2/2016). Parents or legally authorized representatives provided written informed consent prior to enrolment.

To all participants, complementary feeding was recommended after the age of 4 months, without restriction of possible allergenic foods and with intact protein milk formulas in case of insufficient breast milk. To compare the allergy-preventive effect of eHF vs. IPF, all infants were followed-up 6-monthly until 3 years of chronological age, for evidence of atopic dermatitis, asthma, and IgE-mediated food allergies according to a validated parental questionnaire (Comprehensive Early Childhood Allergy Questionnaire) (16). Food sensitization, based on positive skin prick tests at 3 years of chronological age, was also investigated.

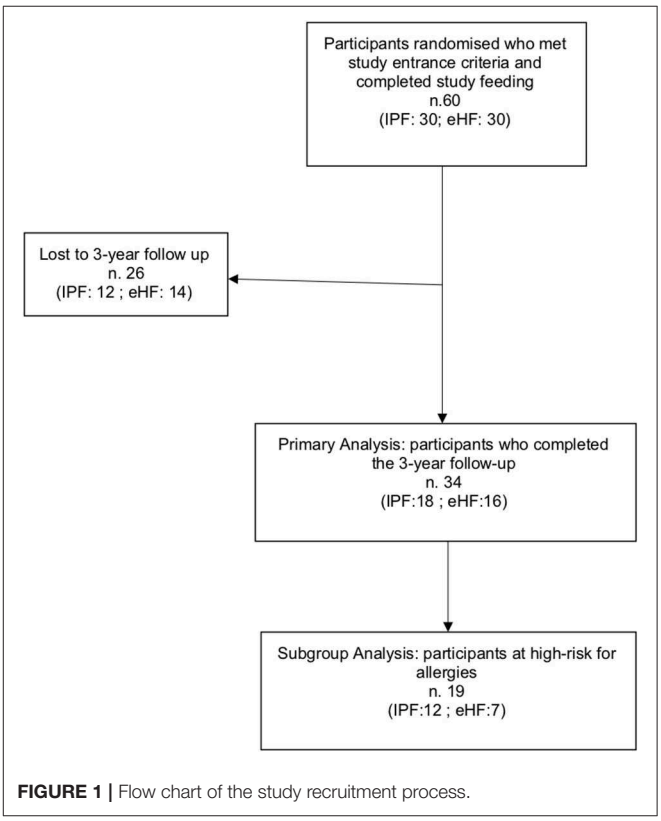

FIGURE 1 | Flow chart of the study recruitment process.

Statistical Analysis

Participant characteristics at enrolment were compared by Student *t*-test (gestational age and birth weight) or chi-square test (gender, birth type, cesarean section). Outcomes such as evidence of atopic dermatitis, asthma, and IgE-mediated food allergies were analyzed by chi-square test. All participants who met study entrance criteria and completed the 3 years follow-up period were evaluated. A subset analysis was carried out to assess participants at high-risk for allergy. High-risk infants were defined as having at least one parent or a single first-degree relative with a history of allergic disease. All tests were conducted at $\alpha = 0.05$. All analyses were conducted using IBM® SPSS® Statistics 23.

RESULTS

A total of 34/60 (56.6%) participants completed the 3-year follow-up study (IPF: 18; eHF: 16) and were included in the primary analysis. Dropouts occurred in 26 children due to protocol violation (3 patients) and voluntary withdrawal by parents during the follow-up period (23 patient) (**Figure 1**).

A subset analysis among infants at high risk for allergy included 19 participants: IPF $n = 12$ (66.7%); eHF $n = 7$ (43.8%); $p = 0.17$. Study flow chart is shown in **Figure 1**.

Infant characteristics at 3 years of chronological age were similar between groups (**Table 1**).

TABLE 1 | Infant characteristics.

	IPF	eHF	*p*
Gender, *n* (%)			0.774
Male	11 (61%)	9 (56%)	
Female	7 (39%)	7 (44%)	
Birth type, *n* (%)			0.311
Singleton	7 (39%)	9 (56%)	
Twin	11 (61%)	7 (44%)	
Gestational age, weeks, mean (*SD*)	30 (1,7)	29,8 (2)	0.860
Birth weight g, mean (*SD*)	1303 (254)	1334 (244)	0.723
High risk for allergy, *n* (%)	12 (67%)	7 (44%)	0.179
Breastfeeding duration, *n* (%)			0.725
Exclusively formula	2 (11%)	2 (14%)	
Breastfeeding <4 months	12 (67%)	8 (53%)	
Breastfeeding >4 months	4 (22%)	5 (33%)	

No difference in incidence of atopic dermatitis, asthma, IgE-mediated food allergies and food sensitization (positive skin prick test) were detected (primary or subset analysis) between groups (**Table 2**).

DISCUSSION

The results of our study indicated that eHFs did not provide any preventive effects of allergy in preterm infants. Despite the low number of patients and the inadequate sample size, our findings are in keeping with the most recent meta-analysis regarding HF effect on allergy prevention in term neonates. In 2015, Boyle et al. found no consistent evidence to support the use of HF formula for reducing risk of allergic diseases (17). Similarly, Osborn et al. in a 2018 Cochrane found no difference in allergic diseases such as asthma, eczema, rhinitis, and food allergy in infants fed with a HF compared to IPF (18).

Only another randomized, double-blind study was conducted in high-risk preterm infants by Szajewska et al. (19). They failed to demonstrate a decrease in the incidence of allergic diseases, yet highlighted only a preventive effect of eHF on atopic dermatitis (AD) during a short follow-up of 12 months.

Conversely, several studies have been conducted in the last decades to investigate the preventive role of HFs on allergy development in term infants (**Table 3**).

A preventive effect of eHF both on food allergy and sensitization was first outlined in 1992, in the RCT conducted by Zeiger et al. on 288 high-risk infants (20).

This result, despite the lack of strong evidence, was later confirmed by Odelram et al. (24) and Oldaeus et al. (25). Mallet et al. in their study on 177 high-risk infants found a preventive effect on eczema linked to the early use of eHF, without any effect on asthma (21).

Similar results on eczema were found by Vandenplas et al., in a RCT on 58 high-risk infants (23).

Another RCT on 158 high-risk infants was conducted by Halken et al., who found a reduction of CMA in the first 6 months of life associated to the early use of eHFs (whey or casein) (22).

Subsequently these results were confirmed by the same authors in a larger study on 595 high-risk infants randomized to receive eHF-W, eHF-C, or pHF (26).

The German Infant Nutritional Intervention (GINI) study is to date the largest, spontaneous, quasi-randomized trial in which 2,252 children (with a family history of allergic diseases) were randomized to receive extensively hydrolyzed

TABLE 2 | Study outcomes.

Outcomes	Primary analysis		*p*-value Fisher Exact Test	Subset analysis		*p*-value Fisher Exact Test
	IPF n. 18	eHF n. 16		IPF n.12	eHF n. 7	
Atopic dermatitis, *n* (%)	3 (17%)	3 (18%)	0.61	3 (25%)	0 (0%)	0.22
Asthma, *n* (%)	2 (11%)	2 (12%)	0.65	2 (17%)	1 (14%)	0.70
IgE-mediated food, *n* (%)	1 (6%)	1 (6%)	0.72	1 (8%)	1 (14%)	0.61
Food sensitization (positive skin prick test), *n* (%)	2 (11%)	2 (12%)	0.65	2 (17%)	2 (29%)	0.47

TABLE 3 | Summary of RCTs assessing the role of different HF on allergic diseases.

First author and year	Characteristics of population	Primary outcome	Secondary outcomes, if any	Types of the milk formula used	Techniques used	Main results
Zeiger et al. (20)	288 high-risk infants	To evaluate the effect of: Maternal or infant food allergen avoidance on AM Sensitization and serum IgE levels at 3 and 4 years of age IgG BLG and IgG OVA responses from birth to 2 years of age	To detect the interaction between genetics and environment on atopy development	eHF-C (Nutramigen)	SPTs Total IgE sIgE Nasal eosinophil determination Specific IgG BLG and IgG OVA DBPCFC	Maternal or infant food allergen avoidance decreased the cumulative prevalence of both food allergy and food sensitization (SPTs), conversely The period prevalence of food allergy or food SPT at 3 and 4 years of age Did not undergo any changes Cow milk avoidance associated with the use of breast milk and/or casein hydrolysate formula from birth to 1 year of age, reduced significantly IgG BLG response from 4 months to 2 years of age Egg avoidance has been slightly more effective in reducing IgG response to OVA, compared to standard feeding practices, which was evident at 2 years of age Influence of genetic and environmental factors toward serum IgE levels
Mallet et al. (21)	177 high-risk infants	To assess the allergy preventive effect of eHF-C in high-risk infant (evaluated at 4,12, 24, and 48 months of age)		eHF-C CMF	Total IgE sIgE	A preventive effect on the prevalence of eczema but not of asthma in eHF-C group
Halken et al. (22)	158 high-risk infants	To examine whether eHF-W (Profylac) is as protective as eHF-C (Nutramigen) considering the development of CMA and AM until 18 months of age		eHF-C (Nutramigen) eHF-W (Profylac)	Oral challenge	The use of eHF-W and eHF-C during the first 6 months of life reduces the incidence of CMA until 18 months of life
Vandenplas et al. (23)	58 high-risk infants	To evaluate the long term effect of pHF-W on the prophylaxis of AM		pHF-W CMF	-SPTs -Total IgE -sIgE	Reduced prevalence of eczema and incidence of diarrhea in the first 6 months of life in the group fed with pHF-W
Odelram et al. (24)	91 high-risk infants	To compare eHF-W with CMF in preventing the development of atopy	To compare growth with the use of eHF-W (Profylac) and CMF	eHF-W (Profylac) CMF	-SPTs -total IgE -sIgE	Despite the lack of strong evidence in the prevention of AM and sensitization, eHF-W seems to be a valid aid in the first 6 months of age No differences between eHF-W (Profylac) and CMF considering weight gain and growth
Oldaeus et al. (25)	155 high-risk infants	To compare AD incidence and allergic sensitization during the first 18 months of life in high-risk infants, fed with eHF, pHF, or CMF from weaning up to 9 months of age		pHF-W eHF-C (Nutramigen) CMF	-SPTs -sIgE	Allergy preventive effect of eHF but not of pHF during the first 18 months of life
Halken et al. (26)	595 high-risk infants	To compare the allergy preventive effect of pHF–W with two eHFs: eHF-C (Nutramigen), and eHF-W (Profylac)	To confirm the absence of differences in the allergy preventive effect of the two eHFs, eHF-C (Nutramigen), and eHF-W (Profylac)	pHF-W eHF-C (Nutramigen) eHF-W (Profylac)	-SPTs -sIgE	Less effectiveness of pHF-W than eHFs in the prevention of CMA No difference regarding the development of AM between the two eHFs, eHF-C (Nutramigen) and eHF-W (Profylac)

(Continued)

TABLE 3 | Continued

First author and year	Characteristics of population	Primary outcome	Secondary outcomes, if any	Types of the milk formula used	Techniques used	Main results
von Berg et al. (27)	2,252 high-risk TERM infants (hereditary risk of atopy)	To evaluate the preventive role of HFs compared with standard CMF in the development of AM at 1 year of age (AD, FA-GIT, and urticaria)		eHF-C eHF-W pHF-W CMF	SCORAD method SPT sIgE Oral challenge	Protective effect of HFs (eHF-C and pHF-W) against AD and AM in the first year of life
Szajewska et al. (19)	122 high-risk PRETERM infants (hereditary risk of atopy)	To evaluate whether the use of HFs may prevent the development of AM (AD, GI symptoms, or wheezing) within 2–5 and 12 months of age	To measure the percentages of preterm neonates who interrupted the intervention due to formula non-acceptance or were lost for any reason at the follow-up	e-HF pHF Standard preterm formula	Total IgE sIgE	Preventive effect of eHF on AD at 12 months Increased risk for preterm infants who had been fed with eHF and then interrupted for any reason and particularly for non-acceptance of the formula
Lowe et al. (28)	620 high-risk infants (hereditary risk of atopy)	To determine whether pHF-W or soy formula modify the risk of the development of AM (eczema and food reaction) up to 2 years of age	To assess the individual incidence of eczema and food reaction in the first 2 years of life and SPT reactivity at 6, 12, and 24 months To detect the prevalence of AR, eczema and asthma at 6 and 7 years of age	pHF-W Soy formula CMF	SPTs to a *milk, egg, peanut, dust mite, rye grass, and cat dander* performed at 6, 12, and 24 months	No evidence that pHW-F or soy formula reduced the risk of AM in the first 2 years of life No evidence of reduced risk of SPT reactivity No evidence of reduced risk for AR, eczema, and asthma at 6 and 7 years of age
von Berg et al. (29)	1,451 (from the original total of 2,252 high-risk TERM infants)	To evaluate the effect of the early use of HFs on the development of AM (AD, AR, and asthma) at school age		eHF-C eHF-W pHF-W CMF	ISAAC questionnaire SCORAD method sIgE	Significant reduction of only AD at 10 years of age in children who had been fed with eHF-C or pHF-W in the early stage of their life, without a preventive effect on asthma or AR
von Berg et al. (30)	1,377 (from the original total of 2,252 high-risk TERM infants)	To assess the relationship between early use of HFs and the development of AR, asthma, and eczema up to adolescence	To detect allergic sensitization through sIgE and respiratory function (spirometry)	eHF-C eHF-W pHF-W CMF	ISAAC questionnaire SCORAD method sIgE Spirometry	Preventive effect of eHF-C (mainly) and pHF-W on eczema, AR, and asthma up to adolescence

CMF, cow's milk formula; CMA, cow's milk allergy; eHF-W, extensively hydrolyzed whey formula; eHF-C, extensively hydrolyzed casein formula; pHF-W, partially hydrolyzed whey formula; AM, allergic manifestations; AD, atopic dermatitis; AR, allergic rhinitis; FA-GIT, food allergy with manifestation in the gastrointestinal tract; GI, gastrointestinal; ISAAC, International Study on Asthma and Allergy in Childhood; sIgE, specific IgE; SPTs, skin prick tests; OFC, Oral Food Challenge; CMF, cow's milk formula; DBPCFC, double-blind placebo-controlled food challenges.

casein formula (eHF-C), extensively hydrolyzed whey formula (eHF-W), partially hydrolyzed whey formula (pHF-W), or standard cow's milk formula, in order to evaluate the possible preventive role of these formulas in the development of allergic diseases during a long term follow-up (27, 29–32). They found a protective effect of eHF-C and pHF-W against AD at 1 year follow-up and a significant reduction of AD at 7–10 years of age in children. However, no preventive effect against asthma or AR was shown. These results were confirmed in the 15-year follow-up study, where the authors reported also fewer diagnoses of AR and asthma in those children fed with hydrolysates (mainly eHF-C) in their early stages of life, as if the preventive effects of HF on these two pathologies had occurred later in time. Throughout the follow-up period eHF-W did not show any preventive effects toward allergic diseases and none of the HF had influence on IgE sensitization.

Differently from GINI study, the Melbourne Atopic Cohort Study, conducted on 620 high-risk infants randomized to pHF-W, soy formula or cow's milk formula, showed neither significant difference in allergic outcomes (eczema and food reactions) in the first 2 years of life nor evidence of reduced risk of SPT reactivity and of lower risk for AR, eczema and asthma up to 6–7 years of age (28).

Furthermore, in an observational population-based study, Goldsmith et al. examined the possible association between the development of food allergy at 1 year of age and either duration of exclusive breastfeeding or use of pHF (33). They found that the incidence of food allergy was not reduced by either the duration of exclusive breastfeeding or by the use of pHF, suggesting that allergen avoidance may not be helpful in allergy prevention.

Pooling data on HF in meta-analyses is problematic due to the heterogeneity of HF products and the different sources of proteins, hence different HF should not be considered equivalent. For this reason, Szajewska et al. conducted a meta-analysis taking into consideration exclusively studies using a unique 100%-whey pHF. They found a preventive effect against all allergic diseases and eczema (34). Based on all data from the literature, use of pHF-W formula has been considered safe in healthy term neonates (35, 36).

At present, the conclusions of guidelines and meta-analysis on the role of HF for prevention of allergic diseases differ in term of recommendations, outcomes, and target population. According to some pediatric Societies the use of pHF is still indicated in infants at high risk of allergy, when mother's milk is not available or is insufficient (37, 38). Differently, other Societies, based on emerging evidence, changed their previous recommendations and no longer proposed HF for prevention of allergic diseases (39–41).

The aim of this paper was to analyse the effect of HF on allergy prevention in preterm infants using follow-up data of a previous randomized, triple-blind, placebo-controlled study. To our knowledge, this is the first long-term follow-up paper concerning allergy prevention with HF in preterm infants. We acknowledge a possible limitation of our literature search due to the search restriction for preterm infants. In fact, using Medical Subject Headings-Terms for preterm infants, we could have missed studies in which subgroup analysis of preterm infants have been carried out, but not mentioned in the title or abstract. However, in our view, publication bias could be negligible. The main limitations of the present study can be considered the underpowered number of preterm enrolled, the short period of eHF administration, the drop-out rate and the lack of quantitative diagnostic methods used to diagnose allergy other than a validate questionnaire and SPT.

To sum up, further well-designed large studies in preterm infants should be conducted to address the preventive effects of HF for allergic diseases and the nutritional non-inferiority of preterm HF compared to modern preterm IPF in these vulnerable population. Moreover, data on long term safety of HF in preterm infants and cost/benefit ratio analysis are needed.

Evidences of hypersensitivity have been described also as predisposing factor in patients with functional gastrointestinal diseases (42), whose high prevalence have been recently found in preterm newborns (43). Despite self-limited diseases, a preventive intervention for FGIDs, especially for high-risk population, might have important clinical, and socioeconomical effects (44, 45). HFs have been investigated as dietary modification for management of these conditions with inconsistent evidences, despite some authors suggested a decreased incidence in infants fed with pHF (46, 47).

Finally, bio-effective agents such as probiotic have been recently added to HF to enhance their putative role in allergic disease prevention (48) and should be evaluated since preterm infants could be considered a strategy population for their well-known dysbiosis-related conditions (49, 50).

CONCLUSION

To date many rigorous systematic reviews and meta-analyses evaluating term infants concluded that evidence is not robust to support the use of HF to prevent atopic diseases. Our data did not show any allergy preventive effect of eHF in a small cohort of preterm infants and highlighted the need of further large studies to better clarify the possible role of HF for allergy disease prevention in this population.

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 Policlinico di Bari Ethics Committee. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

MB conceptualized and designed the study. MT and NLae assessed study participants and collected study data. MF

performed statistical analyses. AD interpreted data and drafted the initial manuscript. GB and AZ performed literature search. MC, LP, SS, and RP revised the manuscript. NLaf coordinated and supervised all activities. All authors contributed to the intellectual content, reviewed and revised the manuscript, and approved the final version. All authors contributed to the article and approved the submitted version.

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Current Insights on Early Life Nutrition and Prevention of Allergy

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The incidence of allergic diseases in childhood appears to have significantly increased over the last decades. Since environmental factors, including diet, have been thought to play a significant role in the development of these diseases, there is great interest in identifying prevention strategies related to early nutritional interventions. Breastfeeding is critical for the immune development of newborns and infants through immune-modulating properties and it impacts the establishment of a healthy gut microbiota. However, the evidence for a protective role of breastfeeding against the development of food allergy in childhood is controversial, and there is little evidence to support the benefits of an antigen avoidance diet during lactation. Although it is not possible to draw a definitive conclusion about the protective role of breast milk against allergic diseases, exclusive breastfeeding is still recommended throughout the first 6 months of life due to associated health benefits. Furthermore, recommendations regarding complementary feeding in infancy have been significantly modified over the last few decades. Several studies have shown that delayed exposure to allergenic foods does not have a role in allergy prevention and recent guidelines recommend against delaying the introduction of complementary foods after 6 months of age, both in high- and low-risk infants. However, trials investigating this dietary approach have reported equivocal results so far. This review summarizes the available high-quality evidence regarding the efficacy of the principal dietary interventions proposed in early life to prevent allergic diseases in children.

Keywords: nutrition, allergy, prevention, breastfeeding, microbiota, diet, complementary feeding

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INTRODUCTION

Environmental exposures, such as nutritional intake during the critical stages of pregnancy and in the early postnatal period, play a significant role in the development of infant immune system and it has been suspected that may also be involved in the origin of childhood atopic diseases (1).

Many studies have investigated the association between maternal diet and the development of childhood allergic diseases, however, to date, the results have been largely inconclusive and controversial (2). Exclusive breastfeeding is recommended throughout the first 6 months of life, due to associated health benefits, but it is also apparent that breastfeeding should be continued beyond 6 months after the introduction of complementary foods (CF). Duration of breastfeeding rather than exclusivity may be important in prevention of allergic diseases. Nonetheless, while the evidence for a protective role of breastfeeding against the development of food allergy is controversial, the recommendations regarding introduction of complementary foods in infancy have significantly changed over the last decades.

This mini review summarizes the current high-quality evidence regarding the principal early life dietary interventions in preventing allergic diseases in children, particularly in regard to maternal diet during pregnancy, breastfeeding, and introduction of complementary foods.

MATERNAL DIET DURING PREGNANCY: DOES IT INFLUENCE THE RISK OF ALLERGIC DISEASES IN OFFSPRING?

Maternal diet during pregnancy represents the earliest nutritional exposure to allergens for the fetus. Most studies to date have demonstrated an equivocal correlation between maternal diet during pregnancy and the development of childhood allergic diseases. A Cochrane systematic review did not support allergen avoidance or nutrient supplementation during pregnancy as a means to prevent allergic diseases in offspring (3). However, evidence from mother consumption of peanut and tree nuts suggests that fetal allergen exposure through maternal diet may actually increase tolerance and reduce risk of developing these childhood food allergies (4). Therefore, further studies are needed to elucidate the role of maternal nutrition in the development of food allergies in offspring.

Fatty Acids

A causal relationship between early maternal consumption of polyunsaturated fatty acids (PUFAs) and childhood allergic diseases is debated (5). The T-helper shift from type 1 to type 2 may derive from a higher n-6: n-3 fatty acids ratio during pregnancy, leading to an increased risk of allergic rhinitis in offspring (6). It is thought that N-3 PUFAs probably limit cytokine cascade (7), decrease n-6 PUFA inflammatory effects, regulate T cell function (cell membrane fluidity, signaling, and gene transcription), and promote long-term effects through epigenetic mechanisms (8). Therefore, daily maternal supplementation of n-3 PUFAs could reduce the risk of food allergies and IgE-associated eczema in infants with a family history of allergy (9). However, findings about the role of n-3 PUFA supplementation in pregnancy for reducing the overall incidence of allergic outcomes in offspring are still insufficient.

Antioxidants

Antioxidants (i.e., vitamin E, flavonoids, selenium, and copper) intake during pregnancy may be beneficial, as they have immunomodulatory properties and could play a role on fetal lung development and on respiratory health later in life, thus reducing the risk of wheezing (10).

Vitamin D

The association between vitamin D status of pregnant women and the development of atopic diseases in childhood is not clear. For this reason, prenatal vitamin D supplementation for the prevention of allergic diseases in offspring is not currently recommended. We know 25-hydroxyvitamin D3 levels in cord blood are directly associated with lower mononuclear cell cytokine responses to allergens and reduced risk of eczema in the first 12 months of life (11). In addition, higher vitamin D intake during pregnancy has been associated with lower risk of wheezing and eczema in offspring at 16–24 months of age (12). However, follow-up of the same cohort showed an increased risk of eczema later in life (13). The immunomodulatory mechanism of vitamin D seems to have an influence on Th2 cells differentiation, but its benefits are still unclear and the literature is conflicting (14).

Foods

A recent prospective birth cohort study showed an association between prenatal maternal intake of certain foods and the risk of allergic diseases in offspring by the age of 3 years (10). Namely, consumption of green vegetables, eggs, and grains were found to play a protective role against respiratory allergic diseases, whereas higher meat intake in the preconception period was positively associated with an increased risk of wheezing, allergic rhinitis, and eczema. In a previous prospective cohort study, a diet rich in vegetables during pregnancy has been associated with reduced risk of childhood asthma in offspring (15). A systematic review and meta-analysis concluded that maternal fish consumption during pregnancy did not reduce the risk of allergic outcome in offspring (16).

BREASTFEEDING: DOES HUMAN MILK PLAY A PROTECTIVE ROLE?

Current guidelines recommend human milk (HM) as the "gold standard" for infant nutrition (17). Breastfeeding prolongs the interaction with the mother's immune system and may influence oral tolerance and the risk of allergies in childhood. Pivotal studies clearly confirm the relevant role of breastfeeding for short-term infant health (e.g., growth, immune function, protection against infections) as well as for potential long-term advantages (e.g., neurocognitive development, prevention of malignancies, and non-communicable diseases) (18). Nevertheless, the evidence for the role of HM for the prevention of allergic diseases remains limited and controversial. This is due to variable definitions of allergic outcomes and to the lack of randomized controlled trials with detailed information about the maternal diet in breastfeeding. While many studies emphasize a protective effect, others even suspect HM may promote allergies (19). The balance between oral tolerance and skin sensitization may affect food allergy risk among infants with eczema: additional evidence suggests that colostrum has a prophylactic role in maintaining oral tolerance, but in case of severe cutaneous barrier dysfunction the protective effect of prolonged breastfeeding is lost (20). Early oral exposure to aeroallergens through HM intake could increase the risk of sensitization in offspring (21). On the other hand, exclusive breastfeeding seems to reduce the incidence of eczema in the first 2 years of life and the risk of asthma in the first 5 years (22, 23), or even 10 years with weaker evidence (24).

Several reasonable explanations account for HM protective effects on allergy susceptibility in children: stimulation of immune development (direct interactions with infant immune cells), epigenetic actions (DNA methylation, and non-coding RNAs), modulation of gut microbiota.

Breast milk is a living tissue and various bioactive factors appear to be involved. For instance, TGF-β is a regulatory cytokine crucial for long-lasting Treg-mediated food tolerance (25). Maternal antigen immune complexes (IgG-IC) in breast milk may interact with the neonatal crystallizable fragment receptor (FcRn) on infant dendritic cells (26), favoring oral tolerance. In addition, breast milk contains up to 10^5 bacterial cells/ml: HM microbiota plays a role in bacterial colonization of the infant intestinal tract that could contribute to modulate allergy susceptibility early and later in life. Finally, human milk oligosaccharides (HMOs) can play a key role in neonatal mucosal and systemic immunity. HMOs represent an antimicrobial barrier acting as soluble decoy receptors that block adhesion of various pathogens, promote intestinal development, and stimulate immunomodulation acting as signal molecules for the host cells. Some HMOs have been proven to play a crucial role in shaping gut microbiota and regulating early life immune development, but the precise mechanism is currently unknown. Furthermore, specific HMOs profiles have been associated with lower risk of cow's milk allergy (27).

Different formulas are available for newborns and infants who cannot be breastfed. Among them, hydrolyzed formulas have been specifically proposed for infants at risk of allergies; however, their role in allergy prevention is still under debate (28).

COMPLEMENTARY FEEDING: DOES TIMING OF FOOD INTRODUCTION INFLUENCE THE RISK OF ALLERGIES?

In past years, it has been traditionally recommended to delay the introduction of foods recognized as potentially allergenic, based on the theory that the gut structural and functional immaturity and increased permeability determines an increased risk of allergic sensitization (29). Nevertheless, early exposure to these allergens may be critical to achieve food tolerance, which is an antigen-driven process as suggested by animal models (30). According to the so-called "dual-allergen exposure hypothesis," oral administration of food allergens favors the establishment of tolerance via the expansion of Th1 and Treg populations, whereas exposure to food allergens through skin barrier disruptions favors sensitization via Th2 switch and cytokines production (29).

Current evidence suggests that oral allergen exposure may start at 4 months onwards, although proper timing of this "critical window" is still not clear (31).

In 2016, the Enquiring About Tolerance trial showed that, in a general population of exclusively breastfed infants, the early (i.e., between 3 and 6 months of age) introduction of potentially allergenic foods (cow's milk, cooked egg, peanut, fish, wheat, and sesame) was effective for prevention of food allergy (32). In particular, while the incidence of food allergy at 3 years of age was not significantly different between the early introduction group compared to the control group in the intention-to-treat analysis, the per-protocol analysis showed a significant reduction of overall incidence of food allergy, peanut allergy, and egg allergy in the early introduction group. However, the high rate of non-adherence in the early introduction group (68.1%) limits the reliability of the study results. Results from the Learning Early About Peanut Allergy trial suggested that peanut should be introduced between 4 and 11 months in infants at high risk for allergy (33). According to a recent systematic review, this practice may reduce the risk of peanut allergy, with the strongest evidence supporting a benefit to high risk infants (i.e., with severe atopic dermatitis or egg allergy) but it is also applicable to infants at lower risk (34). In addition to these findings, there is limited high quality evidence suggesting the lack of a relationship between consumption of peanut during weaning and the risk of atopic dermatitis/eczema and asthma, while not enough evidence supports the causal relationship between consuming peanut and developing allergic rhinitis at 2, 5, or 6 years of age (34).

Many studies addressing the effect of egg introduction on the risk of developing allergy, have provided conflicting results. This is likely due to unaccounted variables from different study populations, as well as variations in dose and form of the eggs used (raw or cooked) (35–39). However, a recent systematic review provided moderate evidence that introducing egg at 4–6 months of age may reduce the risk of developing egg allergy (34). In the same study, limited evidence showed the lack of association between the timing of egg introduction and the development of atopic dermatitis/eczema and asthma, while not enough evidence supported a relationship with risk of allergic rhinitis in the first 5 years of life (34).

Previous observational studies have also investigated the effect of the timing of fish introduction on the risk of allergic diseases (40, 41). A recent systematic review provided limited evidence to suggest that the introduction of fish between 3 and 8 months of life may lower the risk of atopic dermatitis/eczema (34). However, at present there is not enough evidence of a link between fish consumption and food allergy, asthma, or allergic rhinitis (42).

With regard to cow-milk, while previous observational studies reported conflicting results on the risk of cow's milk allergy (43, 44), more recently a randomized clinical trial demonstrated that the risk of sensitization to cow's milk as well as the risk of immediate food allergy were decreased by avoiding supplementation with cow's milk formula for at least the first 3 days of life (45). In addition, a recent systematic review provided limited high quality evidence suggesting the lack of a relationship between age of introduction and risk of food allergy and atopic dermatitis/eczema. However, there was not enough evidence to support an association between cow's milk formula supplementation and the development of asthma and allergic rhinitis (34).

Overall, these findings suggest that there is not a clear optimal timing of introduction of potential allergenic foods for preventing allergic diseases, either for infants in the general population or for high-risk infants (**Table 1**). The latest recommendations issued by the World Health Organization (46), the American Academy of Pediatrics (22), the European Academy of Allergy and Clinical Immunology (47), the European Society for Paediatric Gastroenterology Hepatology and Nutrition (30), the European Food Safety Authority (48), and the British Society for Allergy, and Clinical Immunology (49) have all emphasized the lack of scientific data to support the introduction of the commonly acknowledged allergenic foods

TABLE 1 | Timing of introduction of CF and risk of allergic diseases in childhood.

Type of CF	Allergic disease			
	Food allergy	**Atopic dermatitis/eczema**	**Asthma**	**Allergic rhinitis**
Peanut	Introduction between 4 and 11 months may decrease the risk of peanut allergy in infants at high and low risk (34*, 35#)	No relationship between consumption during weaning and the risk of atopic dermatitis/eczema (35#)	No relationship between consumption during weaning and the risk of asthma (35#)	No relationship between consumption during weaning and developing allergic rhinitis (35#)
Egg	Introduction between 4 to 8 months does not prevent egg allergy in high risk-infants (37*, 39*) Introduction between 4 to 6 months does not prevent egg allergy in low and high risk-infants (36*, 38*) Introduction between 3 to 9 months is effective in preventing egg allergy in high risk-infants (40*) Introduction between 4 and 6 months may decrease the risk of allergy to egg (35 #)	No relationship between consumption during weaning and the risk of atopic dermatitis/eczema (35#)	No relationship between consumption during weaning and the risk of asthma (35#)	No relationship between consumption during weaning and developing allergic rhinitis (35#)
Fish	Fish consumption during weaning is not associated with increased risk of food allergy (35#)	Introduction between 3 and 8 months of age may decrease the risk of atopic dermatitis/eczema (35#, 42§)	No relationship between consumption during weaning and the risk of asthma (35#, 43#)	Introduction before 9 months of age may reduce the risk of allergic rhinitis (41§) No relationship between consumption during weaning and the risk of allergic rhinitis (35 #)
Cow-milk products	Introduction within the first few days of life is associated with an increased risk of developing cow's milk allergy (44§) Introduction within the first 2 weeks of life reduces the risk of cow's milk allergy, whereas introduction between 4 to 6 months increased this risk (45§) Avoiding supplementation with cow's milk formula for at least 3 days of life decreases the risk of cow's milk allergy (46*) No relationship between age of introduction and risk of food allergy (35 #)	No relationship between age of introduction and risk of atopic dermatitis/eczema (35#)	No relationship between age of introduction and risk of asthma (35#)	No relationship between age of introduction and risk of allergic rhinitis (35#)

§*observational study.*
**randomized control study.*
#systematic review.

before 4 months of age, but also the absence of evidence to justify a delayed introduction of CF after 6 months of age to prevent allergy, both in high and low-risk infants (**Supplementary Table 1**).

DIET-INDUCED CHANGES IN GUT MICROBIOTA: DO THEY INFLUENCE ALLERGY RISK?

Pregnancy

Evidence suggests that early life gut microbial dysbiosis precedes atopy development and that the gut microbiota of allergic children may show a depleted diversity (50, 51). In particular, a reduction of certain bacteria (e.g., *Bifidobacterium, Akkermansia,* and *Faecalibacterium*) could drive CD4+ cell dysfunction, thereby increasing the risk of atopic diseases (52). It has been recently suggested that diet during pregnancy may indirectly affect tolerance acting on the microbiota diversity. In particular, fiber consumption appears to lead to changes in the maternal gut microbiota metabolism by increasing the production of short-chain fatty acids (SCFAs), which represent the first metabolites of gut commensal microbiota. Therefore, SCFAs that cross the placenta, influence gene transcription in fetal lung and enhance oral tolerance to allergens by promoting epithelial integrity, T regulatory (Treg) cell differentiation, and IgA release from plasma cells, with long-lasting effects on health (53). Furthermore, it seems that

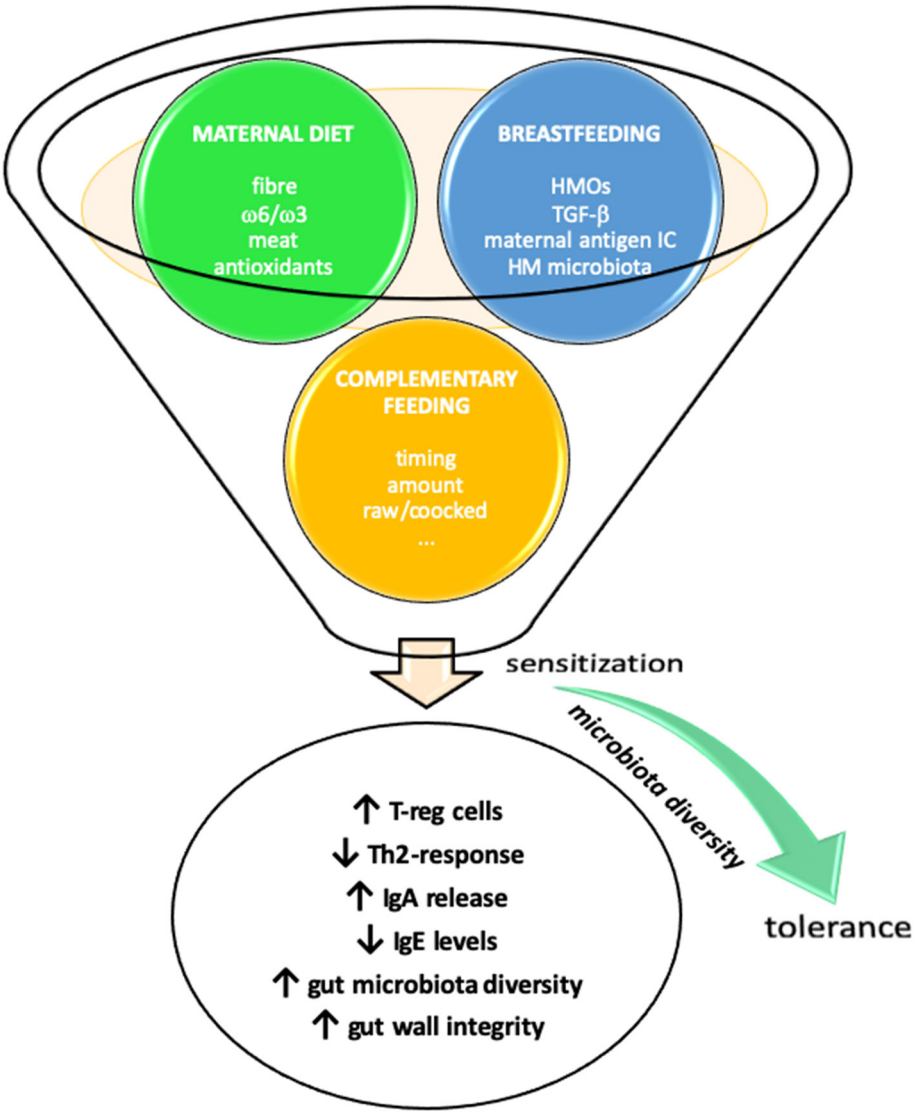

FIGURE 1 | Early life nutrition and prevention of allergic diseases. Maternal diet during pregnancy and lactation and introduction of complementary foods may influence susceptibility to allergic diseases through several diverse mechanisms.

fetal Treg cells may be informed by substantial numbers of maternal cells crossing the placenta and inducing antigen-specific tolerance. *In utero* transfer of microbial antigens during fetal development would enable a balanced immune response of the newborn to the rapidly developing microbiota *post-partum* (54).

Early Life

In recent years there has been increased scientific interest and studies into the role of CF introduction to the infant diet and the risk of allergy. Investigations have sought to determine if there is a cause–effect relationship between diet-induced changes of infant's microbiota and the development of allergic diseases.

Indeed, during weaning, a dramatic change of intestinal microbiota occurs, which is reflected in the transitioning nature of stool composition (55). Thus, it has been suggested that the resident microbiota of the gastrointestinal tract, interacting with solid foods, may be able to modulate the immune system development in early life (56).

In breastfed infants, a rapid rise in the number of *Enterobacteria* and *Enterococci* has been demonstrated (57), along with an increased amount of *Bifidobacterium* and *Lactobacillus* spp. (58). Conversely, in formula-fed infants, a greater abundance of *Bacteroides* and *Clostridium* spp. has been observed, this difference could have implications for the subsequent development of atopic diseases (57, 58). The introduction of CF drives the gut microbiota composition favoring bacteria within the *Bacteroidetes* and *Firmicutes phyla* (58). Most of the reported changes in gut microbiota appear to occur after weaning, from age 9–18 months until the 3rd year of life, when the microbiota stabilizes resembling the adult one (59). Therefore, the window of opportunity for modulating the

intestinal microbiota composition extends up to the first 2 years of life, conversely any dysbiosis in this period could determine an abnormal immune system activation, possibly leading to the development of pathological conditions such as allergy (56).

It has been hypothesized that a more diverse diet leads to a more diverse gut microbiota, which may improve the gut wall integrity and the immune system regulation, supporting the expansion of Treg cells (60), and suppressing IgE levels (61). Indeed, a lower microbiota diversity with an increased number of *Firmicutes* vs. of *Bacteroidetes*, has been observed in children with food sensitization (62). An increased microbial diversity along with the relative abundance of certain bacteria, such as *Lactobacillus* spp. (63), has been correlated with a lower risk of IgE-associated allergic diseases, namely atopic dermatitis and wheezing, through a decreasing Th2-mediated response (64). No significant association between the diversity of complementary foods and allergic rhinitis has been found so far (65).

According to these findings, early infancy does appear as a window of opportunity during which diet intervention may shape the risk of allergic diseases by modulating the composition of the gut microbiota (60). In this context, it has been demonstrated in a murine model that dietary fiber intake leads to marked suppression of the induction of airway allergic disease, by enhancing Treg cell number and function (66). A possible explanation is that SCFAs, which would seem to reduce airway inflammation even in human models, are produced by the microbiota through the metabolism of dietary fibers (65).

A protective effect against the risk of allergic diseases might be also derived from the supplementation with prebiotics which can indirectly promote the production of anti-inflammatory cytokines by increasing the number of *Lactobacillus* and *Bifidobacterium* spp. (67). Finally, it has been suggested that adequate levels of vitamin D during the 1st year of life may lower the risk of developing food allergies, by modulating the gut microbiota composition (68), with increased *Lachnospiraceae* and reduced *Lactococcus* spp. (69).

Overall, these studies have contributed to deepening our knowledge about gut microbiota diversity and species-specific changes. Unfortunately, however, how to manipulate the intestinal microbiota for the prevention of allergic diseases is still a matter of debate.

CONCLUSIONS

The link between early dietary factors and the development of allergy later in life is still not clear. Many dietary factors, from prenatal life through infancy, have been proposed to influence the susceptibility to allergic diseases, by modulating the gut microbiota composition and promoting tolerance to allergens. None of these, other than early introduction of allergens, has been proven effective (**Figure 1**).

Current high-quality evidence for the efficacy of the principal dietary interventions demonstrates that maternal avoidance of allergenic foods during pregnancy and lactation is not effective in preventing allergic diseases. Some evidence suggests that a maternal diet rich in fibers, antioxidants, and n-3 PUFAs might promote a protective immunomodulatory role, also mediated by changes in microbiota. However, further studies are needed to elucidate the role of maternal nutrition in the development of food allergy in offspring.

Evidence for an overall protective role of breastfeeding against allergic diseases is still controversial, even though exclusive breastfeeding is recommended as the "gold standard" for infant nutrition during the first 6 months of life.

With regard to complementary feeding, there is no clear evidence to support a specific timing to the introduction of potentially allergenic foods for preventing allergic diseases, either for infants in the general population or for high-risk infants. Nonetheless, complementary feeding should not be delayed after 6 months of age, nor should breastfeeding be discontinued.

The diversity of the intestinal microbiota in early life is likely associated with a reduced risk of allergies. Indeed, it has been suggested that poor gut microbial diversity may increase the risk of developing allergic diseases. Thus, early infancy does appear as a window of opportunity during which dietary intervention may influence the risk of allergic diseases.

To better identify efficacious dietary strategies for primary prevention of allergies in childhood, future research should implement longitudinal interventional studies in cohorts of pregnant women and their offspring, as well as randomized controlled trials to clarify the potential role of complementary foods and their optimal timing of introduction.

AUTHOR CONTRIBUTIONS

GF, MC, and MG: conceptualization. GF, MC, CM, VN, and MG: writing original draft. GC and MG: review and editing. All the authors read and approved the final version of the manuscript.

SUPPLEMENTARY MATERIAL

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REVIEW
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Dietary Prevention of Atopic March in Pediatric Subjects With Cow's Milk Allergy

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Cow's milk allergy (CMA) is one of the most prevalent food allergies and the most expensive allergic diseases in the pediatric age. There is no cure for CMA, and actual disease management is based on strict avoidance of cow milk protein-containing foods, access to rescue medication, and use of substitutive formulas. Early-life CMA could be one of the first steps of the "allergic march" (AM), leading to the occurrence of other atopic manifestations later in the life, including asthma and oculorhinitis, with subsequent further increase of costs for health care systems and families of affected children. In the last years, diet is emerged as a relevant strategy to prevent allergic diseases through, at least in part, epigenetic modulation of immune system. We provide an overview of studies that investigate the potential role of different dietary strategies in preventing the AM in pediatric patients with CMA.

Keywords: allergic march, food allergy, breast milk, infant formula, gut microbiota, epigenetics

INTRODUCTION

Affecting up to 3% of children worldwide, cow's milk allergy (CMA) is one of the earliest and most prevalent food allergies (FA) in the pediatric age. It is also responsible for the vast majority of food-induced anaphylaxis cases in the Italian pediatric population, with significant costs for the healthcare system and families, and it emerged as one of the most expensive allergic diseases (1–8).

Although most subjects with CMA naturally outgrow it over time, studies evidence a wide range of ages and rates of resolution with an increased risk of persistence in recent decades, mainly due to negative gene–environment interaction leading to the breakdown of immune tolerance mechanisms (9–12). In addition, evidence suggests that early-life CMA could be one of the first steps of the "allergic march" (AM), leading to the occurrence of other allergic disorders during childhood. Indeed, the occurrence of allergic sensitization in these children increases the risk of later developing asthma and allergic oculorhinitis (AR), in particular when sensitization occurs along with atopic dermatitis (AD) (13–15). **Figure 1** depicts the natural history of AM in CMA children. According to data from several clinical

studies, up to 45% of CMA children develop other atopic manifestations later in the life, also after the immune tolerance acquisition to cow milk proteins (3, 5, 16–18). The development of AM is driven by genetic predisposition, but environmental factors may play a key role in its clinical expression. Indeed, as shown by longitudinal studies, only a minority of children follow the classic pathway of AM (starting from AD and followed by sequential development of FA, asthma, and AR) (19, 20). Earlier recognition of at-risk infants, regardless of CMA temporal appearance, allows fielding effective strategies to limit the occurrence of other atopic manifestations later in the life.

There is no cure for CMA, and actual disease management is based on strict avoidance of cow's milk protein-containing foods, access to rescue medication, and use of substitutive formulas (21–25).

Due to the increasing prevalence, persistence, and risk for developing other atopic manifestations in children with CMA, preventive strategies are highly advocated. In the last years, diet is emerging as a relevant strategy to prevent allergic diseases through the active modulation of the immune system (26). This review is focused on the potential role of different dietary strategies in preventing the AM in pediatric patients with CMA.

THE POTENTIAL OF BREASTFEEDING

Breastfeeding is the best dietary strategy for newborn infants due to its optimal nutritional properties and several bioactive compounds that influence health status. Studies suggest a protective role on the onset of FA, asthma, and AD, both in low- and high-risk infants breastfed for at least 3–4 months (27–33). A WHO report suggests that allergic diseases are lower in exclusively breastfed compared to non-breastfed infants (34). A reduction of about 4% in FA risk for every additional month of exclusive breastfeeding has also been estimated (35). Unfortunately, most available data on breastfeeding and allergic diseases are based on observational, retrospective, underpowered studies, and present several confounding factors, such as the inclusion of partially breastfed infants (36, 37). Another limiting aspect is that the protective mechanisms against FA and other atopic manifestations are still not completely characterized. Breast milk contains several potential protective factors against allergy. Some compounds could be able to exert an indirect effect on immune system through a modulation of infant gut microbioma (GM), whereas other components could exert a direct modulatory effect on the infant immune system toward a protection against allergic diseases (38, 39) (**Table 1**).

The GM is emerging as a pivotal regulator of immune tolerance development (6). Breastfeeding shapes infant GM, both by direct transition of the human milk bacteria (HMBs), and indirectly through milk compounds such as human milk oligosaccharides (HMOs), secretory IgA, and antimicrobial factors, which could impact bacterial growth and metabolism (40). Studies have suggested that breast milk owns unique microbiome, including beneficial commensal and potentially

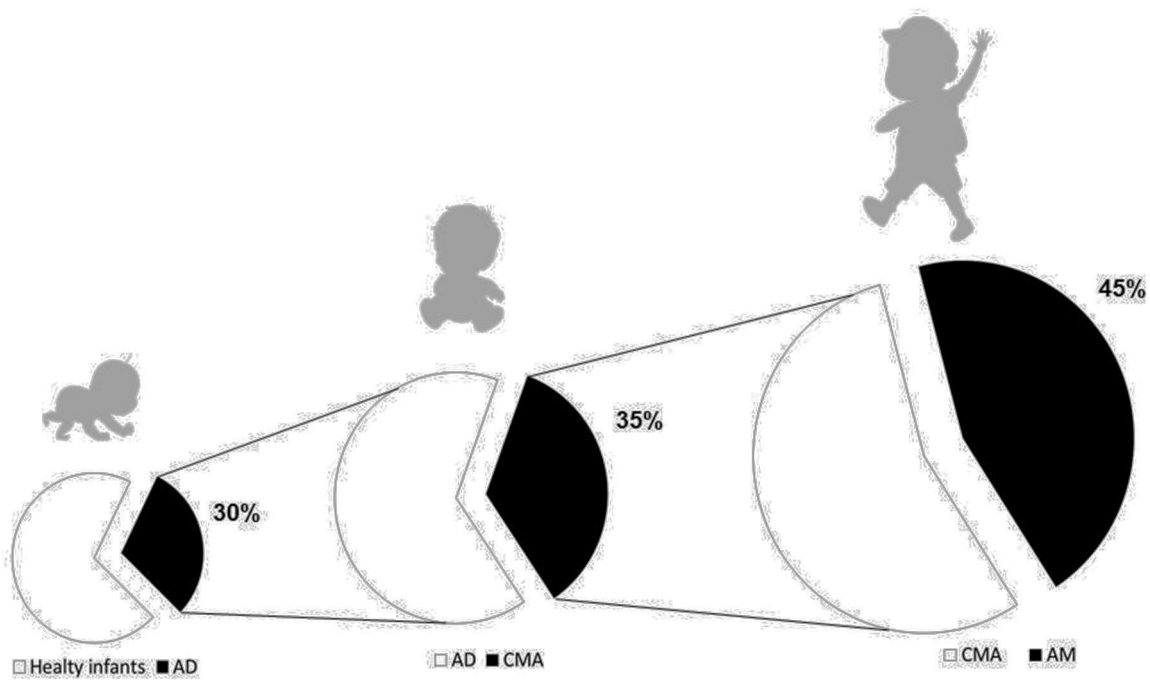

FIGURE 1 | The atopic march in pediatric patients with cow's milk allergy. Atopic dermatitis (AD) is commonly considered the first step of the atopic march (AM), however, AD and cow's milk allergy (CMA) could co-exist, particularly in those with early onset, severe, and persistent atopic eczema. CMA affects about 1/3 of patients with AD. Data from several clinical studies demonstrate that up to 45% of children affected by CMA will develop other atopic manifestations later in the life, also after the immune tolerance acquisition to cow's milk proteins.

TABLE 1 | Main immunomodulatory factors in human milk.

Regulation of infant's immune system through a direct interaction with immune cells	Regulation of infant's immune system through a modulation of gut microbiome
Cytokines	Lactoferrin
Bacterial DNA	Lisozyme
miRNAs	Secretory IgA
Short chain fatty acids	Human milk bacteria
Human milk oligosaccharides	Human milk oligosaccharides
Omega-3 fatty acids	

probiotic bacteria (41). HMBs can originate from maternal skin, newborn oral cavity, or mostly from the maternal gut (the "entero-mammary pathway") and are influenced by mode of delivery, with a lower bacteria variety and abundance in cesarean compared to vaginal delivery (42, 43). Breast milk is considered the second source of microbes to infant GM, and it has been estimated that breastfed infants could receive from human milk microbiota up to 8×10^5 bacteria daily (44). Considering the pivotal role of GM in influencing the infant immune system function against CMA (45), it is possible to hypothesize that HMBs could be an innovative target of intervention. Interestingly, it has been demonstrated that in the milk of allergic mothers the bifidobacteria counts were significantly lower than in the milk of non-allergic mothers (46).

Regarding breast milk non-microbial components, human milk oligosaccharides (HMOs) are a group of non-digestible carbohydrates that are able to regulate the immune system function in a direct or indirect way. The HMO composition in breast milk is influenced by environmental (such as maternal diet) and genetic factors, and a possible role in FA has been suggested (47). Recent studies reported an association between different genetically induced HMO composition and the development of CMA and FA (47, 48). Interestingly, one recent study highlighted the ability of specific HMOs, pulled from human milk, to induce the maturation of human monocyte-derived dendritic cells (DC) (moDC). The derived HMO moDC are able to promote T reg induction from native $CD4^+$ T cells, with a final tolerogenic effect on the infant's immune system (49); but the best characterized HMO properties are related to the prebiotic modulation of early microbial gut colonization with bifidobacteria and *lactobacilli*, which are involved in the production of tolerogenic metabolites short-chain fatty acids (SCFA), in particular, butyrate (41, 50–54). Supporting this view, it has been reported that the GM of allergic infants lacks genes encoding key enzymes for HMO metabolization with the consequent impairment of butyrate production (55).

Butyrate may prevent allergy diseases though different ways, involving a regulation of the epithelial barrier (at skin, gut, and respiratory tract level), a direct effect on Th1/Th2 cytokine expression, and the activation of regulatory T cells (Tregs) (56–60). Many effects are mediated by the epigenetic modulation of gene expression, suggesting the possibility of a long-lasting regulatory effect on immune tolerance network (6).

TABLE 2 | Available data on butyrate concentrations in human milk.

References	N° samples	N° mothers	Min value (mM)	Max value (mM)	Methods
Maria et al. (68)	150	30	0.1	0.23	GC-MS utilizing a lipase assisted sample preparation (deuterated butyric acid (BA-D7) as an internal standard)
Schwab et al. (70)	19	19	1.36	5.7	HPLC-RI with external standard
Prentice et al. (69)	102	102	0.0	0.4	H-NMR and GC-MS
Dai et al. (67)	180	60	0.29	0.48	Methyl esterification of SCFAs and GC analysis

GC-MS, gas chromatography-mass spectrometry; HPLC-RI, high performance liquid chromatography with refractive index detection; H-NMR, nuclear magnetic resonance spectroscopy; SCFAs, short chain fatty acids.

The origin of butyrate in breast milk is still largely undefined. The mammalian gland is able to regulate the concentration of several macro- and micronutrients in human milk. Thus, it is possible to hypothesize that some mechanisms of regulation could modulate the butyrate content in human milk. However, recent evidence supports the hypothesis that, at least in part, human milk butyrate could be produced by the HMBs. The hypothesis of a pivotal contribution by mammalian gland/breast milk microbiota in butyrate production is supported by recent observations demonstrating the presence of potential butyrate-producer bugs (54, 61–65).

An example of a potential pathway in butyrate production in breast milk could be derived by HMO metabolization by selected bacteria, as recently demonstrated by others (62, 66).

Of note, increasing observations demonstrate the presence of significant butyrate concentrations in breast milk, ranging from 0.01 to >5.0 mM (67–70) (**Table 2**).

In line with these data, our preliminary observation from 109 healthy mothers show a median butyrate concentration in mature human milk of 0.75 mM (range 0.16–1.97 mM) (58). Interestingly, several preclinical data show that this butyrate concentration is able to modulate several components of immune tolerance network mainly through epigenetic mechanisms (6, 56–60).

Altogether, these data strongly suggest the potential pivotal role of a modulation of breast milk composition for innovate preventive strategies against CMA and against the occurrence of AM in CMA children.

THE POTENTIAL OF FORMULA CHOICE

The first evidence on the possible role of infant formulas in preventing AM in CMA infants was provided about 10 years ago. In a prospective cohort study of 119 children with IgE-mediated CMA, a multivariate analysis of risk factors for the occurrence

TABLE 3 | The studies exploring the potential of formula choice in preventing atopic march in pediatric patients affected by cow's milk allergy.

References	Study design/population	Age	Sample size	Intervention/duration	Outcomes	Results
Berni Canani et al. (5)	Parallel-arm RCT/IgE-mediated CMA	1–12 months	$N = 220$ $I = 110$ $C = 110$	I = EHCF+ LGG C = EHCF; for 36 months	The occurrence of any atopic manifestation (eczema, urticaria, asthma, oculo-rhinitis) during the 36 months of the study.	The ARD of any atopic manifestation for EHCF+LGG vs. EHCF was: (1) −0.23 [95% CI −0.36 to −0.10, $p < 0.001$] at CCA; (2) −0.22 [95% CI −0.35 to −0.09, $p < 0.001$] at SA-EQS; (3) −0.33 [95% CI −0.45 to −0.21, $p < 0.001$] at SA-BCS; (4) −0.08 [95% CI −0.21 to 0.04, $p = 0.5$] at SA-WCS. The SA-EQS estimate was very similar to the CCA estimate. On absolute grounds, the SA-BCS was 10% higher and the SA-WCS was 15% lower than the CCA estimate. Even under the worst case scenario, a difference in favor of EHCF+LGG was still present (8%). Using the CCA estimate of the ARD, the NNT was 4 (95% CI 3 to 10).
Sánchez-Valverde et al. (71)	Observational cohort study/ IgE-mediated CMA	4 ± 2.63 months	$N = 119$	More extensively hydrolyzed high grade hydrolysates (+EH/HGH), which are those in which >95% of peptides are of < 1,000 kDa, and less extensively hydrolyzed hydrolysates and soya milk formulas.	To evaluate factors that could predict development of atopic march in children with IgE-mediated CMA	Multivariate analysis of risk factor, for the occurrence of AM revealed that EHCF use represented a protective factor for other allergic diseases compared to other hypoallergenic formulas or soy-based formula (OR 0.76; 95% CI: 0.149–0.945, $p = 0.038$).
Gil et al. (72)	Retrospective observational cohort study/ only IgE-mediated CMA	Mean age at diagnosis 5.07 ± 2.67 months Mean age at the end of follow up 14.41 ± 5.42 years	$N = 211$	Five groups, based on formula composition: vegetable-based formulas (rice or soy), high grade EHF in which >95% peptides were 1,000 kDa, high-grade EHF + LGG, low-grade EHF in which higher proportion of peptides > 1,000 kDa, or amino acid–based formulas.	To evaluate if a new scoring system could determine the risk of developing allergic march	The risk of AM occurrence decreased in those treated with high grade EHF (OR 0.42; 95% CI 0.20–0.87, $p = 0.02$), and these results were stronger in patients treated with high-grade EHF + LGG (OR 0.30; 95% CI 0.09–0.98, $p = 0.048$).
Guest and Fuller (73)	Retrospective cohort study/ IgE- and non IgE-mediated CMA	Mean age I = 4.2 ± 2.7 months C = 5.4 ± 2.9 months	$N = 940$ $I = 470$ $C = 470$	I = EHCF+LGG C = EHWF	The occurrence of any allergic manifestations over a period of 24 months from the start of formula	Binary logistic regression analysis showed that infants fed with EHWF had a significant higher relative risk at 24 months of atopic dermatitis (OR: 3.438; 95% CI: 1.975–5.985; $p < 0.001$) and asthma (OR: 2.651; 95% CI: 1.242–5.660; $p < 0.02$) compared with those fed with EHCF+LGG.

RCT, randomized controlled trial; N, total number of subjects; I, intervention; C, control; EHF, extensively hydrolyzed formula; EHCF, extensively hydrolyzed casein formula; LGG, Lactobacillus rhamnosus GG; EHWF, extensively hydrolyzed whey formula; eHF, extensively hydrolyzed formula; IgE, immunoglobulin E; CMA, cow's milk allergy; AM, atopic march; ARD, absolute risk difference; CCA, complete case analysis; SA-EQS, sensitivity analysis—equal worst outcome scenario; SA-BCS, sensitivity analysis—best case scenario; SA-WCS, sensitivity analysis-worst case scenario; NNT, Number needed to treat; 95% CI, 95% confidence interval; OR, odds ratio.

of AM revealed that the use of an extensively hydrolyzed casein-based formula (EHCF) represented a protective factor for other allergic diseases, compared to other hypoallergenic formulas or soy-based formulas (OR 0.76; 95% CI: 0.149–0.945, $p = 0.038$) (71).

To our knowledge, to date, only one randomized controlled trial was performed to test the potential of a formula-based dietary intervention on AM prevention in CMA pediatric patients (5). In this prospective trial, a total of 220 infants with IgE-mediated CMA (67% males, median age 5.0 months) were randomized into two dietary groups: 110 subjects were placed on EHCF-based diet, and 110 children were placed on EHCF + probiotic *Lactobacillus rhamnosus* GG (LGG)-based diet. Patients were followed up for 36 months. In the complete case analysis (CCA), the absolute risk difference (ARD) for the occurrence of at least one atopic manifestation over 36 months was −0.23 (95% CI −0.36 to −0.10, $p < 0.001$). Even under the worst-case scenario, a difference in favor of EHCF+LGG was still detected. Using the CCA estimate of the ARD, the number needed to treat was 4 (95% CI 3–10) (5). These findings are consistent with those of recent studies revealing that the first-line approach with EHCF+LGG for CMA infants may slow down the AM, compared to infants treated with other formulas. A retrospective observational study on 211 subjects with CMA was conducted for new score validation for the risk of developing AM, using selected clinical and laboratory data (72). The authors found that the type of substitutive formula for CMA treatment may influence the natural history of these children. They divided the patients into five groups, based on formula composition: vegetable-based formulas (rice or soy), high-grade extensively hydrolyzed formula (EHF) for those in which >95% of peptides were 1,000 kDa, high-grade

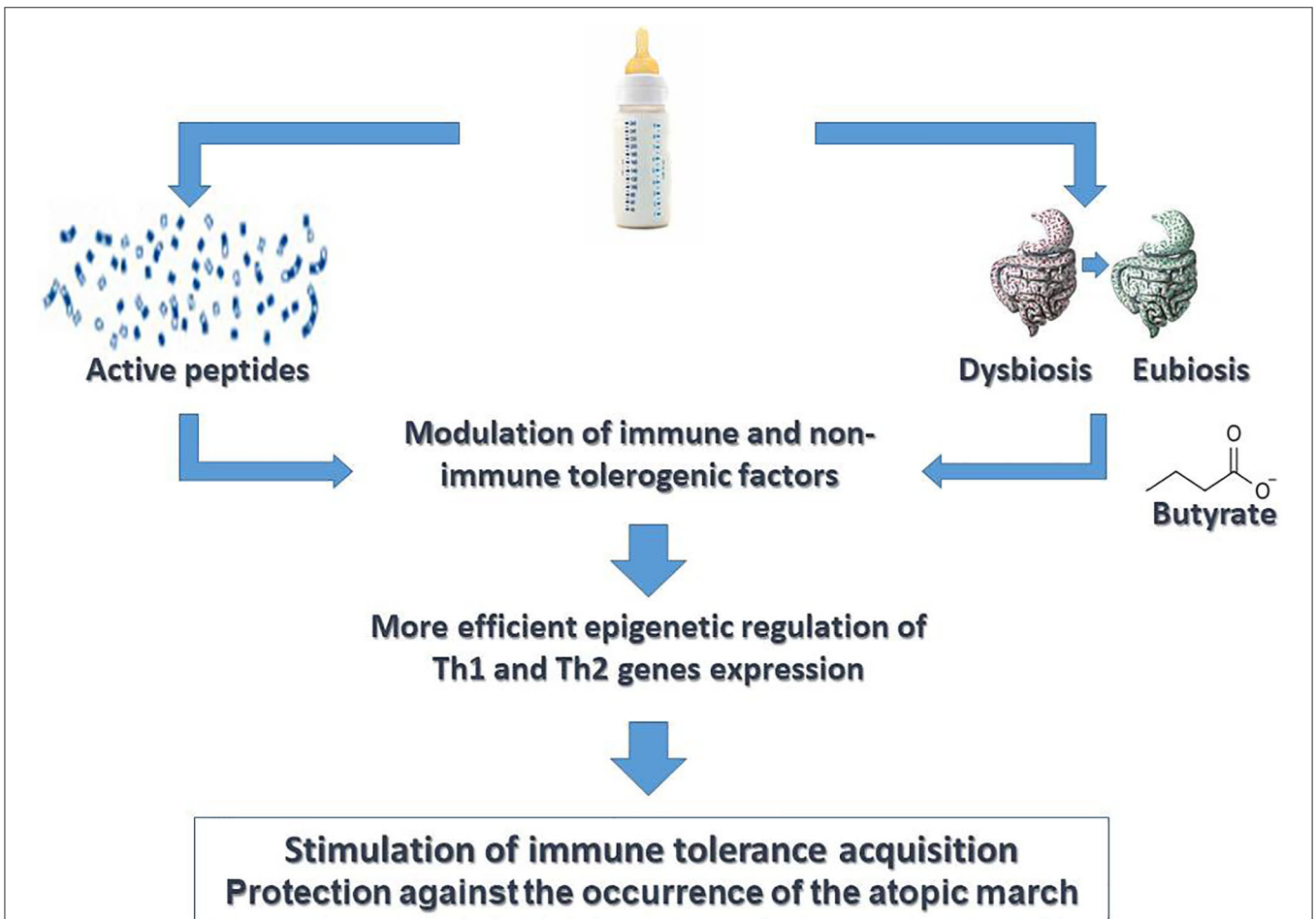

FIGURE 2 | Active diet therapy in pediatric patients with cow's milk allergy. "Active diet therapy" means the possibility to influence the cow's milk allergy (CMA) disease course and to limit the occurrence of other atopic manifestations later in the life. Emerging evidence suggests the importance of formula choice for the management of CMA pediatric patients. It has been demonstrated that the use of extensively hydrolyzed casein formula (EHCF) containing the probiotic *L. rhamnosus* GG (LGG) could exert a modulation of immune tolerance network mediated by the activity of selected casein hydrolysis-derived peptides and by activity of LGG on gut microbiota structure and function leading to an increased production of the short chain fatty acid butyrate. Several non-immune (gut barrier integrity) and immune (cytokines, immune cells) tolerogenic factors are involved in such modulatory action. Many effects are mediated by epigenetic mechanisms. Altogether these mechanisms are able to stimulate a faster acquisition of immune tolerance to cow's milk peptides and to limit the occurrence of atopic march.

EHF plus LGG (EHF+LGG), low-grade EHF for those with a higher proportion of peptides (>1,000 kDa), or amino acid–based formulas. Authors found that the risk of AM occurrence decreased in those treated with high-grade EHF (OR 0.42; 95% CI 0.20–0.87, $p = 0.02$), and these results were stronger in patients treated with high-grade EHF+LGG (OR 0.30; 95% CI 0.09–0.98, $p = 0.048$). The authors concluded that the first-line approach with EHF may be beneficial to prevent the occurrence of AM, and LGG implementation strengthened this trend. They supposed that the hypoallergenic composition of this high-grade EHF and the GM may have helped to positively influence the immune tolerance network, decreasing the risk of developing AM (72). Similarly, in a recent retrospective cohort study of 940 infants with CMA, a binary logistic regression analysis showed that infants fed with extensively hydrolyzed whey formula (EHWF) had a significantly higher relative risk at 24 months of AD (OR: 3.438; 95% CI: 1.975–5.985; $p < 0.001$) and asthma (OR: 2.651; 95% CI: 1.242–5.660; $p < 0.02$) compared with those fed with EHCF+LGG. The authors concluded that the first-line therapeutic approach for newly diagnosed CMA children with EHCF+LGG, reducing the development of other allergic diseases later in life, may slow down the AM (73). Current guidelines provided by scientific societies (EAACI, DRACMA, NICE, ESPGHAN, NIAID, BSACI) strongly suggest avoiding unmodified animal milk proteins for CMA dietary treatment. In addition, there is no evidence supporting the potential role of such mammalian milks in preventing AM in FA patients (74). All available studies focused on the potential role of formulas in preventing AM are summarized in **Table 3**.

POTENTIAL MECHANISMS OF ACTION OF INFANT FORMULAS

It has been suggested that selected milk protein hydrolysates used for CMA management may be able to not only avoid allergic symptoms in CMA infants due to the breakdown of IgE antigens but also play a role in immune system modulation, inducing tolerance and preventing allergic sensitization (75–79). These peptides are able to interact with TLR2 and TLR4, modulating cytokine release by epithelial and immune cells (80). It has also been demonstrated that specific peptides from casein hydrolysates, driving T cell switching from Th2 to Th1 or to Tregs subtype, could exert a protective effect for FA (77, 81). Animal studies have demonstrated that these peptides can suppress Th2 response through an IL-10 up regulation and IL-2 down-regulation (75). Moreover, the production of the tolerogenic cytokine IL-10 was higher in Jurkat T cells that underwent a casein hydrolysate stimulus (79). Preliminary data by our group suggest that formula choice is able to induce immune system modulation through epigenetic mechanisms in CMA infants (17, 82, 83); specifically, evidence suggests that EHCF+LGG is able to modulate GM, raising the abundance of selected genera (*Roseburia, Coprococcus*, and *Blautia*) with increased production of butyrate (16). A significant difference in DNA methylation of Th2 and Th1 cytokine (IL-4, IL-5, IL-10, and IFN-γ) genes and of FoxP3, the transcription factor that modulates the fate of Tregs, was observed in infants treated with EHCF+LGG who develop immune tolerance compared to children who received other formulas (82, 83). A DNA methylation status of all allergy-related genes in infants treated with EHCF+LGG

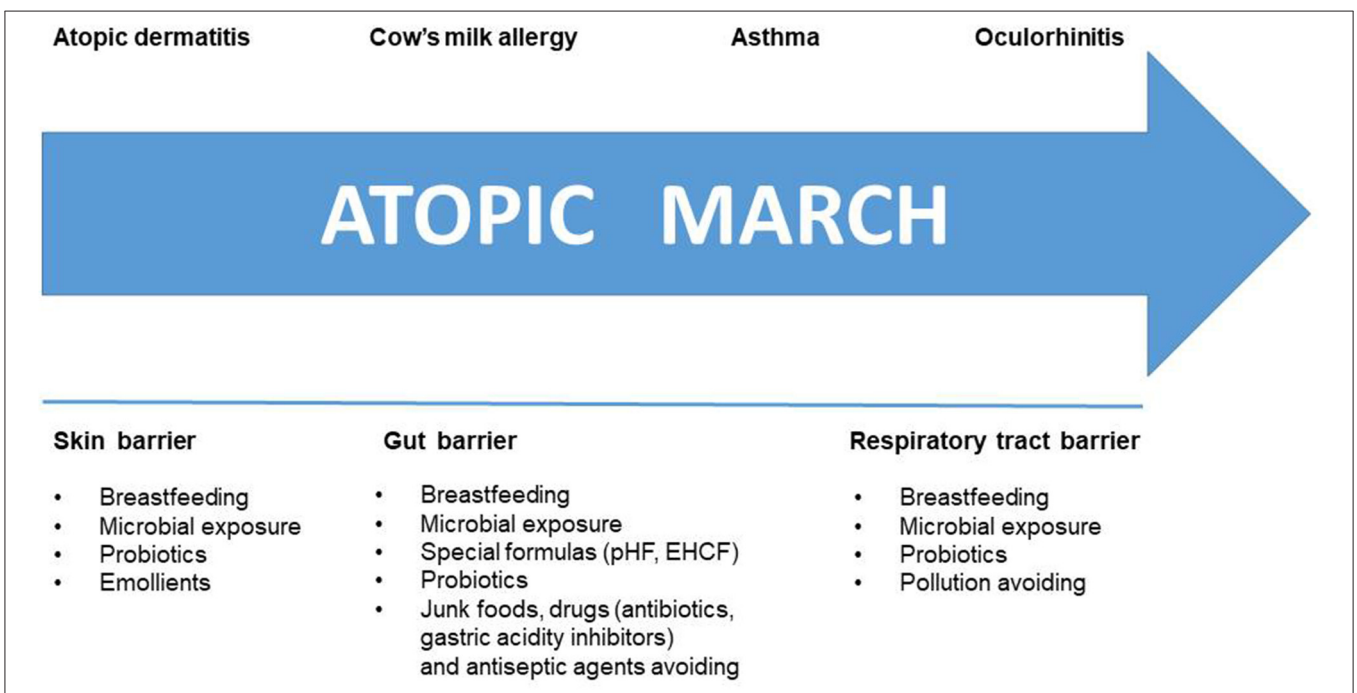

FIGURE 3 | Halting the Atopic March. Several strategies are available to counteract step by step the atopic march. These strategies are targeting the skin, gut, and respiratory tract barrier.

was closer to that observed in healthy children. Analyzing the potential factors able to modulate DNA methylation status in tolerant children, the authors found that the variable that greatly influenced the DNA methylation status was EHCF+LGG formula use (82, 83). A longitudinal study, the EPICMA trial, compared the DNA methylation of FoxP3, Th1/Th2 cytokine genes, and allergy-related microRNAs (miRNAs) profile in IgE-mediated CMA infants taking EHCF+LGG compared to soy formula. This study demonstrated that treatment with EHCF+LGG is characterized by a more pronounced effect on FoxP3 demethylation compared to soy formula and by a higher methylation status of IL-4 and IL-5 and a lower methylation status of IL-10 and IFN-γ (17). Moreover, children treated with EHCF+LGG showed a selected miRNA expression toward a Th1-oriented response, leading to the activation of immune tolerance mechanisms (17). However, the impact of diet on epigenetic mechanisms may not only be direct but also mediated by the GM (84). So, the Diet-GM-Epigenetic axis creates a coherent picture that may be useful for developing potential strategies against AM in CMA children (**Figure 2**). Altogether, these data highlight the relevance of "immune-nutrition management" able to reduce disease duration and to protect against the occurrence of other atopic manifestations the CMA children.

CONCLUSIONS

During the last years, much has changed about AM knowledge. The actual strategies to halt the AM are depicted in **Figure 3**. Despite the lack of cure, novelties about CMA dietary management are moving from "passive" elimination diet to an "active diet-therapy" able to reduce disease duration and to protect against the occurrence of AM. The latter strategy is supported by better knowledge on the role of diet, breastfeeding, gut microbiome, and tolerogenic mechanisms. Thus, an active diet-therapy able to modulate the GM composition, restoring microbial equilibrium and optimal butyrate production, is a positive example of the potential of such strategy. The best nutritional choice for CMA infants is breastfeeding, but recent evidence suggests that breast milk composition could be influenced by environmental factors including maternal diet that could represent relevant target of intervention for preventive strategy against AM in CMA infants. If breastfeeding is not possible, evidence suggests that casein hydrolysate-based infant formula with the adjunction of the probiotic LGG could be able to stimulate immune tolerance acquisition and to reduce the incidence of AM in children with CMA.

AUTHOR CONTRIBUTIONS

RB designed and structured the review, wrote, and read the manuscript. LC and RN analyzed literature, wrote, and read the manuscript. LP and CD analyzed literature and read the manuscript. All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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Dietary Interventions and Nutritional Factors in the Prevention of Pediatric Asthma

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Asthma is the most frequent chronic disease in children, and its pathogenesis involves genetic, epigenetic, and environmental factors. The rapid rise in the prevalence of asthma registered over the last few decades has stressed the need to identify the environmental and modifiable factors associated with the development of the disease. In particular, there is increasing interest in the role of modifiable nutritional factors specific to both the prenatal and post-natal early life as, during this time, the immune system is particularly vulnerable to exogenous interferences. Several dietary factors, including maternal diet during pregnancy, the duration of breastfeeding, the use of special milk formulas, the timing of the introduction of complementary foods, and prenatal and early life supplementation with vitamins and probiotics/prebiotics, have been addressed as potential targets for the prevention of asthma. In this review, we outline recent findings on the potential role of prenatal and perinatal dietary and nutritional interventions for the primary prevention of pediatric asthma. Moreover, we addressed unmet needs and areas for future research in the prevention of childhood-onset asthma.

Keywords: asthma, breastfeeding, children, complementary foods, omega-3 long-chain polyunsaturated fatty acids (LCPUFAs), primary prevention, probiotics, Vitamin D

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INTRODUCTION

Asthma is the most frequent chronic disease in children and is associated with significant morbidity and potential impairment of lung function in adulthood (1). Both genetic, epigenetic, and environmental determinants are involved in the pathogenesis of asthma. Childhood-onset asthma, as opposed to adult-onset asthma, is typically featured by a history of atopy and related markers of type-2 allergic inflammation (2). From 1960, the prevalence of allergy and asthma has progressively increased worldwide, reaching a plateau in some developed countries (3), while continuing to rise in low-income and mid-income countries (4). As a result, over the last few decades, there has been increasing interest in the identification of risk factors for allergy and asthma (5, 6). Recent research has focused on tackling both prenatal and postnatal modifiable factors for the prevention of allergic diseases, as these factors can influence the immune system in a crucial phase of its development (7). A relationship between environmental tobacco smoke, air pollution, respiratory infections, and the development of asthma has been demonstrated and extensively reviewed elsewhere (8, 9). On the contrary, the influence of *in utero* and early-life dietary and nutritional drivers needs to be more fully elucidated. The role of several dietary factors, including maternal diet and vitamin status,

composition of the microbiome, duration of breastfeeding, the use of hydrolyzed formulas and the introduction of complementary foods, has been investigated in recent clinical trials, to identify potential targets for the prevention of childhood-onset asthma (10).

This review focuses on the latest findings on the role of prenatal and perinatal dietary factors in the development of asthma, and whether the modulation of such factors could contribute to the primary prevention of childhood-onset asthma.

MATERNAL DIET DURING PREGNANCY

Given the central role of the first 1,000 days of life for the development of the immune system, numerous studies have investigated the role of intrauterine exposures in the pathogenesis of allergic diseases (11). Recent evidence supports the hypothesis that colonization by a healthy gut microbiome during early infancy can affect the immune system development and the predisposition to immune- mediated diseases later in life, including asthma (12, 13). It has been shown that the maternal diet during pregnancy could influence the composition of the gut microbiome and the immune system development of the neonate, and therefore potentially affect the predisposition to asthma and allergies in childhood (14, 15). There is conflicting evidence on the role of prenatal maternal intake of certain foods and nutrients and the development of asthma and allergies during childhood. In 2015 Beckhaus et al. (16) reported that maternal *in-utero* intake of vitamin D (VD), vitamin E, and zinc had a protective effect against early life wheezing in offspring, but not on childhood-onset asthma or other atopic conditions. One recently published study, assessing the impact of pre-pregnancy diet on the risk of allergic outcomes in children (17), highlighted that the consumption of specific foods during pregnancy, such as cooked green vegetables and eggs, may protect against pediatric wheezing and asthma, while higher maternal intake of meat may increase the risk of wheezing, allergic rhinitis, and atopic dermatitis in children. Further studies are needed to confirm these results and understand the mechanisms related to maternal nutrition and the development of asthma and allergic disorders. Understanding the connection between maternal diet during pregnancy and neonatal microbiome composition may help identify effective prevention strategies, such as providing pregnant women and women desiring pregnancy with nutrition recommendations, particularly regarding products that may influence the development of allergic diseases.

MATERNAL VITAMINS INTAKE DURING PREGNANCY

The recent increase in the prevalence of asthma and allergic disorders in Westernized countries has closely paralleled a VD deficiency epidemic (18). Experimental evidence shows that VD contributes to the fetal-neonatal lung growth and modulates both innate and adaptive immune responses, inhibiting some pro-inflammatory responses associated with allergy and asthma (19). These findings have supported the hypothesis that maternal VD status may play a role in the development of pediatric asthma. Three observational studies found that the risk of asthma in school-age decreases as maternal VD levels increase (20–22), although a recent meta-analysis of observational studies showed that lower prenatal exposure to VD was associated with an increased risk of respiratory infections, but not with asthma and allergic rhinitis development (23). In an early randomized controlled trial (RCT), Goldring et al. (24) randomized 180 women at 27 weeks of gestation to receive either no VD, 800 IU/VD2 daily until delivery or single oral bolus of 200,000 IU/VD3, reporting no reduction in wheezing in offspring at 3 years of age. In the more recent Vitamin D Antenatal Asthma Reduction Trial (VDAART), 881 pregnant women with high atopy risk were randomized to receive either 4,400 IU VD3/daily (active group) or 400 IU VD3/daily (control group), during the 2nd and 3rd semesters of gestation (25). The offspring of the active group showed a clinically relevant, although not statistically significant, reduction (20% or greater) in the risk of asthma/recurrent wheezing by age 3 years, but not by age 6 years (25, 26). These results are in line with those reported by a similar 6-year follow-up trial, the COPSAC study (27, 28), in which pregnant women from an unselected cohort were randomized to receive either 2,400 IU or 400 IU VD3/daily during the 3rd trimester of gestation. These findings show that increasing VD supplementation during pregnancy may reduce the incidence of transient pre-school wheezing, but is not sufficient to prevent school-age asthma, which is usually an allergic-type of asthma (29) (**Table 1**). Further studies are needed to address the influence of VD maternal status on the development of asthma in children before high dose VD supplementation could be recommended during pregnancy.

Regarding other vitamins, Stone et al. (32) showed that higher levels of vitamin E, particularly in its alpha-tocopherol isoform, in postpartum maternal plasma concentration were associated with a decreased likelihood of asthma and wheezing over 2 years.

BREASTFEEDING

Breastfeeding is the most relevant postnatal factor that supports microbial colonization and drives the immune system development of the newborn (33, 34). Compared with formula feeding, breastfeeding has been related to lower morbidity and mortality in infants and decreased incidence of allergic diseases, including asthma (35). Increasing evidence shows that breast milk plays a central role in the development of tolerogenic immune responses during the first years of life, due to its content in immunoglobulins, vitamin A and transforming growth-factors, which promote the gut mucosal barrier integrity and homeostasis (35, 36). Significantly, it has been observed that certain aeroallergens such as dust mite allergens, when found in breast milk, can increase the risk for developing food allergy, as they could disrupt intestinal immune homeostasis through their protease activity and prevent the induction of oral tolerance to food allergens (37). Regarding asthma, breast milk appears to have a protective, dose-dependent impact on

TABLE 1 | Randomized controlled trials on vitamin D supplementation for the prevention of childhood wheezing/asthma.

Authors, Years	Population (N), characteristics	Time of exposure	Interventions	Outcomes
Goldring et al. (24)	180 pregnant women	Prenatal	No VD vs. 800 IU VD2 daily from 27 weeks of gestation until delivery vs. single oral bolus of 200,000 IU VD3 at week 27 of gestation	Wheezing illnesses (assessed by validated questionnaire) in offspring at age 3 years
Litonjua et al. (25) Litonjua et al. (26)	876, pregnant women (18–39 years) high-risk cohort for asthma	Prenatal	4,400 IU VD3/d vs. 400 IU VD3/d starting at 10–18 weeks of gestation until delivery	Asthma or recurrent wheezing in offspring at age 3 years Asthma or recurrent wheezing in offspring at age 6 years
Chawes et al. (27) Brustad et al. (28)	623, pregnant women, unselected cohort	Prenatal	2,400 IU VD3/d vs. 400 IU VD3/d (control) starting at 24 weeks of gestation until delivery	Persistent wheeze and asthma in offspring at age 3 years Asthma in offspring at age 6 years
Grant et al. (30)	260, pregnant women and their infants	Pre and postnatal	Woman-Infant pairs receiving: Placebo-placebo vs. 1,000 IU - 400 IU VD3/d vs. 2,000 IU - 800 IU VD3/d from 27 weeks gestation to birth, and then to offspring for the first 6 months of life	Aeroallergen sensitization and healthcare visit for acute respiratory illness in offspring at age 18 months
Hibbs et al. (31)	300, black premature infants (born at 28–36 weeks' gestation)	Postnatal	400 IU VD3/d vs. diet-limited supplementation daily from birth to 6 months of life	Recurrent wheezing in offspring by 12 months' adjusted age

IU, International Units; VD, vitamin D.

respiratory health, especially for preschool wheezing, although the mechanisms of how breastfeeding reduces the risk of childhood wheezing/asthma is not fully elucidated (38). A systematic review by Dogaru et al. (39) showed that children who were breastfed longer had a lower risk of developing childhood wheezing/asthma. This finding was more significant at ages 0–2 years, and diminished over time, although it remained evident at school age (39).

Other systematic reviews and meta-analyses reported similar findings, suggesting that breastfeeding is protective against pre-school wheezing, which is commonly triggered by viral respiratory infections, whereas this protection tended to wane in older children when heterogeneous factors can influence respiratory morbidity (38, 40). Although there is a growing body of evidence on the role of breast milk in the development of immune tolerance during the first months of life, the impact of breastfeeding on asthma pathogenesis remains controversial.

HYDROLYZED FORMULA FEEDING

The role of partially and extensively hydrolyzed milk formulas (pHF and eHF, respectively) in asthma prevention is still controversial. Two early RCTs showed no significant difference between pHF and eHF in the prevention of allergic diseases in children, including asthma (41, 42). In a prospective, double-blind RCT, high-risk children that could not be breastfed were randomized to receive whey-based pHF or eHF, casein-based eHF, or standard cow's milk formula (CMF) for the first 4 months of life. Hydrolysate nutrition did not have any preventive effect either on asthma or early and late wheezing (43). After 10 years of follow-up, the authors observed no effects on the development of asthma (44), while, between 11 and 15 years of age, the prevalence of asthma was lower in the casein-based eHF group than in the CMF group. No significant effect was found in the whey-based eHF group on any manifestation, nor was there any effect on sensitization with any formula (45).

In a recent birth cohort study, infants received breast milk only, pHF with or without a hypoallergenic label, or non-hydrolyzed formula. The use of the pHF-with hypoallergenic label, compared to non-hydrolyzed formula, had no protective effect on the risk of asthma up to 2 years of age and was related to a higher risk of wheezing at 1 year in high-risk infants (46). A recent Cochrane review concluded that the use of hydrolyzed formula in the early days of infancy, compared to exclusive breastfeeding, showed no significant differences in terms of infant allergy prevention. In particular, the authors found no evidence

to support the use of pHF compared to CMF to prevent allergic diseases among non- exclusively breastfed infants (47).

POSTNATAL VITAMIN D INTAKE

The hypothesis that VD status in childhood might influence the susceptibility to childhood asthma and allergy is supported by the evidence on the role of VD as a key modulator of lung growth and innate and adaptive anti-inflammatory immune responses (48–50). Experimental data have shown that low VD levels are associated with increased type 2-mediated responses, interleukin-10 production, and reduced T-regulatory cells (48). In addition, recent data have shown that early postnatal colonization of the airways by pathogenic bacteria, a risk factor for the development of asthma, is influenced by VD status (50). VD may also affect airway remodeling by direct inhibition of airway smooth muscle cell growth and contractility and fibroblast proliferation (51). Observational studies have shown that VD deficiency in early life is associated with the occurrence and persistence of childhood asthma (19, 52). In a high-risk Australian cohort, VD deficiency in early childhood was associated with a higher risk for persistent asthma at 10 years of age (53). VD deficiency in infancy was also associated with increased risk of early allergic sensitization and susceptibility to respiratory infections (53), which are known risk factors for both preschool and school-age asthma (54).

Interestingly, VD supplementation during pregnancy and infancy has been related to a reduced risk of sensitization to house dust mites at age 18 months (30).

The results of the D-Wheeze trial, in which 300 black infants born prematurely were randomized to receive either a sustained supplementation with 400 IU/day of VD (active group) or a diet-limited supplementation (control group) showed a 34% reduced risk for recurrent wheezing by 12 months in the intervention group (31). There is also evidence that VD supplementation can decrease susceptibility to respiratory viral infection in older children (55). Taken together, the results from *in-utero* (29) and post- natal VD supplementation trials support a role for VD in reducing susceptibility to preschool viral wheezing illnesses. However, there are insufficient data to address whether postnatal VD supplementation may help in the primary prevention of persistent school-age asthma (56), and proper intervention trials with long-term follow-up are needed (**Table 1**). Intervention trials assessing the combination of prenatal and postnatal VD supplementation would also be needed before VD supplementation can be recommended for the primary prevention of pediatric wheezing and asthma.

PROBIOTICS AND PREBIOTICS

There is mounting evidence showing the relationship between the composition of the early-life gut microbiome and the risk of asthma in children (57, 58), which has promoted studies on the modulation of the gut microbiome as a means of preventing asthma. However, RCTs of probiotic and prebiotic supplementation for the prevention of pediatric asthma have shown mixed efficacy outcomes (**Table 2**). An early systematic review and meta-analysis showed no protective role of oral probiotic supplementation during pregnancy or early life on the development of childhood wheeze and asthma (68). A study by Peldan et al. (61) in high-atopy risk children showed that prevalence of asthma at 5 and 10 years of age was similar in the group receiving oral probiotics mixture for the first 6 months of life (also including maternal supplementation starting at week 36 of gestation) and the placebo group. A recent meta-analysis of RCTs concluded that probiotic supplementation during pregnancy or early life did not influence the incidence of wheeze or asthma in infants, but seemed to reduce wheezing incidence among the subgroup of infants with atopic disease (69).

In a 2-year follow-up RCT involving 132 infants at risk of atopy, infants that were fed with a formula containing a mixture of prebiotic oligosaccharides reported a lower incidence for recurrent wheezing compared to the placebo group (59). In a 1-year follow-up RCT involving 113 preterm infants, supplementation of non-human neutral and acidic oligosaccharides during the neonatal period did not reduce the incidence of allergies, bronchial hyper-reactivity, and respiratory infections (60). In 2013, a Cochrane review reported no significant effect of oral prebiotics for the prevention of childhood- onset asthma (70). A more recent meta-analysis of RCTs concluded that the role of prebiotics in the prevention of allergies is still uncertain (71).

Taken together, the evidence on the effects of oral probiotics and prebiotics for the prevention of pediatric asthma is so controversial that no definitive recommendation can be made. Differences in the probiotic strain specificity, the population treated, the timing of administration, and the duration of the intervention all contribute to the heterogeneity of the meta-analysis and of RCT outcomes.

OMEGA-3 LONG-CHAIN POLYUNSATURATED FATTY ACIDS

The supplementation with omega-3 long-chain polyunsaturated fatty acids (LCPUFA) during pregnancy and early life, through the administration of fish oil, has been proposed for the prevention of allergic sensitization and atopic disease, including asthma (72, 73). LCPUFA influence the membrane structure and function, potentially modulating the function of the cells involved in the immune and inflammatory response (74). Most of the studies on maternal fish oil supplementation during pregnancy showed a reduced risk of allergic sensitization to both foods and aeroallergens in children (62, 75–78). In some of these studies, also a lower incidence of eczema was reported (77, 78), while other authors reported no differences in the incidence of allergic diseases (76, 79). Two meta-analyses reported a beneficial effect of maternal supplementation with fish oil on the reduction of allergic sensitization and eczema (80), and on the sensitization to egg and peanut (81), respectively. However, a recent Cochrane review concluded that there is "limited evidence" to support that supplementation with LCPUFA during pregnancy and lactation could reduce the incidence

TABLE 2 | Randomized controlled trials on prebiotics/probiotics and LCPUFA supplementation for the prevention of childhood wheezing/asthma.

	Authors, Years	Population (N), characteristics	Time of exposure	Interventions	Outcomes
Prebiotics Probiotics	Arslanoglu et al. (59)	134, high-risk infants	Postnatal	8 g/L scGOS/lcFOS vs. placebo during the first 6 months of life	Allergic manifestations and infections during the first 2 years of life
	Niele et al. (60)	113, preterm infants	Postnatal	prebiotic mixture of 80% scGOS/lcFOS and 20% pAOS vs. placebo between days 3 and 30 of life	Allergic manifestations and infections during the first year of life
	Peldan et al. (61)	1,223, pregnant women and their offspring	Pre and postnatal	mixed of LGG, L. rhamnosus LC705, Bifidobacterium breve Bb99 and Propionibacterium freudenreichii ssp. shermanii JS vs. placebo (prebiotics) twice daily from 36 weeks of gestation until delivery and in infants daily for the first 6 months of life	Allergic manifestations during the first 10 years of life
LCPUFA	Dunstan et al. (62)	98, pregnant atopic women	Prenatal	3.7 g n-3 PUFAs vs. placebo daily from 20 weeks of gestation until delivery	Allergy, including asthma at 1 year of age
	Bisgaard et al. (63)	736, pregnant women	Prenatal	2.4 g n-3 LCPUFA (fish oil) vs. placebo (olive oil) daily from 24 weeks of gestation until delivery	Persistent asthma and wheezing at 5 years of age
	Olsen et al. (64)	533, pregnant women	Prenatal	2.7 g n-3 PUFAs vs. capsules with olive oil vs. no oil capsules from 30 weeks of gestation until delivery	Asthma at 16 years of age
	D'Vaz et al. (65)	420, high-risk infants	Postnatal	280 mg docosahexaenoic acid and 110 mg eicosapentaenoic acid vs. placebo (olive oil) from birth to age 6 months	Eczema, food allergy and asthma at 1 year of age
	Mihrshahi et al. (66)	376, high-risk infants	Postnatal	tuna fish oil and omega-3-rich margarine and cooking oils vs. placebo (polyunsaturated margarine and cooking oils) from 6 months of life (or at the start of formula feeding)	Allergic sensitization and asthma/wheezing at 18 months of age
	Marks et al. (67)	516, high-risk children	Postnatal	House dust mite avoidance (mattress cover) vs. placebo Dietary fatty acid modification (see (66)) vs. placebo	Asthma, allergic sensitization and eczema at 5 years of age

Cfu, colony forming units; lcFOS, longchain fructo oligosaccharides; LCPUFA, omega-3 long-chain polyunsaturated fatty acids; scGOS, short-chain galacto-oligosaccharides; pAOS, pectin-derived acidic oligosaccharides.

of allergies in children (82). Regarding the prevention of wheeze/asthma, the literature data available show mixed efficacy results for the supplementation of LCPUFA during pregnancy (62–64) (**Table 2**). Indeed, a recent meta-analysis concluded that LCPUFA supplementation during pregnancy is not associated with a significant protective effect on wheeze/asthma in offspring (81).

Conflicting results have been found in studies investigating fish oil supplementation in infants and children for the prevention of allergic sensitization and asthma (65–67) (**Table 2**). A meta-analysis of RCTs found no evidence of a protective role of LCPUFA supplementation in infants and children in the prevention of asthma (83). Overall, the available studies show methodological heterogeneity and risk for suboptimal adherence bias. Therefore, further trials are needed to clarify the role of LCPUFA supplementation during pregnancy and early life in the prevention of pediatric asthma.

TIMING OF COMPLEMENTARY FEEDING

Recent advances in the field of allergy prevention showed that early 2000s recommendation to delay the introduction of solid allergenic foods to the infant's diet is not an effective approach to reduce the risk of allergic sensitization and atopic diseases in children (84–88). More recently, high-quality clinical trials showed that the early introduction of some food allergens, such as peanut and egg, is associated with a reduced risk of food allergy to those foods (84). There is conflicting evidence regarding early complementary feeding and the prevention of pediatric asthma, with some observational studies reporting that the early introduction of some solid foods (before 1 year of age), such as oat, fruit, vegetables, and fish, was associated with a reduced prevalence of wheezing and asthma in childhood (89–91), while others reporting no such association (92).

The timing of the introduction of fish is of particular interest to the purpose of primary prevention of asthma, given its high content in LCPUFA. Despite the heterogeneity in the methods of analysis and outcome measures, the early introduction of fish has been associated, in many observational studies, with a reduced risk of allergic sensitization (93, 94). However, the protective effect of early introduction of fish on the development of infant wheezing and asthma seems more controversial, with some observational studies reporting such association (93, 95–98), while others did not confirm this protective effect (90, 99). Indeed, two recent meta-analyses conclude that introducing fish before the age of 9 months is associated with low-to-very low evidence of reduced allergic sensitization and rhinitis (94), but there is limited evidence that early introduction of fish could reduce the risk of developing wheezing and asthma in childhood (100).

CONCLUSION

The significant increase in the prevalence of asthma and allergic diseases registered in recent years has promoted research on the identification of modifiable risk factors for the prevention of such disorders. It is well-acknowledged that respiratory health is determined by a complex interaction between genetic factors and environmental drivers that occur during prenatal and early postnatal life, including dietary factors. However, it remains difficult to define the contribution of specific dietary supplements and nutritional food sources to the risk of developing pediatric asthma, due to the heterogeneous pathogenesis of this disease and the limitations of the currently available evidence, in terms of study design, type and duration of interventions and outcomes measures.

Further research is needed to accurately identify dietary and nutritional modifiable risk factors for asthma and to address whether the modulation of such factors, either alone or in combination, could contribute to the primary prevention strategies of pediatric asthma.

AUTHOR CONTRIBUTIONS

DP, GN, IT, GC, and PC made substantial contributions to conception, design, and acquisition of data. GC, IT, GN, and PC drafted the initial manuscript. DP, EV, ED'A and PC critically reviewed it for important intellectual content. All authors approved the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Role of Vitamin D in Prevention of Food Allergy in Infants

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The prevalence of food allergy is increasing over the last decades. The role of vitamin D in the prevention of food allergy has been largely investigated. Its role on the physiology of calcium and bone is known, but calcitriol (active form of the vitamin D) also influences the epithelial cells, T cells, B cells, macrophages, and dendritic cells. Almost all cells of the adaptive immune system express the vitamin D receptor, making them also capable of being vitamin responsive. Specifically considering the potential role of vitamins in food allergy, vitamin D has been shown to affect several mechanisms that promote immunologic tolerance, including the T regulatory cell function and the induction of tolerogenic dendritic cells. The target of our review is to evaluate the role of vitamin D in the prevention of food allergy in children. There are contradictory data on the relationship among the vitamin D deficiency and the developing of food allergy. Some studies associate lower exposure to sunlight to food allergy; on the other hand, further research has found that higher vitamin D levels could increase the likelihood of allergic sensitization and food allergy. Therefore, there is an urgent need for well-planned randomized controlled trials on vitamin D supplementation, with particular regard to the prevention of food allergy. The role of vitamin D beyond bone and calcium metabolism is not fully understood.

Keywords: vitamin D, food allergy, prevention, allergic sensitization, immune system

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KEY POINTS

- Vitamin D is a hormone with pleiotropic effects, essential not only for calcium homeostasis and bone mineralization but also for the proper functioning of the immune system.
- However, some patients do not benefit from vitamin D supplementation owing to genetic alterations in metabolism rather than absorption.
- The association between vitamin D and development of food allergy is contradictory.
- There is a potential association between lower sunlight exposure and food allergy, but on the other side, it appears that higher levels of vitamin D might raise the probability of allergic sensitization and food allergy.
- Vitamin D must be considered as a further chance in comprehension and treatment of atopic diseases.
- There is an urgent need for well-planned randomized controlled trials on vitamin D supplementation in food allergy.

INTRODUCTION

In recent decades, the occurrence of food allergies has recorded a significant increase in many developed countries worldwide, probably as a result of environmental and lifestyle changes (1, 2). Vitamin D is a hormone with pleiotropic effects, essential not only for calcium homeostasis and bone mineralization but also for the regulatory effects on the immune system (**Figure 1**) (3).

Vitamin D receptors (VDRs) are expressed in almost all the tissues of the human body. There is a significant association between vitamin D levels and the risk of immunologic, metabolic, or neoplastic disorders (4). Moreover, epidemiological evidence suggests a role of vitamin D in food allergy pathogenesis (5). Vitamin D impacts the function of macrophages, dendritic cells, B cells, T cells, and epithelial cells, playing a key role in immune response, both innate and adaptive (6, 7). All these cells convert the circulating prohormone form into the active one. Thanks to vitamin D, the innate response is capable to express antimicrobial peptides—such as cathelicidin—and plays an important role for keeping mucosal integrity and reinforce epithelial barrier by stimulating junction genes (8, 9). Regarding the adaptive immune response, it was found that VDR agonists affect the function of Th1 and Th2 cells, stimulate tolerogenic dendritic cells and regulatory $CD4^+CD25^+$ $Foxp3^+$ T cells, avoid dendritic cell maturation, and abolish allergen-specific IgE synthesis (10, 11). In *in vitro* studies, by exposing human CD4 T cells to $1,25(OH)_2D$, their conversion into IL-10 secreting Treg cells has been proven; at the same time, there is a suppression of IgE making by B cells, with production of antiphlogistic and tolerogenic cytokines (12, 13).

In our study, we reviewed the current literature to evaluate the role of vitamin D in food allergy in children.

VITAMIN D PHYSIOLOGY

In humans, the major source of vitamin D (90%) is the exposure to solar UVB radiation (290–315 nm wavelengths),

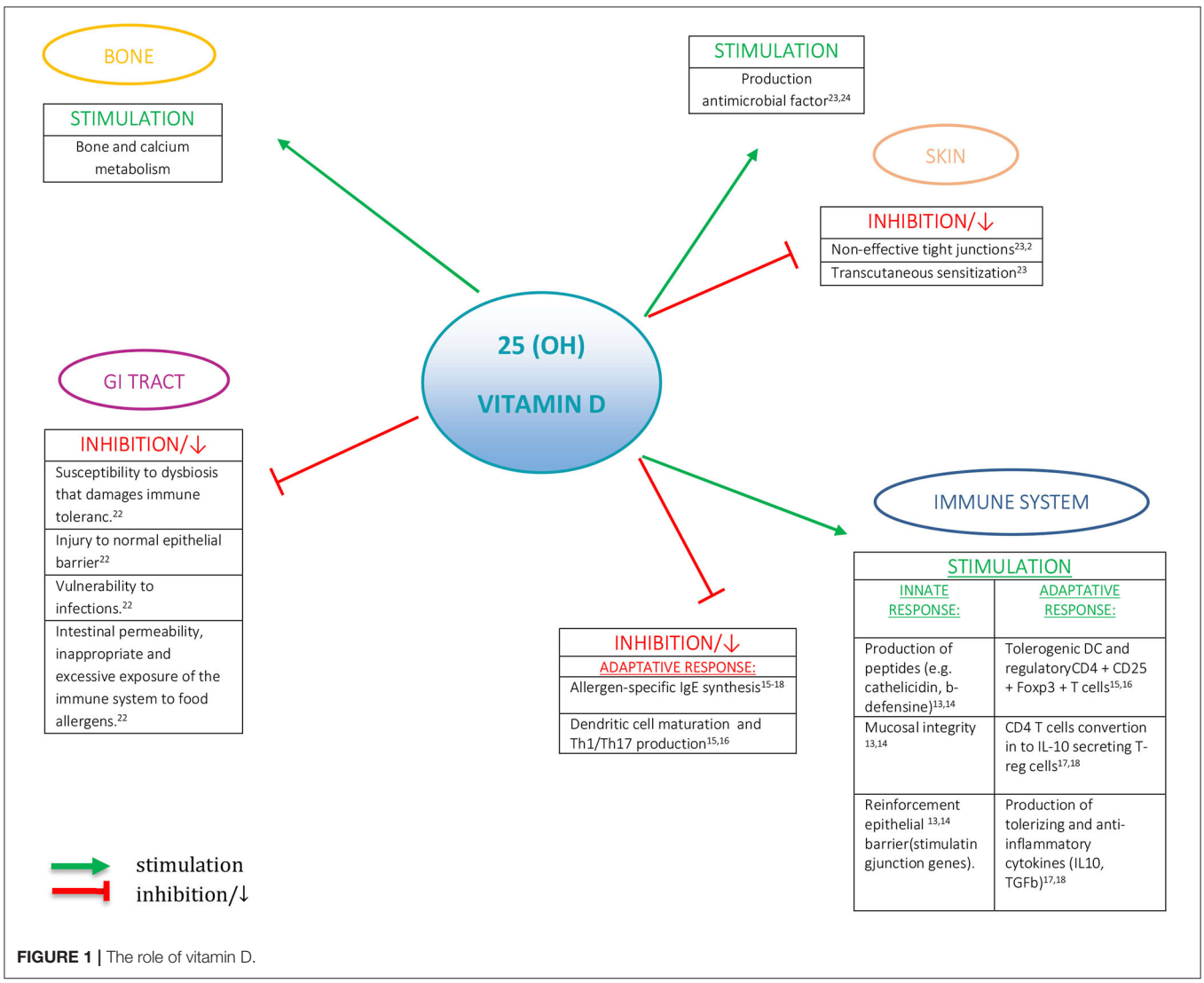

FIGURE 1 | The role of vitamin D.

which determines the formation of cholecalciferol in the skin, which is then metabolized in the liver to 25-hydroxyvitamin D (25-OH-D_3) and finally carried to the kidneys, where it is transformed into the active form [1,25-dihydroxyvitamin D, 1,25-$(OH)_2D$] (3, 14). Only 10% of vitamin D is obtained through food ingestion.

The best indicator of vitamin D status is serum 25$(OH)D_3$ levels, which reflect the whole intake of vitamin D, comprehensive sun exposure, integrations, and food intake. In recent decades, it has been observed that there is an increasing evidence of a global vitamin D deficiency (VDD) for all ages (15, 16) owing to a combination of extrinsic and intrinsic factors. In the first group, we can include the intensity of exposure to UVB determined by seasons, latitude, altered eating habits, and behavioral factors, without forgetting campaigns to prevent skin cancer, whereas in the latter we might mention the individual level of skin melanin content and the intestinal absorption of vitamin D. Obese people have a greater risk of VDD, caused by their lifestyle and also probably as a consequence of the uptake in the adipose tissue of this liposoluble vitamin (17). However, some patients do not benefit from vitamin D supplementation owing to genetic alterations in metabolism rather than absorption. In a recent literature review, 35 genes putatively associated with abnormal serum 25 $(OH)D_3$ were identified (18).

POTENTIAL ROLE OF VITAMIN D IN THE DEVELOPMENT OF FOOD ALLERGY

Hypothesis on Pathogenic Mechanisms

The cellular and molecular mechanisms involved in the pathogenesis of food allergy are very complex and encompass genetic, epigenetic, and environmental factors (19–21). Several mechanisms have been proposed aimed at clarifying the role played by VDD in the food allergy pathogenesis. VDD, at a particular time of life, might increase the susceptibility to colonization by abnormal intestinal microbial flora, contributing to increased intestinal permeability, leading to an inappropriate and excessive exposure of the immune system to food allergens. On the other hand, VDD might cause a disequilibrium at the intestinal level that damages immune tolerance, destroys the normal epithelial barrier, and increases the susceptibility to infections (22). Food allergen sensitization can also be driven by percutaneous sensitization, which may be important particularly in children with VDD (23). It can be speculated that decreased antimicrobial factor and non-effective tight junctions caused by VDD may determine, in the skin, an anomalous exposition and thereby a boost of the immune system, driving to allergic sensitization eczema (24) and the onset of food allergy (23), in addition to an important increase in the severity of atopic dermatitis (25).

The current studies in the literature on the possible role of vitamin D in the development of food allergy have been reported in **Tables 1**, **2**, which are detailed below.

Evidence on VDD and Anaphylaxis

The first reports regarding a possible association between food allergy and VDD came from the observation that there was a direct relationship among increasing latitude and cases of anaphylaxis, prescription of epinephrine autoinjector, or food allergy–related admissions (30, 40–44). Contrasting results have been reported about the correlation between vitamin D status and atopic dermatitis severity (45). A recent Korean study (28) compared incidence of food-induced anaphylaxis (FIA) and vitamin D serum levels between two regions of high and low solar radiation, finding that, in the region of lower solar radiation, vitamin D levels were lower, with concomitantly higher FIA incidence. These findings suggested the possible causal function of vitamin D levels in food allergy, but data sources of FIA and vitamin D used in the study differed (27, 28, 30, 40–44, 46). Kim et al. (28) designed a study that included 2,814 patients with FIA and 15,367 people with available serum vitamin D measurements. After stratification by age, sex, and area of residence, lower solar radiation region had higher FIA incidence (2.2 per 100,000 person-years vs. 1.8 per 100,000 person-years) and lower vitamin D values (16.5 vs. 17.8 ng/ml) than higher solar radiation region. Camargo et al. (42) examined regional rates of epinephrine autoinjector (EpiPen) prescription in the United States, finding a strong north–south gradient. Mullins et al. (44) evaluated epinephrine autoinjector prescriptions and anaphylaxis hospital admission rates in Australia, used as surrogate markers of anaphylaxis. Both in an unadjusted and adjusted model of children from birth to the age of 4 years, they found a decrease in EpiPen prescription as decreasing absolute latitude. The anaphylaxis admission rates also showed a similar gradient. These data provided additional support and etiologic clues for a possible role of vitamin D in anaphylaxis pathogenesis. However, we cannot demonstrate that food allergy is linked to vitamin D levels and not to any other geographic, seasonal, or sunlight-derived factor (47).

Evidence on VDD and Season of Birth

Other studies showed a relationship between less sunny season of birth and increase of food allergy prevalence. Seasonal differences in UVB exposition result in reduced 25$(OH)D_3$ levels in autumn and winter months; in the higher latitudes, there is no sufficient UVB intensity in the cooler months for proper synthesis of 25$(OH)D_3$ to occur, irrespective of sunlight exposure (27). In addition, there are various data that hypothesize the possible link between season of birth and food allergy. The potential mechanisms are consequential to VDD owing to a paucity of UVB exposure. Matsui et al. (59), in their recent review, proposed the hypothesis that autumn and winter birth could worsen eczema, with the risk of excessive food antigen exposure and sensitization. Moreover, the deficiency of UVB exposure may lead to inadequate Treg expansion, potentially responsible for impaired food tolerance regardless of VDD. VDD resulting from an inadequate vitamin D synthesis from skin could compromise the intestinal epithelial barrier and antimicrobial peptides, with the risk of intestinal dysbiosis. Lastly, VDD could also modulate immune response and lead to sensitization and impaired food tolerance. Limitations of considered studies are a precise definition of vitamin D deficiency and the presence of co-factors in the population, such as eczema, skin color, race, residence, skin color, gender, and age. The apparent immune

TABLE 1 | Summary of studies on the possible role of vitamin D in the development of food allergy.

References	Study	Age, sample	Results	Definition of vitamin D deficiency
Sharief et al. (26)	Retrospective study	3,136 children/adolescents and 3,454 adults	25(OH)D levels <15 ng/ml associated with peanut allergy, no consistent associations seen in adults	25(OH)D deficiency <15 ng/ml, insufficiency 15–29 ng/ml
Mullins et al. (27)	Retrospective study	115 peanut allergic patients younger than 72 months	Non-linear relationship between neonatal 25(OH)D_3 levels and peanut allergy in children under 6 months of age, slightly higher levels (75–99.9 nmol/L) linked with lower vs. those in the reference group (50–74.9 nmol/L)	Neonatal concentration of 25(OH)D divided into four groups: <50, 50–74.9, 75–99.9, and >100 nmol/L. The reference group was considered between 50 and 74.9 nmol/L
Kim et al. (28)	Retrospective study	18,181 patients 10 years or older (2,814 patients with food-induced anaphylaxis and 15,367 people with available serum vitamin D measurements)	Higher incidence of food-induced anaphylaxis in regions with lower vitamin D levels in the population	Not defined
Kull et al. (29)	Prospective birth cohort	4,089 newborn infants were followed for 4 years	Water-soluble form increased the risk of allergic disease in children up to the age of 4 years compared with supplementation of same vitamin given in peanut oil	Not defined
Camargo et al. (30)	Prospective pre-birth cohort study	1,194 mother–child pairs followed up through age 3 years	Higher maternal intake of vitamin D during pregnancy may decrease the risk of recurrent wheeze in early childhood	Not defined
Nwaru et al. (31)	Prospective cohort study	971 children with 5-year follow-up	It was found that maternal intake of vitamin D was inversely associated with sensitization to food allergens	Not defined
Liu et al. (32)	Prospective birth cohort study	649 children who were enrolled at birth and followed from birth onward	Vitamin D deficiency may increase the risk of food sensitization among individuals with certain genotypes	Cord blood 25(OH)D_3 <11 ng/ml
Jones et al. (33)	Prospective birth cohort study	231 mother–child pairs, derived from a larger (n = 669) prospective birth cohort, followed up until 1 year of age	Reduced fetal exposure to vitamin D increases the risk of eczema in infants by 12 months of age	25(OH)D_3 levels cutoffs were divided in <50 nmol/L, 50–74.99 nmol/L, >75 nmol/L
Weisse et al. (34)	Prospective	378 mother–child pairs followed up until 2 years of age	High vitamin D levels in pregnancy and at birth may contribute to a higher risk for food allergy	The assay detection limit was defined as 6.7 ng/ml for maternal 25(OH)D_3 and 5.2 ng/ml for maternal 25(OH)D_2. Detection limit for cord blood 25(OH)D_3 and D_2 was 3 ng/ml
Allen et al. (35)	Australian large prospective cohort study	577 infants, 1 year of age	Vitamin D insufficiency more likely associated with peanut and/or egg allergy. Vitamin D insufficiency linked to multiple food allergies (≥2) rather than a single food allergy	Vitamin D insufficiency: ≤50 nmol/L
Chiu et al. (36)	Prospective study	186 children (0–4 years)	Cord blood 25(OH)D levels inversely linked with the risk of milk sensitization at 2 years of age	Low vitamin D levels <20 ng/ml
Chawes et al. (37)	Prospective clinical study	257 children	Cord blood 25(OH) vitamin D levels defined as <50 nmol/L was not associated with allergic sensitization	Cord blood 25(OH)-Vitamin D: deficient, 50 nmol/L; insufficient, 50–75 nmol/L; sufficient, >75 nmol/L
Hennessy et al. (38)	Prospective Cork BASELINE Birth Cohort Study	Vitamin D was measured in maternal sera at 15 weeks of gestation (n = 1,537) and umbilical cord blood (n = 1,050)	The investigators did not observe any association between vitamin D during pregnancy or at birth with allergic disease outcomes at 2 and 5 years old	Maternal 25 (OH) D divided into <30 nmol/L; 30–49.9 nmol/L; 50–74.9 nmol/L; ≥75 nmol/L
Ercan et al. (39)	Prospective, observational, case–control study	111 children <2 years of age	No statistically significant relationship between the CMPA group and healthy controls in terms of 25(OH)D levels	Vitamin D deficiency ≤20 ng/ml, insufficiency 21–29 ng/ml, adequate ≥30 ng/ml

(Continued)

TABLE 1 | Continued

References	Study	Age, sample	Results	Definition of vitamin D deficiency
Sardecka et al. (48)	Prospective two-stage study	138 infants with CMA and 101 healthy infants	Children with increased Foxp3mRNA expression (predictive of faster gain of tolerance in infants with CMA) have lower serum vitamin D levels than healthy children	25 (OH)D concentration sufficient ≥30 ng/ml for the Polish population
Baek et al. (49)	Cross-sectional study	226 children aged 3–24 months with atopic dermatitis or suspected food allergy	VDD increased the risk of food allergen sensitization especially to milk and wheat. The polysensitization group had significantly lower levels of 25(OH)D than the non-sensitization and monosensitization group	Serum 25(OH)D levels: deficiency, <20.0 ng/ml; insufficiency, 20.0–29.0 ng/ml; and sufficiency, ≥30.0 ng/ml
Rosendahl et al. (50)	Randomized controlled study	975 infants followed up until 12 months of age	No differences between the vitamin D supplementation groups in food sensitization at 12 months. Possible adverse effect of high concentrations of vitamin D	25 $(OH)D_2$ considered sufficient for concentrations ≥50 nmol/L
Guo et al. (51)	Large observational study	2,642 children followed up until 2 years of age	No evidence found supporting the link between low levels of 25 (OH)D and allergic sensitization to various allergens	25(OH)D concentrations insufficient <75 nmol/L and sufficient otherwise
Thorisdottir et al. (52)	Longitudinal Icelandic study	144 children followed up until 6 years of age	At 12 months, IgE-sensitized children had a lower intake of vitamin D, but no significant difference in mean serum 25(OH)D was found between IgE-sensitized and non-sensitized children, nor at 12 months or 6 years	Vitamin D deficient: <30 nmol/L and vitamin D intake from diet and supplements combined did not exceed 25 μg/day in infancy or at 6 years

suppressive effect of ultraviolet radiation is not limited to the supposed inverse relationship between vitamin D levels and FIA rate, but it is also thought to be involved in the development of immune-related disorders, such as type 1 diabetes mellitus (43).

Evidence on VDD and Allergic Sensitization

The milestone NHANES study (26), based on extensive nationally representative samples from the United States of more than 3,000 children and adolescents, found an association between VDD and higher levels of specific IgE, and thereby allergic sensitization to several allergens, both environmental and food, in children and adolescents, but not in adults. At the same time, the relationship between increased IgE and excessively high vitamin D levels was not confirmed. By contrast, Hypponnen et al. (60), in an adult population study, showed that both low and excessive circulating $25(OH)D_3$ levels were correlated with an increase in IgE in a non-linear relationship. Furthermore, higher rates of food sensitization have been seen in infants born to mothers with low vitamin D intake in pregnancy. Nwaru et al. (31) examined the effect of maternal diet during pregnancy on allergic sensitization in a population-based cohort study with 5-years follow-up, by evaluating 971 children with human leukocyte antigen–caused predisposition to type 1 diabetes, for whom maternal pregnancy dietary survey and allergen-specific IgE measurements at 5 years were recorded. The data showed an inverse correlation between sensitization to food allergens and maternal intake of vitamin D, whereas intake of citrus fruits in childbearing might raise the risk of developing allergic sensitization in the sons. A Korean cross-sectional study (49), which included 226 infants [168 infants with atopic dermatitis (74.3%) and 58 with suspected food allergy without atopic dermatitis (25.7%), aged 3–24 months], demonstrated that VDD increased the risk of food allergen sensitization, especially to milk and wheat, but in this work, the diagnosis of food allergy was only suspected and not confirmed by oral food challenge. An Australian large prospective cohort study proved that infants with low vitamin D levels ($25(OH)D_3$ <50 nmol/L) at 12 months of age were more likely to be affected by challenge-proven food allergy, in particular to peanuts and egg, and to have multiple food allergies, in comparison with those who had appropriate vitamin D levels. Curiously, this connection was clear only among infants of Australian-born parents, hinting a gene–environment interaction (35). However, other studies did not support this evidence (55). Kull et al., in a prospective birth cohort of 4,089 infants, showed that vitamin D in water-soluble form increased the risk of allergic disease in children up to the age of 4 years, compared with supplementation of same vitamin given in peanut oil, but vitamin D levels were not measured at baseline nor follow-up (29).

In a prospective, observational, case–control study that involved 111 children <2 years of age, Ercan et al. (39) assessed the possible link between cow's milk protein allergy (CMPA) and $25(OH)D_3$ levels in infants with an initial diagnosis of CMPA. Moreover, they also evaluated the association between $25(OH)D_3$ levels and skin prick test induration size, specific IgE to milk and specific IgE to casein. For the study purpose, they considered vitamin D deficiency if $25(OH)D_3$ values were

TABLE 2 | Summary of review on the possible role of vitamin D in the development of food allergy.

References	Study	Age, sample	Results	Definition of vitamin D deficiency
De-Regil et al. (53)	Cochrane review	Variable	Vitamin D supplementation, during pregnancy, both single-dose or continued, increased 25(OH)D_3 levels at term; however, the clinical implication of improving the vitamin D concentration and the possible use of this intervention strategy as part of the routine antenatal care are yet to be evaluated	Variable
Mirzakhani et al. (54)	Review	Variable	Well-designed and well-powered clinical trials are needed to determine whether supplementation of vitamin D should be recommended in allergic diseases	Variable
Willits et al. (55)	Review	Variable	No association between food allergy and vitamin D level	Variable
Yepes-Nuñez et al. (56)	Systematic review of randomized and non-randomized studies	Variable	Vitamin D supplementation for pregnant women, breastfeeding women, and infants may not decrease the risk of developing allergic diseases, such as atopic dermatitis (in pregnant women), allergic rhinitis (in pregnant women and infants), asthma and/or wheezing (in pregnant women, breastfeeding women, and infants), or food allergies (in pregnant women). However, they conclude that the potential impact of vitamin D on food allergy remains uncertain	Variable
Saggese et al. (57)	Review	Variable	In food allergies, the role of vitamin D remains controversial	Variable
Hawrylowicz et al. (58)	Review	Variable	Longitudinal studies of vitamin D requirements *in utero* and post-natally, better understanding of factors that influence bioavailability of vitamin D, and mechanistic insights into vitamin D effects on neonatal-specific immune pathways are awaited	Variable
Matsui et al. (59)	Review	Variable	Fall and winter birth could worse food sensitization	Variable

$\leq$20 ng/ml, insufficiency if they were between 21 and 29 ng/ml and adequate when they were $\geq$30 ng/ml. No statistically significant relationship was found between the CMPA group and healthy controls in terms of 25(OH)D_3 levels, nor even milk antigen induration diameter and vitamin D levels of CMPA infants. Hence, they concluded that, at the starting diagnosis of infants with CMPA, routine workup of vitamin D levels may have no benefit. Sardecka et al. (48) examined the relationship between Foxp3mRNA expression (the best marker for Treg lymphocytes) and serum concentration of vitamins D and C, and the development of different phenotypes of tolerance in children with CMPA. The results suggest that increased of Foxp3mRNA expression can predict faster tolerance acquisition in infants with CMA. Regardless of whether they acquire tolerance, children with CMPA have lower serum vitamin D levels than healthy children. Recently, also Guo et al. (51) performed a large observational study involving 2,642 children with the aim of evaluating the correlation between serum 25(OH)D_3 and allergic sensitization among childhood 0–2 years of age. Vitamin D was considered insufficient when serum concentration of 25(OH)D_3 was <75 nmol/L and sufficient otherwise. They did not find evidence supporting the link between low levels of 25(OH)D_3 and allergic sensitization to various allergens.

Evidence on VDD, Pre-natal Data, and Birth Cohort Studies

Another matter investigated by some authors has also been the potential link between 25(OH)D_3 concentration in newborns as a marker of risk of future development of food allergies. In the study of Mullins et al. (61) on 115 patients younger than 72 months, it was found that there is an association between peanut allergy and neonatal 25(OH)D_3 levels. In comparison with the reference group (50–74.9 nmol/L), 25(OH)D_3 levels of 75 to 99.9 nmol/L were linked to a reduced risk of peanut allergy. At levels of 100 nmol/L or higher, no additional reduction was found, whereas the probability of peanut allergy at levels lower than 50 nmol/L was substantially equal to that of the reference group. The risk of peanut allergy at levels <50 nmol/L was also not significantly different from the reference group. Jones et al. (33) studied the association among cord blood 25(OH)D_3 and allergic sensitization, eczema, and food allergy at 1 year of age. The lower vitamin D levels at birth were associated to higher likelihood of eczema at 12 months, without significant differences between IgE-mediated and non–IgE-mediated eczema. Although in this high-risk subset there was a greater likelihood of IgE-mediated food allergy and allergen sensitization after the first year of life, the probability to develop IgE-mediated food allergy was not linked to cord blood 25(OH)D_3 (33, 37). Chawes et al. (37), in their Copenhagen Prospective Studies on Asthma in Childhood (COPSAC2000) at-risk mother–child cohort, analyzed the relationship between cord blood 25(OH)vitamin D and asthma and allergy-related conditions during pre-school age in 257 children. After adjusting for season of birth, deficient cord blood 25(OH) vitamin D levels, defined as <50 nmol/L, were not associated with allergic sensitization. On the other hand, Chiu et al. (36), considering a birth cohort of Taiwanese children, found an inverse relationship between cord blood 25(OH)D_3 levels and milk sensitization at the age of 2 years. One hundred eighty-six children aged 0 through 4 years were enrolled and regularly followed up for 4 years. The average cord blood 25(OH)D_3 level was 23.8 $\pm$ 9.5 ng/ml, with a high occurrence of VDD (<20 ng/ml) at birth (42%). A trend was found between low cord blood 25(OH)D_3 levels and higher risk of milk sensitization throughout childhood. At the same time, cord blood 25(OH)D_3 levels showed an inverse relationship to the risk of milk sensitization at 2 years old, an age

at which a greater occurrence of milk sensitization was markedly associated to the risk of asthma development and allergic rhinitis at the age of 4 years. Nonetheless, low cord blood vitamin D levels do not seem linked to a higher risk of allergic rhinitis, eczema, or asthma in early childhood. Hennessy et al. (38), in their Cork BASELINE Birth Cohort Study, investigated associations between intrauterine vitamin D status and atopic outcomes in an extensively characterized, disease-specific, maternal-infant cohort. In this study, the diagnosis of food allergy was made for all children during the 24-months clinical evaluation visit using skin prick tests. The panel of food allergens included cow's milk, eggs, peanuts, cod, soybeans, and wheat. In the case of wheals with a diameter of ≥3 mm, a blinded oral food challenge was completed, if the food had not been eaten previously or if there was a story that suggested the risk of food allergy. The investigators did not observe any association between vitamin D during pregnancy or at birth (measured in maternal sera at 15 weeks of gestation and umbilical cord blood) with allergic disease outcomes (eczema, food allergy, asthma, allergic rhinitis) at 2 and 5 years old. A German study (34) focused on the effects of newborn and maternal vitamin D levels and their influence on the development of food allergy in children, and considered 378 mother–child pairs during pregnancy and at childbirth atopic manifestations during the first 2 years of life by using questionnaires filled out by the parents during pregnancy and annually thereafter. They demonstrated that high vitamin D levels in pregnancy and at birth might lead to a greater risk of food allergy, suggesting that, to prevent atopy, integration is not required. Recently, Rosendahl et al. (50) realized a randomized controlled trial of daily vitamin D supplementation of 10 or 30 μg from the age of 2 weeks, measuring food and aeroallergen IgE antibodies at 12 months of age. It was demonstrated that high-dose vitamin D supplementation did not prevent allergic sensitization and allergic diseases during the first year of life. On the other hand, it was observed that there is an increased risk of milk allergy in infants randomized to the higher vitamin D supplementation and an increased risk of milk allergy in infants with high cord blood vitamin D status, thereby suggesting a possible adverse effect of high concentrations of vitamin D. A Cochrane review (53) proved that vitamin D supplementation, during pregnancy, both single-dose or continued, increased $25(OH)D_3$ levels at term; however, the clinical implication of enhancing the vitamin D concentration and the possible role of this approach in the standard antenatal care are still to be evaluated because of the limited number of trials and outcomes to deduce implications on safety and efficacy. The presence of mixed results may be a consequence of the gaps in our knowledge about the precise role of vitamin D in the development of food allergy (47). Yepes-Nuñez et al. (56), in a recent systematic review including randomized and non-randomized studies, showed that vitamin D integration for pregnant women, breastfeeding women, and infants might not reduce the probability of allergic disease development, such as food allergies (in pregnant women), asthma and/or wheezing (in pregnant women, breastfeeding women, and infants), allergic rhinitis (in pregnant women and infants), or atopic dermatitis (in pregnant women). However, they concluded that the potential impact of vitamin D on food allergy remains uncertain. Liu et al. (32) evaluated the association among cord and maternal vitamin D level (VDD if cord blood $25(OH)D_3$ <11 ng/ml) and atopic outcomes in 649 children recruited at birth and followed from then on, and found that VDD alone was not related with food sensitization. If examined together with SNPs, a significant interaction was found between VDD and IL-4 gene polymorphism. VDD raised the risk of food sensitization among children carrying CC/CT genotypes; comparable but lower relationships were seen for the SNPs. The conclusion of their study is that VDD may enhance the risk of food sensitization among people with specific genotypes. A recent longitudinal Icelandic study, involving 144 children followed up for 6 years, compared infant feeding with particular regard to vitamin D supplementation and $25(OH)_3$ levels between IgE-sensitized and non-sensitized children at 6 years. They found that, at 1 year of age, IgE-sensitized children had a reduced intake of vitamin D, partially explained by a reduced, but non-significant, vitamin D supplement use and reduced consumption of vitamin D fortified formula. At 6 years, less IgE-sensitized children used vitamin D supplements regularly. Equally, vitamin D integration at 6 years decreased the ratio of IgE sensitization (52). Nonetheless, the authors did not record a difference in mean serum $25(OH)D_3$ between IgE-sensitized and non-sensitized children, nor at 12 months (96.8 ± 33.6 vs. 99.3 ± 32.2 nmol/L, respectively) or 6 years (59.3 ± 15.9 vs. 56.0 ± 16.7 nmol/L, respectively). In conclusion, their data encouraged, for Nordic infants and children, vitamin D intake from diet and supplements.

Further issues are about the definition of optimum, deficiency, and insufficiency vitamin D serum levels, not worldwide recognized and rather specific for bone outcomes, but not for global health effects (4, 6). Low vitamin D levels are common in healthy newborns (33, 62) and are independently associated with various factors (skin color, diet, maternal levels and intake, supplements, and seasonality) and strengthen the controversy on the benefits of providing vitamin D integration during infancy.

CURRENT SCENARIO

The current reviews (54, 56–59) conclude that further studies are needed to evaluate the association between allergy and vitamin D. Vitamin D supplementation controlled studies aimed to clarify its role in food allergy development are still lacking.

Currently, there are more evidence to support vitamin D supplementation in pregnancy and infancy, in light of its positive effects. In fact, VDD may boost the risk of food allergy and sensitization among people with particular genotypes (32). Nevertheless, when studied alone, VDD was not associated with food allergy whereas, on the other hand, it was significantly related with specific gene polymorphisms, supplying evidence on food allergy. Also, the role of vitamin D beyond bone and calcium metabolism is not fully understood.

CONCLUSIONS

The role of vitamin D beyond bone and calcium metabolism is alluring but not fully understood. The association between

vitamin D and development of food allergy is contradictory (**Tables 1**, **2**).

Potential relationships come from ecologic studies that associate lower sunlight exposure to food allergies. On the other hand, further research found that higher levels of vitamin D might raise the probability of allergic sensitization and food allergy. However, in light of a large literature linking the vitamin D levels to the onset of eczema and allergic diseases, this hormone must be considered as a further chance in the comprehension and treatment of atopic diseases. For this reason, there is an urgent need for well-planned randomized controlled trials on vitamin D supplementation, with particular regard to food allergy, to demonstrate that vitamin D might actually contribute to the prevention of allergic diseases.

AUTHOR CONTRIBUTIONS

GR, AG, and LB: conceptualization. AG, LB, and JC: resources. GR, AG, and RM: methodology and writing—review and editing. AG, LB, JC, MG, RM, and GR: writing—original draft preparation. RM and GR: supervision. All authors contributed to the article and approved the submitted version.

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REVIEW
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Nutrition and Avoidance Diets in Children With Food Allergy

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Food allergy (FA) is a significant health issue which considerably influences the quality of life of both children and their family. The increasing prevalence of FA, documented in the last 3 decades, has led to the reassessment of FA prevention strategies and particularly to giving up the approach based on delaying the introduction of potential food allergens. Several observational and interventional studies demonstrated a potential effectiveness of the early food introduction strategy in FA prevention, although strong evidence from randomized controlled trials are lacking and, sometimes, contrasting. The current approach to FA is mainly based on avoidance diet and the use of rescue medications in case of allergic reaction, although active allergen immunotherapy has recently become an increasingly important therapeutic strategy to approach IgE-mediated FA, potentially able to induce improvement through desensitization to a specific food. This review provides an overview on the historical evolution of recommendations about FA and on evidence published in the last 15 years on nutritional intervention strategy, i.e., early introduction of allergen or avoidance diet, in the prevention and management of IgE-mediated and non-IgE-mediated FA in children.

Keywords: early-introduction, elimination diet, food allergen, non-IgE-mediated food allergy, tolerance, immunotherapy

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INTRODUCTION

Food allergy (FA) is a significant health issue with an increasing prevalence in the last 30 years, affecting up to 6–8% of children worldwide (1–4) and up to 10% in high-income countries (5). FA presents a very heterogeneous clinical spectrum, which varies from mild and self-limited reactions to severe anaphylaxis, and it is often encumbered by a significant reduction in the quality of life (QoL) of both patients and their family (6, 7). Therefore, the development and improvement of FA treatment has become a public health priority, mainly in cases of potentially life-threatening reactions (8).

FA can be categorized in (a) IgE-mediated allergic reactions, characterized by an acute onset of symptoms, usually within a few minutes or a few hours after exposure to food antigen; (b) non-IgE mediated reactions, where there is a delayed onset of symptoms, mainly gastrointestinal ones; and (c) mixed IgE and non-IgE mediated food allergy.

The natural history of FA is usually characterized by presentation in the early stages of allergic march along with atopic dermatitis, sometimes associated to more severe allergic reactions, and by spontaneous tolerance for food allergens such as cow's milk (CM) and hen's egg (HE) within early school-age years. However, in some cases, FA persists over time with a negative impact on the QoL of patients and their family (9). The current approach to FA is mainly based on avoidance diet

and the use of rescue medications in the case of an allergic reaction. Alternatively, active allergen immunotherapy (AIT), the only available treatment able to potentially induce a resolution of FA, could be performed in selected patients followed up in highly specialized centers (8).

The increased prevalence of FA has led to the reassessment of prevention strategies and, in particular, to give up the approach based on delaying the introduction of potential food allergens in infants at high risk of atopy, which results in unsuccessful FA prevention and potentially negatively affects FA natural history. Therefore, in the last decade, several studies have evaluated an early food introduction approach for the prevention of FA, demonstrating the potential effectiveness of this strategy in FA prevention. This review focuses on the most recent studies, published in the last 15 years, that investigated nutrition intervention strategy, i.e., early introduction of allergen or avoidance diet, in the prevention and management of IgE-mediated and non-IgE-mediated FA in children. The possible role of vitamin D, pro- or prebiotics, or short-chain fatty acids as nutritional strategies against FA will not be discussed in this review. Research was carried out through MEDLINE via PubMed (http://www.ncbi.nlm.nih.gov/pubmed/), Embase, CINAHL, and Cochrane Library, based on the combinations of two or more of the following keywords: (Food allergy) AND (nutrition OR diet OR prevention OR avoidance diet OR early introduction) AND (milk OR egg OR peanut OR fish OR cereal OR tree nut) AND (tolerance OR immunotherapy) AND (IgE-mediated OR non-IgE-mediated) AND (children OR pediatrics). Research included articles written in English belonging to the categories of clinical trial, observational study, meta-analysis, multicenter study, randomized controlled trial, or review.

HISTORICAL OVERVIEW

Over the last 40 years, the prevention and treatment of FA has been extensively investigated due to its high social and healthcare cost (10).

Between the late 1900's and early 2000's, several recommendations indicated the delay of dietary allergen introductions in infants, based on the hypothesis that exposure to solid foods in early infancy could increase the risk for allergic sensitization. In 1974, the *Present-Day Practice in Infant Feeding* guidelines (11) discouraged both the early introduction of cereals and other solid food into babies' diet before 4 months of age. This recommendation was based on the assumption that an early and increased incidence of celiac disease in Britain, between the 1960's and early 1970's, had been related to precocious introduction of gluten. This assumption was supported by a subsequent decline of incidence, between 1974 and 1979, that had been observed after the changes in infant feeding practices (12). By the late 1990's, the World Health Organization (WHO) recommended a further delay of first exposure to solid foods to 6 months of age, and to delay eggs and peanut introduction to 10 months and 3 years of age, respectively (13). In the early 2000's, similar indications were proposed by American guidelines that recommended the introduction of dairy products and egg at 12 and 24 months of age, respectively, and peanut, tree nut, fish, and seafood at 36 months of age, in particular in infants with a family history of atopy in first-degree relatives (14, 15).

These recommendations, based on insufficient evidence, did not determine a reduction in FA incidence, as expected, but, on the contrary, probably promoted a further increase of FA prevalence in association with genetic, epigenetic, and environmental factors. The most relevant theories trying to explain the role played by environmental factors are: the *hygiene hypothesis*, sustaining the implication of early-life microbial exposure to antigens in the regulation of immune response and in the prevention of allergic diseases; the *vitamin D hypothesis*, supporting the action of vitamin D deficiency in furthering the development of FA; and the *dual-barrier hypothesis,* suggesting the potential role of transcutaneous early exposure to food allergens in FA pathogenesis (16).

Consistent with the dual-barrier hypothesis, several studies demonstrated the close relationship between disrupted skin barrier and FA in infants. Transcutaneous sensitization to peanut protein in children with inflamed skin through topical peanut-oil emollient application (17), as well as other environmental exposure, have been associated with higher risk of peanut allergy (18, 19). Kelleher et al. documented that neonatal skin barrier alterations predicted FA at 2 years of age (20). These results were supported by studies on murine models and filaggrin mutations. Noti et al. demonstrated in murine models that transcutaneous exposure to ovalbumin or peanut through an atopic dermatitis-like skin lesion promoted immunological mechanisms related to FA development, specifically by an increase of Th2 cytokine response, antigen-specific serum IgE levels, and localization of mast cells in the intestine (21). Strid et al. concluded with similar results, highlighting that epicutaneous exposure to peanut protein selectively promoted Th2 immune response and prevented the induction of oral tolerance in murine models (22). Patients with filaggrin loss-of-function mutations were more likely to develop sensitization to food allergens and FA (23, 24).

Moreover, maternal solid allergen-free diet during pregnancy and lactation in the prevention of FA in infants has been confirmed ineffective (18, 25–28), and on the contrary, it seems to be able to induce sensitization (29).

Evidence and results of other observational studies (30, 31) definitively clarified that early-life avoidance strategy to food allergens does not prevent FA; instead, it could contribute to promote the increasing prevalence of FA in association with genetic, epigenetic, and environmental factors. Accordingly, American guidelines recommended the introduction of

Abbreviations: FA, Food allergy; AIT, active immunotherapy; QoL, quality of life; CM, cow's milk; HE, hen's Egg; WHO, World Health Organization; DBRCT, double-blind randomized controlled trial; EAT, Enquiring About Tolerance; PETIT, Prevention of Egg Allergy with Tiny Amount Intake; STAR, Solid Timing for Allergy Research; HEAP, Hen's Egg Allergy Prevention; STEP, Study Starting Time of Egg Protein; BEAT, Beating Egg Allergy Trial; PA, peanut allergy; LEAP, *Learning Early About Peanut Allergy*; OFC, oral food challenge; FPIES, food protein induced enterocolitis syndrome; FPIAP, food protein–induced allergic proctocolitis; and FPE, food protein–induced enteropathy; FTT, failure to thrive; SPT, skin prick test; OIT, oral immunotherapy; SLIT, sublingual immunotherapy; SCIT, subcutaneous immunotherapy; EPIT, epicutaneous immunotherapy; CTs, controlled trials.

complementary foods between 4 and 6 months of age (32, 33). However, guidelines specifying the timing of introduction of potential allergenic solid foods are not available (34), except for peanut (35).

CURRENT STATUS FOR IGE-MEDIATED FOOD ALLERGY

An increasing number of observational and interventional studies have been carried out to assess the timing of first exposure to allergens and to evaluate the role of the early introduction of food allergens in the prevention of FA. Even though observational studies support that early allergen ingestion can be effective in FA prevention, strong evidence from randomized controlled trials (RCT) are lacking.

Milk

Observational studies suggested that avoidance diet and the delayed introduction of CM proteins did not prevent CM allergy, and on the contrary, early introduction could promote tolerance. A large prospective birth cohort study evaluated the association between age of CM and other foods' first introduction and infant atopic manifestations and sensitization (specific IgE levels) in the first 2 years of life (36). Authors demonstrated that the delayed introduction of CM proteins and other solid foods was associated with an increased risk for atopic manifestations, such as eczema and recurrent wheeze (36). In another large-scale population-based prospective study, a significantly lower frequency of IgE-mediated CM allergy was documented in infants precociously exposed to CM proteins (within 14 days of life) compared to delayed introduction (between 105 and 194 days of life), allowing authors to conclude that early exposure to CM proteins, in association to breastfeeding, might promote tolerance (37). Consistently, in a double-blind, randomized controlled trial (DBRCT) involving children with a family history of allergic diseases, partially hydrolyzed whey-dominant formula supplemented with a specific oligosaccharide mixture was ineffective to prevent eczema at 12 months of life compared to the standard CM formula (38).

The *Enquiring About Tolerance* (EAT) trial is the only available interventional study evaluating the early introduction of CM proteins (39). In this randomized controlled trial (RCT), CM proteins (yogurt) were the first administered allergen to 3-month-old breast-fed infants, followed by other six allergens (cooked HE, peanut, white-fish, sesame, wheat) (39). Even though the low rate of per-protocol adherence to allergen assumption in the early-introduction group influenced study result interpretation as a whole, the adherence rate for CM was acceptable (85.2%). In the per-protocol analysis, CM allergy rate was not significantly different between groups, although it was lower in the early-introduction group (3–6 months of age) than in the standard-introduction group (after 6 months of age). Similarly, in the intention-to-treat analysis, at 3 years of age, CM allergy rate as well as allergy rate to other allergens was not significantly lower in the early-introduction group compared to the standard-introduction group (39).

Egg

Available data about the efficacy of HE early introduction to prevent sensitization and allergy are contrasting (34). A large cross-sectional study documented a higher risk to develop HE allergy in children who underwent delayed introduction of cooked egg (≥10 months of age) than those who received HE at 4–6 months of age (40). In accordance with this result, in the per-protocol analysis of EAT trial a significantly lower prevalence of HE allergy was documented in early-introduction group (rate of adherence 43.1%) than in controls, and the consumption of at least 4 grams per week of egg protein was associated with a significantly lower prevalence of egg allergy than less consumption (39) (**Table 1**).

The results of *Prevention of Egg Allergy with Tiny Amount Intake* (PETIT) DBRCT also supported an early introduction of allergen for HE allergy prevention, although findings should be interpreted taking into consideration the considerable drop-out (41). More specifically, a cohort of 4–5-month-old infants with eczema, never orally exposed to HE before, were enrolled to undergo a stepwise introduction of egg protein (in the form of heated egg powder) or placebo from 6 months of age (41) (**Table 1**). At 12 months, a significant lower prevalence of HE allergy in the active group compared to the placebo control group has been documented. Moreover, authors reported a significantly lower level of ovomucoid-specific IgE and a higher concentration of ovomucoid-specific IgG1, IgG4, and IgA in the active group compared to placebo group (41).

Conversely, the *Solid Timing for Allergy Research* (STAR), the *Hen's Egg Allergy Prevention* (HEAP), the *Study Starting Time of Egg Protein* (STEP), and the *Beating Egg Allergy Trial* (BEAT) trials did not demonstrate that early consumption of HE was able to prevent HE sensitization and allergy (42–45). The STAR DBRCT evaluated the IgE-mediated HE allergy rate in infants with moderate-to-severe eczema daily receiving a teaspoon of pasteurized raw whole egg powder (active group) or placebo (control group) from 4 to 8 months of age (42) (**Table 1**). At 12 months of age, egg allergy incidence was not significantly different between groups; however, 31% of active group patients stopped egg powder ingestion due to allergic reactions (42).

The HEAP RCT included no-sensitized to HE infants who were randomized to receive pasteurized egg white powder (cases) or placebo (controls) from age 4–6 months until 12 months (43) (**Table 1**). At 12 months of age, sensitized or allergic subjects were not significantly different between cases and controls (43) (**Table 1**).

The STEP RCT included infants with a family history for allergy and without eczema, who were randomized to receive pasteurized raw whole egg or placebo from age 4 to 6.5 until 10 months (44) (**Table 1**). At 12 months of age, there was no significant difference between groups in the percentage of infants with IgE-mediated egg allergy (44) (**Table 1**).

Finally, the BEAT DBRCT included no-sensitized to HE infants with a family history of allergy who were randomized to receive whole-egg powder or placebo from age 4 to 6 until 8 months (45) (**Table 1**). At 12 months of age, there were no differences in egg allergy prevalence between groups. However, authors highlighted that early exposure to whole egg reduced

TABLE 1 | Overview of RCTs evaluated with early introduction of hen's egg.

Trial	Study design	Primary outcome	Active group selection criteria	Active group/controls (n) included in the primary analysis	Allergen formulation (vehicle)	Intervention (control)	Limitations
EAT (39)	RCT	Prevalence of challenge-proven FA to HE or to other 5 foods in early-introduction group between 1 year and 3 years of age	3-month-old exclusively breast-fed infants	652/651	Whole hard-boiled egg (not specified)	Early-introduction group (3–6 months of age): 4 g of egg protein/week (equivalent to 2 g of egg-white protein) (Standard-introduction group: introduced the same egg proteins amount after 6 month of age)	Low rate of per-protocol adherence in the early-introduction group.
PETIT (41)	DBRCT	Prevalence of HE allergy confirmed by OFC at 12 months of age	4–5-month-old infants with AD, never orally exposed before to HE	60/61	Heated egg powder (squash, Japanese pumpkins)	Stepwise introduction of allergen From 6 to 9 months of age: 25 mg of egg proteins/daily; From 9 to 12 months of age: 125 mg of egg proteins/daily (Placebo from 6 to 12 months of age)	The study was early stopped because of a large group difference at the planned interim analysis.
STAR (42)	DBRCT	Diagnosis of IgE-mediated HE allergy by SPT and OFC at 12 months of age	4-month-old infants with moderate-to-severe AD, never orally exposed before to HE	42/35	Pasteurized raw whole egg powder (infant rice cereal)	From 4 to 8 months of age: 0.9 g of egg protein/daily From 8 months of age: medically supervised cooked egg exposure (Placebo from 4 to 8 months of age)	- Recruitment was early stopped for logistic reason, without reaching the sample size originally estimated. - 31% of active group patients stopped egg powder ingestion due to allergic reactions.
HEAP (43)	RCT	Defined HE sensitization by sIgE at 12 months of age	4–6-month-old infants from general population with HE sIgE levels < 0.35 kUA/L	156/142	Pasteurized egg white powder (solid baby food)	From recruitment to 12 months of age, 3 times/week: - 0.8 g in the first week - 1.6 g in the second week - 2.5 g from the third week of intervention to 12 months of age. (Placebo from recruitment to 12 months of age)	- Recruitment was early stopped for allergic reaction in active group at first exposure to allergen.
STEP (44)	RCT	Diagnosis of IgE-mediated HE allergy and sensitization by OFC and SPT, respectively, at age 12 months	4–6.5-month-old infants with atopic mothers and without history of allergic disease or previous egg ingestion	407/413	Pasteurized raw whole egg powder (carrot, pineapple, and rice powders)	From recruitment to 10 months of age: 0.4 g egg protein/daily From 10 months of age: cooked egg and egg-containing foods were included in diet of both groups. (Placebo from recruitment to 10 months of age)	- Inability to reach the planned sample size. - Relatively small amount of dietary egg.
BEAT (45)	DBRCT	Prevalence of HE sensitization confirmed by SPT at 12 months of age	4–6-month-old infants no-sensitized to HE with a family history for allergy (at least one first-degree relative with allergic diseases)	165/154	Pasteurized whole egg powder (not specified)	From recruitment to 8 months of age: 350 mg egg protein/daily From 8 months of age: liberalized diet in active group and controls. (Placebo from recruitment to 8 months of age)	- Relatively small amount of dietary egg. - Impossibility to challenge all infants with possible egg allergy at 12 months of age.

FA, Food allergy; HE, hen's egg; DBRCT, double-blind randomized controlled trial; RCT, randomized controlled trial; sIgE, specific IgE; EAT, Enquiring About Tolerance; PETIT, Prevention of Egg Allergy with Tiny Amount Intake; STAR, Solid Timing for Allergy Research; HEAP, Hen's Egg Allergy Prevention; STEP, Study Starting Time of Egg Protein; BEAT, Beating Egg Allergy Trial; OFC, oral food challenge; AD, atopic dermatitis; SPT, skin prick test.

sensitization to egg and induced egg-specific IgG4 production in high-risk infants (45).

Peanut

Peanut allergy (PA), although lower in prevalence compared to CM and HE allergy, is burdened by a higher prevalence of severe allergic reactions and persistency over time. In infants affected by PA, a 10-fold higher environmental exposure to peanut was reported during the first year of life compared to atopic infants without PA (46). Available trials produced good evidence on a probable role of early peanut introduction in reducing the risk of PA in high-risk infants.

In the per-protocol analysis of the EAT trial, PA prevalence was significantly lower in the early-introduction group than in the standard-introduction group and the consumption of at least 2 g per week of peanut protein was associated with a significantly lower prevalence of PA than less consumption (39). These results should be interpreted in the light of a rate of adherence for peanut assumption of 61.9% (39).

The *Learning Early About Peanut Allergy* (LEAP) RCT evaluated which approach between early peanut introduction and avoidance diet was the most efficacious in PA prevention in infants at high risk for allergy (47). Specifically, infants with severe eczema and/or egg allergy were randomized in an avoidance group and an active group, consuming at least 6 g of peanut protein a week through peanut products for at least 3 time a week, from age 4–11 months (median 7.8 months, interquartile range 6.3–9.1) until 60 months (47). In the intention-to-treat evaluation, PA prevalence at 60 months of age, documented by an oral food challenge (OFC), was significantly lower in the active group compared to the avoidance group, independently from the sensitization or not of active group infants at baseline (on the basis of a skin prick test and specific IgE levels). Moreover, children belonging to the avoidance group had a significantly higher levels of peanut-specific IgE and lower levels of peanut-specific IgG4 than active group children (47). Results of this study pointed out important considerations concerning the continuous adaptation and evolution of immune system response. In particular, the efficacy of peanut introduction at a median age of 7.8 months in preventing PA suggested that the immunotolerance window is not likely limited between 4 and 6 months of age. Moreover, the higher significant difference of PA incidence at 5 years of age between the active group and the placebo group (3 vs. 17%) suggested the possibility of a progressive adaptation of the immune system, differently by studies evaluating CM and HE prevalence at 12 months of age. These design peculiarities could justify the better outcome reported in LEAP RCT compared to those of the CTs evaluating CM and HE early introduction.

In the LEAP-On study, 88.5% of participants in both groups from the LEAP study were instructed to avoid peanut consumption for 12 months (48). Authors demonstrated a significantly lower PA prevalence in children who had been in the active group during the LEAP study compared to children who had been assigned to the avoidance group (48), suggesting the beneficial role of peanut early introduction to achieve sustained tolerance despite the 12-month avoidance in the peanut consumption group children.

These findings significantly stimulated the development of Addendum Guidelines to specifically address the prevention of peanut allergy (35).

Cereals and Fish

An observational study, carried out in infants from birth to a mean age of 4.7 years, prospectively evaluated the association between the timing of first exposure to cereals (oats, wheat, barley, or rye) and the development of wheat allergy based on parent report (49). At the end of follow-up, 1% of children were defined as having a wheat allergy. The probability to report a wheat allergy was increased 4-fold in children who first introduced cereals after 6 months of age compared to the ones who consumed cereals before 6 months of age, after controlling for family history of allergic diseases and history of food allergy before 6 months of age (49). Moreover, having a history of other food allergy before 6 months of age or a family history of allergic diseases (asthma, eczema, or hives) in a first-degree relative was independently associated with an increased risk of developing wheat allergy (49). Authors concluded that delayed first exposure to cereals after 6 months of age has not demonstrated a protective role in wheat allergy prevention but, on the contrary, could increase the risk of wheat allergy development (49). In another large observational Finnish birth cohort, introduction of wheat, rye, oats, or barley at 5–5.5 months, as well as introduction of fish at 6–9 months, has been related to a likely reduced risk of having asthma, allergic rhinitis, and atopic sensitization at 5 years of age, documented by a validated questionnaire (50).

With regard to fish allergy, an observational study, prospectively following infants from birth to 4 years of age, documented that regular fish consumption before 12 months of age was associated with a reduced risk of allergic disease and sensitization at 4 years of age (51). Consistently, results from another meta-analysis, aiming to specifically clarify the role of fish intake on different asthma outcomes in children, demonstrated that early introduction (between 6 and 9 months of age) and regular consumption of fish (at least once a week) decreased the risk, prevalence, and symptoms of asthma in children up to 14 years of age (52). Conversely, a recent meta-analysis reported both low-certainty evidence of association between fish introduction before age 6–12 months and reduction of allergic rhinitis, and very low-certainty evidence between fish introduction before age 6–9 months and reduction of allergic sensitization (53).

These findings should be interpreted considering the limitation due to the observational design of the studies that cannot demonstrate a specific cause–effect linkage between the timing of first ingestion of allergen and allergic symptoms.

EAT RCT also evaluated the effect of early introduction (before 6 months of age) of white-fish and wheat in FA prevention (39). In the per-protocol analysis, fish allergy rate was not significantly higher in the early-introduction group compared to the standard-introduction group, while there were no cases of wheat allergy in either group (39).

CURRENT STATUS FOR NON-IgE-MEDIATED FOOD ALLERGY

Recently, an increasing interest in diagnosis and management of non-IgE mediated FA has been developed. Typically, non-IgE-mediated FA has a delayed onset of allergic symptoms after culprit food ingestion. Non-IgE-mediated FAs include a wide range of disorders principally characterized by gastrointestinal symptoms. Management of non-IgE-mediated FAs is based on avoidance of the suspected trigger food and support to prevent nutritional deficiencies (**Figure 1**).

We focused on the nutritional and avoidance approach in food protein-induced enterocolitis syndrome (FPIES), food protein-induced allergic proctocolitis (FPIAP) and food protein-induced enteropathy (FPE), while eosinophilic esophagitis will not be discussed in this review.

CM and soy are the main allergens involved in non-IgE-mediated FAs, although they could be induced by rice, oats, and other foods (barley, chicken, turkey, egg, peanut, vegetables, fish, and mollusks) in relation to genetic, epigenetic, and environmental (e.g., age of introduction of the specific food into the diet) factors.

FPIES

In the last decade, the clinical pattern, diagnosis, management, and natural history of FPIES have been extensively investigated (54). Clinically, in the majority of patients, FPIES is characterized by acute symptom onset, including repetitive protracted vomiting, ∼1–4 h after trigger food ingestion, usually associated with pallor and lethargy and sometimes with watery diarrhea within 5 up to 24 h. Severe cases progress to hypothermia, methemoglobinemia, acidemia, dehydration, hypotension, and shock. Rarely, FPIES has a chronic trend associated with failure to thrive (FTT). The diagnosis is primarily based on the typical clinical picture and symptom resolution by avoidance diet. OFC, performed in a specialized setting, could be necessary in those cases with unclear clinical history (54). The first-line approach in FPIES treatment is a strict avoidance diet to offending trigger foods; however, long-term management should be tailored for every patient. Dietary management of FPIES follows empirical recommendation based on trigger food, possible cross-reactions with other food, and nutritional needs (55, 56). Breastfeeding should be recommended and maternal dietary elimination of trigger foods should not be routinely advised except for those cases in which allergic reaction occurs after breastfeeding, and it is associated to FTT in exclusively breast-fed infants (54, 57). In non-breast-fed infants with CM/soy-induced FPIES, an extensively hydrolyzed formula or amino acid-based formula (required in about 20% of patients) has been indicated (58, 59). Soy formula is not recommended before 6 months of age in CM-induced FPIES infants, whereas it may be thereafter considered in weaned infants, *vice versa* in soy-induce FPIES infants, even though a possible cross-reactivity between allergens has to be always considered (57, 58). In CM-induced FPIES infants, other animal milks (e.g., sheep and goat milk) should be avoided because of possible cross-reactions and insufficient nutritional value. Culprit food should be avoided also in baked and processed forms, unless baked products are already included in the diet, based on the assumption that high temperature does not destroy sequential allergenic epitopes recognized by T-lymphocytes (56). However, conclusive data evaluating baked food tolerance in FPIES are not available (55, 58). Due to the latency of symptoms onset and to possible reduction of required trigger dose in repeated episodes, it is difficult to establish a threshold dose able to provoke allergic reaction. This could partially justify the difficulty to plan an oral immunotherapy approach of FPIES (55).

Up to 80% of FPIES children have a single food allergy; therefore, delayed introduction of complementary food over 6 months of age is not recommended because of FPIES (10). Clinicians play a pivotal role to promote implementation of normal dietary variety, to prevent unnecessary avoidance, and to regularly monitor children growth.

The natural history of FPIES varies principally according to food trigger, feeding habits, and coexistence of IgE-mediated allergy; therefore, the timing for OFC to evaluate achieved tolerance varies accordingly. Conventionally, OFC is advised within 12–18 months after the most recent allergic reaction (56). Most FPIES patients achieve tolerance spontaneously within 5 years of age. CM tolerance has been reported in up to 85% of children by 3 years of age (57), whereas the average reported ages of tolerance are 12, 35, and 42 months for soy, grains and other solid foods, respectively (55, 58). However, these data do not derive from studies specifically designed to evaluate tolerance achievement; therefore, they may be biased by other factors.

FPIAP

FPIAP is one of the most frequent causes of rectal bleeding in healthy infants, which histologically appears as an eosinophilic colitis (60–62). In healthy formula-fed infants with bloody-streaked stools, FPIAP is estimated to occur in up to 60%, while it is reported in up to 10% of extensively hydrolyzed-fed infants, although prevalence is not exactly determined (61, 63). FPIAP is a benign and self-limiting disease, associated to an excellent prognosis that starts in the first 2 weeks to 6 months of life and spontaneously resolves within 12 months of life in the majority of patients (64). FTT is not a peculiarity of FPIAP, while diarrhea, vomiting, abdominal pain, and anorexia could be present. CM is the most commonly involved trigger food. FPIAP onset is usually insidious, and it could depend on the timing of introduction of allergens in diet, even though up to 60% develop during exclusive breastfeeding. Culprit food elimination from child and mother diet usually determines symptom improvement in infants within 3 days (62). However, up to 20% of breastfed infants have a spontaneous resolution of bloody stools without the mother's avoidance diet; therefore, avoidance diet is not univocally advised in FPAIP management (65). In formula-fed infants, an extensive hydrolysate formula may be necessary (65). Jang et al. reported a failure of elimination diets in determining rectal bleeding resolution in 20% of infants of their cohort with histologic findings consistent with FPIAP (66). Arvola et al. assessed the effect of a CM-elimination diet on rectal bleeding duration in 40 infants, prevalently breast-fed (68%), with age between 4 weeks and 6 months (67). They randomized infants

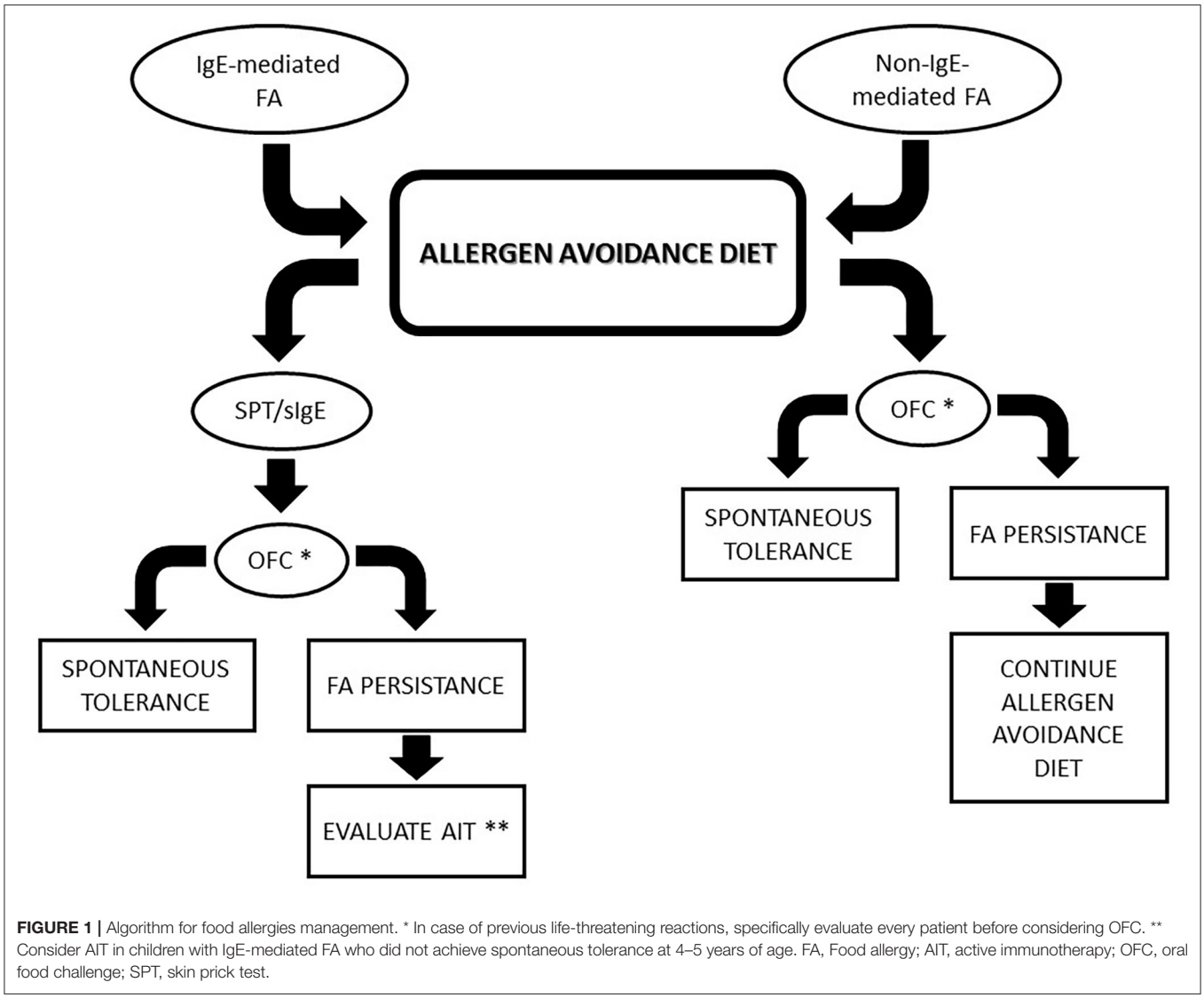

FIGURE 1 | Algorithm for food allergies management. * In case of previous life-threatening reactions, specifically evaluate every patient before considering OFC. ** Consider AIT in children with IgE-mediated FA who did not achieve spontaneous tolerance at 4–5 years of age. FA, Food allergy; AIT, active immunotherapy; OFC, oral food challenge; SPT, skin prick test.

to the CM-elimination diet (amino acid-derived formula or CM-elimination diet in lactating mothers) or non-elimination diet for 1 month. CM IgE-mediated allergy was documented in 18% of infants by an OFC. Authors demonstrated that a CM-elimination diet did not significantly affect the duration or severity of rectal bleeding during follow-up; however, elimination diet seemed to shorten the duration of rectal bleeding in infants with CM IgE-mediated allergy (67). Therefore, after a limited period of CM-free diet, they suggested to perform a CM challenge, preceded by skin prick test (SPT) to CM proteins, to confirm diagnosis in those infants who had symptom resolution during the avoidance diet (67).

In a prospective population-based study, Elizur et al. evaluated the outcome of CM-free diet in 21 infants with rectal bleeding (19% exclusively breast-fed) (61). CM-free diet was carried out for a mean of 3 months, followed by the recovery of diet consumed before the initiation of rectal bleeding. All but one infant experienced a resolution of symptoms during CM-free diet. Also these authors highlighted that CM protein reintroduction, following symptom resolution, is often well-tolerated and is recommended to confirm the diagnosis and to avoid a prolonged unnecessary elimination diet (61). Moreover, this approach may be reinforced by evidence of negative effect of elimination diet on the possible switching toward CM IgE-mediated allergy (37).

Miceli Sopo et al. proposed a specific approach for infants with suspected FPIAP based on available evidence. In particular, they suggested waiting for a spontaneous resolution without an elimination diet in case of rectal bleeding $\leq$1 month, or starting an CM elimination diet in case of rectal bleeding >1 month, followed by CM challenge if rectal bleeding disappears (65). If hematochezia returns, these authors suggested restarting the elimination diet for 3 months further and to perform specific SPT before the next CM challenge (65).

FPE

FPE starts in the first year of life, a few weeks after the introduction of an allergen, and it resolves within 2 years of

age in the majority of patients. Protracted diarrhea is the typical onset symptom and could be associated with abdominal pain and distension, early satiety, emesis, malabsorption with steatorrhea, and FTT (62, 68). The clinical picture of FPE could resemble a post-enteritis syndrome; however, FPE could effectively develop after an infectious gastroenteritis (62). Histologically, FPE is characterized by lymph nodular hyperplasia in the duodenal bulb and villous structure alterations (68). CM is the most common trigger, but soy, rice, poultry, fish, and shellfish have also been reported as triggers. FPE has not been reported in exclusively breastfed infants (64).

Data on FPE nutritional management are lacking. Avoidance of culprit food determines the resolution of symptoms in 1–3 weeks, and the association of extensively hydrolyzed formula has been suggested (64). In cases with malabsorption and FTT, parenteral nutrition may be necessary (68).

LOOKING AT TOLERANCE

Tolerance is considered the achievement of a goal to safely consume a normal serving of food containing the trigger allergen, previously counted harmful, despite a period of absence of exposure (8). Tolerance could be spontaneously achieved as in the majority of children affected by CM, HE, wheat, and soy protein IgE-mediated allergy. On the other hand, patients allergic to peanut, tree nut, and fish have a natural history of allergy that is quite disappointing with persistence of symptoms. Persistence of allergy could negatively affect the QoL of children and their family. Therefore, patients with FA should be regularly followed up to avoid an inappropriate or unnecessarily prolonged elimination diet that possibly conditions social life, dietary nutritional intakes, and growth. In this perspective, a specific management for every patient should be programmed. Specific IgE testing (*in vitro* or SPT) could be the first step to assess allergen sensitization decrease and to address subsequent steps, since decreasing specific IgE testing response over time seems to be related with clinical tolerance; however, specific IgE testing has limited value in guiding the timing of OFC.

OFC is able to demonstrate an achievement of tolerance and it should be performed at regular intervals, based on clinical patient history, trigger allergen, SPT, and/or specific IgE results. Currently, re-testing OFC has been suggested every 6–12 months from the last allergic reaction in patients with CM or HE allergy and every 2 years in cases of peanut and tree nut allergy in the absence of reaction due to accidental ingestion of trigger food (10).

On the way toward tolerance, it has been demonstrated that extensively heated allergens are tolerated earlier than raw allergens in IgE-mediated HE allergy and, to some extent, CM allergy. In these patients, introduction of baked products containing CM and HE proteins is supported by current evidence because it is safe, convenient, and well-accepted by patients, and it seems to accelerate tolerance achievement of raw allergens (69).

In recent years, AIT became an increasingly important therapeutic strategy to approach FA, potentially able to induce improvement through desensitization to a specific food (70). AIT is indicated in patients with confirmed diagnosis of IgE-mediated FA in whom spontaneous tolerance did not develop, and an avoidance approach is ineffective or causes severe limitations to a patient's QoL (8) (**Figure 1**). Currently, it is recommended to wait for the acquisition of tolerance until about 4–5 years of age before considering AIT, although a clinical picture of every patient will be specifically evaluated.

AIT is able to increase the threshold of trigger food intake amount, reducing allergic symptoms and the incidence of life-threatening reactions, and, in selected patients, to attain post-desensitization effectiveness (8). In light of available evidence, desensitization is a more feasible objective. In that condition, allergic symptoms may occur, with the same characteristics or attenuated, when administration of AIT is interrupted.

AIT is burdened by side effects, including systemic and potentially life-threatening reactions. In addition, it is usually a long-term treatment, carried out at a specialized center. Therefore, patients who underwent AIT should be carefully selected.

The most common approaches for AIT is the oral immunotherapy (OIT), consisting in oral administration and prompt ingestion of food allergen. The use of sublingual immunotherapy (SLIT), subcutaneous immunotherapy (SCIT), and epicutaneous immunotherapy (EPIT) is less common.

A meta-analysis of RCT and non-randomized controlled trials (CTs) evaluating different allergens documented that both OIT and SLIT are efficacious in terms of desensitization in children. Based on the results of RCTs, this meta-analysis suggested, but did not confirm, post-desensitization effectiveness (or sustained unresponsiveness) in children treated with OIT (71).

More recently, in a phase 3 trial, carried out in subjects who were highly allergic to peanut, OIT with AR101 (a peanut-derived investigational biologic oral immunotherapy drug) resulted in desensitization in children and adolescents (72). In particular, a significantly higher percentage of active group patients were able to ingest a single dose of at least 600 mg of peanut protein (cumulative dose $\geq$ 1043 mg) during the exit food challenge, with no dose-limiting symptoms and lower symptom severity, compared to the placebo group, although desensitization was evaluated after only 6 months of maintenance regimen (72).

Interestingly, OIT has been evaluated in subjects affected by tree nut allergy, a condition characterized by a very frequent cross-reaction between allergens (about 86% of children with a tree nut allergy develop sensitization to another tree nut by adolescence) that are rarely outgrown spontaneously, that is burdened by a high risk of life-threatening reactions and often heavily and negatively affects the QoL of patients and their family, for which no CTs on the role of early allergen introduction in tree nut allergy prevention have been conducted. Particularly, in a preliminary, prospective cohort study involving subjects with tree nut co-allergies, OIT to walnut resulted in desensitization to walnut as well as cross-desensitization to other tree nuts, with a reasonable safety profile (73). This promising result supports the possibility to simultaneously induce desensitization to cross-reactive allergens by OIT, but it should be confirmed by RCT in a larger cohort of patients (73).

TABLE 2 | Key points.

		Key points
IgE-mediated FA	Milk	The effectiveness of CM early introduction in allergy prevention has not been demonstrated by the only one interventional study evaluating CM early introduction.
	Egg	Although two interventional studies suggested a positive effect of the early introduction strategy for HE allergy prevention, the other four did not confirm this result.
	Peanut	Two interventional studies suggested a positive effect of peanut early introduction in PA prevention.
	Cereal and fish	Effectiveness of early introduction in fish allergy prevention has not been demonstrated by the only one interventional study evaluating fish early introduction. In the same study, no cases of wheat allergy were reported.
Non-IgE-mediated FA	FPIES	Currently, the first-line approach is the strict avoidance diet of offending foods, followed by periodic re-evaluation of tolerance achievement by OFC, according to allergen and patient clinical history.
	FPIAP	Avoidance diet should not be univocally advised in breastfed infants with FPIAP and their mothers; spontaneous resolution of symptoms could be expected if rectal bleeding has lasted < 1 month.
	FPE	Avoidance of culprit food, sometimes associated to extensively hydrolyzed formula, determines the rapid resolution of symptoms.

FA, Food allergy; CM, cow's milk; HE, hen's egg; PA, peanut allergy; OFC, oral food challenge; FPIES, food protein induced enterocolitis syndrome; FPIAP, food protein–induced allergic proctocolitis; and FPE, food protein–induced enteropathy.

OIT is usually carried out with fresh or natural raw food or, alternatively, with different processed foods in those patients with a history of severe allergic reactions. However, the concentration of an allergen and, consequently, its allergenic potential, changes in relation to food form (raw, cooked, or processed food) and type of processing (homogenization, hydrolysis, irradiation, etc.) in case of processed foods.

Many issues are still unsolved in the management of AIT, including the standardization of food and shared protocol(s) employed in OIT, identification of predictive biomarkers, and long-term effectiveness (permanent tolerance).

CONCLUSIONS

In summary, delayed food introduction as well as assumption of allergenic foods before 4 months of age has been proven ineffective in FA prevention, while early introduction of potential trigger food, between 4 and 6 months of age, has been suggested as a preventive strategy for FA, although reliable evidence is available only for peanut allergy. The current approach to FPIES, FPIAP, and FPE is based on elimination diet and nutritional counseling; however, strong supporting evidence on dietary management are lacking. Key points of FA discussed above are outlined in **Table 2**.

In this context, clinicians play a central role in promoting dietary variety and preventing unnecessary or prolonged avoidance diet in children affected by FA.

As suggested by the results of the most recent CTs, several aspects, including study design, age of allergen introduction, quantity and form of assumed food (e.g., raw or cooked food), have to be considered in outcome interpretation, since they can affect the effectiveness of early introduction strategy for FA prevention. Based on available data, we recommend, both in infants at high-risk for allergies and at normal-risk, to begin the introduction of complementary foods between 4 and 6 months of age, continuing progressively with different foods, in accordance with infant development and familial and cultural customs, carrying on breastfeeding up to 6 months or beyond.

Infants who have been diagnosed with FA and/or are affected by atopic dermatitis with positive SPT to a specific food should undergo OFC under medical supervision in a specialized center to evaluate the achievement of spontaneous tolerance before reintroducing the culprit food into the diet.

In patients with persistent IgE-mediated FA, in which elimination diet represents often a heavy therapeutic option and, accidental exposure could provoke severe adverse reactions, AIT represents an emerging reality potentially able to actively treat FA inducing desensitization.

AUTHOR CONTRIBUTIONS

GP conceived, reviewed, and revised the manuscript. DC drafted and wrote the manuscript. TA and MW were involved in literature search and revised the manuscript. LC and FL prepared the table and the figure. All authors approved the final manuscript as submitted and agreed to be accountable for all aspects of the work.

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REVIEW
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The Role of Diet Diversity and Diet Indices on Allergy Outcomes

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Nutrients in foods are not eaten in isolation and food intake interacts in a complex manner, affecting health and disease outcomes. For this reason, focusing on the whole "pattern" of dietary intake instead of the single nutrients or groups of nutrients when studying diseases outcomes is increasingly appealing and growing. Diet diversity refers to the variety of foods being eaten, and the terms, diversity or variety, are often used interchangeably. When the overall diet is characterized by healthy foods, diet diversity will reflect a diversity/variety of healthy foods eaten over a period of time. The introduction of solid foods in the 1st year of life is considered a measure of increased diet diversity. Consuming a diverse range of foods and food allergens in the first year of life may increase intake of important nutrients and positively affect the gut microbiome structure and function. Intake of omega-3 fatty acids and fibers/prebiotics may be particularly important but more information is required about dose and which individuals are most likely to benefit. Increased diet diversity in the first year of life is also associated with reduced food allergy outcomes. In addition to diet diversity, diet indices are considered measures of overall diet quality and can be used as a simple assessment of dietary intake. The focus of this paper is to review and critically address the current knowledge of the association between diet diversity and diet indices and allergy outcomes. Based on the current evidence, we recommend the introduction of solid foods, including common allergenic solids, during the 1st year of life, according to the infant's neuro-developmental abilities and familial or cultural habits. For infants with severe AD and/or FA, medical assessment may be advisable before introducing common food allergens into the diet. Limited evidence exist about the role of diet indices in pregnancy and allergic disease in the offspring, and the most promising results indicate a reduction in childhood wheeze and/or asthma intake.

Keywords: diet diversity, diet indices, pregnancy, allergy outcomes, microbiome

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INTRODUCTION

Diet diversity is defined as the variety of food being eaten; the term "variety" can be used instead of "diversity" (1). If the diet consists of healthy foods, diet diversity will reflect a diversity/variety of healthy foods eaten over a period of time. Diet diversity may include the number of foods/food groups and the period and the frequency of consumption (2). In this review, we consider the introduction of solid foods in the first year of life as a measure of increased diet diversity. A more

diverse diet in the 1st years of life may increase exposure to food allergens, thereby promoting tolerance development (3–7). Diet diversity may also promote an increased intake of nutrients which can be associated with allergic disease prevention. Finally, diet diversity may play a role in allergy prevention by modifying the gut microbiome. During introduction of solid food in the weaning period, higher diet diversity may increase gut microbiome diversity (8). Data regarding the effect of diet diversity in the 1st year of life and atopic dermatitis, rhinitis, and asthma development are conflicting (2).

In this review, we include studies investigating diet diversity, the development of clinical allergy outcomes, and sensitization to aeroallergens and/or foods. In addition to diet diversity, diet indices are considered measures of overall diet quality and offer a simple assessment of dietary intake. In the few last decades, several indices have been developed and employed (9). We focus on diet indices and subsequent development of allergy, with particular emphasis on the Mediterranean diet. We used a combined search strategy using search terms from three EAACI papers (2, 10, 11).

Aim of the Review

In part 1, we summarize the road map leading to current recommendations on food allergen introduction in the 1st year of life. In part 2, we focus on the effect of diet diversity and diet indices in pregnancy, lactation, and 1st year of life on allergy development.

INTRODUCTION OF SOLID FOODS IN THE FIRST YEAR OF LIFE

History of Introduction of Solid Foods and Food Allergens

Recommendations regarding solid food introduction in early life have changed dramatically in the past two decades. In the early 2000s, the American Academy of Pediatrics (AAP) proposed commencing complementary feeding after first six months and to delay introduction of food allergens until after one year of age in infants considered at high risk for allergy development, such as those having a first-degree relative with a history of allergic diseases. The AAP suggested introduction of milk containing foods after the age 1 year, egg after age two years, and peanuts, tree nuts, and fish after age 3 years (12).

This advice was mainly based on the evidence from two studies; the former (13) showed that early introduction of allergenic foods at 3 months increased risk of atopic disease, and the latter demonstrated a correlation existing between diet diversity before 4 months of life and risk of developing eczema later (14).

In 2006, the American College of Allergy, Asthma, and Immunology (ACAAI) suggested delayed introduction of potential allergenic foods also for children without a risk of atopy/allergic diseases (15). Despite these recommendations, the prevalence of food allergy (FA) continued to increase in Western countries (16). Many observational studies highlighted that postponing the introduction into the diet of foods with an allergenic potential may cause an increased risk of IgE-sensitization and FA (3, 17–22), especially to peanut (3) and egg (19). In support of these studies, the dual allergen hypothesis proposed that early oral food allergen introduction opposed to exposure via the skin might be protective against food allergy (23). This hypothesis is sustained by the fact that skin exposure to food allergens in infants with eczematous skin may favor a Th2 response leading to allergic sensitization (24), whereas oral exposure leads to tolerance.

In 2008, the AAP updated their previous recommendations highlighting that there was not sufficient evidence to postpone introduction into the infants' diet of potential allergenic foods (25). No recommendations were made at that stage about the timing of the introduction of foods. The lack of clear information about food allergen intake at the time was addressed by a number of randomized controlled trials.

Early Introduction of Allergenic Foods and Food Allergy Prevention

Single Allergenic Foods

The Learning Early About Peanut Allergy (LEAP) study by Du Toit et al. (4) demonstrated that introduction of peanut in high-risk atopic infants younger than 1 year old suffering from severe atopic dermatitis and/or egg allergy could reduce the development of peanut allergy. In this study, 640 infants were randomly divided into two groups: some were assigned to consume peanuts, other to avoid peanuts up to 60 months of age. Development of peanut allergy was then tested by an oral food challenge.

The intention to threat analysis showed a significantly lower prevalence of PA in the intervention group than in the control group both in the group with a negative SPT to peanut at the beginning of the study and in children with SPT results of 1–4 mm. Noteworthy, infants (7/640) who had never been fed peanuts previously had positive oral peanut challenge at enrolment both in the case of positive SPTs (6 out of 47, 12.8%) and negative SPTs (1 out of 272, 0.4%).

The authors used the term "early introduction" reflecting introduction between 4 and 11 months, differently from the delayed introduction (after 2 years of age), previously recommended in international guidelines (12), indicating the importance of introducing peanut in the 1st year of life and continuing with regular peanut intake once introduced.

The LEAP-On follow-up study showed that cessation of peanut intake for 1 year, after consumption for 5 years, did not lead to a significant increase of peanut allergy by 6 years of age (26).

The ongoing Preventing Peanut Allergy in Atopic Dermatitis (PEAAD) trial is evaluating if peanut ingestion for one year, in infants and children aged 5–30 months and suffering from AD, may have an effect on the development of peanut allergy (27).

Several randomized controlled trials (RCTs) focused on clarifying whether the earlier introduction (before 6 months of age) of other potential allergenic foods (e.g., egg) into diet may prevent the occurrence of developing allergic sensitization

TABLE 1 | Food allergy prevention via early introduction of allergenic food: list of RCTs.

Trial, Year, Country	Study type	Food type	Inclusion criteria	Type of intervention (no. of subjects)	Outcomes	Results
STAR (30), 2013, Australia	DBPCRCT	Egg (raw white pasteurized)	4 m.o. infants at risk for allergy (moderate-severe eczema—SCORAD ≥15)	Intervention (33): 0.9 g of egg protein per day from 4 to 8 months Control (34): placebo	Primary: prevalence of OFC-diagnosed EA at 1 year of age	A lower but not significant proportion of infants in the egg group (33%) had EA compared with the control group (51%; relative risk, 0.65; 95% CI, 0.38–1.11; $P = 0.11$)
EAT (32), 2014, UK	RCT	Milk (yogurt), cooked egg, wheat, peanut, sesame, fish	3 m.o. infants exclusively breastfed, not at risk for allergy	Intervention (652): from 3 to 6 months sequential introduction of the six foods (milk always first) 4 g proteins/week Control (651): only breastfed from birth to 6 months of age	Primary: prevalence of OFC-diagnosed FA to one of the six foods at 1–3 years of age	In the intention-to-treat analysis, no statistically significant difference between early introduction group and standard introduction group
LEAP (4), 2015, UK	RCT	Peanut (snack/butter)	4–11 m.o. infants at risk for allergy (moderate-severe eczema and/or EA, SPT ≤ 4 mm)	Intervention (319): 6 g peanut protein/week, ≥ 3 times/week, up to 5 years of age Control (321): peanut avoidance	Primary: prevalence OFC-diagnosed PA at 5 years of age	Among SPT-negative infants, prevalence of PA at 60 months of age was 13.7% in the avoidance group and 1.9% in the consumption group ($P < 0.001$) Among SPT-positive infants, prevalence of PA was 35.3% in the avoidance group and 10.6% in the consumption group ($P = 0.004$)
HEAP (28), 2017, Germany	DBPCRCT	Egg (raw white pasteurized)	4–6 m.o. infants not at risk for allergy (egg s-IgE <0.35 kU/L)	Intervention (184): 2.5 g of egg protein at least 3 times per week starting from 4 to 6 months until 12 months of age Control (199): placebo	Primary: egg s-IgE positivity Secondary: prevalence of OFC-diagnosed EA at 1 year of age	5.6% of the children in the egg group were hen's egg sensitized and 2.1% were confirmed to have hen's EA vs. 2.6 and 0.6%, respectively, in the placebo group (For sensitization: RR, 2.20; 95% CI, 0.68–7.14; $P = 0.24$) (For allergy: RR, 3.30; 95% CI, 0.35–31.32; $P = 0.35$)
STEP (31), 2017, Australia	DBPCRCT	Egg (raw whole pasteurized)	4–6 m.o. infants at risk for allergy (maternal atopy history, no eczema), who have never taken egg	Intervention (407): 0.4 g of egg protein per day starting from 4 to 6 months until 10 months of age Control (413): placebo From 10 months, no restriction for egg introduction in both intervention and placebo groups	Primary: prevalence of OFC-diagnosed EA at 1 year of age	No difference in term of IgE-mediated EA (egg 7.0% vs. control 10.3%; adjusted relative risk, 0.75; 95% CI, 0.48–1.17; $P = 0.20$)
BEAT (35), 2017, Australia	DBPCRCT	Egg (raw whole pasteurized)	4 m.o. infants at risk for allergy (≥1 first degree relative with allergy)	Intervention (165): 0.35 g of egg protein per day starting from 4 to 8 months of age Control (154): placebo From 8 months, no restriction for egg introduction in both intervention and placebo groups	Primary: egg white SPT positivity Secondary: prevalence of OFC-diagnosed EA at 1 year of age.	Sensitization to EW at 12 months was 20 and 11% in infants randomized to placebo and egg, respectively (odds ratio, 0.46; 95% CI, 0.22–0.95; $P = 0.03$, χ^2 test)
PETIT (36), 2017, Japan	DBPCRCT	Egg (cooked lyophilized)	4–5 m.o. infants at risk (eczema), who have never taken egg	Intervention (37): 0.025 g of egg protein per day between 6 and 9 months, followed by 0.12 g between 9 and 12 months of age Control (38): placebo	Primary: prevalence of OFC-diagnosed EA at 1 year of age	8% of egg group had an EA compared to 38% of placebo group (RR 0.221 [0.090–0.543]; $p = 0.0001$)

DBPC, Double-blind, placebo-controlled; EA, Egg allergy; EW, Egg White; OFC, Oral Food Challenge; PA, Peanut allergy; RCT, Randomized Clinical Trial; RR, Relative risk.

and FA (4, 26, 28–32). These RCTs are summarized in **Table 1**.

Studies evaluating the effects of early introduction of egg gave contrasting results, probably related to variation in the study design (populations, outcomes) and in the dose and the form of egg used (i.e., cooked vs. raw egg; **Table 1**).

The Solid Timing for Allergy Research (STAR Study) (30) trial reported that early introduction of egg had no protective effect on the development of EA in high risk infants who had never eaten egg. The study was discontinued because of a high rate of allergic reactions to pasteurized raw egg. Since then, two RCTs showed no difference in the development of egg sensitization or EA in high risk infants consuming or avoiding egg.

The Australian Study Starting Time of Egg Protein (STEP) trial (31) considered 820 at risk infants (e.g., infants with atopic mothers) who had never consumed egg and did not show allergic symptoms. Infants were randomly allocated to consume pasteurized raw egg or placebo from 4 to 6 months of life until 10 months. No differences were found at the intention-to-treat (ITT) analysis between the active and placebo groups in terms of OFC-diagnosed EA development (7% active vs. 10.3% control) and in cutaneous sensitization, defined by positive egg SPT (10.8% active vs. 15.1% control, $P = 0.15$) at 12 months.

The Beat Egg Allergy Trial (BEAT) (35) randomized children at high risk (having at least a first-degree relative with atopic disease), not sensitized, to receive pasteurized whole raw egg powder or placebo from 4 to 8 months of age. At 12 months, no difference was observed for the percentages of positive challenge between the two groups (10.5% active vs. 6.2% placebo), despite a lower prevalence of sensitization to egg white in the active group.

In the Hen's Egg Allergy Prevention (HEAP) (28) infants aged 4–6 months without risk factors for allergy development were recruited and randomly assigned to be fed pasteurized raw egg white powder or placebo. At 12 months of age, no difference was observed in the prevalence of EA or egg specific-IgE between the two groups.

The most recent Prevention of Egg allergy with Tiny Amount Intake (PETIT) (36) trial assessed the safety and efficacy of a stepwise introduction of heated egg (equivalent to 0.2 g of whole egg boiled for 15 min) in a sample of infants with mild to severe atopic dermatitis, no immediate allergic reaction to egg, and no history of immediate allergic reaction to egg or to any type of food. All infants were treated with topical treatment for AD during the study.

The results of the study showed a statistically significant decrease of OFC-diagnosed EA at 12 months of age in the study group (8% intervention vs. 38% control, $p < 0.0001$).

However, it should be pointed out that only per protocol analysis was conducted and the primary outcome (OFC-diagnosed EA) was not established in 26 (17%) infants; thus, these findings need to be treated with caution.

A recent meta-analysis of RCTs (39) (**Table 1**) showed with moderate evidence that egg introduction at the age of 4–6 months reduces EA occurrence. However, the conclusions of this meta-analysis mainly relied on the results of the PETIT study (36), in which the stepwise introduction of cooked egg from six months of age seemed to be effective in reducing EA prevalence.

A recent RCT showed that avoiding cow's milk formula supplementation in newborns for the first 3 days of life reduce sensitization to cow's milk and food allergy (40).

Similarly, a Finnish cohort study ($n = 6{,}200$ infants), with a follow-up of 18–34 months, found that exposure to cow's milk (CM) proteins within the first few days of life increased the risk for CM allergy (34). In contrast, a cohort study from Israel reported that the introduction of CM proteins within the first 2 weeks of life was associated to a lower risk of CM allergy development, and introduction between 4 and 6 months of life was associated to a higher risk (40).

Omega-3 fatty acids (present in fatty fish) have anti-inflammatory properties. Observational studies investigated if a relationship exists between the timing of fish introduction and the risk of subsequent asthma and atopic diseases (41–45). Pooling the results of these studies showed that there is limited evidence to support early fish introduction (before 9 months of life) for reducing the risk of allergic sensitization, rhinitis (35), and asthma (46). Nevertheless, fish contains important nutrients and omega-3 fatty acids play an important role in development of the central nervous system (47, 48). We feel reasonable to recommend fish introduction during the second semester of life, timing based on local tradition, weaning approach, and family preferences (49, 50).

In summary, the introduction of peanuts from 4 to 11 months of age in infants at high risk of developing PA may be beneficial to prevent peanut allergy. The same may be true for introduction of heated egg. With regard to early introduction of other allergenic foods (milk, fish, and cereals) most of the available data are from observational studies and do not prove a cause-effect relationship.

Multiple Allergenic Foods

The Enquiring About Tolerance (EAT) is the only intervention trial which aimed to investigate the effects on allergy development of early ingestion of different food allergens (i.e., milk, peanut, egg, wheat, fish, and sesame) in exclusively breastfed infants with unknown risk of allergy status (32).

The per protocol analysis revealed a statistically significant decrease of overall FA (2.4 vs. 7.3%, $p = 0.01$), PA (0 vs. 2.5%, $p = 0.003$; NNT 40) in the early introduction group compared to the control; the ITT analysis did not show a different occurrence of FA to at least one of the six foods at three years follow-up. In the per protocol analysis, the NNT was very high. The findings of this study suggested a possible effect on FA prevention with introduction of foods and food allergens between 3 and 6 months of age. However, non-adherence rate in the intervention group was substantial (68.1%) and may lead to bias in the per-protocol analysis (51). It also indicated the difficulty for parents to introduce so many allergens at such an early age (33). Of note, the lowest adherence rate was reported for the introduction of egg (43.1%).

In many of these studies, infants were sensitized just before introducing the allergenic food/s (4, 24, 26, 27). This suggests that other factors, including genetics, epigenetics, and gut flora, could take part in FA development before weaning (52–56).

TABLE 2 | Timing of the introduction of potential food allergens.

Medical societies and scientific societies	Year	Recommendation
AAP (25)	2008	No evidence that delaying introduction of solid food beyond 4–6 months of age has a significant protective effect on the development of atopic disease regardless of whether infants are fed cow milk protein formula or human milk.
AAAAI (62)	2013	Complementary foods can be introduced between 4 and 6 months of age.
ASCIA (57)	2016	Complementary food to be introduced at around 6 months, but not before 4 months
ESPGHAN (63)	2017	Complementary foods (i.e., solid foods and liquids other than breast milk or infant formula) should not be introduced before 4 months but should not be delayed beyond 6 months. Allergenic foods may be introduced when complementary foods is commenced any time after 4 months.
NIAID (58)	2017	For peanut Severe eczema and/or egg allergy: between 4–6 months Mild to moderate eczema: around 6 months in infants No eczema or any food allergies: according to family and cultural feeding practices
BSACI/BDA (64)	2018	No risk factors for food allergy: introduce solid foods at around 6 months of age but not before 4 months, including peanut, egg, and other foods eaten as part of the family's normal diet. Eczema or existing food allergy: consider introducing solid foods including cooked egg and then peanut from age 4 months, alongside other solids
AAP (61)	2019	There is no evidence that delaying the introduction of allergenic foods, including peanuts, eggs, and fish, beyond 4 to 6 months prevents atopic disease.

AAP, American Academy of Pediatrics; AAAAI, American Academy of Allergy, Asthma and Immunology; ASCIA, Australasian Society of Clinical Immunology and Allergy; BDA, British Dietetic Association; BSACI, British Society for Allergy and Clinical Immunology; ESPGHAN, European Society for Pediatric Gastroenterology, Hepatology, and Nutrition; NIAID, National Institute of Allergy and Infectious Diseases.

Current Recommendations

In response to the Learning Early About Peanut (LEAP) study (4) and a number of RCTs on egg and multiple allergen introductions, guidelines around the world were adapted. The first were the ASCIA (57) guidelines suggesting that peanut, cooked egg, wheat, and dairy foods should be introduced into infants' diet in the 1st year of life. The NIAID (USA) guidelines (2017) (58) suggested different peanut introduction schedules depending on the degree of risk: in infants affected by severe atopic dermatitis and/or EA from 4 to 6 months while around 6 months in infants with mild to moderate eczema and that family and cultural feeding practices should be followed in infants with no eczema or any food allergies. The latest COT report (UK) (59) and BSACI guidance (60) suggest to introduce complementary foods, in the general population, from around 6 months of age, including peanut and egg. The BSACI guidance suggests that in *high risk* infants, parents may wish to start complementary feeding around 4 months of age. Parents may also consider to include egg and peanut, but current guidelines are in disagreement about the feasibility and need for assessment prior to introduction.

The most recent update AAP guidelines (61) state that there is no evidence for postponing food allergen introduction beyond 4–6 months, including allergenic foods.

In summary: Evidence suggests introduction of peanut and egg before 11 months of age in high risk infants, after medical assessment. These foods can be given also as part of the weaning diet in low risk infants, ideally before 1 year. There is no/limited evidence regarding the other food allergens but it does not suggest to delay introduction of these foods unnecessarily (**Table 2**).

DIET DIVERSITY IN PREGNANCY

The role of diet diversity in pregnancy and offspring outcomes has not been studied.

DIET DIVERSITY IN THE 1ST YEAR OF LIFE AND ALLERGY OUTCOMES

The PASTEUR/EFRAIM Study by Carole Roduit et al. (37) is the first study specifically describing the role of diet variety in early life and its effect on food allergy development. This prospective, multicenter study evaluated the association between complementary food introduction in the 1st year of life and allergy sensitization or clinical outcomes up to 6 years of age. In this study, diet variety was defined by investigators in two ways: Definition 1: group of 15 different foods frequently assumed by 80% of the study population in the first 12 months of age. Definition 2: group of the 6 major foods introduced in the first 6 months or first 12 months of life. The risk of sensitization to food allergens at the age of 4.5 or 6 years was higher in the group of children with lower diet diversity. The same study also showed a reduction in reported doctor-diagnosed food allergy up to 6 years of age associated with increased introduction of vegetables/fruits, cereals, bread, meat, cake, and yogurt within the first 6 months or first 12 months of life.

In addition to this study, Venter et al. (38) recently reported an association between increased diet diversity in the first year of life and reduced odds of food allergy over the first decade of life. In particular, they showed that the introduction of each additional food at 6 months of age reduced by 10.8% the odds of developing food allergy over the first 10 years of life. Moreover, for each additional food allergen introduced by 12 months, it reported a

TABLE 3 | Allergy prevention via diet diversity increase: list of studies.

Study/Author, Year, Country	Study type	Sample size	Food diversity assessment	Outcomes	Results
PASTURE (37), 2014, Austria, Finland, France, Germany, Switzerland	Prospective birth cohort	Baseline: 1,133 Analytic: 856	Parent self-administered questionnaire on dietary intake at 2, 12, 18, and 24 months of age and then yearly up to age 6 years	Food allergy (parental report of ever doctor-diagnosed food allergy up to 6 years of life)	Increased DD in the 1st year of life associated with reduced risk of reported doctor-diagnosed food allergy up to 6 years
				Asthma (at least one either doctor-diagnosed asthma or at least 2 doctor-diagnosed episodes of obstructive bronchitis in the last 12 months in the year 4, 5, or 6 questionnaires independent of a diagnosis reported in the first 3 years of life)	Increased DD in first year of life associated with reduction of reported asthma (for each successive food introduced: 26% reduction)
				Allergic rhinitis (reported presence of symptoms or doctor-diagnosed allergic rhinitis in the 6-year questionnaire)	No effects of DD on allergic rhinitis development
				Atopic dermatitis (parental report of doctor-diagnosis up to 4 years and/or positive SCORAD score during medical examination at the age of 1 year)	Increased DD within first year of life associated with reduction of AD development up to 4 years of age (for each successive food introduced in 1st year: 25% reduction)
Finnish Type I Diabetes Prediction and Prevention Study (67), 2014, Finland	Prospective Cohort Study	Baseline: 4,074 Analytic: 3,781	Age-specific dietary questionnaires at ages 3, 4, 6, and 12 months	Atopic dermatitis (parental reports of doctor-diagnosis ever up to 5 years)	No effects of DD at 3 and 4 months of age and AD, development up to 5 years. DD in 1st year of life positively associated with reduction in asthma up to 5 years. DD in first year of life not associated with reduction in AR up to 5 years. Reduced DD at 6 months associated with increased risk of AD development up to 5 years
LISA Study, 2006-2008 (21, 22), Germany	Prospective birth cohort study	2612 up to 2 years, 2073 up to 6 years	Parental interview on infant's diet at 6 and 12 months	Atopic dermatitis (parental reports on doctor-diagnosis and symptoms of AD, questionnaires at birth, 0.5, 1, 1.5, 2, 4, and 6 years)	Increased DD in the first 6 months associated with reduced risk of AD development at 2 years No effects of DD at 4 months and AD risk Excluded infants/children with early symptoms of allergy, increased DD associated with an increased risk of doctor-diagnosis of AD, but no symptoms of AD at 6 years
GINI Study (17), 2007, Germany	Prospective Birth Cohort Study	4,753	Parental interview on infant's diet at 12 months	Parental reports on doctor—diagnosis and symptoms of AD, yearly up to 4 year	No significant association between AD (both doctor-diagnosed or symptomatic) and diversity of solids
GINIplus and LISAplus study (68), 2011, Germany	Prospective Birth Cohort Study	9,088	Parental report at 4 and 6 months	See LISA and GINA study	No association between DD at the age of 4 and 6 months and AD (doctor-diagnosed) in the first 4 y of life High DD before 17 weeks of age may be associated with an increased allergy development Increased DD at 4 months associated with higher risk of symptomatic AD at 2 years, doctor-diagnosed AD at 6 years, but not at 4 years
LISAplus (69), 2017, Germany	Prospective Birth Cohort Study	Baseline: 3,097 Analytic: 2,518	Self-administered parental questionnaires from birth to 2 years of age and at 4 and 6 years	Doctor-diagnosed eczema, asthma, and allergic rhinitis assessed at 1 year of age	Children in the highest quartile who were introduced to all eight food groups during the 1st year of life had lower odds of developing eczema up to age 15 years compared with children in the lowest quartile with a maximum of five food groups

(Continued)

TABLE 3 | Continued

Study/Author, Year, Country	Study type	Sample size	Food diversity assessment	Outcomes	Results
Turati (70), 2017, Italy	Case-control study	451 (+451 controls)	Face to face questionnaire for number of items introduced in the infant's diet at 4 and 5 months	Dermatologist-diagnosed AD between 3 and 24 months	The introduction of a high number of different solid foods at 4 and 5 months of age reduced the risk of AD
Fergusson, 1981-1994 (14, 71–73), New Zeland	Prospective Birth Cohort Study	1,265–1,067	Parent interview and food diary assessed at 4 months	Maternal report, doctor report	Increased DD at 4 months was associated with increased eczema at 2 years, increased risk for AD at 2 years, increased risk for AD up to 3 years, increased risk of recurrent/chronic AD up to 10 years

significant reduction of 33.2% in the likelihood of food allergy over the first 10 years of life. These studies may indicate that diet diversity is associated with increased nutrient intake, including those nutrients which could have a protective role on allergy development (omega-3 fatty acids and non-digestible fibers) (65, 66). Other studies investigated the effect of diet diversity in early life and allergy outcomes are summarized in **Table 3**.

Omega-3 Fatty Acids

Omega-3 long-chain polyunsaturated fatty acids (LCPUFAs) are essential nutrients found in many foods such as fatty/oily fish, fish oil, and nuts (66, 74, 75). Evidence from *in vitro* and *in vivo* studies have shown that omega-3 fatty acids are able to lower pro-inflammatory cytokines and antagonize IgE responses and mast cells degranulation (66, 74). An imbalance in omega-6 fatty acid intake vs. omega-3 fatty acid in people eating a Western Diet (76, 77) has been considered as a possible reason for the increase in allergic diseases. This may be due to the pro-inflammatory activity of omega-6 fatty which favors a Th2 immune response (78).

However, other than some effect on allergen sensitization, evidence from RCTS is not conclusive to support omega-3 fatty acid supplementation for offspring allergy prevention during pregnancy and/or lactation (65, 66). Omega-3 fatty acid supplementation has also been studied in infancy and childhood with conflicting results. Using house dust mite (HDM) avoidance and dietary fatty acid modification during the first 5 years of life, the Childhood Asthma Prevention Study (CAPS) showed modification of the plasma fatty acids status (increasing omega-3/omega-6 ratio) at age 5 years, but no clinical effects (79).

D'Vaz et al. (80) showed that in infants at high risk of atopy, supplementation of omega 3 fatty acids for the first 6 months of life had beneficial effects on preventing sensitization, eczema, and food allergy. Similarly, Birch et al. (81) demonstrated that infant formula supplemented with omega-3 fatty acids in healthy infants is protective against allergic disease (wheezing/asthma, wheezing/asthma/atopic dermatitis, or any allergy) throughout three years of life. The difference in findings of these studies may be explained by differences in the underlying diet, high risk/low risk populations, dose of supplements used, timing and duration of supplementation, or serum levels of omega-3 intake at the start of the trial (75, 79, 80, 82, 83).

Non Digestible Fibers/Prebiotics

Prebiotics are defined as a "substrate that is selectively utilized by host microorganisms conferring a health benefit" (84). They naturally occur in foods or can be artificially produced as galactooligosaccharides (GOS) and fructooligosaccharides (FOS). In the large bowel, prebiotics undergo fermentation by local bacteria modulating the microbiota composition. Microbiota can, in turn, produce beneficial metabolites such as short-chain fatty acids with known anti-inflammatory effects. Human milk contains more than different 400 oligosaccharides which act also as prebiotics (66) and can shape infant gut microbiota composition (85). Following from this, prebiotics have been added to infant formula trying to mimic the beneficial effect of breastmilk (85).

A recent metaanalysis including 22 studies assessed the effect of supplementing prebiotics in infants on the risk of development of allergic symptoms. Studies on infants at high risk and normal risk of allergy were included. Most of these studies evaluated FOS with GOS supplementation added to infant formula. The authors concluded that the evidence for supplementation of prebiotics for the prevention of allergies are not strong enough to make any clear recommendations (86).

Focusing on the whole "pattern" of dietary intake instead of the single nutrients or groups of nutrients when studying diseases outcomes is therefore increasingly appealing and growing. This is because nutrients and foods are not eaten in isolation. All food intake interacts in a complex manner to determine well-being or disease.

DIET DIVERSITY AND THE MICROBIOME

If there is a place for diet diversity in allergy prevention, then the mechanisms of action need to be clarified. Diet diversity may affect the gut microbiome by providing a more diverse food intake which may increase intake of fiber and other nutrients affecting the gut microbiome. Very few studies have compared diet diversity to gut microbiome diversity. The first study conducted by Claesson et al. (87) showed that increased diet diversity in the elderly was associated with increased gut microbial diversity. If introduction of solid food is considered an increase in diet diversity, then this would be another example of how diet diversity increases gut microbiome diversity. This was reflected by increased protein intake, carbohydrate, and fiber intake, as well as in increased intake of family foods (88). Increased gut microbial diversity has been related to reduced

TABLE 4 | Diet indices in pregnancy and allergy outcomes in the offspring.

References	Exposure	Outcomes
Castro-Rodriguez et al. (97)	Mediterranean diet score Spain *N* = 1,000 Age assessed: 1.5 and 4 years	Current wheezing, rhinitis, and dermatitis MedDiet scores during pregnancy was not a protective factor for current wheezing, rhinitis, or dermatitis in preschoolers.
Chatzi et al. (98)	EPIC scores used with the addition of milk and removal of alcohol Spain, Greece *N* = 1,771 Age assessed: 1 year	Wheeze and Eczema EPIc scores was not associated with the risk of wheeze and eczema in any cohort.
Chatzi et al. (99)	EPIC scores used with the addition of milk and removal of alcohol Spain *N* = 460 Age assessed: 6.5 years	Persistent wheeze, atopic wheeze, atopy (sensitization to ≥1/6 common aeroallergens) Higher EPIC scores were negatively associated with persistent wheeze OR 0.23 (0.09–0.60), atopic wheeze OR 0.34 (0.12–0.97) and atopy OR 0.55 (0.32–1.97)
Bedard et al. (100)	Mediterranean diet score United Kingdom Avon Longitudinal Study of Parents and Children (ALSPAC) *N* = 8,907 Age assessed = 7.5 years	Asthma, eczema, maximal mid-expiratory flow The maternal Mediterranean diet score was not associated with asthma or other allergic outcomes Weak positive associations were found between maternal Mediterranean diet score and childhood maximal mid-expiratory flow after controlling for confounders Higher Mediterranean diet scores were associated with increased $FEF_{25-75\%}$ *z*-scores adjusted for age, height, and sex (β 0.06, 95% CI 0.01–0.12; $p = 0.03$, comparing a score of 4-7 vs. a score of 0–3)
Lange et al. (93)	Mediterranean diet score and Alternate Healthy Eating Index modified for pregnancy (AHEI-P) USA *N* = 1376 Age assessed: 3 years	Recurrent wheeze None of these indices, recurrent wheeze in children Secondary outcomes: Doctor's diagnosis of asthma, eczema, lower respiratory tract infections, or atopy at any time (0–3 years); in the adjusted models, neither diet score was associated with any of the secondary outcomes
Moonesinghe et al. (94)	Alternate Healthy Eating Index modified for pregnancy (AHEI-P) UK *N* = 937 Age assessed: 3 and 10 years	Atopy (sensitization to any food and/or aero-allergen), reported allergic diseases (asthma, eczema, allergic rhinitis, and food allergy) AHEI-P was not associated with atopy or allergic diseases
Chen et al. (95)	Diet inflammatory index and Healthy Eating index (HEI-2015) Ireland *N* = 862 Age assessed: first 10 years of life	Asthma for the first 10 years of life: Higher diet inflammatory diet scores were associated with higher risk of offspring asthma (OR: 1.35; 95% CI: 1.10, 1.65). Higher HEI-2015 scores were associated with lower risk of asthma (OR: 0.77; 95% CI: 0.64, 0.93) (both $P < 0.01$); persisted in adjusted models
Hanson et al. (96)	Diet inflammatory index USA *N* = 1,424 Age assessed: first 9 years of life	Ever asthma and wheezing in the past year (early childhood and mid childhood); current asthma and lung function (mid childhood), and wheeze trajectory during 1–9 years Higher diet inflammatory scores were associated with an early vs. never wheeze trajectory (OR, 1.89; 95% CI, 1.14–3.13) (adjusted models) and lower forced expiratory flow (forced expiratory flow at 25-75%) in mid childhood (beta, −132 mL; 95% CI, −249 to −14). Ever asthma, were not related to diet inflammatory scores

TABLE 5 | Diet indices in childhood and allergy outcomes.

References	Exposure	Outcomes
KIDMED—Mediterranean Diet Quality Index for children and teenagers		
Grigoropoulou et al. (106)	Greece $N = 1,125$ Age assessed: 10–12 years	Asthma 1-unit increase in the Kidmed index was associated with 16% lower likelihood of having asthma symptoms Greater adherence to MD was inversely associated with "ever wheeze" (O: 0.88; 95% CI, 0.78, 0.98) and wheeze when exercise w (OR: 0.79; 95% CI, 0.67, 0.93).
Chatzi et al. (99)	Spain $N = 460$ Age assessed: 6.5 years	Persistent wheeze, atopic wheeze (current wheeze and atopy), atopy (sensitization to $\geq$1/6 common aeroallergens) No statistical significant effect was seen.
Alphantonogeorgos et al. (101)	Greece Urban (Athens, $n = 700$) or rural environment ($n = 425$) Age assessed: 10–12 years	Asthma, any asthmatic symptom Adherence to the Kidmed index was negatively associated with asthma symptoms (standardized beta = -0.224, $p < 0.001$).
Arvaniti et al. (102)	Greece $N = 700$ Age assessed: 10–12 years	Ever asthma, any asthma symptoms, ever wheeze, exercise induced wheeze, night cough Greater adherence to MD inversely associated with ever asthma ($p = 0.002$), any asthma symptoms ($P < 0.001$), ever wheeze ($p < 0.001$), exercise induced wheeze ($p = 0.004$). One-unit increase in KidMed score was associated with 14% lower likelihood of having asthma.
Chatzi et al. (104)	Greece $N = 690$ Age assessed: 7–18 years	Respiratory and allergic symptoms over the past 12 months, skin prick tests to 10 aeroallergens, any wheezing in the past, atopic wheeze, current wheezing, nocturnal dry cough, any rhinitis in the past, atopic rhinitis, current allergic rhinitis, current seasonal rhinitis, atopy High level of adherence to MD was inversely related to Allergic rhintis ever OR 0.34 (0.18–0.64) $p < 0.01$ Allergic rhinitis with atopy OR 0.39 (0.13–0.97) Current allergic h = rhinitis OR 0.49 (0.24–0.99) $p < 0.05$ Nocturnal cough apart from cold in the last 12 months OR 0.49 (0.23–0.96) No significant effect seen for wheezing and atopy
EPIC—European Prospective Investigation into Cancer and Nutrition Cohort—Mediterranean diet score		
Castro-Rodriguez et al. (107)	Spain $N = 1,784$ Age assessed: 08 $\pm$ 0.8 years	Current wheezing Highest quartile of EPIC scores associated with a reduction in current wheeze 0.54 (0.33–0.88)
de Batlle et al. (108)	Mexico $N = 1,476$ Age assessed: 6–7 years	Asthma ever, wheeze ever, current wheeze, rhinitis ever, sneezing ever, current sneezing, current itchy-watery eyes. rhinitis related outcomes by ISAAC questionnaire Adherence to the EPIC scores (2nd and 3rd tertile compared with 1st tertile) inversely associated with asthma ever OR 0.60 (0.40–0.91), wheezing ever OR 0.64 (0.47–0.87), current Sneezing OR 0.71 (0.52–0.96) and current itchy-watery eyes OR 0.63 (0.42–0.95)
Garcia-Marcos L et al. (109)	Spain $N = 20,106$ Age assessed: 6–7 years	Current occasional asthma, current severe asthma, rhinoconjunctivitis Every 1 unit increase in EPIC score showed a protective effect on current severe asthma in girls (adjusted OR 0.90, 95% CI: 0.82–0.98)
Suarez-Varela et al. (110)	Spain $N = 20,106$ Age assessed: 6–7 years	Atopic dermatitis No association between EPIC diet scores and atopic dermatitis
Tamay et al. (111)	Turkey $N = 9,875$ Age assessed: 6–7 years	Allergic rhinitis, lifetime rhinitis, current rhinitis, current rhinoconjunctivitis, physician-diagnosed allergic rhinitis No association between EPIC diet scores and any of the outcomes studied
Akcay et al. (112)	Turkey $N = 9,991$ Age assessed: 13–14 years	Wheeze ever, wheezing in last 12 months, lifetime doctor diagnosed asthma prevalence No association between EPIC diet scores and any of the outcomes studied
Rice et al. (113)	Peru $N = 287$ asthmatic + 96 controls Age assessed: 9–19 years	Asthma status (asthma control, FEV1), allergic rhinitis, atopy No association between EPIC scores and asthma control, FEV1, allergic rhinitis, or atopic status
Romieu et al. (114)	Mexico $N = 158$ asthmatic + 50 controls Age assessed: 6–14 years	Pulmonary function was measured and nasal lavage collected and analyzed. No significant difference between the asthmatic and the non-asthmatic children.
Gonzalez et al. (115)	Spain $N = 7,454$ Age assessed: 6–7 years $N = 7,391$ Age assessed: 13–14 years	6–7 years: Asthma current asthma, severe asthma, and exercise-induced asthma 13–14 years: Asthma current asthma, severe asthma, and exercise-induced asthma 6–7 years: Increased EPIC diet scores were associated with a higher risk of severe asthma (odds ratios = 2.26, 95% CI: 1.21–4.22 in the 2nd quartile, but not in the 3rd and 4th) in girls. There was no significant relationship for the other asthma categories

(Continued)

TABLE 5 | Continued

Study, Year	Exposure	Outcomes
Diet inflammatory index		
De Castro et al. (116)	Portugal $N = 501$ Age assessed: 7–12 years	Asthma The effect of indoor pollution on asthma outcomes was more severe in those with a pro-inflammatory diet (OR = 1.44, 95% CI: 1.01–2.21; and OR = 1.29, 95% CI: 1.03–1.68, respectively) compared to those having an anti-inflammatory diet. No direct effect of DII on asthma outcomes were reported.
Han et al. (117)	USA $N = 8,175$ Age assessed: 6–17 years	Current asthma, current wheeze, lung function measures Higher Diet inflammatory index scores were associated with high fractional exhaled nitric oxide (a marker of eosinophilic airway inflammation; OR = 2.38, 95% CI = 1.13–5.02; $P_{trend} = 0.05$) in children. The DII was not associated with lung function or current asthma

allergen sensitization (89) and allergy outcomes in both children and adults (90–92).

DIET INDICES IN PREGNANCY

Three diet indices in pregnancy have been studied in relation to allergy outcomes in infants, the healthy eating index (93–95), the diet inflammatory index (95, 96), and the Mediterranean diet index (93, 97–100) (**Table 4**).

The Healthy Eating Index

Three studies have studied the role of healthy eating in pregnancy and childhood allergy outcomes. In one study (Food Allergy and Research Study, UK), the Alternate Healthy Eating Index modified for pregnancy (AHEI-P) was used to examine associations with allergic outcomes in the offspring at 3 and 10 years (94). This study found no association between the AHEI-P and atopy (defined as sensitization to any food and/or aero-allergen) or reported allergic diseases at 3 or 10 years. Lange et al. (93) used data from the Project Viva cohort (US) and found no association between the HEI and recurrent wheeze in infants at the age of 3 years. In contract, Chen et al. (95) using data from an Irish cohort found that higher HEI-2015 scores were associated with lower risk of asthma (OR: 0.77; 95% CI: 0.64, 0.93) (both $P < 0.01$) and the effect persisted in adjusted models.

The Diet Inflammatory Index

Two studies have looked at the association between a pro-inflammatory diet in pregnancy and asthma, wheeze, or lung function outcomes through childhood (95, 96). One recent study from Ireland (95) showed an association between DII scores and asthma outcomes over 10 years. The Project viva (US) study showed an association of DII scores with wheeze trajectories, but not asthma, up to 7.5 years of age (96).

The Mediterranean Diet Index

The associations between the Mediterranean diet index and allergy outcomes have been studied in by five cohorts (93, 97–99) (two from Spain, one from Greece, one from the USA, and one from the UK). Four studies investigated wheeze in the infant (93, 97–99), four studies investigated rhinitis, atopic dermatitis, and/or eczema (97–100), two studies included sensitization to food/aero-allergens (93, 99), and two studies investigated asthma in the child (93). Four of the five studies found no association between the Mediterranean diet index and the allergic outcomes studied (93, 97, 98, 100). Childhood persistent wheeze, atopic wheeze, and atopy was associated with the Mediterranean diet index in only one of the studies (99). One study (100) found an association between the Mediterranean diet score and childhood maximal mid-expiratory flow as well as $FEF_{25-75\%}$ z-scores.

Based on the current evidence, limited recommendations can be made about the role of diet indices in pregnancy and allergy outcomes in the offspring. The most potential of these indices may be in the prevention of lung function, wheeze, or asthma outcomes in the offspring.

DIET INDICES IN CHILDHOOD

The role of diet indices in infancy vs. subsequent development of allergic outcomes in later childhood have not been conducted. A number of studies have however looked at the Mediterranean diet in childhood vs. allergy outcomes, using either the KIDMED mediterranean score (99, 101–106) or the adult EPIC score (107–115) and the diet inflammatory index (116, 117) (**Table 5**).

The Diet Inflammatory Index

Two studies investigated the role of the diet inflammatory index on allergy development in the children (116, 117) and neither one of these could find an association between diet inflammatory scores and asthma in the child. One study found that indoor pollution on asthma outcomes was more severe in those with a pro-inflammatory diet than those following an anti-inflammatory diet (116).

The Mediterranean Diet Index

Three studies found reduced allergy outcomes with an increase in mediterranean diet scores: reduced current wheeze (107), asthma ever, wheezing ever, current sneezing, and current itchy-watery eyes (108), and severe asthma in girls (109). Five studies found no effect on any of the outcomes studied (110–114) and one study found an increased risk of severe asthma, with increased mediterranean diet scores (115).

CONCLUSIONS

Based on the current evidence, we recommend the introduction of solid foods, including common allergenic foods, during the

1st year of life, according to the infant's neuro-developmental abilities and familial or cultural habits.

In infants with severe AD and/or FA, medical assessment may be advisable before introducing common food allergens into the diet. Consuming a diverse range of foods in the 1st year of life may increase intake of nutrients and positively affect the gut microbiome composition. Intake of omega-3 fatty acids and fibers/prebiotics may be particularly important, but more information is required about dose and which individuals are most likely to benefit. Increased diet variety in the first year of life is also associated with reduced food allergy outcomes. Limited evidence exist about the role of diet indices in pregnancy and allergic disease in the offspring. The most promising results indicate a reduction in childhood wheeze and/or asthma. Further studies are warranted to investigate the effects of diet diversity during pregnancy and lactation and diet indices in early life on the development of allergic diseases in infants and children.

AUTHOR CONTRIBUTIONS

ED'A conceptualized the paper, wrote the introduction, and the section on diet diversity. DP critically reviewed the draft and proofread the paper. MS wrote the abstract and made tables on diet diversity. EV contributed to write the section on diet diversity. GZ made final revisions of the draft. CV wrote the section on diet indices, made tables on diet indices, and critically reviewed the paper. All authors contributed to the article and approved the submitted version.

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Use of Soy-Based Formulas and Cow's Milk Allergy: Lights and Shadows

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Soybean (*Glycine max*) is a species of legume native to East Asia and used in childhood diet for over 2,000 years in the East. Soy protein formulas have been available for almost a century. Nowadays, the increase in cow's milk allergy and vegetarian dietary preferences are driving consumers toward cow's milk alternatives. In this paper, we reviewed the nutritional composition of soy-based infant formula and discussed their possible use in pediatric age, mainly focusing on prevention and treatment of cow's milk allergy. Protein quality is determined by digestibility and amino acid content. Purified or concentrated vegetable proteins (e.g., soy protein and gluten) have high digestibility (>95%), similar to those of animal ones. For some intact vegetable products (e.g., whole cereals and pulses), protein digestibility is lower (80–90%). Food processing and heat treatment also influence protein digestibility. Considering these data, we tried to evaluate the possible use of soybean and derivatives in pediatric age, including the nutritional composition of soy formulas and the clinical indications for their use. Moreover, since plant-based beverages are being perceived as healthy by consumers and their use is growing on the market, we recommend that soy drink should not be used as a substitute for infant formulas or cow's milk in children younger than 24 months.

Keywords: soy-based formula, infant nutrition, cow's milk allergy, vegetables beverages, soybean, nutritional status

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INTRODUCTION

The soybean (*Glycine max* L.) is a legume crop of East Asian origin, but its use has nowadays spread worldwide due to its nutritional value (i.e., high protein and oil contents). In the East, soybeans are used to produce traditional foods such as miso, tofu, natto, tempeh, soymilk, soy sauce, and soy paste. Conversely, in the West, soybeans are mainly processed to obtain full-fat flakes that are then defatted by using organic solvents and pressed into soybean meal, a high-quality protein source. This is subsequently used to obtain texturized vegetable protein, soy concentrate, and soy isolates, used as a protein supplement for various foods, including infant formulas (1, 2).

In the present paper, we reviewed the nutritional composition of soy-based infant formula (SIF) and discussed their possible use in pediatric age, mainly focusing on prevention and treatment

of cow's milk allergy (CMA). This paper could be very useful for all health-care professionals (especially pediatricians and nutritionists) who deal with giving practical advice to families, based on scientific evidence.

NUTRIENT CONTENT OF SOYBEAN

Understanding the nutritional composition of soybean in terms of macronutrients, micronutrients, and many minor bioactive compounds is crucial to evaluate the nutritional properties and limits (**Table 1**, **Figures 1**, **2**).

Proteins

Soybeans contain a high quantity of proteins of up to ∼40–41% of their dry weight. About the 65–80% of this amount is represented by storage proteins. Among these, glycinin and β-conglycinin are the major ones, the former being richer in sulfur-containing amino acids (methionine and cysteine) than the latter. The remaining proteins are mainly used by the seed itself to mobilize stored nutrients and defend it against microorganisms/macroorganisms, to assure proper growth (2).

Proteins: Nutritional Quality

Since 2013, in order to assess the protein quality, the World Health Organization/Food and Agriculture Organization (WHO/FAO) adopted an evaluation methodology based on protein digestibility (increased by heating and fermenting soy) and the ability to provide an adequate amount of indispensable amino acid to meet organism requirement [digestible indispensable amino acid scores (DIAAS)] (9, 10).

As previously mentioned, purified or concentrated vegetable proteins have high digestibility (>95%), while for some intact vegetable products, protein digestibility is lower (around 80–90%). Moreover, one of the most used methods by agencies around the world to evaluate protein's nutritional quality is the protein digestibility-corrected amino acid score (PDCAAS). This rating measures the quality of specific food protein comparing against a standard food protein based on the amino acid requirements of a preschooler adjusted for digestibility. Isolated soy protein has a PDCAAS of 1.0, which is the highest value that any protein can achieve and the same score as milk protein and egg white (11, 12).

The high nutritional quality of soybean protein is reaffirmed by the fact that it is high in lysine, and therefore, it may be a valuable supplement to cereal foods, which, conversely, have a low content of lysine (1).

Proteins: Enzymes and Antinutritional Factors

A small part of soybean proteins is represented by soybean protease inhibitors. Among these, the best known are the trypsin–chymotrypsin inhibitor and trypsin inhibitor (13). Various studies have demonstrated how these proteins can inhibit pancreatic enzymes, thus reducing the digestibility of proteins (1, 2). Among these antinutritional factors, lectins also deserve to be mentioned as they interfere with the absorption of micronutrients. Soybean protease inhibitors and lectins are both inactivated by heat treatment and fermentation (2, 9). As for enzymes, lipo-oxygenases are not antinutritional factors but give the soybean their characteristic, undesirable, beany flavor (14, 15), whereas urease is used mainly in production processes as an indicator of adequate heat treatment (1, 16).

Lipids

Soy contains various types of lipids; among these, the most numerous are triglycerides (96%). Smaller percentages are represented by phospholipids (2%), unsaponifiable lipids (1.6%), free fatty acids (0.5%), and traces of carotenoid pigments (1). The lipid content of soy is important because soy oil is rich in polyunsaturated fatty acids (PUFAs), linoleic and linolenic acids, which are essential fatty acids (EFAs) that cannot be synthesized by mammals and therefore need to be obtained from the diet (2). These EFAs are metabolized in long-chain PUFAs (LCPUFAs), in particular, linoleic acid (LA; C18:2 $n-6$), which is the progenitor of $n-6$ long-chain LCPUFA series [arachidonic acid (AA), C20:4 $n-6$], while alpha-linolenic acid (ALA; C18:3 $n-3$) is the progenitor of $n-3$ LCPUFA series eicosapentaenoic acid (EPA; C20:5 $n-3$) and docosahexaenoic acid (DHA; C22:6 $n-3$). It is now known that PUFAs provide important health functions, especially in cardiovascular health, mainly by improving plasma lipid profile and thereby lowering cardiovascular risk (17, 18). Notably, LA dietary intake is inversely correlated with cardiovascular risk by modulating several cardiometabolic factors, such as the cholesterol-lowering effect and beneficial effect on glucose metabolism (6).

Carbohydrates

In terms of composition, the second macronutrients more represented are carbohydrates, whose percentage is around 35% of dry seed weight (a mature soybean seed contains approximately 40% of proteins, 20% of lipids, 35% of carbohydrates, and 5% of minerals of dry weight).

Soy contains two groups of carbohydrates: soluble sugars (sucrose 5%, stachyose 4%, and raffinose 1%) and insoluble fibers (20%), which are structural carbohydrates. Sucrose is an important carbohydrate because it makes soy more palatable, giving it a very sweet flavor (2, 19). Raffinose and stachyose are unwelcome components in soybean seeds because they are the major cause of soybean flatulence, a challenging problem associated with the consumption of soybeans. Since humans do not have enzymes capable of processing them, they reach the intestine undigested. At that level, being non-nutritional factors, they are fermented by intestinal flora bacteria, which, as a consequence, produce gas and flatulence (1, 2, 19).

Minerals

Minerals represent 5% of dry seed weight. The main constituents are potassium, calcium, and magnesium, while others, such as iron, zinc, and copper, are present just in traces, thus requiring supplementation (1).

Other Constituents

Two other important constituents of soybeans are phytic acid and isoflavones. Phytic acid is found in soybeans as phytin salts, which are not available to humans and can chelate a

TABLE 1 | Main constituents of soybean, mature seeds, and raw dry soybeans.

	Nutritional value for 100 g	Comments
Energy	1,866 kJ (446 kcal)	
Protein	36.49 g	
Essential amino acids:		Soy protein, although plant-derived, is a complete protein; it contains a great quantitative of lysine, while methionine is a limiting amino acid
Histidine	1.097 g	
Isoleucine	1.971 g	
Leucine	3.309 g	
Lysine	2.706 g	
Methionine	0.547 g	
Phenylalanine	2.122 g	
Threonine	1.766 g	
Tryptophan	0.591 g	
Valine	2.029 g	
Other proteins:		
Enzymes		
Lipo-oxygenase		The unpleasant bean flavor typical of soybeans is due to the content of lipo-oxygenase
Urease		Urease is present in uncooked soybean and is progressively destroyed by heat, so it can be used as an indicator of adequate heat treatment
Antinutritional factors:		
Soy protease inhibitors		Soy protease inhibitors reduce protein digestibility
Lectins		Lectins can interfere with micronutrient absorption
Fats[a]	19.94 g	The fat content consists mainly of unsaturated fatty acids, with smaller amounts of saturated fatty acids and no cholesterol
Saturated	2.884 g	
Monounsaturated	4.404 g	Soy is high in polyunsaturated fatty acids, which are essential fatty acids with a hypocholesterolemic effect
Polyunsaturated[b]	11.255 g	Omega-3 ALA 0.72–2.16% Omega-6 LA 6.48–11.6%
Carbohydrates	30.16 g	
Sugars	7.33 g	Soluble sugars increase palatability, thus potentially causing flatulence
Dietary fiber	9.3 g	Dietary fiber is a non-digestible portion of food. In soybean, it is composed of lignin, enzyme-resistant starch and oligosaccharides, and cell wall polysaccharides (cellulose, hemicellulose, and pectins)
Minerals[c]		Although soybean's mineral content is high, bioavailability is slow since phytate reduces the absorption of dietary minerals, particularly for zinc and iron
Potassium	1.797 mg	
Calcium	277 mg	
Magnesium	280 mg	
Copper	1.658 mg	
Iron	15.7 mg	
Zinc	4.89 mg	
Other constituents		
Phytic acid		Phytic acid impairs mineral absorption and may promote mineral deficiencies
Isoflavone[d]		Plant compounds have lower estrogenic activity than the human female hormone 17-β-estradiol and other estrogenic receptor-independent effects, which can play a role in normal sexual development and reproductive function

Table 1 *source: Reference (3).*
[a]*Source: reference (4).*
[b]*Source: reference (5).*
[c]*Source: reference (6).*
[d]*Source: reference (7).*
ALA, alpha-linolenic acid; LA, linoleic acid.

series of micronutrients among which especially zinc, calcium, magnesium, and iron, thus preventing their mucosal absorption (20). Therefore, it is considered an important antinutritional factor. Soy is also rich in isoflavones (daidzein, genistein, and glycitein), also known as phytoestrogens since their structure is similar to that of estrogens. They are capable of binding estrogen

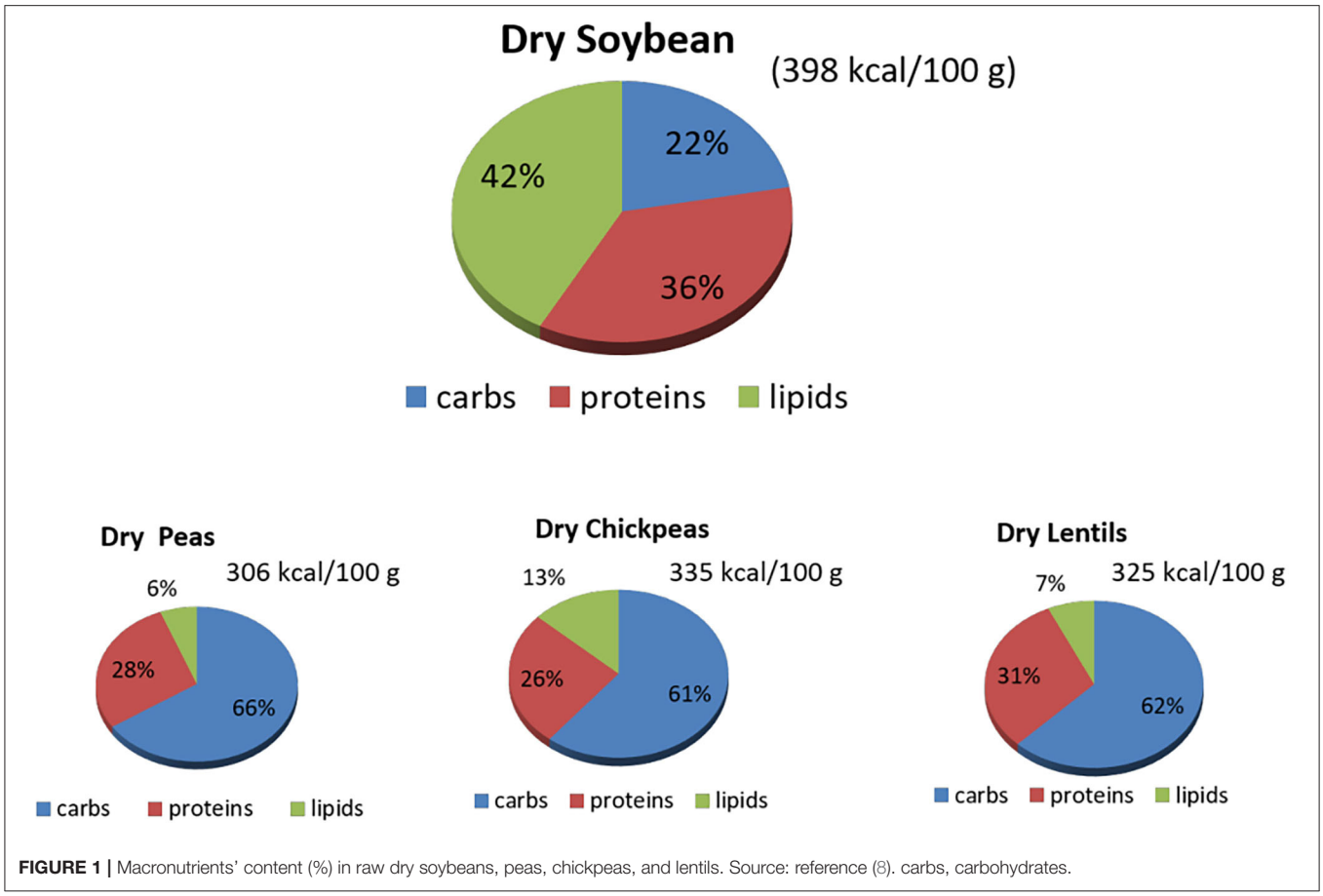

FIGURE 1 | Macronutrients' content (%) in raw dry soybeans, peas, chickpeas, and lentils. Source: reference (8). carbs, carbohydrates.

receptors (ERs), although less strongly, increasing circulating estrogens levels. Current evidence on a possible link between isoflavone consumption and various diseases remains dubious, especially in terms of their capacity to prevent cardiovascular pathologies and osteoporosis and to promote cancers (2).

ROLE OF SOY IN INFANT FEEDING

Various factors contributed to the extensive use of soy-based formulas and products among children. Among others, the most important is the high nutritional value and palatability (21). Soy-based formulas were first introduced to the western market almost 100 years ago. In particular, their appearance in the USA dates back to 1909 when they were proposed as an alternative for feeding children with an allergy to cow's milk proteins (CMA) (22). At the time of their first introduction, soy-based formulas were made from soy flour. Soon, however, various limiting factors such as low digestibility and high quantity of fibers and phytates induced the market to find alternative solutions and led to the development of soy-based formulas containing proteins isolated from soy (soy protein isolate) (23, 24). Soy protein isolate is extracted from the flake using different solvents to obtain a final proteic source with higher digestibility and nutritional value since it contains higher amounts of essential amino acids (23).

Throughout history, soy-based formulas have undergone various rearrangements. In particular, in the 1970s, amino acids such as methionine (an essential amino acid), taurine, and carnitine, which are poorly contained in soy protein, were added to formulas (23). In 2000, soy-based formulas also started being supplemented with LCPUFAs (24).

Soy-Based Infant Formula Indications and Nutritional Safety

Soy-based formulas currently on the market do not contain cow's milk proteins and lactose (25). Therefore, the main current indications for their use in infancy are allergy/intolerance to cow's milk-based formulas (CMF), hereditary lactase deficiency, and galactosemia (23, 25). Lactose-free formulas can be considered in the management of acute gastroenteritis in hospitalized children age <5 years, but their routine use is not recommended in an outpatient setting (26).

Soy-Based Infant Formula Nutritional Composition

The nutritional composition of formulas based on soy protein isolates, as compared with human milk (HM) and cow's milk, is shown in **Table 2**. The protein source is represented by soy protein isolates with supplementations in methionine, carnitine, and taurine. The fat content is made up mainly by vegetable oils, in particular soy, palm, sunflower, olein, safflower, and

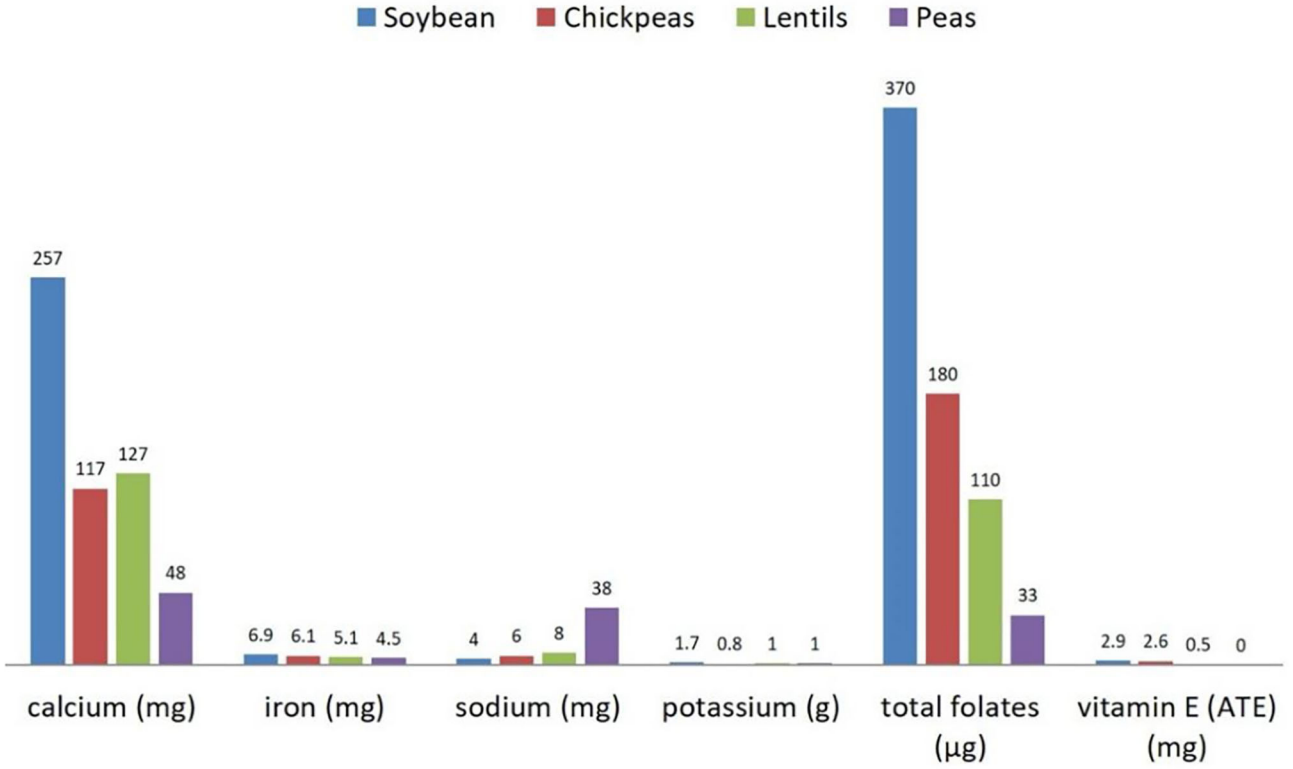

FIGURE 2 | Micronutrients' content (per 100 g) in raw dry soybeans, peas, chickpeas, and lentils. Source: reference (8).

coconut oil. Currently, DHA and AA are added to all formulas based on soy protein isolates (25). Phosphorus and calcium are present at concentrations 20% higher than those present in CMF. Currently, soy-based formulas undergo a heating process to eliminate protease inhibitors, thus increasing digestibility. This heating process neutralizes up to 90% of protease inhibitors. Another issue with soy-based formulas is the high quantity of phytates and fibers, which can bind zinc and iron. Over time, this issue has been resolved by fortifying them with iron and zinc (23, 25).

Nutritional Safety of Soy-Based Infant Formula

Given the many pros and cons of soy, the health effects of soy-based formulas have been intensely investigated over the last few years, primarily for their high phytoestrogen content and, thus, their impact on children's health and chronic disease risk (31).

A systematic review and meta-analysis by Vandenplas et al. (23) evaluated the safety of soy-based formulas in chronic administration, analyzing cross-sectional, case-control, cohort studies, and clinical trials published until 2013 in which the sexual development of SF formula-fed infants was compared with that of children fed with other formulas. The authors concluded that SF is a safe alternative to CMF, given that, although some differences could be detected, such as values of genistein and daidzein, which were higher in children fed SIF than in children fed CMF or HM, the long-term effects of isoflavones on the main reproductive functions in humans were clinically irrelevant. In particular, anthropometric patterns, bone mineral content, and neurological, reproductive, immune, and endocrinological outcomes in SF formula-fed infants were similar to those in CMF-fed infants (32–34). According to these data, a recent review of Messina et al. (31) shows that soy can be safely incorporated into the children's diet, always following the principles of variety and moderation. There is little evidence that soy exerts adverse hormonal effects in children, but data are very limited.

Some studies show that soy consumption in early childhood can be related to altered thyroid function, although there are very few studies in children, and most of the conclusions are based on old case reports, published before the introduction of iodine-supplemented SF (25, 35, 36). A retrospective study of Fort et al. (37) evaluated the prevalence of autoimmune thyroid disease in breast-fed and SF-fed children, obtaining a history of feeding practices in 59 children with autoimmune thyroid disease, their 76 healthy siblings, and 54 healthy non-related control children. The frequency of feedings with SF in early life was significantly higher in children with autoimmune thyroid disease (prevalence 31%) than in their siblings (prevalence 12%) and healthy non-related control children (prevalence 13%). In a more recent study, Conrad et al. (38) analyzed the effect of childhood diet on thyroid function in children with congenital hypothyroidism, testing the hypothesis that SF-based feeding in infants with congenital hypothyroidism leads to a prolonged

TABLE 2 | Nutritional composition of human milk (HM), cow's milk (CM), soy-based beverages (SB), soy-based infant formula as regulated by EU (SIF-EU), and Italian commercialized soy-based formula (SF).

	HM[a]	CM[a]	SB[b]	SIF-EU[c]	SF[d]
COMPOSITION IN 100 g					
Energy (kcal)	70	62	32	60–70	67–68
Water (g)	87.5	87.7	89.7		
Total protein (g)	1.0	3.3	2.9	1.35–1.96	1.6–1.7
Total fat (g)	4.4	3.3	1.9	2.64–4.2	3.3–3.5
Lactose	6.9	4.7			
MINERALS					
Calcium (mg)	32	112	13	30–98	55–68
Iron (mg)		0.1	0.4	0.18–0.91	0.9–1.1
Magnesium (mg)	3	11		3–10.5	
Phosphorus (mg)	14	91		15–63	39–40
Potassium (mg)	51	145	120	48–112	68–77
Sodium (mg)	17	42	32	15–42	20–27
Zinc (mg)	0.2	0.4	0.2	0.3–0.7	
Copper (μg)	100			36–70	
Selenium (μg)	1.8	1.8		1.8–6.02	
Manganese (μg)		8		−70	

[a]*Source: reference (27).*
[b]*Source: reference (28).*
[c]*Source: reference (29).*
[d]*Source: reference (30).*

increase of thyroid-stimulating hormone (TSH). They evaluated eight children in the soy diet group and 70 in the non-soy diet group, finding that SF-fed children had a prolonged increase of TSH than had children fed with non-soy formula. As a result, not all the issues regarding the safety of SF in infants have been solved.

This field is still highly controversial because it is still difficult to assess whether soyfood consumption early in life (<24 months of life) is completely safe with no hormonal effects at all; thus, further research is needed to establish that SF given early in life is safe and beneficial from a nutritional point of view.

SOY-PROTEIN FORMULAS AND COW'S MILK ALLERGY

Soy-protein formulas are widely used for feeding children with CMA. Cow's milk allergy is an adverse immune response associated with a clinical reaction due to the binding of specific immunoglobulin (IgE) to antigens/proteins in cow's milk (IgE), which could induce an allergic reaction in sensitized individuals (39). It is the most common type of food allergy, affecting <2% of children under 4 years of age (40).

The general treatment for CMA consists of avoiding exposure to the implicated allergens: patients must avoid cow's milk and cow's milk protein-based products. If breastfeeding is not feasible, replacement with a substitute appropriate formula is mandatory (41). Extensively hydrolyzed formulas (eHFs), in which milk proteins have been fragmented (hydrolyzation) to make them less allergenic, are the first choice in children diagnosed with CMA. They are nutritionally adequate and well-tolerated by children allergic to cow's milk proteins, although among their main drawbacks is poor palatability, high costs, and the remote potential to cause anaphylaxis. From this point of view, amino-acid formulas (AAF) are safe but very expensive, unpalatable, and not widely available (42). Therefore, the choice for a different milk formula increasingly falls on soy formulas (SF) and rice-hydrolyzed formulas (RHFs), which are well-tolerated and considered a second-line resource in infants with IgE-mediated CMA (43).

In the last few decades, soy formulas have been changed over the years to improve digestibility, nutritional values, and protein quality. Contemporary soy formulas are supplemented with appropriate amounts of amino acids such as methionine, taurine, and carnitine, and they are not deficient in zinc, calcium, or phosphorus. Nevertheless, despite all these benefits, two potential drawbacks remain for the use of soy-based formulas in infants with CMA.

One important concern regard phytoestrogens in the form of isoflavones present in soy protein and their possible hormonal effects on the reproductive system. Indeed, phytoestrogens are plant compounds with structure and function similar to those of 17-β-estradiol that, thanks to this particular chemical structure, are able to recognize and to bind ERs and thus exert an agonistic or antagonistic effect on this hormone. These chemical agents perform an estrogenic action either directly (binding the ERs, enhancing aromatase activity, and increasing sensitivity to estrogens) or indirectly, through their effect on GnRH, thus leading to an increase in endogenous production of estrogens. They are present in a very large amount in soy-based foods, although their stimulus on the receptor is 100–1,000 times less than the human female hormone 17-β-estradiol.

Over 94% of the phytoestrogens contained in SF are present as beta-glycosylated isoflavones, such as genistein, daidzein, and glycitein, which are biologically inactive and very poorly absorbed when in this form (44). Their activation occurs in the gut, even if the number of active compounds that are absorbed and enter in the blood varies from subject to subject, primarily according to the composition of the gut microbiota. Moreover, the liver plays a central role in determining the concentration of active isoflavones in the blood because a large portion of them are metabolized, thus reducing the number of compounds that can exert an estrogen-like activity (45). However, phytoestrogens have other ER-independent effects, such as the alteration of epigenetic marks and the inhibition of estradiol, which can play a role in the development of early puberty in females and sexual disorders in males (7).

Although data are limited since relatively few studies have been conducted in children, some authors currently support the possible correlation between phytoestrogens and the development of permanent modifications of reproductive system function (46, 47). A Korean case-control study involving 150 girls found that genistein blood concentration in girls with precocious puberty was significantly higher than in the

control group (48). In contrast with these findings, most of the studies about soy and pubertal development show no conclusive evidence that SF has hormonal negative effects on children or affect pubertal development, as is confirmed in a systematic review with meta-analysis by Vandenplas et al. (23). Sinai et al. also confirmed this by conducting the first prospective, physical examination-based study, demonstrating no association between SIF consumption and growth and puberty parameters (49). Given that data are conflicting on these points, as a precaution, the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) recommends that children under 6 months of life should not be fed with soy milk as their only source of nutrition (31, 50).

The other concern about SF is the use of transgenic soybeans. Although available data suggest no deleterious effects on the human genome, concerns about the use of transgenic food persist (51). Since soy has a long history of successful use in managing CMA in infants, to better predict the usefulness of soy proteins for controlling food allergies, it is important to understand the relative allergenic reactivity of soy compared with other major food. The antigenicity of soy has been suspected since 1934 (52) and documented in 1982 by Eastham et al. (53). Several studies have measured the proportion of infants with documented CMA that developed soy allergy when SF was used as a replacement for cow's milk formula. Zeiger and Sampson (54) evaluated 93 children <3.5 years of age with documented IgE-associated CMA by double-blind, placebo-controlled food challenge: soy allergy occurs in 14% of children with IgE-associated CMA, whereas Businco et al. (55) implicated soy allergy in 4% of 143 children. Bock and Adkins (56) reported that 7% of CMA infants developed soy allergy when switched to SF. In the study by Klemola et al., 10% of CMA infants developed SF allergy, and adverse reactions to soy occurred more frequently in children under 6 months of age than those of 6–12 months (5 out of 20 vs. 3 out of 60) (57). These studies show that SF can be a safe alternative to cow's milk in children with IgE-associated CMA and reported that infants allergic to cow's milk could be effectively managed with SF if soy tolerance is proved at the time of introduction of SF.

This overall performance approaches the clinical standard for hypoallergenic formula, where a hypoallergenic formula is a formula that meets the criterion characterized by a clinical tolerance of 90% in children with certain CMA (95% confidence that 90% of allergic infants will not react) (58). Nevertheless, given all these pros and cons, the ESPGHAN recommends not using soy in infants with food allergy during the first 6 months of life and not in preterm infants (50). For the management of CMA, it is preferable to use eHFs of cow's milk proteins in the first instance (59). Growth patterns, bone health, and metabolic, reproductive, endocrinological, immune, and neurological outcomes of SF-fed infants are similar to those observed in children fed with CMF (23). Therefore, after the first 6 months of life, SFs may be considered if tolerance to soy proteins is established, and SF can be suggested as a first-choice alternative for infants >6 months of age with CMA.

Could Soy-Protein Formulas Be Used for the Prevention of Cow's Milk Allergy?

Prevention of allergic diseases in high-risk children is a difficult challenge, and at the moment, only breastfeeding is suggested as useful in these patients; if breastfeeding is not possible, the recommended approach is a diet with CM eHF, although rice or soy infant formula can be considered as a second option (60, 61). A study on SFs used for the prevention of CMA published in 1953 reported that SFs prevented the onset of a food allergy if given to atopic children (62), although the results were criticized mainly because of the lack of a control group. Among other studies published later, some have concluded recommending the use of SFs because subjects showed fewer allergic reactions than did infants fed with cow's milk formulas, while others found a similar frequency of allergic manifestations with both formulas. Bardare et al. (63) confirmed the preventive effect of SFs in a large prospective study including 391 atopic infants. At the end of the first step (1 year of life), 13% of the participants in the study group and 29% of the participants in the control group had an atopic disease.

On the contrary, a meta-analysis by Osborne et al. published in 2004 showed contradictory results: feeding with an SF should not be recommended for the prevention of allergy or food intolerance in infants at high risk for these diseases (64).

The argument is still greatly controversial, mainly because most of the studies that have been conducted either lacked scientific criteria for diagnosing soy allergy or misinterpreted the conclusions. Thus, further studies are necessary to study not only the prevalence of soy allergy in children with CMA and the entire pediatric population but also the preventive effect of soybean on allergic disease development. In addition, we suggest planning double-blind placebo-controlled oral food challenge studies in larger cohorts of children to compare the efficacy and safety of SFs in children with IgE-mediated CMA and to determine further ways to prevent CMA in atopic and allergic children.

USE OF SOYBEAN AND ITS DERIVATES IN PLANT-BASED DIETS

One of the main factors that has influenced the popularity of soy foods is the ever-growing interest of the population toward plant-based diets and their many health benefits, together with the recognized high protein quality of soy (31, 65–67).

Plant-Based Diets

The term "plant-based diets" includes many different dietary patterns that comprise higher quotas of plant products (vegetables, fruits, whole grains, legumes, nuts, and seeds) than animal ones (66). Specifically, there is evidence that soy foods may reduce low-density lipoprotein (LDL)-cholesterol levels and modestly lower blood pressure, thus reducing the risk of coronary heart disease (68). Nevertheless, further research is required before unequivocal conclusions can be made. By definition, a vegetarian diet is one that excludes the consumption of meat and fish. Its most common variant is lacto-ovo-vegetarianism, which allows the consumption of eggs, milk, and cheese. Conversely,

the most restrictive variant of plant-based diet is veganism, which prohibits all animal-derived food (65, 69, 70).

It is important to emphasize that the health benefits gained from adhering to a vegetarian or vegan diet can be obtained also through an omnivorous diet, involving the reduction of meat and an increase in the intake of plant foods (66, 69). Together with the well-known health benefits, plant-based diets are associated with less environmental impact. However, a sustainable diet can also be achieved with dietary patterns characterized by low to moderate amounts of animal-based food and a high intake of plant-based food, such as the Mediterranean diet (66).

In Italy, the percentage of people following a vegetarian diet (vegan or lacto-ovo-vegetarian) has doubled in the past 5 years and is around 7.3% (71). Although it is not possible to know the exact number of vegetarian children, vegetarian parents frequently choose vegetarian diets for their children (72).

Plant-Based Diets and Nutritional Requirements for Children

Well-planned plant-based diets can provide adequate nutrition requirements throughout all stages of life. Nevertheless, special attention should be paid to children following a vegetarian or vegan diet, especially during complementary feeding (73, 74). Indeed, although theoretically a vegan diet can meet nutritional requirements when medical and dietary advice regarding supplementations is followed, the risk of severe deficiency, especially for vitamin B12, is present. Therefore, if parents choose a vegan diet for the complementary feeding of their infant, regular medical and dietetic supervision is necessary to avoid the risk of irreversible cognitive damage.

Diets derived from the VegPlate Junior (VPJ) method, based on six foods including grains, protein-rich foods, vegetables, fruits, nuts and seeds, and fats, have recently been considered well-planned plant-based diets (75). Regarding protein-rich foods, since soy and its derivatives have essential amino acids in a proportion similar to those in animal foods, their consumption should be encouraged.

Another criterion to define a plant-based diet as well-planned is to consume a wide variety of plant foods (73). Being plant food sources of protein very variable in terms of digestibility, quality, and composition of essential amino acids, to reach adequate protein requirement, it is important to combine different plant foods to provide all the essential amino acids. In the case of soy, for example, since methionine is an essential limiting amino acid, it would be optimal to combine it with cereals that are rich in methionine but lack lysine.

The risk of nutritional deficiencies in plant-based diets increases the more restrictive the diet is and the younger the child (69). In the first year of life, the infant can be fed either breast milk or formula milk, and at this age, the only alternative to cow's milk formulas are plant-based formulas such as soy-based formula (72).

Soy-Based Beverages and Foods

Despite being called soy milk on the market, it is important to highlight that soy beverages are not nutritionally comparable with milk (69) (**Table 2**). Regarding this issue in Europe, to fight misleading labels, Council Regulation 1234/2007 prohibits the use of the word "milk" for drinks that are not made from mammary secretion (76).

Since they can cause severe nutritional deficit (77), these drinks should not be chosen as a cow's milk alternative in children younger than 24 months (78). Indeed, soy beverages have lower energetic levels and contain fewer carbohydrates, fats, calcium, and vitamin B12, than have cow's milk. However, compared with CM, they are a good source of trans fats, monounsaturated fatty acids (MUFAs), and PUFAs (ALA and LA). In addition to the above-mentioned advantages, soy drinks have a higher protein quality than other plant-based drinks; furthermore, they are lactose and cholesterol free (79).

Soy may have a role in childhood nutrition also during complementary feeding, such as tofu, which is obtained by curdling the liquid extracted from pressed soybeans. Many vegetarian or Asian mothers choose to use tofu because it has a soft consistency that makes it easy to blend with other cereals, has great palatability, and is rich in calcium, which makes it nutritionally adequate for a baby or a toddler (21).

SOY-BASED FORMULA AND PRETERM INFANTS

Although the soy-based formula is considered safe for healthy and full-term infants, both the American Academy of Pediatrics (AAP) and the ESPGHAN do not recommend its use in preterm infants (25, 50, 80).

Aluminum Content and Risk of Osteopenia in Preterm Infants

Taking into account that aluminum competes with calcium absorption, preceding studies have reported that the consumption of soy-based formulas by preterm infants could increase the risk of osteopenia due to their higher aluminum content as compared with cow's milk-based formula and breast milk (17, 18, 24, 81). To solve this issue, modern soy-based formulas have been supplemented with high amounts of phosphorus and calcium, while the aluminum content has been maintained lower than the tolerable intake, according to the Joint WHO/FAO Expert Committee on Food Additives (21, 82). However, the long-term effects of early aluminum exposure have not been fully elucidated yet (23, 25). In a random, controlled study of very low birth weight (VLBW) infants from 3 to 8 weeks of age, Hall et al. (83) reported that VLBW infants randomized to be soy-based formula-fed supplemented with calcium, phosphorus, and vitamin D, showed a lower weight gain and serum protein concentration than those randomized to be whey-predominant premature infant formula-fed. In light of the specific high nutritional requirements and vulnerability of preterm infants, further insights are needed before their use can be proposed in preterm infants (12).

KEY MESSAGES

Modern soy-based formulas are adequate to ensure normal growth patterns in healthy infants, but they have few indications as a substitute for cow's milk formulas: allergy or intolerance to cow's milk, galactosemia, severe persistent lactose intolerance, post-infection diarrhea, and vegan diet.

Formulations have changed over the years to improve digestibility, nutritional values, availability of minerals, and protein quality, and the data suggest that modern soy-based formulas are well-tolerated and support normal growth patterns and nutritional status in healthy term infants.

In infants with cow's milk allergy, soy-based formulas may be considered as a first choice alternative to CMF only after 6 months of life, if tolerance to soy protein is established, although soy-based drinks should not be used as a substitute for cow's milk in children <24 months old.

The ESPGHAN and AAP recommend not using soy in infants with food allergy during the first 6 months of life and not using soy in preterm infants.

It is still unclear if the routine use of soy-based formulas may have roles in the prevention of allergic diseases, and further large-scale studies are required to clarify the safety of soy and its use for the treatment of cow's milk allergy.

CONCLUSIONS

Since their first use as cow's milk substitute formulas for children with CMA, many changes have occurred over the years to improve digestibility, nutritional values, and protein quality of soy-based formulas.

Modern soy-based formulas appear to be adequate to ensure normal growth patterns and development in healthy infants, although they do not appear to have nutritional advantages over cow's milk formulas. Indeed, in term infants, although isolated soy-protein based formulas may be used to provide nutrition, there are few indications for their use as a substitute of cow's milk formulas: indications include allergy/intolerance to cow's milk, galactosemia, severe persistent lactose intolerance, post-infection diarrhea, or vegan diet. Beyond these, there are no valid indications for replacing cow's milk with soy-based drinks, also remembering that, up to 6 months of life, HM remains the best way to feed infants. It is still unclear if soy protein formulas may have roles in the prevention of allergic diseases, but, for CMA infants, they certainly represent a valid, economic, and well-tolerated alternative to eHFs.

Worries are related to the use of soy in children regarding phytoestrogens and the use of transgenic soy, with their possible hormonal effects on the reproductive system, neurodevelopment, obesity, and gut microbiota development. There is no conclusive evidence that soy isoflavones can adversely affect development, especially the reproductive system and endocrine function, although there are little data available on the potential effects of phytoestrogens in young children on subsequent sexual and reproductive development. Similar considerations hold for use of transgenic soy: the available data suggest no deleterious effects on the human genome. Concerning growth patterns, clinical studies have shown that during the first years of life, there are no significant differences between infants fed SF and those fed cow's milk formulas. As the matter is still debated, the ESPGHAN and AAP recommend not using soy in infants with food allergy during the first 6 months of life and not using soy formulas in preterm infants. After 6 months of life, SFs may be considered if tolerance to soy proteins is established and SF can be recommended as a first-choice replacement for infants >6 months of age with cow's milk allergy.

Although significant advancements have been made in recent years in our understanding of soy properties, substantial gaps in our knowledge still exist; for many reasons, it is still difficult to establish whether soy-based food consumption early in life is safe and beneficial; thus, we recommend that soy drinks should not be used as a substitute for infant formulas or cow's milk in children younger than 24 months. Further additional studies will be needed to clarify the effects of soy on the reproductive system, long-term effects on neurodevelopment, the effects of glyphosate, effects on the microbiome, and, generally, all the long-term consequences of soy.

AUTHOR CONTRIBUTIONS

EV, LC, GN, and DP made a substantial contribution to conception, design, and acquisition of data. EV, LC, GN, LR, GV, ED'A, MG, GZ, and DP drafted the manuscript and critically reviewed it for important intellectual content. All authors gave the final approval of the version to be published.

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REVIEW
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The Role of Prebiotics and Probiotics in Prevention of Allergic Diseases in Infants

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Allergic diseases have been linked to genetic and/or environmental factors, such as antibiotic use, westernized high fat and low fiber diet, which lead to early intestinal dysbiosis, and account for the rise in allergy prevalence, especially in western countries. Allergic diseases have shown reduced microbial diversity, including fewer lactobacilli and bifidobacteria, within the neonatal microbiota, before the onset of atopic diseases. Raised interest in microbiota manipulating strategies to restore the microbial balance for atopic disease prevention, through prebiotics, probiotics, or synbiotics supplementation, has been reported. We reviewed and discussed the role of prebiotics and/or probiotics supplementation for allergy prevention in infants. We searched PubMed and the Cochrane Database using keywords relating to "allergy" OR "allergic disorders," "prevention" AND "prebiotics" OR "probiotics" OR "synbiotics." We limited our evaluation to papers of English language including children aged 0–2 years old. Different products or strains used, different period of intervention, duration of supplementation, has hampered the draw of definitive conclusions on the clinical impact of probiotics and/or prebiotics for prevention of allergic diseases in infants, except for atopic dermatitis in infants at high-risk. This preventive effect on eczema in high-risk infants is supported by clear evidence for probiotics but only moderate evidence for prebiotic supplementation. However, the optimal prebiotic or strain of probiotic, dose, duration, and timing of intervention remains uncertain. Particularly, a combined pre- and post-natal intervention appeared of stronger benefit, although the definition of the optimal intervention starting time during gestation, the timing, and duration in the post-natal period, as well as the best target population, are still an unmet need.

Keywords: prebiotics, probiotics, prevention, atopic dermatitis, eczema, synbiotics, allergic diseases

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INTRODUCTION

Allergic diseases represent a medical challenge and a worldwide burden, in particular in the most developed countries, where the frequency of affected subjects overcome 30% and is still growing (1, 2). Allergic diseases are the result of a complex interaction genome-environment which leads to an alteration of the immune system (3, 4). A lot of genes, HLA, and specific genes identified by genome-wide association studies, have been identified for asthma (5, 6), food allergy (7), and atopic dermatitis (8, 9). In infants, allergic disease prevalence has been associated with the allergic status of the parents, being ∼10% in those with a negative family history of atopic disorders and 20–30% in those with allergy in their first-degree relatives (10). Although genetic factors can affect the tendency to the development of allergic diseases, the rapid rise of allergic diseases in the last two decades can be explained by environmental factors (11). A lot of factors related to the environment have been called in cause to explain the rise, especially in western countries. These include mode of delivery, with cesarean delivery representing a risk for atopy, food allergy and asthma (12), antibiotic use (13), westernized high fat and low fiber diet (14, 15), reduction of omega-3-polyunsaturated fatty acids and vitamin D insufficiency or deficiency (16). All the above act on microbiota (**Figure 1**), which an increasing body of evidence suggests to play a central role in shaping the normal development, and maturation of the immune system (17). Some of the effects on immune programming are thought to be due to epigenetic effects on the expression of genes (18, 19).

In a healthy state, the gut microbiota is in eubiotic status; in contrast, gut dysbiosis, an imbalance in the composition and/or function of the gut microbiota, has been associated with allergic diseases, such as eczema, asthma, and food allergy (20–23). Animal and human studies have found that subjects with allergic disease are carriers of reduced microbial diversity and different proportions of certain microbial species (24).

The establishment of an altered gut microbiota seems to occur in the early stage of development, as demonstrated by studies that have shown that atopic infants vs. non-atopic infants at 1 year of age had different gut composition at 3 weeks of age and 3 months (25). These differences, with allergic diseases showing reduced microbial diversity, including fewer lactobacilli and bifidobacteria, were observed before the onset of clinical symptoms, supporting their possible causative role in allergic diseases (24, 26–28). In agreement with these observations, more recently West et al. (29) reported that the development of atopic eczema is influenced by lack of immune system modulation after birth, mediated by the gut microbiota. The majority of molecular data suggest that gut colonization occurs through contamination shortly after delivery (30, 31) although some recent experiments suggest that it might take places already *in utero* and then further shaped post-natally (32). Therefore, it has been speculated that the recent increase in the prevalence of allergy may be consequent to early intestinal dysbiosis (33). The above hypothesis and observations aroused the interest of research for shaping gut microbiota in the early stages to prevent the development of allergic diseases. Different strategies have been studied, including probiotic, prebiotic, synbiotic supplementation pre-natally, post-natally, or both (34).

Probiotics are "living microorganisms that, at certain doses, may provide health benefits" (10). Probiotics affect phagocytosis and synthesis of pro-inflammatory cytokines, and thus have been proposed as modulators of the allergic response and advocated as therapeutic and preventive interventions for allergic diseases (35, 36). Prebiotics are "non-digestible food components that selectively promote the growth of intestinal microbes with positive effects on host health" (37, 38), specifically stimulating the expansion of bifidobacteria and lactobacilli species (39). Altering the intake with foods of these components or supplementing the diet with prebiotics can modify in a positive manner the proportions and the activity of certain intestinal microbial species (39). Synbiotics are "combinations of prebiotics and probiotics" (40).

The aim of this narrative review was to provide researchers an updated overview on the use of prebiotics, probiotics, and synbiotics for the primary prevention of allergy in infants, highlighting the controversies, current research gaps, and potential developments in the field. This review considered the administration of probiotics as supplements, excluding the possible exposure through common food, naturally containing probiotics (such as fermented milk, yogurt). We searched the Cochrane library and PubMed, (Embase, Medline,) during the last 20 years, up to March 2020, using as keywords the following: "allergic diseases," "food allergy," "allergy prevention," "allergic proctocolitis," "atopic dermatitis," "wheezing," "eczema," "allergic rhinitis," "atopic disease," "prebiotics," "probiotics," "synbiotics," "prevention." We limited our evaluation by age ("children," "aged 0–2 years") and languages (English); however, to be more inclusive, the operators "AND" "OR" were also used. Intervention controlled trials, reviews, meta-analyses, and guidelines on prebiotics and probiotics were considered, as well as the following populations for possible supplementation with probiotics: pregnant women, breastfeeding mothers, and infants, regardless of exclusive breastfeeding. All types of prebiotics and probiotics and doses of supplementation were evaluated.

MECHANISMS OF PROBIOTICS/PREBIOTICS IN MODULATING GUT MICROBIOTA

Diet is recognized as one of the most important factors which may modulate the gut microbiota composition and function (41). It is well-known that changes in the composition of the diet, such as westernized high fat and low fiber diet, can modify the prevalence and types of intestinal microbial species, as certain species are more suitable to utilize specific substrates (42). An association between low-fiber diet and non-communicable chronic diseases, including allergic diseases have been hypothesized on the basis of observational studies (43). The positive effects of the diet on gut microbiota are hypothesized to be due to the prebiotic component.

As anticipated, prebiotics "are non-digestible food components" that selectively trigger the growth in the gut

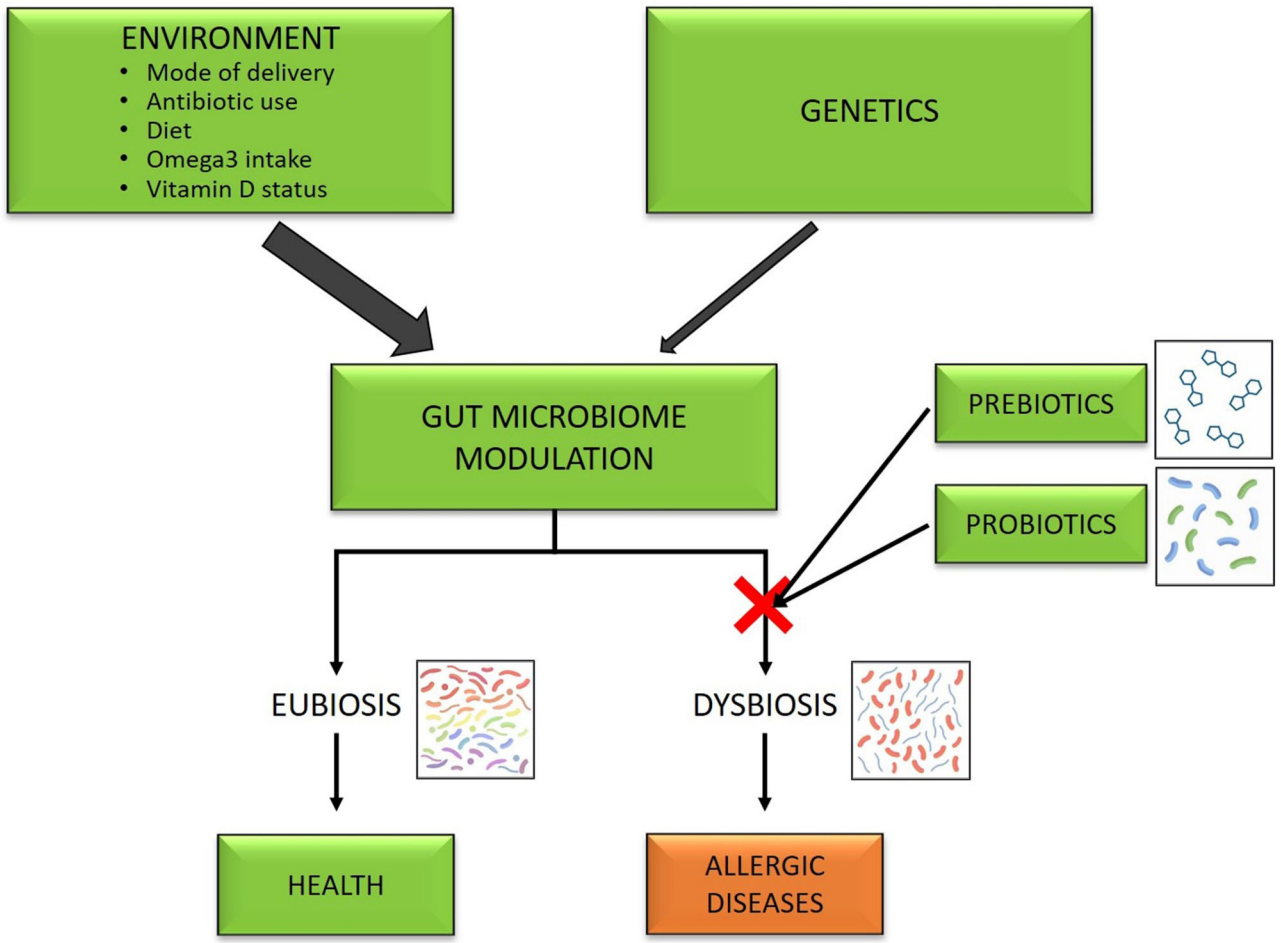

FIGURE 1 | Gut microbiota as a target of prevention of allergic diseases.

of microbes with positive effects for host health (38): not being the target of upper gastrointestinal digestive enzymes, they typically reach intact the colon, where they are fermented by intestinal microbes (main endproducts of their fermentation are short-chain fatty acids) and selectively stimulate the growth of those intestinal microorganisms (bifidobacteria and lactobacilli species) that are associated with host health and well-being (44). Indeed, they are the favorite meal of the saccharolytic bacteria living in the human gut, as different bacteria prefer other energy sources. Prebiotics are naturally contained in cereals, fruits, vegetables, etc. (non-digestible oligosaccharides), or can be produced by industry (38, 45). Consequently, by modifying the intake of foods containing these products or by supplementation with prebiotics, diet can be used as a powerful tool to direct the gut microbial population (46, 47).

Firstly, prebiotics are naturally present in human milk, who contains at least 200 human milk oligosaccharides (HMO), while oligosaccharides are virtually absent from cow's milk, which explains the increase of gut bifidobacteria observed in breastfed infants compared with standard formula-fed (SF) (48, 49). Human milk oligosaccharides (among the widest components in human milk together with lactose and fats), may represent an excellent meal for beneficial species and prevent the adhesion of pathogens, contributing to the shift of the infant gut microbiota, influencing the immune system (50) and infants health (51). Different HMOs have different properties and functions (52); their molecular structure differs in size and sequence among women (53), being influenced by certain factors (lactation period, secretor status, maternal Lewis Blood Group, etc.), and giving the infant a different degree of protection. A recent study showed infants receiving human milk with a low Lacto-N-fucopentaose III (LNFP) content were more prone to develop Cow's Milk allergy (CMA) compared to infants fed with milk containing a high concentration of LNFP III (OR: 6.7, 95% CI 2.0–22) (54). However, the role of breastfeeding (BF) in preventing allergic diseases is still debated (55), with studies showing no protective effect or even an increased risk for AD with prolonged exclusive breastfeeding (40, 56–58), while other studies/systematic reviews reporting positive effects on prevention, mostly of AD (39, 59). From a mechanistic point of view, BF is thought to prevent

allergy development through its content of allergens and immune mediators, absent in artificial milk (55, 60), as well as of HMOs known to stimulate a gut microbiota that might induce tolerance (61). Consequently, when breastfeeding is not possible, trying to reproduce the functional effects of HMOs, infant formulas have been supplemented with galactooligosaccharides and/or fructooligosaccharides. Studies in term and preterm infants indicate that a short-chain galacto-oligosaccharides (scGOS)/long-chain fructo-oligosaccharides (lcFOS) mixture has prebiotic activities, producing a gut microbiota similar to that of breastfed infants (62–64).

Moreover, in murine models, galacto-oligosaccharides (GOS) have been shown to improve the skin lesions of atopic dermatitis, by inducing the production of IL-10 and blocking the production of pro-inflammatory cytokines, such as IL-17, (65). In addition, prebiotics were reported to decrease Ig free light chain (Ig-fLC) concentrations in infants at high-risk for allergies (66): Ig-fLC might play a role in the pathophysiology of the allergic disease since increased Ig-fLC content was found in patients with AD, allergic rhinitis, asthma or cow's milk allergy. However, overall, the mechanism of action of prebiotics seems mostly due to the previously described indirect effects on gut microbiota.

Another approach used for shaping gut microbiota is the supplementation of the diet with probiotics. Among ligands for "pattern recognition receptors," Toll-like receptors (TLRs), able to activate the immune system, such as virus and the recently identified virus-derived synthetic RNA-DNA hybrids, Bacteria are considered the most powerful immunomodulating factors (39).

The possible mechanism of action of probiotics in this regard includes influences on the maturation of intestinal barrier and on immune response by rebalancing Th1 and Th2 response while suppressing Th17 cells, promoting Tolerogenic Dendritic and Regulatory T (Treg) cell development, and pattern recognition receptor (TLR) stimulation (67). In fact, dendritic cells within the gut mucosa play a key role in the differentiation of regulatory T cells (Treg) which are known to be important in the development of immune tolerance (68). Alterations in Treg functions are associated with the development of allergic diseases (69) and evidence indicates that the gut microbiota acquired early in life is essential for the right development of Treg and Th1/th2 balance (70). The possible mechanisms whereby probiotics may obtain atopy prevention include the stimulation of Th1 response and a decrease in the secretion of Th2 cytokines, such as interleukin (IL)-4, IL-5, and IL-13, a decrease in IgE, and a rise of C-reactive protein and IgA (41). In addition, a murine model of asthma showed that neonatal supplementation with probiotics inhibits the development of allergic sensitization and airway disease by inducing regulatory T cells (Tregs) and producing transforming growth factor-B (71).

Indeed, selected strain of probiotics (such as *Lactobacillus* GG) provides maturational signals for the gut-associated lymphoid tissues (GALT) and development of Tolerogenic dendritic and regulatory T (Treg) cell differentiation, which will induce intestinal barrier maturation and reduce the prevalence of the allergic reactions (72). Therefore, by improving the barrier function and reducing the leakage of antigens through the mucosa probiotics may reduce the potential exposure to allergens (73). Moreover, specific probiotics demonstrated local and systemic anti-inflammatory effects referred to increased secretion of IL-10 (67). Other researchers suggested as a possible mechanism of action of probiotics, in regard to protection against allergic diseases, also the stimulation of Toll-like receptors, which induce the production of mediators, e.g., IL-6, and further IgA secretion (74).

In addition, through increased production of secretory IgA, which contributes to the exclusion of antigens from the intestinal mucosa (75) probiotics may obtain direct modulation of the immune system and eventually prevention of allergic diseases (76).

Moreover, colonizing the mother pre-natally by probiotics supplementation, together with subsequent changes in her breast milk composition and cytokines pattern, with an increased concentration of transforming growth factor-beta (TGF-b), could be beneficial for the infant regarding allergy development (77) and acquisition of immunotolerance (78).

In summary, the probiotics could potentially produce local effects, such as permeability reduction and thus systemic antigens penetration, increased local IgA production, and tolerance induction. Moreover, anti-inflammatory effects mediated by Toll-like receptors, the stimulation of Th1 response to allergens, the activation of tolerogenic dendritic cells, and the production of Treg are among the systemic effects of probiotics (75, 79).

However, despite the evidence on possible mechanisms of action of different preventive strategies, studies evaluating the efficacy of prebiotic and/or probiotic supplementation in the prevention of allergic diseases have yielded conflicting results.

PREBIOTICS TO PREVENT ALLERGIC DISEASES

The bifidogenic effect of human milk (rich in oligosaccharides) is well-known. Prebiotics have long been added to infant milk formulas to mimic these functional characteristics of breast milk (52, 80, 81). A combination of galacto-oligosaccharide (GOS) and fructo-oligosaccharide (FOS) (scGOS 90% plus lcFOS 10%) was prebiotic of choice in a number of intervention trials. Acidic oligosaccharides (AOS), polydextrose (PDX) (with or without lactulose), different content of lactose, oligofructose plus inulin have also been tested (**Table 1**). Modification of intestinal microbiota represents the principal way by which this effect has been orchestrated (93) and has been reported in several studies (82, 90, 92, 94, 95). The 2′-fucosyllactose (2′-FL) human milk oligosaccharide (HMO), the most plentiful HMO in most human milk, has been recently synthesized and is now commercially available in few supplemented infant formulas, bringing the composition closer to human milk (95).

We summarize the evidence on the preventive effects of different prebiotic administration in **Table 1**. The majority of these studies evaluated the effects of prebiotics on atopic dermatitis (AD); other allergic manifestations were much less investigated; however, it remains unclear whether prebiotics supplementation can prevent allergic diseases

TABLE 1 | Prebiotics administration in prevention of allergic disorders.

(A) Prebiotic + Standard formula (or prebiotic of human milk).

References	Study	Enrolled patients	Prebiotic + Standard formula (or prebiotic of human milk)	Prebiotic substance, Beginning of treatment (S), End of treatment (E).	Outcomes	Follow-up (duration)
Ziegler et al. (82)	double-blind, randomized, controlled, parallel-group, prospective trial	226 healthy term infants in 3 groups: - 58 in control group: control formula only - 58 in PG4 group: control formula +4 g/L prebiotic mixture - 48 in PGL8 group: control formula +8 g/L prebiotic mixture	Control formula added with a prebiotic mixture (4 g/L) of PDX and GOS, 50:50 ratio (PG4 group) Control formula containing a prebiotic mixture (8 g/L) of PDX, GOS, and LOS, 50:33:17 ratio). (PGL8 group)	S: 14 days of age E: 120 days of age	Infants fed formula containing a prebiotic mixture achieved normal growth and stool characteristics more similar to those of breast-fed infants (softer, looser) compared to infants fed an unsupplemented formula. Statistical difference among adverse events: - Eczema (PG4 vs. control: 18 vs. 7%, $P = 0.046$; PG4 vs. PGL8: 18 vs. 4%, $P = 0.008$) - Diarrhea: control vs. PG4: 4 vs. 18%, $P = 0.008$) - Irritability: control vs. PGL8, 4 vs. 16%, $P = 0.027$)	120 days
Niele et al. (83)	Double-blind, randomized placebo controlled trial	113 preterm infants (GA<32 weeks or Wt <1.500 gr) 94/98 infants eligible at the corrected age of 1 year participated in the follow-up study	Prebiotic mixture: 80% scGOS/lcFOS and 20% pAOS Placebo mixture: maltodextrin in increasing dose for 30 days. After discharge, all infants received Human Milk or Nenatal Start or Nenatal 1 (both without oligosaccharides or probiotics)	S: <3days of life E: 30 days of life	Supplementation with non-human neutral and acidic oligosaccharides during the neonatal period in preterm infants did not significantly decrease the incidence of allergic and infectious diseases during the 1st year of life (AD at 1 year: 15 vs. 19%)	12 months
Gruber et al. (84)	double-blind, placebo-controlled, randomized prospective nutritional intervention study	Healthy term infants with low atopy risk: - 414 infants in prebiotic group (PG) - 416 infants in control group (CG). - 300 infants in breast-feeding group (BG)	PG: regular formula containing a specific mixture of neutral oligosaccharides [scGOS/lcFOS, ratio 9:1, (85 wt%),] and pectin-derived acidic oligosaccharides OS) (15wt%) CG: Standard formula without oligosaccharides. BG: Breast milk	S: before post-natal age of 8 weeks E: 12 months	Formula containing a mixture of neutral oligosaccharides was effective as primary prevention of atopic dermatitis in low atopy risk infants (5.7% in PG vs. 9.7% in CG, $P = 0.04$; 7.3% in BG)	1 year
Gruber et al. (85)	double-blind, controlled, randomized prospective nutritional intervention study	Healthy term infants with low atopy risk: - 232 infants in prebiotic formula group (PG) - 243 infants in control formula group (CG) - 197 infants in breast-feeding group (BG)	PG: regular formula containing aspecific mixture of neutral oligosaccharides [scGOS/lcFOS, ratio 9:1, (85 wt%),] and pectin-derived acidic oligosaccharides OS)(15wt%) CG: Standard formula without oligosaccharides. BG: Breast milk	S: before post-natal age of 8 weeks E: 12 months	The cumulative incidence of AD up to age 5 years was 18.2% (PG) 20.2% (CG) and 23.9% (BG), therefore in this follow up study there was no sustained statistically significant effect of prebiotics added to infant diet against the occurrence of early AD at preschool age	5 years

(Continued)

TABLE 1 | Continued

References	Study	Enrolled patients	Prebiotic + Standard formula (or prebiotic of human milk)	Prebiotic substance, Beginning of treatment (S), End of treatment (E).	Outcomes	Follow-up (duration)
Pontes et al. (86)	double-blind, randomized, controlled trial	healthy children (1–4 years of age) 125: CMBB with DHA,PDX,GOS, β-glucan, and other key nutrients 131: control	Cow's Milk-Based Beverage (CMBB) containing DHA, the prebiotics polydextrose (PDX) and galactooligosaccharides (GOS), β-glucan, and other nutrients including zinc, vitamin A and iron	S: 1–4 years of age E: 28 weeks later	CMBB was associated with fewer episodes of allergic manifestations (atopic dermatitis, wheezing, allergic rhinitis) compared to controls ($p = 0.021$)	28 weeks.
Ranucci et al. (87)	randomized, double-blind, placebo-controlled trial	118/201 infants who received a prebiotic (GOS/PDX)-enriched formula (PF) completed the study 104/199 infants who received an SF until 48 weeks of life completed the study 123/140 infants who remained on exclusive breastfeeding until six months of age completed the study	prebiotic (mixture of 4 g/L of GOS/PDX)-enriched standard formula (PF) vs. identical standard formula without prebiotic	S: birth E: 48 weeks of life	There were not significant differences in the cumulative incidence, intensity and duration of AD among groups. However, the risk of AD in PF was reduced by 35% compared with SF. Bifidobacteria and Clostridium clusterI colonization increased in the PF group. Bifidobacteria was associated with RIs protection, whereas Clostridium cluster I had a protective role in atopy development	96 weeks

(B) Prebiotic +Hydrolyzed/ amino acid-based formulas.

References	Study	Enrolled patients	Hydrolyzed/ amino acid-based formulas+ Prebiotic substance	Prebiotic substance, Beginning of treatment (S), End of treatment (E).	Outcomes	Follow-up (duration)
Moro et al. (80)	Prospective randomized, double-blind placebo controlled trial	206/259 infants at high risk of atopy completed the study: 102 infants in the prebiotic group; 104 infants in the placebo group	Extensively hydrolysed cows'milk whey protein formula supplemented either with 8 g/L scGOS/lcFOS / (prebiotic group) or a 8 g/L maltodextrin (placebo group)	8 g/L scGOS/lcFOS S: within the first 2 weeks of life E: 6 months	The cumulative incidence of AD atopic dermatitis was significantly reduced at 6 months of age by prebiotics supplementation (9.8 vs. 23.1%, $p < 0.05$)	6 months
Arslanoglu et al. (88)	Prospective randomized, double-blind placebo controlled trial	134/152 infants at high risk of atopy completed the study 66 in the prebiotic group 68 in the placebo group	Extensively hydrolysed cows'milk whey protein formula supplemented either with 8 g/L scGOS/lcFOS / (prebiotic group) or a 8 g/L maltodextrin (placebo group).	8 g/L scGOS/lcFOS S: within the first 2 weeks of life E: 6 months	Cumulative incidences of AD, recurrent wheezing, and allergic urticaria were significantly reduced at 2 years of age by prebiotics supplementation (13.6%, 7.6%, and 1.5 vs. 27.9%, 20.6% and 10.3% respectively, $p < 0.05$)	2 years
Arslanoglu et al. (89)	Prospective randomized, double-blind placebo controlled trial,	92 infants at high risk of atopy completed the study 42 in the prebiotic group 50 in the placebo group	Extensively hydrolysed cows'milk whey protein formula supplemented either with 8 g/L scGOS/lcFOS / (prebiotic group) or a 8 g/L maltodextrin (placebo group).	8 g/L scGOS/lcFOS S: within the first 2 weeks of life E: 6 months	Cumulative incidences of any allergic manifestations and atopic dermatitis were significantly reduced at 5 years of age by prebiotics supplementation (30.9%, and 19.1 vs. 66 and 38%, respectively, $p < 0.05$)	5 years

(Continued)

TABLE 1 | Continued

References	Study	Enrolled patients	Hydrolyzed/ amino acid-based formulas+ Prebiotic substance	Prebiotic substance, Beginning of treatment (S), End of treatment (E).	Outcomes	Follow-up (duration)
Francavilla et al. (90)	Prospective two-phases clinical trial (cross-over design)	21 infants with a confirmed CMA 15 healthy breast-fed infants as controls	Phase 1: extensively hydrolyzed formula without lactose for 2 months Phase 2: an identical extensively hydrolyzed formula containing lactose (3.8%) for an additional 2 months	3.8% Lactose	The addition of lactose to an extensively hydrolyzed formula increased the total fecal counts of Lactobacillus/Bifidobacteria, the concentration of total short-chain fatty acids, mostly acetic and butyric acids and decreased the counts of Bacteroides/Clostridia	4 months
Boyle et al. (91)	double-blind, randomized, controlled parallel-group nutritional intervention trial	863 high-risk infants: - 432 infants in the prebiotic group (PG) - 431 Infants fed with standard foumula (CG	PG: partially hydrolysed whey-based infant formula containing a specific mixture of neutral scGOS and lcFOS (9: 1; 85 weight per cent, 0.68 g/100 ml) and acidic pAOS (15 weight per cent, 0.12 g/100 ml acidic) (pHF-OS)	S: before 18 weeks of life E: 6 months	pHF-OS did not prevent eczema in high-risk infants in the first 12 months (Eczema occurred in 30.8% pHF-OS vs. 30.3% control in all infants (OR 0.99 95% CI 0.71, 1.37; $P = 0.94$). as well as by 18 months. However, pHF-OS reduced cow's milk-specific IgG1 ($P < 0.0001$)	12 and 18 months
Wopereis et al. (92)	Double-blind, randomized, controlled, parallel group nutritional intervention trial	138 Infants at high risk: - 51 infants in the prebiotic group (PG) - 57 Infants fed with standard foumula (CG) - 30 infants in the breast-fed group (BG)	PG: Partially hydrolyzed formula containing short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides (9:1; 0.68 g/100 mL) and pectin-derived acidic oligosaccharides (0.12 g/100 mL) CG: standard formula BG: breast milk	S: before 18 weeks of life E: 26 weeks of age	Infants with eczema at 18 months: 32% in CG, 39% in PG and 47% in BG Infants presenting eczema at 18 months showed a decrease in acquisition of *Eubacterium* and *Anaerostipes* species with increased lactate and reduced butyrate levels	18 months

Cow's milk protein allergy (CMA), Healthy controls (HC), Human milk oligosaccharides (HMO), short-chain galactooligosaccharides (scGOS), long-chain fructooligosaccharides(lcFOS), human milk oligosaccharides (HMOS), prebiotics polydextrose (PDX), galactooligosaccharides (GOS), galacto-oligosaccharide/polydextrose (GOS/PDX), Probiotic formula (PF), atopic dermatitis (AD), cow's milk-based beverage (CMBB).
Standard formula (SF), Breast feeding (BF).

due to heterogeneity of the studies and type of prebiotics. In a longitudinal cohort study enrolling 259 high-risk infants, Moro et al. (80) found that a hydrolyzed protein cow's milk-based formula supplemented with 90% scGOS−10% lcFOS, (8g/L) significantly reduced AD at the age of 6 months [intervention group: 9.8 vs. 23.1% placebo group ($P < 0.05$)] and increased the number of fecal bifidobacteria. Long term beneficial effect on allergy prevention (i.e., atopic dermatitis, rhinoconjunctivitis, and allergic urticaria) was also noted during the follow-up period, at 2 and 5 years of age compared to the placebo group (88, 89). In another RCT study (84) a 44% lower incidence of AD was reported at 1 year of life in infants at low risk of allergy fed an intact protein formula supplemented with GOS/FOS and specific pectin-derived acidic oligosaccharide compared to infants fed standard formula. It is noteworthy that the rate of AD in the prebiotic group was similar to that of fully breastfed babies (5.7 vs. 7.3%) but the protective effect vanished at preschool age (85).

Supplementation with prebiotics also showed a beneficial effect when used in children aged 1–4 years old. In a double-blind, randomized, controlled trial (86), 125 children were given cow's milk-based beverage (CMBB) containing DHA, the prebiotics polydextrose (PDX) and galactooligosaccharides (GOS), beta-glucan, zinc, iron, vitamins A and D, and were compared to 131 children fed with standard cow's milk for 28 weeks. Children who consumed CMBB had significantly reduced episodes of allergic manifestation, including eczema and urticaria, allergic rhinitis or conjunctivitis, wheezing, and allergic cough, compared to the control group. A meta-analysis (96) evaluating different types of prebiotics, duration of administration, and length of follow-up concluded for an overall 32% reduced risk of eczema and dermatitis (RR:0.68, 95% CI: 0.48–0.97; NNT 25), but not of asthma.

However, other trials did not confirm these positive results (83, 87, 91, 97–100). In a study (83), evaluating preterm, low birth weight infants fed with a formula containing a prebiotic mixture (GOS/FOS plus acidic oligosaccharides), there was no difference in the prevalence of AD and bronchial hyper-reactivity. In another study (91) a partially hydrolyzed formula supplemented with specific oligosaccharides (pHF-OS) induced immunomodulatory effects, such as increased regulatory T-cell numbers, in infants at increased risk of allergy, but was not able to reduce AD incidence at 12 or 18 months compared with standard formula-fed infants.

In 2011, the Nutrition Committee of the European Society for Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) found insufficient evidence to recommend supplementing with prebiotics in infant formulas to prevent atopic disease (98).

In 2016, the World Allergy Organization (99), using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach, was in favor to use prebiotic supplementation in not-exclusively breastfed infants but reporting very low certainty of evidence. No significant difference in eczema (RR: 0.57, 95% CI: 0.30–1.08) emerged after the meta-analysis of five studies (1,313 infants), while the meta-analysis of two studies (249 infants) found a reduction in recurrent wheeze or asthma (RR: 0.37, 95% CI: 0.17–0.80) in the prebiotic group of infants. Only one study assessed the risk of food allergy and found a reduced risk (RR: 0.28, 95% CI 0.08–1.00) in infants supplemented with prebiotics.

In 2017 a systematic review performed by Cuello-Garcia et al. (100) found not enough evidence to reject or to support the use of prebiotics for allergy prevention in infants analyzing the risk of AD (RR: 0.68, 95% CI: 0.40–1.15), asthma/wheezing (RR, 0.37; 95% CI: 0.17–0.80), and food allergy (RR: 0.28, 95% CI: 0.08–1.00). No evidence of an increased risk of any adverse effects was also noted in supplemented infants (RR: 1.01, 95% CI: 0.92–1.10). In 2018 infants with a positive family history of allergy were randomized to receive a GOS/PDX-formula (PF) or standard formula (SF) until 48 weeks of life while 140 infants were exclusively breastfed (BF): even if there was a 35% reduction in AD risk in PF compared with SF, there was no a statistically significant difference in any AD analyzed variables between the two groups at 36, 48, and 96 weeks (87).

Interestingly, in the same year, a systematic review (101) on HMOs reported a preventive effect on cow's milk allergy (CMA) at 18 months of age.

Therefore, at present, despite some promising results with specific prebiotics on the gut microbiota (102), the heterogeneity and the limited numbers of studies do not allow to draw any definitive conclusions on the clinical impact of prebiotics for allergy prevention.

As it has been suggested (103), since a large amount of prebiotics are already present in human milk, more carefully conducted RCTs in formula-fed infants, at high as well at low risk of allergy, are still needed before routine prebiotic supplementation can be recommended for allergy prevention.

PROBIOTICS TO PREVENT ALLERGIC DISEASES

Recent evidence suggests that exposure to beneficial bacteria in early life may have a role in the prevention of allergy (72). A number of studies first demonstrated that infants born vaginally and breastfed are colonized by Lactobacilli and Bifidobacteria whilst infants born through cesarean section and fed with standard formula show a significantly lower prevalence of Bifidobacteria and more Bacteroides and Coliforms (72) associated with increased prevalence of respiratory allergies (104). Thus, probiotic supplementation during pregnancy was considered to transfer beneficial bacteria to the infant during delivery and after birth. Secondly, the gut is highly exposed to microbial exposure and immune stimulation (105) and probiotic supplementation early in life may facilitate the maturation of the immune system (106, 107). Based on these hypotheses, most trials evaluating probiotics for prevention of allergy are based on supplementation during pregnancy, lactation; and/or post-natally. The route of administration varied from oral preparation (capsules; oil droplets; and suspension), addition to infant formula, maternal intake in breastfed babies, or a combination of the above (108). Various microbial species have been tested, in primis *Lactobacillus* and *Bifidobacterium*, alone

or in combination with other species, such as *Propionibacterium*, *Streptococcus*, *Lactococcus*, and *Escherichia coli* (**Table 2**).

At present, the strains of probiotics tested for prevention of allergy are considered as generally safe during pregnancy and in infancy (81) although adverse effects have not been fully assessed in all studies. We hereby summarize studies and meta-analysis evaluating the efficacy of probiotics on prevention of atopic dermatitis (the most relevant reported outcome) and other allergic manifestations (rhinitis, rhinoconjunctivitis, asthma and/or wheezing, food allergy, and/or their combination).

Evidence on Prevention of Atopic Dermatitis (AD)

The pioneering study using *Lactobacillus* GG probiotic supplementation in pregnant women, breastfeeding mothers, and infants at high risk of allergy, demonstrated a reduced prevalence of early AD in children compared to the control group (109). Noteworthy, specific Toll-like receptor genetic variations were associated with the protection of eczema by two probiotic strains (*Lactobacillus rhamnosus* HN001 and *Bifidobacterium lactis* HN019), suggesting that individual genetic factors might influence the efficacy and outcome of probiotic supplementation (110).

Table 2 shows details of published RCTs on this topic, with several studies supporting (109, 111–126), while others providing no evidence (127–139), for recommending probiotics in primary prevention of atopic disease.

Conflicting results and conclusions also emerged from reviews, meta-analyses, and guidelines in the last 12 years. Two Cochrane reviews dated 2007 and 2011 did not provide guidance and showed many uncertainties (140, 141). Osborn's first meta-analysis (140) recognized an effect on the prevention of atopic dermatitis, but heterogeneity across studies hampered the draw of definitive conclusions. Afterward, the meta-analysis by Lee et al. (142) analyzed data from a total of 1,581 patients for perinatal administration and showed a preventive effect with a RR of 0.69 (CI: 0.57–0.83). Betsi and colleagues (143) analyzed three studies (584 patients) reporting a significantly decreased incidence of dermatitis in two of them. In 2012 Doege et al. (144), analyzed seven RCTs that reported a modest preventive effect on AD (RR: 0.82, CI: 0.71–0.95; 2,843 patients) with *Lactobacilli*, but not with mixtures of probiotics (128, 129). In the same year, a larger meta-analysis of 13 studies documented a significant preventive effect (RR: 0.79, CI: 0.71–0.88) (145). No difference was found between specific strains nor for target populations (pregnant mothers, breastfeeding mothers, or infants). One year later, a systematic review of 9 studies reported a reduced risk of AD with estimated efficacy ranging from 30 to 70% (146).

In 2015 the WAO (10) reviewed 23 RCTs: in 7 trials the supplementation of probiotics was only in infants (117, 118, 131, 147–150), in 8 trials was in pregnant women and infants [(136, 149, 151–154), while in the other 8 was in pregnant women, breastfeeding mothers and infants (109, 112, 112, 114, 116, 130, 136)]. Fifteen randomized trials of probiotics given to infants measured development of eczema (106, 109, 114, 116–118, 129, 130, 134, 136, 148–150, 155, 156). When used during pregnancy, probiotics were usually supplemented in the last 3 months (10) resulting in a decreased risk of eczema in children, compared to placebo (RR: 0.72, 95%, CI: 0.61–0.85). According to these results, the WAO guideline concluded that (tested) probiotics assumed by pregnant women provide a clear benefit, primarily for the prevention of eczema, in high-risk infants; however, it was a "conditional recommendation," based on "very low-quality evidence" (10). The same conclusion (conditional recommendation, very low-quality evidence) was drawn in favor of probiotics considering the reduced rate of eczema when compared to placebo (RR 0.61, 95% CI from 0.50 to 0.64) in breastfeeding mothers (10) and in infants (RR 0.81, 95% CI 0.70–0.94).

Two other meta-analyses published in 2015 documented a clear benefit of probiotics only for primary prevention of eczema but did not report significant preventive effects of any other allergic manifestations (108, 151). Zuccotti et al. (151) analyzed 17 studies (4,755 children) and found that probiotics supplementation was associated with a significantly lower relative risk (RR) for developing eczema compared with placebo (RR 0.78; 95% CI: 0.69–0.89), and the most pronounced effect was obtained in particular when heterogeneous mixtures of probiotic strains were used (RR 0.54; 95% CI: 0.43–0.68) (but no with *Lactobacilli* or *Bifidobacteria* alone).

The metanalysis by Cuello-Garcia et al. (108) evaluating 29 studies reported a reduced risk of eczema (follow-up period until 2 years of age) when probiotics were given in the last 3 months of pregnancy (RR 0.71; 95% CI, 0.60–0.84), in breastfeeding mothers (RR 0.57; 95% CI, 0.47–0.69), or both to infants and mothers (RR, 0.80; 95% CI, 0.68–0.94), but not when administered only to infants (RR, 0.83; 95% CI, 0.58–1.19). However, using the GRADE approach, there was a low or very low certainty of evidence due to the "risk of bias, inconsistency and imprecision of results, and indirectness of available research" (108). Results supporting a stronger efficacy of combined perinatal supplementation were reported by two subsequent reviews (34, 153). In particular, according to the Italian review (34), there was "a moderate but constant effect across studies available in the literature for the prevention of atopic dermatitis among children at risk of allergy with the administration of probiotics to the mother during pregnancy and/or after delivery, and to their child during the first 6 months of life." Similarly, in the most recent review and meta-analysis by Li et al. (153), assessing 28 studies, probiotic supplementation was reported as protective against atopic eczema (OR: 0.69, 95% CI: 0.58–0.82, $P < 0.0001$) and only pre-natal combined with post-natal supplementation obtained a significant reduction. However, it was still open to question when during the gestation the supplementation should start and for how long the intervention should continue in the post-natal period (103, 152).

Moreover, many other clinical studies and meta-analyses reported conflicting results (109, 140, 144, 145, 150, 154–159). These discrepancies could be likely related to different study designs, populations, probiotic strains, and dosages used. As a single strain, LGG showed the most beneficial effect (157) in particular on reducing total and specific immunoglobulin E (IgE) sensitization (158). Conversely,

TABLE 2 | Probiotics administration in prevention of allergic disorders.

(A) Probiotic given orally (eg droplets, suspensions, capsules) or with breastfeeding/ standard formula.

References	Study	Enrolled patients	Probiotic + Standard formula/breast Feeding	Probiotic strain, Beginning of treatment (S), End of treatment (E).	Pre-natal administration (duration)	Post-natal administration (duration)	Outcomes	Follow-up (duration)
Kalliomaki et al. (109)	double-blind, randomized, placebo-controlled trial	- 159 Pregnant woman who had at least one first-degree relative (or partner) with atopic disease - breastfeeding mothers - their infants, post-natally if not breast-fed	Placebo group ($n = 82$): two capsules of placebo (microcrystalline cellulose) Probiotic group ($n = 77$): two capsules of 1×10^{10} CFU of *Lactobacillus* GG daily: for infants contents were mixed with water and given by spoon	Pregnant woman: S: 2–4 weeks before expected delivery E: at delivery or 6 months later if breastfeeding mothers Infants: S: birth E: 6 months	2–4 weeks before expected delivery	6 months	There was a halving in frequency of atopic eczema in the probiotic group compared with the placebo group (15/64 [23%] vs. 31/68 [46%]; relative risk 0.51 [95% CI 0.32–0.84]). The number needed to treat was 4.5 (95% CI 2.6–15.6)	2 years
Rautava et al. (111)	parallel, double-blind placebo-controlled trial	205 pregnant women with allergic disease and atopic sensitization	Probiotic groups: - 1 sachet of L.rhamnosus LPR (1×109 CFU) and B. longum BL999 (1×109 CFU ($N = 73$) daily or - L paracasei ST11 (1×109 CFU) and B longum BL999 (1×109 CFU) daily ($N = 70$) Placebo group ($n = 62$): the same sachet without probiotics	S: 2 months before expeted delivery E: 2 months after delivery (during breast-feeding)	2 months before expeted delivery to delivery	2 months	There was a significantly reduced risk of developing eczema in infants of mothers receiving LPR1BL999 (odds ratio [OR], 0.17; 95% CI, 0.08-0.35; $P < .001$) and ST111BL999 (OR, 0.16; 95% CI, 0.08–0.35; $P < .001$)	2 years
Kalliomäki et al. (112)	double-blind, randomi-zed, placebo-controlled trial	- 132 Pregnant woman who had at least one first-degree relative (or partner) with atopic disease - breastfeeding mothers - their infants, post-natally if not breast-fed	Placebo group ($n = 53$): two capsules of placebo (microcrystalline cellulose) Probiotic group ($n = 54$): two capsules of 1×10^{10} CFU of *Lactobacillus* GG daily: for infants contents were mixed with water and given by spoon	Pregnant woman: S: 2–4 weeks before expected delivery E: at delivery or 6 months later if breastfeeding mothers Infants: S: birth E: 6 months	2–4 weeks before expected delivery	6 months	There was an extention beyond infancy of the preventive effect of lactobacillus GG on atopic eczema: (14/53 in probiotic group developped eczema vs. 25/54 receiving placebo (relative risk 0.57, 95% CI 0.33–0.97)	4 years

(Continued)

TABLE 2 | Continued

References	Study	Enrolled patients	Probiotic + Standard formula/breast Feeding	Probiotic strain, Beginning of treatment (S), End of treatment (E).	Pre-natal administration (duration)	Post-natal administration (duration)	Outcomes	Follow-up (duration)
Kalliomäki et al. (113)	double-blind, randomized, placebo-controlled trial	- 116 Pregnant woman who had at least one first-degree relative (or partner) with atopic disease - breastfeeding mothers - their infants, post-natally if not breast-fed	Placebo group ($n = 62$): two capsules of placebo (microcrystalline cellulose) Probiotic group ($n = 53$): two capsules of 1×10^{10} CFU of *Lactobacillus* GG daily: for infants contents were mixed with water and given by spoon	Pregnant woman: S: 2–4 weeks before expected delivery E: at delivery or 6 months later if breastfeeding mothers Infants: S: birth E: 6 months	2–4 weeks before expected delivery	6 months	The cumulative risk for developing eczema was significantly lower in the L.GG group than in the placebo group (42.6% vs. 66.1%; RR, 0.64; 95% CI, 0.45-0.92) According to Cox regression, the risk of eczema was significantly reduced in the L. GG group (odds ratio, 0.58; 95% CI, 0.35–0.94; $P = 0.027$)	7 years
Wickens et al. (114)	Double-blind, randomized placebo-controlled trial	- Pregnant women who had at least one first-degree relative (or partner) with atopic disease, - breast feeding mothers - their infants	Two Probiotic groups(capsule powder with): - Lactobacillus rhamnosus HN001 ($N = 170$) - Bifidobacterium animalis subsp lactis strain HN019 ($N = 171$) Placebo group: ($N = 171$): capsule powder without probiotics	Pregnant women: Lactobacillus rhamnosus HN001 (6 x 3 109 CFU /d), Bifidobacterium animalis subsp lactis strain HN019 (9 x 3 109 CFU /d) or placebo daily from 35 weeks gestation until 6 months if breast-feeding Infants: same treatment from day 2-16 of life to 2 years	From 35 weeks gestation	Breast feeding mothers: for 6 months Infants: for 2 years since day 2-16 of life	infants receiving L rhamnosus had a significantly ($P = 0.01$) reduced risk of eczema (hazard ratio [HR], 0.51; 95% CI, 0.30–0.85) compared with placebo, but this was not the case for B animalis subsp lactis (HR, 0.90; 95% CI, 0.58–1.41)	2 years
Dotterud et al. (115)	randomized, double-blind trial	415 pregnant women	Probiotic group ($n = 138$): probiotic milk contained LGG 5 × 1010 CFU, Bb-12 5 × 1010 CFU and La-5. 5 × 109 CFU daily. Placebo group ($N = 140$): the placebo milk contained no probiotic bacteria	S: 4 weeks before expected delivery date E: 3 months after delivery (while breastfeeding)	4 weeks (from 36 weeks of gestation)	3 months while brestfeeding	There was a odds ratio (OR) of 0.51 for the cumulative incidence of AD in the probiotic group compared with the placebo [95% CI, 0.30–0.87; $P = 0.013$]. There were no significant effects on asthma or atopic sensitiza- tion	2 years
Kim et al. (116)	randomized, double-blind, placebo-controlled trial	112 pregnant women and newborns	Probiotics group: mixture of B. bifidum BGN4 [1.6×10^9CFU], B. lactis AD011 (1.6×10^9 CFU), and L. acidophilus AD031 (1.6×10^9 CFU) in 0.72 g of maltodextrin and 0.8 g of alpha-corn once daily Placebo group: maltodextrin and alpha-corn without probiotic bacteria	S (women): 4–8 weeks before expected delivery E (women): 3 months after delivery (during breastfeeding) S (infants): 4 months after delivery E(infants): 6 months	4–8 weeks before expected delivery to delivery	6 months	There was a significant reduction in the cumulative incidence of eczema during the first year in probiotic group (36.4% vs. 62.9%, $p = 0.029$)	1 year

(Continued)

TABLE 2 | Continued

References	Study	Enrolled patients	Probiotic + Standard formula/breast Feeding	Probiotic strain, Beginning of treatment (S), End of treatment (E).	Pre-natal administration (duration)	Post-natal administration (duration)	Outcomes	Follow-up (duration)
West et al. (117)	double-blind, placebo-controlled randomized intervention trial	179 infants during weaning	Probiotic group ($n = 89$): fed cereals with *Lactobacillus* F19 Placebo group($N = 90$): fed cereals without probiotics	S: 4 months E: 13 months	no	9 months	There was a cumulative incidence of eczema of 11% (4–17%, 95% CI) in the probiotic group vs. 22% (13–31%, 95% CI) in the placebo group ($p < 0.05$)	13 months
Lodinova-Zadnikova et al. (118)	controlled clinical trial	158 infants: - $N = 56$ colonized infants of allergic mothers, $N = 57$ control infants of allergic mothers - $N = 45$ control infants of healthy mothers	One milliliter of *E. coli* was administered to infants of allergic mothers	S: within 48 h after birth and subsequently 3 times a week E: 4 weeks	no	4 weeks	There were allergy symptoms in 14 infants of control allergic mothers, in 7 infants of healthy mothers, and in 2 colonized infants of allergic mothers	5 years
Ezaki et al. (119)	Retrospective study	30 newborns after small intestine surgery	Probiotic group ($N = 18$ newborns GA 34.5 (23.5–38.4): suspension of *B. breve* (7.5×10^8 cells/day). Placebo group ($N = 12$ newborn, GA 34.4 (26.4–40.0):	S: After small intestine sugery E: when full enteral feeding (100 ml/kg/day) was reached	no	After small intestine surgery until full enteral feeding (100 ml/kg/day) was reached	Administration of probiotics reduced the incidence of cow's milk protein intolerance (CMPI) after small intestine surgery (one vs. eight, $p < 0.001$)	
Wickens et al. (120)	Double-blind, randomized placebo-controlled trial	- Pregnant women who had at least one first-degree relative (or partner) with atopic disease, - breast feeding mothers - their infants ($N = 425$)	Two Probiotic groups: - Lactobacillus rhamnosus HN001 - Bifidobacterium animalis subsp lactis strain HN019 Placebo group:	Pregnant women: Lactobacillus rhamnosus HN001 (6×3 109 CFU/d), Bifidobacterium animalis subsp lactis strain HN019 (9×3 109 CFU/d) or placebo daily from 35 weeks gestation until 6 months if breast-feeding Infants: same treatment from day 2-16 of life to 2 years	From 35 weeks gestation	Breast feeding mothers: for 6 months Infants: for 2 years since day 2–16 of life	The cumulative prevalence of eczema [Hazard ratio (HR) 0.57 (95% CI 0.39–0.83)] and prevalence of rhinoconjunctivitis [Relative risk 0.38 (95% CI 0.18–0.83)] were significantly reduced in the children taking HN 001; HN 019 did not affect the prevalence of any outcome	4 years

(Continued)

TABLE 2 | Continued

References	Study	Enrolled patients	Probiotic + Standard formula/breast Feeding	Probiotic strain, Beginning of treatment (S), End of treatment (E).	Pre-natal administration (duration)	Post-natal administration (duration)	Outcomes	Follow-up (duration)
Wickens et al. (121)	Double-blind, randomized placebo-controlled trial	- Pregnant women who had at least one first-degree relative (or partner) with atopic disease, - breast feeding mothers - their infants ($N = 425$)	Two Probiotic groups: - Lactobacillus rhamnosus HN001 - Bifidobacterium animalis subsp lactis strain HN019 Placebo group:	Pregnant women: Lactobacillus rhamnosus HN001 (6 × 3 109 CFU/d), Bifidobacterium animalis subsp lactis strain HN019 (9 × 3 109 CFU/d) or placebo daily from 35 weeks gestation until 6 months if breast-feeding Infants: same treatment from day 2-16 of life to 2 years	From 35 weeks gestation	Breast feeding mothers: for 6 months Infants: for 2 years since day 2–16 of life	HN001 was associated with significantly lower cumulative prevalence of eczema (HR = 0.56, 95% CI 0.39–0.80), SCORAD ≥ 10 (HR = 0.69, 0.49-0.98) and SPT sensitization (HR = 0.69, 95% CI 0.48–0.99). HN019 had no significant effect on any outcome	6 years
Wickens et al. (122)	Double-blind, randomized placebo-controlled trial	- Pregnant women who had at least one first-degree relative (or partner) with atopic disease, - breast feeding mothers - their infants	Two Probiotic groups: - Lactobacillus rhamnosus HN001 ($N = 97$) - Bifidobacterium animalis subsp lactis strain HN019 ($N = 104$) Placebo group: ($N = 97$) The capsule powder was either given undiluted to the infant or mixed with water, breast milk, or formula and given via a teaspoon or syringe or sprinkled on food.	Pregnant women: Lactobacillus rhamnosus HN001 (6 × 3 109 colony-forming units/d), Bifidobacterium animalis subsp lactis strain HN019 (9 × 3 109 colony-forming units/d) or placebo daily from 35 weeks gestation until 6 months if breast-feeding Infants: same treatment from day 2-16 of life to 2 years	From 35 weeks gestation	Breast feeding mothers: for 6 months Infants: for 2 years since day 2–16 of life	HN001 significantly reduced the 12-month prevalence of eczema at age 11 years (relative risk [RR] = 0.46, 95% CI 0.25-0.86, $P = 0.015$) and hay fever (RR = 0.73, 95% CI 0.53–1.00, $P = 0.047$). HN001 was associated with a significant reduction in lifetime prevalence of atopic sensitization (hazard ratio [HR] = 0.71, 95% CI 0.51–1.00, $P = 0.048$), eczema (HR = 0.58, 95% CI 0.41–0.82, $P = 0.002$) and wheeze (HR = 0.76, 95% CI 0.57–0.99, $P = 0.046$). HN019 had no significant effect	11 years
Bertelsen et al. (123)	large, prospecti-ve pregnancy cohort study	40,614 mothers and children	probiotic milk products containing *L. acido-philus* LA-5, *B. lactis* Bb12, +/- *L. rhamno-sus* GG	S(mother): during pregnancy S(infants): after 6 months E: 18 months	during pregnancy	Mothers: during breast-feeding Infants: from 6 to 18 months of age	Consumption of probiotic milk in pregnancy was associated with a slightly reduced risk [(adjusted RR (aRR)] of atopic eczema at 6 months aRR=0.94 (95% CI: 0.89, 0.99) and of rhinoconjuctivitis between 18 and 36 months, aRR=0.87 (95% CI: 0.78, 0.98); the adjusted relative risk of rhinoconjunctivitis was aRR=0.80 (95% CI: 0.68, 0.93) when both mother and infant had consumed probiotic milk	36 months

(Continued)

TABLE 2 | Continued

References	Study	Enrolled patients	Probiotic + Standard formula/breast Feeding	Probiotic strain, Beginning of treatment (S), End of treatment (E).	Pre-natal administration (duration)	Post-natal administration (duration)	Outcomes	Follow-up (duration)
Simpson et al. (124)	Double-blinded, randomized placebo-controlled trial	161 pregnant women	Probiotic group ($N = 81$): probiotic milk contained LGG 5 × 1010 CFU, Bb-12 5 × 1010 CFU and La-5. 5 × 109 CFU daily. Placebo group ($N = 80$): the placebo milk contained no probiotic bacteria	S: 4 weeks before expected delivery date E: 3 months after delivery (while breastfeeding)	4 weeks (from 36 weeks of gestation)	3 months while brestfeeding	There was a trend toward a lower cumulative incidence of AD in the probiotic group (OR 0.64, 95 % CI 0.39–1.07, $p = 0.086$; NNT = 10). This finding was statistically significantly in the complete case analysis (OR 0.48, 95 % CI 0.25–0.92, $p = 0.027$, NNT = 6)	6 years
Schmidt et al. (126)	double-blind, placebo-controlled intervention trial	290 infants aged 8 to 14 months (Mean age 10.1 months)	Probiotic group ($N = 144$): B. animalis subsp lactis and L. rhamnosus (10^9 CFU of each) daily + maltodextrin powder Placebo group ($N = 146$): maltodextrin powder	S: up to 12 weeks before expected start in child care. E: after 6 months	no	6 months	A significantly lower incidence of eczema was observed in the probiotic group compared to the placebo group (4.2% vs. 11.5%, $P = 0.036$). The incidence of asthma, rhinitis, conjunctivitis, and sensitization did not differ	6 months
Peldan et al. (127)	double-blinded, placebo-control-led study	1223 mothers with infants at high risk for allergy	445 mothers received probiotic's mixture: LGG (5×10^9 cfu), L rhamnosus LC705 (5×10^9 cfu), B. breve Bb99 (2×10^8 cfu), and Propionibacterium freudenreichii ssp. shermanii JS (2×10^9 cfu) twice daily. Their infants received the same probiotic capsule + syrup containing 0.8 g of galacto-oligosaccharides once daily 446 mothers and infants received capsules containing microcrystalline cellulose, (placebo) and the infants also received syrup without galacto-oligosaccharides	S (women): From 36 weeks of gestation, E (women): at delivery S (infants): birth E (infants): 6 months	From 36 weeks of gestation,	from birth until age 6 months.	the prevalence of allergic rhino-conjunctivitis was greater in the probiotic group compared to the placebo group (36.5% vs. 29.0%, OR: 1.43, 95% CI: 1.06–1.94, $p = 0.03$	5-10 years
Taylor et al. (128)	Randomized, double-blind, placebo-controlled trial	178 newborns at high risk of allergy: - Probiotic group ($n = 89$) - Placebo group($n = 89$)	Probiotic group: 3×10^9 L. acidophilus LAVRI-A1 once a day(in sachet packets) Placebo group: Maltodrexine	S: births E: 6 months	no	6 months	Early probiotic supplementation with L acidophilus did not reduce the risk of AD at 12 months of age (38/88 vs. 34/87 in the placebo) and was associated with increased allergen sensitization (35/88 vs. 21/86)	12 months

(Continued)

TABLE 2 | Continued

References	Study	Enrolled patients	Probiotic + Standard formula/breast Feeding	Probiotic strain, Beginning of treatment (S), End of treatment (E).	Pre-natal administration (duration)	Post-natal administration (duration)	Outcomes	Follow-up (duration)
Abrahamsson et al. (129)	prospective double-blind, placebo-controlled, multicenter trial	188 mothers with allergic disease Their infants continued with the same product	Probiotic group: oil + L reuteri ATCC 55730 (1×10^8 CFU) daily Placebo group: (CFUs): the same oil without probiotics	S (Women): 36 weeks of gestational age E (women): delivery S (infants): at birth E (infants):12 months	from gestational week 36 until delivery.	12 months	The cumulative incidence of eczema was similar, 36% in the treated vs. 34% in the placebo group. The probiotic group had less IgE-associated eczema during the second year, 8% vs. 20% ($P = 0.02$),	2 years
Kopp et al. (130)	Randomized, Double-Blind, Placebo-Controlled Trial	- 94 pregnant women with a family history of atopic disease - 89 breastfeeding mothers - their infants ($n = 94$: 5 not breastfeed infants from birth, 89 from the age of 3 months)	L-GG group: 1 capsule(5×10^9 CFU) of L- GG twice Daily ($N = 50$) Placebo group: capsules of microcrystalline cellulose ($N = 44$)	S (women): 4 to 6 weeks before expected delivery, then during breastfeeding for 3 months; S (infants): 5 infants from birth, 89 from the age of 3 months E(women): at delivery or after 3 months if breastfeeding E (infants): 6 months of age	4-6 weeks	6 months	Supplementation with L- GG neither reduced the incidence of AD (28% vs. 27.3%, $P = 0.93$) nor altered the severity of AD but was associated with an increased rate of recurrent wheezing bronchitis (26% vs. 9.1% $P = 0.03$)	2 years
Prescott et al. (131)	Randomized, double-blind, placebo-controlled trial	153 newborns at high risk of allergy: - Probiotic group ($N = 74$) - Placebo group ($N = 76$)	Probiotic group: 3×10^9 L. acidophilus LAVRI-A1 once a day(in sachet packets) Placebo group: Maltodrexine	S: births E: 6 months	no	6 months	Supplementation with this probiotic did not reduce the risk of dermatitis (31/74, 42%) compared with placebo group (25/76, 34%). There was no significant reduction in any other allergic disease or allergen sensitization	2.5 years

(Continued)

TABLE 2 | Continued

References	Study	Enrolled patients	Probiotic + Standard formula/breast Feeding	Probiotic strain, Beginning of treatment (S), End of treatment (E).	Pre-natal administration (duration)	Post-natal administration (duration)	Outcomes	Follow-up (duration)
Kuitunen et al. (133)	double-blinded, placebo-control-led study	1223 mothers with infants at high risk for allergy	445 mothers received probiotic 's mixture: LGG (5 x10^9 cfu), L rhamnosus LC705 (5 × 10^9 cfu), B. breve Bb99 (2 x10^8 cfu), and Propionibacterium freudenreichii ssp. shermanii JS (2 × 10^9 cfu) twice daily. Their infants received the same probiotic capsule + syrup containing 0.8 g of galacto-oligosaccharides once daily 446 mothers and infants received capsules containing microcrystalline cellulose, (placebo) and the infants also received syrup without galacto-oligosaccharides	S (women): From 36 weeks of gestation, E (women): at delivery S (infants): birth E (infants): 6 months	From 36 weeks of gestation,	from birth until age 6 months	No significant difference appeared in frequencies of eczema (39.3% vs. 43.3%), atopic eczema (24.0% vs. 25.1%), allergic rhinitis (20.7% vs. 19.1%), or asthma (13.0% vs. 14.1%) between groups. However, less IgE-associated allergic disease occurred in cesarean- delivered children receiving probiotics (24.3% vs. 40.5%; odds ratio, 0.47; 95% CI, 0.23% to 0.96%; *P* 5.035)	5 years
Niers et al. (134)	Double-blind, randomized, placebo-controlled trial	98 pregnant women with a family history of allergic diseases and their infants	Probiotic group (*N* = 50): sachets containing B. bifidum (1 × 109 CFU), B. lactis (1 × 109 CFU), and L. lactis (1 × 109 CFU) daily Placebo group (*N* = 48): rice starch and maltodextran	S: last 6 weeks of pregnancy E: 12 months after delivery (to infants)	last 6 weeks of pregnancy	12 months	Cumulative incidence of eczema at 1 and 2 years was 23/50 (intervention) vs. 31/48 (placebo) and 27 (intervention) vs. 34 (placebo), respectively	2 years
Boyle et al. (135)	Randomized controlled trial	250 pregnant women carrying infants at high risk of allergic disease	Probiotic group: Lactobacillus rhamnosus GG (LGG) 1.8 × 10^{10} CFU/day Placebo group	S: 36 weeks of gestation E: at delivery	From 36 weeks of gestation until delivery	no	Pre-natal probiotic treatment was not associated with reduced risk of eczema (34% probiotic, 39% placebo; RR 0.88; 95% CI 0.63, 1.22) or IgE-associated eczema (18% probiotic, 19% placebo; RR 0.94; 95% CI 0.53, 1.68)	
Ou et al. (136)	randomized, double-blind, placebo-controlled trial	191 pregnant women with atopic diseases, breastfeeding mothers or non-breastfeeding neonates,	Probiotic group (*N* = 95):*LGG* ATCC 53103, 1 × 1010 CFU daily Control group (*N* = 96)	S (women): from the second trimester of pregnancy; E: 6 months after delivery (breastfeeding mothers or non-breast-feeding infants from birth)	From the 24 weeks of gestational age to delivery	6 months	There was no significant difference between the cumulative risk of sensitization and developing allergic disease at the age of first 36 months by log-rank test (*P* = 0.86 and *P* = 0.74, respectively)	3 years

(Continued)

TABLE 2 | Continued

References	Study	Enrolled patients	Probiotic + Standard formula/breast Feeding	Probiotic strain, Beginning of treatment (S), End of treatment (E).	Pre-natal administration (duration)	Post-natal administration (duration)	Outcomes	Follow-up (duration)
Damm et al. (137)	Controlled interventional cohort study	527 preterm neonates (<30 weeks of gestation)	Probiotic group ($N = 249$): L. rhamnosus GG (1×10^9) and B. animalis subsp. lactis (BB12) (1×10^8) daily Control group ($N = 278$): not treated with probiotics	S: third day of life E: at discharge from hospital,	no	from the third day of life to discharge from hospital	The prevalence of AD was similar in the two groups (20.9% in the probiotic treated group vs. 17.1% in the not treated group, $p = 0.33$)	2-8 years
Laursen et al. (138)	randomized, double-blind, placebo-controlled study	290 infants aged 8 to 14 months	Probiotic group ($N = 144$ B. animalis subsp lactis and L. rhamnosus (10^9 CFU of each) daily + maltodextrin powde Placebo group ($N = 146$): maltodextrin powder	S: up to 12 weeks before expected start in child care. E: after 6 months		6 months	Probiotic treatment did not reduce the number of days absent from child care due to infections in healthy infants at the time of enrollment in child care	6 months
Murphy et al. (139)	Sub-Sample Analysis From a randomized, controlled, 3-arm trial (115, 116)	- Pregnant women who had at least one first-degree relative (or partner) with atopic disease, - breast feeding mothers - their infants	Two Probiotic groups: - Lactobacillus rhamnosus HN001 ($N = 285$ stools) - Bifidobacterium animalis subsp lactis strain HN019 ($N = 50$ stools) Placebo group: ($N = 315$ stools sample)	Pregnant women: Lactobacillus rhamnosus HN001 (6×3 109 colony-forming units/d), Bifidobacterium animalis subsp lactis strain HN019 (9×3 109 colony-forming units/d) or placebo daily from 35 weeks gestation until 6 months if breast-feeding Infants: same treatment from day 2–16 of life to 2 years	From 35 weeks gestation	Breast feeding mothers: for 6 months Infants: for 2 years since day 2-16 of life	Supplementation with L. rhamnosus HN001 was associated with increased overall glycerol-3 phosphate transport capacity and enrichment of L. rhamnosus. There were no differences in development of eczema by 2 years in either community alpha or beta diversity ($P > 0.05$)	2 years

(B) Probiotic given with hydrolyzed/ amino acid-based formulas.

References	Study	Enrolled Patients	Hydrolyzed/ amino acid-based formulas+probiotic	Probiotic Strain, Beginning of Treatment (S), End of Treatment (E).	Pre-natal administration (if yes: duration)	Post-natal amministration (if yes: duration)	Outcomes	Follow-Up (duration)
Berni Canani et al. (125)	Parallel-arm randomized controlled trial	220 children with cow's milk allergy with a median age of 5.0 months	Probiotic group ($N = 110$): Extensively hydrolyzed casein formula (EHCF) + Lactobacillus rhamnosus GG (LGG) Control group($N = 110$): Extensively hydrolyzed casein formula (EHCF)	Lactobacillus rhamnosus GG (LGG) S: after randomization E: 3 years	no	36 months	EHCF+LGG reduces the incidence of allergic manifestations (AM)(absolute risk difference was 20.23 (95% CI, 20.36 to 20.10; $P < .001$), and speeds up the time to development of oral tolerance in children with IgE-mediated CMA	36 months

Lactobacillus acidophilus has been associated with an increased risk of atopic sensitization (158).

Evidence on Prevention of Allergic Rhinitis (AR)

Development of allergic rhinitis (AR) in the child following supplementation of probiotics in pregnant women has been evaluated in 5 studies (112, 114, 115, 129, 133) reviewed by Fiocchi et al. (10]. No significant effect has been reported (RR 0.86, 95% CI 0.44–1.7).

Three trials evaluated the onset of AR after supplementing with probiotics breastfeeding mothers and infants (112, 114, 115). Again, relatively few events have been observed and the results were inconclusive (RR 0.86, 95% CI 0.21–3.47). Four trials assessed the development of AR following infant supplementation (120, 128, 138, 142) and confirmed the lack of efficacy of probiotics (RR 0.83, 95% CI from 0.39 to 1.79).

However, in a large cohort study (123), the mothers of 40,614 children were asked to consume two brands of milk and yogurt that contain probiotic strains (*L. acido-philus* LA-5, *B. lactis* Bb12, +/– *L. rhamnosus* GG) during pregnancy. A slight reduction of the risk [adjusted RR (aRR) = 0.87)] of rhinoconjunctivitis at 18–36 months was reported (123). The association between rhinoconjunctivitis and probiotics appeared increased in the case of both the mother (during pregnancy) and the child (from 6 months of age) had consumed these probiotics, as compared when only mother or child consumed.

Conversely, in a longitudinal trial, a higher prevalence of allergic rhino-conjunctivitis at the age of 5–10 years was noted in the probiotic group as compared with the placebo group (36.5 vs. 29.0%, $p = 0.03$) (127).

Therefore, at present, there is no clear evidence that probiotics prevent AR (160), with some reports demonstrating even a detrimental effect (99).

Evidence on Prevention of Asthma and/or Wheezing

Several systematic reviews and meta-analyses (10, 161, 162) failed to demonstrate a protective effect of probiotics supplementation during pregnancy or early life in the subsequent development of asthma or wheezing. Surprisingly, even an increase in respiratory infections has been reported in children supplemented with probiotics (161).

In 2014 a systematic review and meta-analysis (162) evaluating pre-and post-natal supplementation with probiotics concluded that there was insufficient data to recommend probiotics for the prevention of asthma and wheezing.

In 2015, the WAO analysis (10) of 8 studies (113–115, 129, 133, 135, 136, 163) focusing on the development of asthma/wheezing in the child following administration of probiotics to pregnant women, did not show differences between probiotic and placebo arms (RR 0.93, 95% CI of 0.76–1.1 5). No differences were recorded between probiotic and placebo arms (RR of 1.05, 95% CI from 0.59 to 1.87) in the 4 studies that evaluated asthma/wheezing (113–115, 136) after supplementation of mothers both during pregnancy and during the breastfeeding period and/or supplementation of the infant, Similarly, no differences between the probiotic and placebo groups (RR 0.98, 95% CI from 0.78 to 1.23) were found in the development of asthma/wheezing in the nine studies that evaluate the effect of infants supplementation (10, 113, 114, 117, 118, 129, 131, 133, 136, 163).

Evidence on Prevention of Food Allergy

A variety of studies provided data that probiotics, including LGG or *L. acidophilus*, do not protect against CMA in infancy (128, 148, 164). Moreover, in a review involving 1,549 infants, Osborn and Sinn (140) stated that the benefit of probiotics in reducing food hypersensitivity is disputable.

In a study conducted by Morisset et al. (150), children at high-risk for the onset of atopic disease were fed with standard infant formula or a fermented infant formula containing heat-killed *Bifidobacterium breve* C50 and *Streptococcus thermophilus* 065. No statistical differences in the incidence of CMA were observed between these two groups, despite infants fed the formula containing probiotics were less sensitized to CMP at skin prick tests (150).

Similarly, a reduced skin prick test sensitivity to CMP or hen's egg protein at age 6 months was reported (165) in children, following supplementation with *Lactobacillus* and *Bifidobacterium* daily to pregnant women (from 36 weeks gestation to delivery) and to infants (from birth through 6 months), when compared to mothers and infants receiving placebo.

However, the results of these studies suggested that probiotics may modulate the development of allergic sensitization to foods, but not necessarily this translates into food allergy prevention (166). Food hypersensitivity is not always associated with symptoms of food allergy, although infants with food sensitization may be more prone to develop a food allergy.

Newborns who received small intestine surgery and antibiotics showed a higher incidence (67%) of CMPI compared to the group supplemented with probiotic treatment (*B. breve)* (119).

Two other studies reported conflicting results with supplementation of LGG (167, 168) (RR 0.88 (95%CI: 0.76–1.03).

Guidelines published in 2014 by the European Academy of Allergy and Clinical Immunology's Taskforce on the prevention of food allergy suggested that there was not enough evidence to support the routine use of probiotics for food allergy prevention (169).

In 2015, the WAO systematic review and meta-analysis (10) reviewed studies evaluating probiotics given to pregnant women (111, 112, 129), breastfeeding mothers (111, 112), and infants (112, 118, 129, 131, 150), and did not document significant effects in reducing the risk of developing food allergy in infants.

Conversely, another meta-analysis (170) in 2016 indicated that probiotics administered pre-natally and post-natally were effective in reducing the risk of atopy and food hypersensitivity (RR 0.77, 95% CI: 0.61–0.98), particularly in families at high risk for allergy. Based on subgroup analyses, the preventive effect was higher when probiotics were administered to both mother and infant, or for a longer duration of the intervention

(170), whilst no effect of post-natal probiotic supplementation alone (direct to child) was observed. Only 1 study (135) used solely pre-natal supplementation, and no significant difference in effect was observed between groups. Interestingly, one trial (133) showed that probiotic and prebiotic supplementation during pregnancy and infancy conferred protection preferably to cesarean-delivered children who could not be exposed to remarkable microbial load from a vaginal delivery.

A few studies showed conflicting results of probiotics (LGG) supplementing an extensively hydrolyzed formula in the acquisition of tolerance in infants with CMA (171, 172). Reducing the duration of CMA would be relevant to decrease the possible related risk of other clinical conditions including functional gastrointestinal disorders (173).

To our knowledge, studies exploring the effects of probiotics on confirmed food allergy are surprisingly scant and did not show evidence of benefit compared to non-intervention (111, 128, 165, 170, 174).

Evidence on Prevention of Whatever Combination of Allergic Diseases Other Than AD

Supplementation with probiotics did not protect against food allergy, asthma, or allergic rhinitis according to two metanalyses published in 2013 (158, 161) and the WAO review (10) that evaluated four randomized trials (106, 131, 136, 156) in 2015 (RR 0.97, 95% CI from 0.85 to 1.12). Two studies evaluating the risk of developing "any allergy" following supplementation in the breastfeeding mother and infant (136, 175) did not report any benefit or harm (RR 1.02, 95% CI 0.71–1.46) (13). The same conclusions were expressed by the other three papers (34, 108, 151). However, Lundelin et al. (176) reported the long-term safety and efficacy of four different strains of probiotics: children receiving LGG perinatally alone or in combination with other strains (*Bifidobacterium lactis* Bb-12, *Lactobacillus paracasei*ST11, and *Bifidobacterium longum*BL999) had a lower risk of developing allergic diseases (allergic rhinitis, eczema, asthma or food allergy) during long-term follow-up (at the age of at least 10 years) compared to the placebo group (47 vs.56%, $p = 0.09$) (176).

A positive effect was also demonstrated in a different RCT (125), involving 220 children (median age of 5 months) with CMA, randomized to either receive extensively hydrolyzed casein formula alone or with L. rhamnosus GG. In the group supplemented with LGG, there was a decrease in the incidence of allergic manifestations (including asthma, eczema, and allergic rhino-conjunctivitis) over a 3-year period and an increased rate of acquisition of tolerance at 36 months (125).

In 2019 a meta-analysis (177) of 17 RCTs (5,264 children) reported there was no significant reduction in the risk of developing asthma after probiotic supplementation compared with controls (RR: 0.86, 95% CI: 0.73–1.01; $p = 0.06$). However, through subgroup analyses, the occurrence of asthma was reduced by L-GG supplementation (RR 0.75; 95% CI: 0.57–0.99; $p = 0.04$) and post-natal only (compared to pre- and post-natal) intervention. The rate of AR, wheeze, and positive aeroallergen SPT results were not different between the two groups. In conclusion, this meta-analysis underlined the importance of specific strain of probiotics and the timing of intervention but also the need for large-sample and high-quality RCTs (177).

Recently, Schmidt et al. (126) examined the effect of supplementation with a mixture of two probiotic strains (*Lactobacillus rhamnosus* and *Bifidobacterium animalis* subsp lactis) in late infancy and early childhood (the mean age at enrollment was 10 months) on the development of allergic diseases and sensitization. As part of the Probicomp Study (138), a double-blind, placebo-controlled intervention trial in which the primary outcome was to reduce infection rate, 290 participants were randomized to either receive a daily mixture of the two probiotic strains ($n = 144$) or placebo ($n = 146$) for 6 months, starting prior to attending daycare. At follow-up (mean age 16.1 months) there was a significantly decreased incidence of eczema in the probiotic group compared to the placebo group (4.2 vs. 11.5%, $P = 0.036$), corresponding to a relative risk of 0.37, but no differences in the incidence of asthma, rhinitis, conjunctivitis, and sensitization across groups were noted (126). However, when the endpoint was grouped as "any allergic disease" (including eczema), 7.6% ($n = 9$) in the probiotic group and 18.9% ($n = 23$) in the placebo group were affected ($P = 0.010$) (126).

Therefore, in conclusion, differences in environmental factors, such as diet or geographic region, in genetic liability as well as in probiotic strains used, timing, and duration of supplementation may be responsible for the heterogeneity in the results of different studies. Overall, diet supplementation with probiotics does not seems to have a beneficial effect in the prevention of allergic manifestations other than AD.

SYNBIOTICS IN THE PREVENTION OF ALLERGIC DISEASES

First of all, recently it has been revealed that breast milk is not sterile since contains live probiotic *Lactobacillus* (mostly salivarius and fermentum), *Bifidobacterium* species (*B. breve*) (178, 179) as well as *Staphylococcus* and *Streptococcus*. Many factors may influence the composition of breast milk microbiota: the composition of the mother's skin and intestinal microbiota, the mother's health state, and exposure to medications, mostly antibiotics. Moreover, we already discussed the presence and the role in human milk of non-digestible milk human oligosaccharides (HMO) (61). Therefore, we can consider breast milk as a natural synbiotic, containing both probiotics and prebiotics (180) and the beneficial effect of breast milk in the prevention of allergy could be associated with a "synbiotic's effect."

Regarding supplementation with synbiotics, there are only two RCTs evaluating their role to prevent AD or FA (**Table 3A**). The first study (156) reported a reduction in the rate of eczema and IgE-associated allergic diseases, including challenge-proven FA, by synbiotic supplementation. The second study documented a reduced eczema risk with synbiotic supplementation but did not study FA (181). However, a meta-analysis of these studies

TABLE 3 | Synbiotics administration in prevention of allergic disorders.

(A) Synbiotic + Standard formula/breastfeeding.

References	Study	Enrolled Patients	Synbiotic + Standard formula/ breast feeding	Prebiotic substance, Beginning of Treatment (S), End of Treatment (E).	Probiotic Strain, Beginning of Treatment (S), End of Treatment (E).	Pre-natal administration (duration)	Post-natal administration (duration)	Outcomes	Follow-Up (duration)
Kukkonen et al. (156)	double-blind randomized, placebo controlled trial	- 1223 pregnant woman carrying high- risk children and their infants: - *N* = 461 mothers-infants received symbiotic - *N* = 464 mothers-infants received placebo	- Synbiotics group: - mothers: 1 capsule containing 4 probotics twice daily - infants received 1 opened capsule containing the same probiotics mixed with galacto-oligosaccharides once daily Placebo group: capsules containing microcrystalline cellulose, and the infants received syrup without galacto-oligosaccharides	Synbiotics group: infants received 1 opened capsule containing the same probiotics mixed with drops of sugar syrup containing 0.8 g of galacto-oligosaccharides once daily S(women): 2–4 weeks before delivery E (Women): at delivery S (infants): birth E (infants): 6 months	1 capsule containing L. rhamnosus GG(ATCC 53103), 5×10^9 cfu; L. LC705 (DSM 7061), 5×10^9 cfu; B. breve Bb99(DSM 13692), 2×10^8 cfu; and P. freudenreichii ssp. shermanii JS(DSM 7076), 2×10^9 cfu, twice daily S(women): 2–4 weeks before delivery E (Women): at delivery S (infants): birth E (infants): 6 months	2–4 weeks before delivery	For 6 months	There was no effect of probiotic supplementation compared with placebo on the cumulative incidence of any allergic disease (OR, 0.85; 95% CI, 0.64–1.12). There was a reduced occurrence of Eczema in the probiotic group (OR, 0.74; 95% CI, 0.55–0.98)	2 years
Roze et al. (181)	double-blind, randomized, multicenter trial	Ninety-seven non-brestfed term neonates:	Symbiotics group (n 48): Standard formula + symbiotics Control group(n 49): standard formula	experimental formula containing the two strains of probiotics +96% galacto-oligosaccharides and 4 % short-chain fructo-oligosaccharides	experimental formula containing L. rhamnosus LCS-742(1.4×10^8), B. longum subsp infantis M63 (1.4×10^8) and prebiotics:	no	For 6 months	Atopic dermatitis was less frequently observed in the experimental group (2.6% vs. 17.8%, $P < 0.05$)	6 months

(B) Synbiotic +Hydrolyzed/ amino acid-based formulas.

Refereences	Study	Enrolled patients	Hydrolyzed/ amino acid-based formulas+synbiotic	Prebiotic substance, Beginning of treatment (S), End of treatment (E).	Probiotic strain, dose Beginning of treatment (S), End of treatment (E).	Pre-natal administration (duration)	Post-natal administration (duration)	Outcomes	Follow-up (duration)
van der et al. (182)	double-blind, placebo-controlled multicentre trial	ninety full-term infants, aged <7 months with AD	Synbiotic group: extensively hydrolyzed whey-based formula with additional synbiotics [B. breve M-16V and a galacto/fructooligosaccharide mixture] Control group: same formula without synbiotics	mixture of 90% scGOS and 10% lcFOS 0.8 g/100 ml S: <7 month E: after 12 weeks	B. breve M-16V (1.3×10^9 cfu/100 ml) S: <7 month E: after 12 weeks	no	12 weeks	The SCORAD score improvement (AD severity) did not differ between the synbiotic and the placebo group. In the synbiotic group there was a significantly higher percentage of bifidobacteria (54.7% vs. 30.1%, $P < 0.001$) and significantly lower percentages of *Clostridium lituseburense*	12 weeks

(Continued)

TABLE 3 | Continued

Refereences	Study	Enrolled patients	Hydrolyzed/ amino acid-based formulas+synbiotic	Prebiotic substance, Beginning of treatment (S), End of treatment (E).	Probiotic strain, dose Beginning of treatment (S), End of treatment (E).	Pre-natal administration (duration)	Post-natal administration (duration)	Outcomes	Follow-up (duration)
								/Clostridium histolyticum (0.5 vs. 1.8, $P = 0.02$) and *Eubacterium rectale /Clostridium coccoides* (7.5 vs. 38.1, $P < 0.001$) after intervention than the placebo group	
van der et al. (183)	double-blind, placebo-controlled multicentre trial	ninety full-term infants, aged <7 months with AD	Synbiotic group: extensively hydrolyzed whey-based formula with additional synbiotics [B. breve M-16V and a galacto/fructooligosaccharide mixture] Control group: same formula without synbiotics	mixture of 90% scGOS and 10% lcFOS 0.8 g/100 ml S: <7 month E: after 12 weeks	B. breve M-16V (1.3×10^9 cfu/100 ml) S: <7 month E: after 12 weeks	no	12 weeks	infants in the synbiotics group have a lower prevalence of asthma-like symptoms (frequent wheezing) and asthma medication use at 1-year follow-up than those who received placebo [13.9% vs. 34.2%, absolute risk reduction (ARR)]20.3%, 95% CI −39.2% to −1.5% and (5.6% vs. 25.6%, ARR−20.1%, 95% CI −35.7% to −4.5%)	1 years
Candy et al. (184)	multicenter, double-blind, randomized controlled trial	Term infants <13 months old, with suspected non-IgE-mediated CMA	Symbiotic group ($N = 35$): amino-acid-based formula (AAF) contained a prebiotic blend and a probiotic strain control group ($N = 36$): commercially available AAF	chicory-derived neutral oligofructose, long-chain inulin (9:1 ratio at a total concentration of 0.63 g/100 ml S: <13 months E: after 8 weeks	*Bifidobacterium breve* M-16V) at a concentration of 1.47×10^9 CFU/100 mL S: <13 months E: after 8 weeks	no	8 weeks	There was a significantly higher median percentage of *Bifidobacteria* w ($p < 0.001$) in the test group than in the control subjects (35.4% vs. 9.7%), whereas a lower percentage of *Eubacterium* rectale/*Clostridium coccoides* group in feces (9.5% vs. 24.2%) and similar to that detected in breastfed infants (55% and 6.5%, respectively). There was no statistically significant changes over 8 weeks in the reported scores for skin symptoms. SCORAD decreased between weeks 0 and 8, from 12.83 ± 18.84 to 9.63 ± 12.45 in the test group and from 14.43 ± 19.74 to 7.06 ± 10.01 in the control group	8 weeks

(Continued)

TABLE 3 | Continued

Refereences	Study	Enrolled patients	Hydrolyzed/ amino acid-based formulas+-synbiotic	Prebiotic substance, Beginning of treatment (S), End of treatment (E).	Probiotic strain, dose Beginning of treatment (S), End of treatment (E).	Pre-natal administration (duration)	Post-natal administration (duration)	Outcomes	Follow-up (duration)
Fox et al. (185)	double-blind, randomized, controlled multicenter trial	Term infants < 13 months old, with suspected non-IgE-mediated CMA	Symbiotic group ($N = 35$): amino-acid-based formula (AAF) contained a prebiotic blend and a probiotic strain control group($N = 36$): commercially available AAF Healthy reference group ($N = 51$)	chicory-derived neutral oligofructose, long-chain inulin (9:1 ratio at a total concentration of 0.63 g/100 ml) S: <13 month E: after 8 weeks	*Bifidobacterium breve* M-16V at a concentration of 1.47 × 10^9 CFU/100 mL S: <13 month E: after 8 weeks	no	8 weeks	The supplementation of AAF with specific synbiotics induced a sustained improvement in gut microbiota composition. The median percentages of bifidobacteria were significantly higher at week 26 in the test group than controls [47.0% vs. 11.8% ($p < 0.001$)], whereas percentages of *ER/CC* were significantly lower [(13.7% vs. 23.6% ($p = 0.003$)]. The use of dermatological medication and reported ear infections were lower in test vs. control, $p =$ 0.019 and 0.011, respectively	26 weeks

labeled these results as not significant, underling the wide CI (186).

Other studies evaluating synbiotic supplementation (182, 184, 185) documented only fecal microbiota changes. Van der Aa et al. (182) reported the effects of a mixture of synbiotics, *B. breve* M-16V, and scGOS/lcFOS, added to an extensively hydrolyzed formula and administered for 3 months to formula-fed infants, in resembling the metabolic profile of breast-fed infants, by modulating the composition and the metabolic activity of gut microbiota. The same synbiotic mixture significantly reduced the prevalence of asthma-like symptoms and of asthma medications use at 1-year follow-up (183) (**Table 3B**). A recent multicenter double-blind RCT (184) documented the effects of an amino acid-based formula (AAF) supplemented with *B. breve* M-16V and fructo-oligosaccharides, in 35 infants with suspected non-IgE-mediated CMA, compared to 36 controls: after 8 weeks of administration, there was a significantly lower median percentage of Bifidobacteria in the control group (9.7 vs. 35.4%), whereas *Eubacterium rectale*/*Clostridium coccoides* group in feces was lower in the synbiotic group (9.5 vs. 24.2%) and similar to breastfed infants (55 and 6.5%, respectively). A subsequent trial with the same study groups and design and formulas confirmed the same changes in the fecal microbiota at 26 weeks (185).

Overall, the limited available data on the role of supplementation with synbiotics for the prevention of allergic diseases cannot allow a definitive conclusion.

DISCUSSION

Even if many studies, reviews, and meta-analyses, and several guidelines are available on this topic, the overall preventive effect of prebiotic/probiotic supplementation on allergic diseases remains unclear. The safety profile of these agents is excellent without significant adverse events in any revised literature. Regarding probiotics, the combined strategy of pre-natal and post-natal supplementation has been demonstrated promising in preventing atopic eczema; however, question when during the gestation and for how long the intervention should continue in the post-natal period is still open. There is no clear evidence that probiotics have a beneficial effect on the development of AR, asthma, and/or wheezing. Thus, routine use of probiotics for preventive purposes cannot be recommended. Future studies focusing on the primary prevention of allergic diseases should investigate the optimal strains, dosing, duration of therapy, and longer follow-up times are warranted (170).

Currently, most guidelines, including those from the European Academy of Allergy and Clinical Immunology (169), and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (98), due to lack of definitive evidence, do not recommend probiotic supplementation to prevent allergic diseases. Conversely, the World Allergy Organization (WAO), recommends probiotics for high-risk infants, for the potential benefits, in pregnant women, in breastfeeding women, and in infants, in preventing AD (10). However, the WAO did not consider specific strains and concluded that available data are not enough to support intervention for preventing any form

of allergic disease by the routine use of probiotics, except for infants at high risk for eczema (10). Indeed, the activity of the probiotics is believed to depend on the bacterial strain type, on the dose, and on the intervention timing. Probiotics have been administered during pregnancy, lactation, to neonates, or later in infancy with different results, and without clear data on the right timing. Furthermore, careful selection of treatment strategy during pregnancy and early infancy is mandatory to identify the best target population, to achieve positive and limit negative outcomes.

The existing evidence on prebiotics is even more limited. The metanalyses and systematic reviews about prebiotics and prevention of allergic diseases concluded that the existing evidence is insufficient to draw conclusions (96, 98) on their preventive effect due to the high heterogeneity of the various studies. The WAO recommends supplementation with prebiotics in infants that are not exclusively breastfed, even if there is a very low quality of evidence (99). Cuello-Garcia et al. (100) showed a potential activity of prebiotic supplementation in infants resulting in asthma or wheezing risk reduction. However, the evidence is very low. The activity of prebiotics on the prevention of atopic eczema is observed, but data are inconclusive. Therefore, the authors (100) stated that available data on supplementation with prebiotics in terms of allergy risk reduction is not so strong to support or reject the concept of benefit or harm with prebiotic.

In addition, after a careful review of the available literature according to the method of administration (**Tables 1**–**3**), we noticed that the positive outcomes in prevention were reported more frequently among the group of studies in which prebiotics or probiotics were given with hydrolyzed/amino acid based formula, compared to those in which were administered alone or with a standard formula, suggesting a possible synergic effect with a hypoallergenic formula that needs to be confirmed with further studies. Moreover, due to the known bifidogenic effect of lactose, its content in different formulas (especially hydrolyzed/aminoacid based formulas) should be taking into account when evaluating the results of prebiotic studies, as might be a possible confounding factor. In addition, it has been recently suggested the possible role of different approaches to complementary feeding in the development of the gut microbiota in early life (87, 187, 188). Therefore, the type of the first solid foods introduced could play a relevant role in shaping the infant's gut microbiota, as well as different intakes of foods naturally containing prebiotic components, all acting as possible confounding factors when evaluating results of pre/probiotic studies.

In conclusion, further RCTs in populations with high or low atopy risk, taking into account a possible synergic effect with other factors, are needed to carefully define the effectiveness of prebiotics/probiotics by itself for allergy prevention. At this time, on the basis of currently available data, supplementation with probiotics for prevention of allergies in children cannot be recommended, even if it is possible to underline the net benefit in high-risk infants in the prevention of eczema, as this effect is predominantly constant across studies available in the literature. However, the optimal strains, dose and timing, and duration of supplementation are still unknown, although a combined pre- and post-natal intervention appeared of stronger benefit. Moreover, the evidence for recommendation of prebiotic supplementation in infants who are not exclusively breastfed is of very low certainty and quality. Therefore, conclusive evidence is still lacking to be able to recommend routine use of pre/probiotics for allergic preventive purposes.

AUTHOR CONTRIBUTIONS

SSe, ED'A, and LP contributed to conception and design of the review, interpretation of data, drafting the article, and final approval of the version to be published. MB, SSa, VT, ES, FT, and DC contributed to interpretation of data, drafting the article, and final approval of the version to be published. All authors contributed to the article and approved the submitted version.

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Nutritional Factors in the Prevention of Atopic Dermatitis in Children

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Atopic dermatitis is one of the most frequent chronic skin diseases worldwide and often develops within the first few years of life. Recent advancements in our knowledge of its pathophysiology have brought to light the role of genetic predisposition and environmental triggers. With the increasing prevalence of allergic diseases, there is a strong need for a better understanding of the various modifiable eliciting factors of such conditions. The concomitant rise in food allergy and insights into the skin barrier function has highlighted the role of nutrition and diet in the prevention and modification of allergic disorders. Furthermore, the identification of the skin as an important route of sensitization, and the risk of progression to asthma later in life, stress the significance of optimizing our management of skin inflammation in the prevention of allergies. Many nutritional factors, including the type of maternal diet during pregnancy, the duration of breastfeeding, the epicutaneous exposure of allergenic food proteins in the first few years of life, the timing of the introduction of complementary foods, the supplementation of vitamins and probiotics/prebiotics during prenatal and early life, have been assessed as potential targets for the prevention of atopy and eczema. Here, we review the latest data addressing prenatal and perinatal nutritional and dietary interventions in the primary prevention of atopic dermatitis. Also, we define knowledge gaps and targets for future research in the prevention of atopic dermatitis.

Keywords: atopic dermatitis, breastfeeding, children, complementary foods, omega-3 long-chain polyunsaturated fatty acids, prevention, probiotics, vitamin D

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INTRODUCTION

Atopic dermatitis (AD) is a common inflammatory skin disease, which affects as many as one-fifth of all individuals (1) and is associated with a high financial and psychosocial burden for patients and their families (2, 3). The prevalence differs greatly in many parts of the world but has been found to have increased significantly in industrialized and developing countries in the last few decades (1, 4, 5). Changes in the exposome, due to urban expansion and socioeconomic growth, have led to greater energy consumption and waste production. The industrial revolution in the nineteenth century has led to increased exposure to air pollutants and chemical hazards, which has had an impact on the integrity of the physical epidermal barrier (6).

Recent findings in the pathogenesis of AD have revealed a complex interplay between impairment of the skin barrier function, environmental and nutritional factors, and immune dysregulation (6–9), which begins in early life. Some evidence suggests that AD is primarily a skin

barrier defect (10, 11), which influences the development of sensitization and atopy (9, 12), and early AD may be a causative factor in developing food allergy (13). Indeed, the condition is often regarded as the first step of the "allergic march," which leads to a progressive course of atopic illness, including food allergy, asthma, and allergic rhinitis.

As a result of the recent rise in allergic diseases worldwide, there has been a growing interest in the exploration of risk factors involved in the development of AD and epidermal barrier dysfunction (14, 15). Recent research has focused on the role of dietary and nutritional intervention in early life for the prevention of allergic diseases, as these factors are modifiable and can influence the immune system maturation in a crucial phase of its development (16).

In this review, we focus on currently available evidence on the nutritional and dietary factors that could be involved in the occurrence of AD and therefore could be targeted for the prevention of this disease (**Figure 1**).

MATERNAL DIET DURING PREGNANCY

Maternal prenatal nutrition and dietary diversity are crucial factors in a child's development. Some of the known health risks associated with intake at this time include obesity, hypertension, and diabetes (17). Several studies have assessed the role of the first 1,000 days after conception and their impact on the pathogenesis of allergic diseases (18). Current literature supports the hypothesis that the process of colonization by a healthy microbiome in the gut, airways, and skin in early life, can affect immune development and maturation, and the susceptibility to immune-mediated disorders later in life, including allergies (19, 20). The prenatal and early infancy period is a critical period for the type of microbiome colonization as well as for the maturation of immune responses, and exposure at this stage can promote immune tolerance (21, 22). The evidence for the prenatal maternal consumption of allergenic foods and their impact on allergic diseases is conflicting, and different for various foods. In addition, there have been many studies assessing the effect of prenatal nutritional exposures on early-life wheezing and asthma, and a paucity of data on AD. An earlier 2007 UK birth cohort found a beneficial effect on maternal oily fish consumption, with eczema at 5 years, but no association was found with other allergenic food groups investigated (23). This was consistent with a previous study by Dunstan et al. (24), which examined the effect of fish oil supplementation during pregnancy on early developing immune responses and outcomes in infants with atopic predisposition. A 2019 review of four longitudinal birth cohort studies found no significant effect of diverse Mediterranean diet patents in pregnancy on atopic outcomes in the offspring (25). Guidelines from Australasia, Germany, and the UK recommend eating fatty fish regularly during pregnancy (26). In 2015 Beckhaus et al. (27) showed that maternal consumption of various supplements (including vitamins C, D, E) had a protective effect on early life wheezing, but this did not extend to other atopic conditions. A recent cohort study found that the higher maternal intake of meat is associated with an increased risk of allergic rhinitis, wheezing, and AD in children (28). Overall, there is conflicting evidence on the effect of prenatal maternal consumption of certain food on the risk of allergy outcomes in childhood (29). Further studies are needed to assess the relationship between maternal dietary intake during pregnancy and long-term allergy outcomes in offspring. Furthermore, dietary diversity needs to be clearly defined to harmonize research into the effect of specific foods, considering geographic and cultural differences.

Abbreviations: AD, Atopic dermatitis; eHF, Extensively hydrolyzed milk formulas; LCPUFA, Long-chain polyunsaturated fatty acids; pHF, Partially hydrolyzed milk formulas; RCT, Randomized controlled trial; SCORAD, Scoring Atopic Dermatitis; CMF, Standard cow's milk formula; VD, Vitamin D.

MATERNAL VITAMIN D INTAKE DURING PREGNANCY

The worldwide rise in allergic diseases has paralleled a vitamin D (VD) deficiency epidemic in Westernized countries (30), which supports the hypothesis that VD might influence the development of allergies (30, 31). VD levels are mainly influenced by sun exposure but also by diet, which makes it an important modifiable factor in allergy prevention. It has been suggested that children born to mothers with low VD intake during pregnancy have an increased prevalence of AD (32). Cross-sectional studies also showed that children born during autumn and winter have a higher prevalence of AD compared with children born in spring and summer (33). In 2015 Beckhaus et al. (27) found that maternal intake of VD, vitamin E, and zinc during pregnancy was associated with a reduced risk of early life wheezing illnesses, but not of childhood-onset asthma or other atopic conditions in offspring. A recent meta-analysis from four prospective cohort studies showed that lower maternal VD serum level during pregnancy was associated with an increased risk of AD in offspring (34). Another recent meta-analysis of observational studies found no significant association between prenatal VD status (i.e., circulating 25-hydroxyvitamin D levels in maternal blood during pregnancy or cord blood at birth) and the risk of AD in offspring from age 1 to 9 years (35). However, a correlation between prenatal VD levels and the risk of AD was found at higher latitude, highlighting the effect of regional and geographic changes (35). More research is needed to analyze the influence of VD maternal status on the occurrence of AD in childhood.

BREASTFEEDING

The is conflicting evidence on the relationship between breastfeeding and allergy risk, with some studies reporting protective effect against AD development, while others showing no effect or even an increased risk for AD occurrence (36). Still, international scientific societies recommend exclusive breastfeeding for at least 4–6 months for primary prevention of allergic disease (37, 38). Breastmilk supports diverse microbial colonization and drives the immune system maturation of

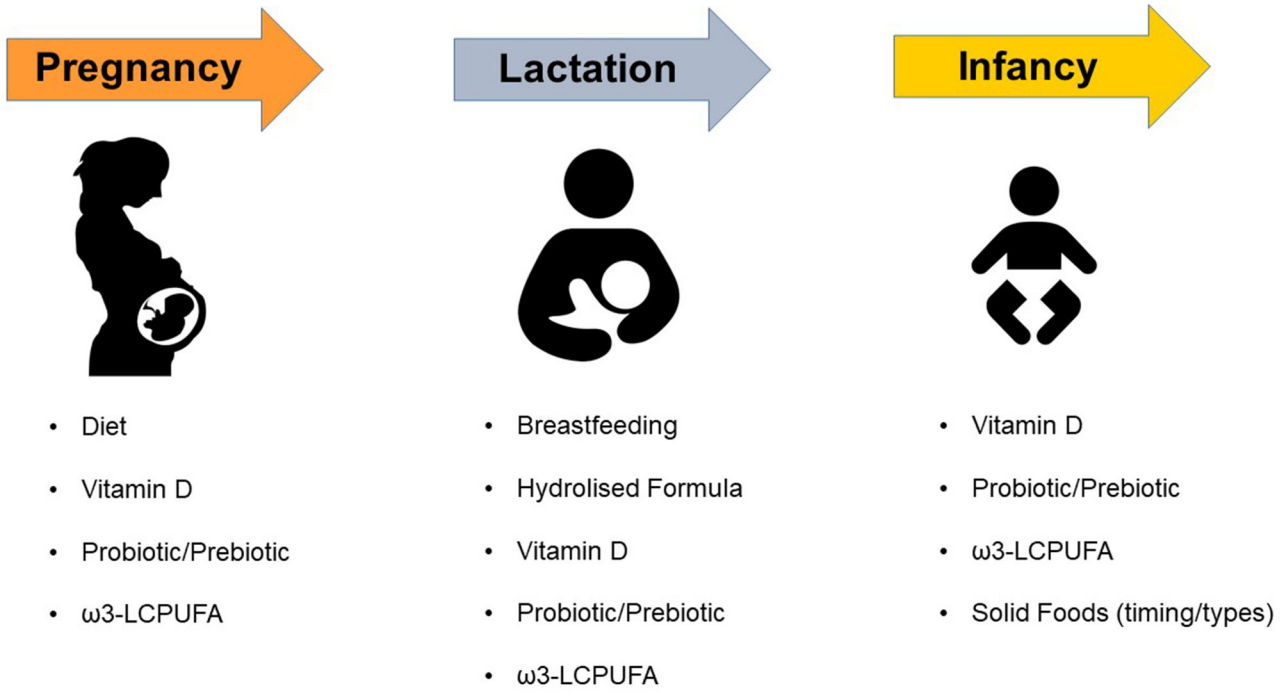

FIGURE 1 | Dietary and Nutritional factors that may affect the risk of atopic dermatitis in children. ω3-LCPUFA, omega-3 long-chain polyunsaturated fatty acids.

the newborn (39–41). Breastfeeding has been associated with decreased morbidity and mortality in infants and lower incidence of allergic diseases (42). A 2005 birth cohort study enrolling 4,089 children showed that exclusive breastfeeding for ≥4 months reduced the risk for developing AD at 4 years of age, irrespective of the concomitant presence of either family history of atopy, allergic sensitization, or asthma (43). A systematic review of 18 prospective studies demonstrated that exclusive breastfeeding for the first 3 months after birth is associated with a lower incidence of AD in childhood, even in the presence of a family history of atopy (44). In contrast, a subsequent systematic review of prospective cohort studies comparing breastfeeding with conventional infant formula feeding or partial breast-feeding in developed countries, revealed that exclusive breast-feeding for at least 3 months was not significantly protective against the development of AD (45). Finally, a recent meta-analysis found that exclusive breastfeeding for 3–4 months was associated with a reduced risk of early life AD (<2 years of age), but the quality of evidence was low (46). In summary, the effect of breastfeeding on the risk of AD remains controversial (36), possibly due to different study populations and designs, and requires more randomized controlled trials (RCTs).

HYDROLYZED FORMULA FEEDING

Elemental cow's milk formula and hydrolyzed cow's milk or soy formulas are often prescribed to infants with the intention to prevent allergic diseases. However, their role in allergy risk reduction is still unclear (47). Partially (pHF) and extensively (eHF) hydrolyzed protein formulas have been widely investigated for their role in allergy and AD prevention. Two earlier RCTs reported no significant difference between pHF and eHF for the prevention of allergic diseases and AD in children (48, 49). This finding was in contrast to an earlier study by Oldaeus et al. (50), who found a lower incidence of AD in at-risk infants fed with a casein-based eHF, compared with those receiving a whey-based pHF or standard cow's milk formula (CMF). The GINI study, a prospective, randomized, double-blind trial, conducted among at-risk children, found a lower risk of AD at 3 and 6 years of life among those children who received a whey-based pHF or a casein-based eHF in their first 4 months, compared to those receiving CMF (51). Interestingly, this finding was exclusive to eczema as hydrolysate nutrition did not have a preventive effect on asthma or childhood wheezing (51). In the nationwide ELFE birth cohort study on infant feeding (comparing breast milk only, pHF with hypoallergenic label [pHF-HA] or without a hypoallergenic label [pHF-non-HA], and CMF), pHF-HA use was not associated with a lower risk of AD (52). The difference in these outcomes could be explained by the fact that the GINI trial was based only on whey-based pHF, whereas the ELFE cohort considered all types on pHF-HA formulas (51, 52). Finally, a recent Cochrane review found that nutrition with hydrolyzed formula, particularly pHF compared to CMF, in the early days of infancy, does not prevent atopic diseases among non-exclusively breastfed infants (47).

POSTNATAL VITAMIN D INTAKE

VD is a pleiotropic hormone and its insufficiency represents a growing global health concern. The VD receptor has been found in numerous immune and non-immune cells, including keratinocytes, and current evidence demonstrates that it modulates the expression of more than 200 genes (53–55). In recent years, the relationship between VD serum levels and the prevalence and severity of AD has been widely studied. Peroni et al. (56) showed that the serum levels of the circulating form of VD, the 25-hydroxyvitamin D, were inversely related to AD severity, although this finding was not confirmed in other studies (57, 58). In a Norwegian study, Byremo et al. (59) randomly selected 30 children from 4 to 13 years of age with severe AD to settle in a tropical zone for 4 weeks, and 26 children with AD to remain in Norway. A significant improvement was observed in disease activity as well as in the quality of life in the group who moved in a tropical zone after 4 weeks and 3 months (59). In a double-blind RCT, in which 60 AD patients aged ≥14 years were randomized to receive either 1,600 IU/day of VD or placebo, authors showed a significant improvement in the active group after 60 days, regardless of the initial severity of AD, which suggests that VD supplementation may improve AD (53). In contrast, Back et al. (60) showed that greater intake of VD during childhood correlated with an increased risk of AD at 6 years of age. VD supplementation in infancy has also been associated with a reduced risk of sensitization to house dust mites at age 18 months, which is an important trigger for the occurrence and severity of AD (61). Even though there are promising results regarding the role and therapeutic use of VD in AD, currently available data are conflicting. RCTs are needed to establish the optimal dose, desired levels, duration of treatment, and effect of VD supplementation in both prevention and treatment of AD.

PROBIOTICS AND PREBIOTICS

It has been hypothesized that an imbalance in the intestinal microbiota composition and metabolic function due to dietary and lifestyle changes may be involved in the pathogenesis of atopic disease (22). The activation of the IL-4/IL-13 axis in AD promotes the skin barrier breakdown and is associated with changes in the gut microbiota (62). Several studies examining the role of oral administration of prebiotics and probiotics in atopy have shown that alterations in gut microbiota composition can precede the occurrence of AD (22, 62). In an earlier case-control study, individuals with AD had lower intestinal concentrations of *Bifidobacterium* compared to healthy controls (63). The *Bifidobacterium* levels were also inversely correlated with AD disease severity, suggesting that intestinal flora might play a role in AD onset and severity (63). The KOALA birth cohort revealed that the presence of *Clostridium difficile* was associated with a higher risk of developing AD and other allergic diseases, while a stronger association was found with *Escherichia coli*, which conferred an exponential risk to AD only (22). Prenatal and postnatal treatment with *Lactobacillus* and *Bifidobacterium* strains have been shown to reduce the risk of AD in infants (62). In a recent double-blind RCT, that included 50 children (aged 4–17 years), Navarro-López et al. (64) reported that a mixture of *Bifidobacterium* strains was effective in reducing AD severity as measured by the Scoring AD (SCORAD) index. A meta-analysis by Huang et al. (65) confirmed this result with improved SCORAD scores in 568 children treated with different strains. In a 2 year follow-up RCT including 132 at atopy risk infants, authors found that the cumulative incidence for AD was lower in the group fed with a formula that contained a mixture of prebiotic oligosaccharides (13.6%) compared to the placebo group (27.9%) (66). A recent Cochrane review, which evaluated the effect of oral prebiotics for the prevention of allergy in infants, reported a significant reduction in AD (67). In summary, supplementation with specific probiotic strains may modulate gut bacteria, which may influence skin inflammation, protect some children against AD development, and be considered a useful therapy in the future (68). However, the strain-specific effects of probiotics make it difficult to make recommendations (**Table 1**). Future studies comparing strains and adopting a common method of outcome measurement (such as SCORAD) will greatly improve our data and clinical recommendations.

OMEGA-3 LONG-CHAIN POLYUNSATURATED FATTY ACIDS

Several birth-cohort studies have reported that increased omega-3 long-chain polyunsaturated fatty acids (LCPUFA) intake during pregnancy may reduce the risk of AD, asthma, and sensitization to house dust mite (69). The supplementation of LCPUFA, through the administration of fish oil during pregnancy and early life, has been proposed for the prevention of allergic sensitization and atopic diseases (70, 71). LCPUFA influence cell membrane structure and function, and potentially modulate inflammatory responses by increasing cell membrane docosahexaenoic acid (DHA; 22:6 ω-3) and eicosapentaenoic acid (EPA; 20:5 ω-3), thus competing with the synthesis of inflammatory arachidonic acid (AA, 20:4, ω-6). This results in a reduction in prostaglandin E synthesis and inhibition of cytokine and immunoglobulin E (IgE) production (72). While some studies showed that maternal fish oil supplementation during pregnancy is associated with a lower incidence of AD in offspring (73, 74), other authors reported no differences in the incidence of allergic diseases (75, 76). Best et al. (77) recently published the long-term follow-up of the DOMInO trial (78), where pregnant women were randomized to receive either fish oil capsules (900 mg of ω-3 LCPUFA) or vegetable oil capsules without ω-3 LCPUFA (control group) daily from the 2nd trimester of gestation until birth. The longitudinal analysis of 706 at-risk offspring from the DOMIno trial showed no significant difference in the progression of allergic diseases between the active and control groups assessed at 1, 3, and 6 years (77, 78). Conversely, a different RCT reported protective effects of prenatal supplementation with ω-3 LCPUFA on the risk of IgE-mediated AD at 1 year, and on follow up at 2 years (73, 74). Finally, a 2015 Cochrane review found that

TABLE 1 | Randomized and non-randomized studies on the use of pre and probiotics for the prevention of atopic dermatitis in children.

References	Study Type	Population (*N*)	Time of exposure	Interventions	Outcomes
Penders et al. (22)	Prospective birth cohort (KOALA birth cohort)	957 infants at 1 month	Postnatal	Detection of gut microbiota composition in stool and total and specific IgE in venous blood	Associations between microbial composition and atopy at 2 years
Watanabe et al. (63)	Case-control	30 AD cases, and 68 controls	Postnatal	Detection of fecal microflora, fecal IgA concentrations, IgA on the skin surface	Differences in fecal microflora between patients with AD and healthy control subjects
Huang et al. (65)	Systematic review and meta-analysis of 13 RCTs	13 RCTs of children <18 years with confirmed AD	Postnatal	Scoring AD (SCORAD) assessment following probiotic administration	Effect of probiotics in the treatment of AD
Arslanoglu et al. (66)	RCT	134, high-risk infants	Postnatal	2 arms: 8 g/L scGOS/lcFOS) or placebo (8 g/L maltodextrin) during the first 6 mo of life	Allergic manifestations and infections during the first 2 years
Osborn and Sinn (67)	Systematic review of RCTs	4 RCTs, including 1428 infants	Postnatal	Prebiotic mixtures in low-risk and high-risk infants	Allergy outcomes in the first 2 years of life
Tam-lim et al. (68)	Systematic review and meta-analysis of RCTs	22 RCTs including 28 different strains	Postnatal	2 arms—strain mixes vs. placebo Mix1 (*Bifidobacterium animalis subsp. lactis* CECT 8145, *Bifidobacterium longum* CECT 7347, and *Lactobacillus casei* CECT 9104); *Lactobacillus casei* DN-114001; and Mix6 (*Bifidobacterium bifidum, Lactobacillus acidophilus, Lactobacillus case*i, and *Lactobacillus salivarius*)	Efficacy of probiotic strains compared to placebo, on pediatric atopic dermatitis

ω-3 LCPUFA supplementation in pregnant or breastfeeding mothers was associated with a reduction in AD in high-atopy risk children aged 12 to 36 months (but not at any other time point) but concluded that there was "limited evidence" to support supplementation with LCPUFA during pregnancy and/or lactation for the prevention of allergic diseases in children (79). In summary, despite the presence of RCTs suggesting protective effects, the data are still inconsistent, and long term follow-ups are crucial to determine whether prenatal and early postnatal ω-3 LCPUFA supplementation may be of benefit as a primary prevention strategy for AD.

TIMING OF COMPLEMENTARY FEEDING

Contrary to previous belief, the delayed introduction of solids in an infant's diet does not reduce the risk of allergic sensitization and atopic diseases (80–83). In a 2011 birth cohort of more than 18,000 newborns and 1,000 AD cases, Chuang et al. (84) found no evidence of a protective effect of delayed introduction of solid foods to infant's diet on the risk of AD at 18 months of age, while a longer duration of breastfeeding increased this risk. A recent case-control study conducted by the HYGIENE Study Group demonstrated that early introduction of solids was inversely related to the risk of AD. Children weaned at 4 months had a lower AD risk (OR = 0.41, 95% CI, 0.20–0.87) compared to those exclusively breastfed, and weaning started at 5 months of age revealed similar results (OR = 0.39, 95% CI, 0.18–0.83) (85, 86). Moreover, findings from the ISAAC Phase II Study found no evidence that prolonged exclusive breastfeeding protected against eczema (87).

The early introduction of fish has been associated with a reduced risk of allergic sensitization in some reports (88, 89), probably due to its high content of LCPUFA (70–72). However, not all the studies confirmed this protective role of fish introduction on the development of allergic diseases (89, 90). Despite the discrepancies in findings, observational studies find that delaying the introduction of solids increases the risk of AD. The difficulty in accepting early weaning to prevent AD is strongly linked with the emphasis given to the nutritional and health benefits of exclusive breastfeeding. Whilst more robust evidence is being sought to specify food types, quantities, and timing, recommendations should be aimed at gradually integrating a more diversified diet from 4 months of age, in addition to breastfeeding.

CONCLUSION

Globally, robust recommendations on dietary intake during pregnancy for the prevention of allergic diseases are sparse. Some guidelines recommend eating fatty fish or taking LCPUFA supplements during pregnancy to reduce AD in the offspring. From our review of common dietary interventional strategies, there is conflicting evidence to support such recommendations.

A most recent systematic review of 17 RCTs and 78 observational studies found no consistent evidence of a clear benefit of nutritional factors in the alteration of the risk of AD in children (91). Long-term follow-up studies are essential to determine the true benefit of prenatal and early life dietary and nutritional interventions as a primary prevention strategy for AD.

AUTHOR CONTRIBUTIONS

TT and PC made substantial contributions to the conception, design, and acquisition of data. TT and PC drafted the initial manuscript. ED'A, DP, and GZ critically reviewed the manuscript for important intellectual content. All authors approved the final version of the manuscript.

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OPINION
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Raw Cow Milk Consumption and the Atopic March

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INTRODUCTION

The article "Dietary Prevention of Atopic March in Pediatric Subjects with Cow's Milk Allergy" in Frontiers of Pediatrics, 11 August 2020, reviews potential solutions to the current worldwide increase in cow's milk allergies. The authors state that "In the last years, diet is emerging as a relevant strategy to prevent allergic diseases through the active modulation of among others the immune system." We agree that there is an urgent need to find new dietary strategies to prevent food allergies as well as the allergic march. However, we feel that the authors of this review miss an important potentially preventive measure, namely raw, unpasteurized farm milk or more gently processed milk.

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RAW MILK IN EARLY CHILDHOOD

There are review studies published about the role of raw milk in asthma and allergy (1–3). In more than 90% of epidemiological studies worldwide there was a protective effect of unprocessed cow's milk consumption on the development of asthma, hay fever and atopic sensitization in both in farm and urban children (1). The meta-analysis of Brick et al. (3) including 12 publications, show consistent protective outcomes of early and current raw milk consumption and asthma in both farm and non-farm children. Evidence was especially strong in the ALEX (4) and the PARSIFAL studies (5) in several regions of continental Europe. The study by Perkin and Strachan (6) in Shropshire, UK clearly demonstrated that raw farm milk was equally, or possibly more, effective in children from non-farming households than in farming children. On the other hand, farm children receiving shop milk or boiled farm milk have increased risk of asthma, allergies and atopy in contrast to those drinking raw farm milk (7).

The above studies have been criticized for their cross-sectional methodology, which may have included various types of biases. However, longitudinal observations of the children included in the European case-control cohort "PASTURE," from 2001 to present, combined with several complementary studies (e.g., the "GABRIELA" studies) supported that consumption of raw milk by the mother and/or the child actually protects against atopic allergy, and this is likely through an early modulation of the immune response (2, 8–10). Epidemiological studies have been evaluated in a meta-analysis and clearly indicate the protective effects of raw milk against asthma and allergies (3).

In addition to the potential tolerance-promoting effect of consumption of raw milk, there are indications that unpasteurized milk may be better tolerated than shop milk by cow's milk allergic children. In a small study eleven children with cow's milk allergy were tested in a double-blind provocation trial with either raw farm milk or pasteurized and homogenized "shop" milk (**Table 1**). Most children tolerated the maximum amount tested of raw milk (50 ml), but far lower levels of

shop milk (8.6 ml on average; $P < 0.01$) (11). In the past 5 years, pre-clinical studies have analyzed various aspects of the raw milk protection against allergic reactions to explain the underlying effects of the protection against the atopic march in children (**Table 1**: 1).

The differences between raw farm milk and shop milk have been tested in sensitization and tolerance studies in mice (**Table 1**: 2; 3; 4). When increasing milk heating from 50 to 80°C was tested, it was shown that the allergic responses were present above 60°C in mice (**Table 1**: 5). Furthermore, in an asthma-mouse model heated raw farm milk (80°C) had a significant impact on the asthma parameters, whereas after raw milk consumption reactions were similar to those observed in the negative control mice (**Table 1**: 8). These preclinical mice studies indicate that the early life allergenic effects of commercial milk are primarily caused by heating of the heat-sensitive whey protein fraction and not by homogenization or fat standardization (13). This, however, does not preclude other additional factors, like the milk fat composition (n3, n6 FA) and of course more clinical validation is needed.

CHANGES IN THE RAW MILK MATRIX

The antigenic properties of raw milk may change after heat treatment. Beta-lactoglobulin (BLG), a protein which is not present in human milk, is considered to be the main allergen in cow's milk. *In vitro* studies showed aggregation and increased antigenicity of BLG when milk is heated above 60°C up to 90°C, while heating above 90°C resulted in a decline of antigenicity, which is known as 'baked-milk' (16, 17). Other whey proteins are also reduced in concentration when milk is heated above 60°C (13). Hence, lower pasteurization temperatures and more gentle

TABLE 1 | Outcomes of pre-clinical studies in allergy and asthma (below) in coherence with a trial in children (above) (abbreviations explained at the bottom of the table).

Test group human	Type of study	Milk comparison	Outcome symptoms	Outcome immunology	Publication
(1) Multiple allergic children, 1½ Y	DBPC Pilot study; Tolerance	Raw, shop	Better tolerance to Raw milk: 50 vs. 8.6 mL of Shop milk	No measurements	Abbring et al. (11) in Clin Exp Allergy. 2019, 00, 1–13; doi: 10.1111/cea.13399
Test group mice	**Type of study**	**Milk comparison**	**Outcome symptoms**	**Outcome immunology**	**Publication**
(2) Female C3H/HeOuj mice, 3-5W	Food allergy; Tolerance	Raw, shop	Raw milk: reduced allergic symptoms (skin, shock, temp)	No measurements	Abbring et al. (12) in Nutrients 2019, 11, 1721; doi: 10.3390/nu11081721
(3) Female C3H/HeOuj mice, 3–5W	Food allergy; Tolerance; Epigenetics	Raw, shop	Raw milk: reduced allergic symptoms (skin, shock, temp)	Epigenetic histone modifications	Abbring et al. (12) in Nutrients 2019, 11, 1721; doi: 10.3390/nu11081721
(4) Female C3H/HeOuj mice, 4W	Cow milk allergy; Sensitization	Raw, 80°-heated, Shop	Raw milk: lower allergic potential and less symptoms (skin, shock, temp); higher risk after 80°C compared to 73°C-heated milk	Lower IgE in Raw milkmice; inhibition of Th2 cytokines	Abbring et al. (11) in Clin Exp Allergy. 2019, 00, 1–13; doi: 10.1111/cea.13399
(5) Female C3H/HeOuj mice, 3W	Food allergy; Tolerance	Raw; Steps from 50°C to 80°C-heated milk/30 min	Milk heated at 65°C and higher was no longer protective in terms of skin response	Immunologically active whey proteins started a decrease in concentration at 60°C; Immunoglobulins denaturation at 60/65°C	Abbring et al. (13) in Food & Function, 11, 4982–93; doi: 10.1039/d0fo01175d
(6) Female C3H/HeOuj mice 3W	Food allergy; Tolerance	Raw, skimmed raw, and pasteurized	Lower allergic potential and less symptoms (skin, shock, temp) after Raw and Skim Raw milk; higher risk after 78°C-heated milk/15 s.	No effects on SCFA in caecum; low IgE and Th2-related cytokines	Abbring et al. (14) in: Nutrients 2019, 11, 1499; doi: 10.3390/nu11071499
(7) Female C3H/HeOuj mice 4W	Cow milk allergy; Sensitization	Native Raw milk whey and 80°C-heated whey	Reduced allergic symptoms (skin, shock, temp) after Raw whey	Reduced Th2 cytokine response in the Raw whey group	Abbring et al. (11) in Clin Exp Allergy. 2019, 00, 1–13; doi: 10.1111/cea.13399
(8) Male BALB/c mice, 6-7W	Asthma: House dust mite allergy	Raw, 80°C-heated Raw	Improved lung function after Raw milk	Reduced number of inflammatory cells	Abbring et al. (15) in Front. Immunol. 8, 1045. doi: 10.3389/fimmu.2017.01045

DBPC, double blind placebo-controlled trial; skin, ear swelling; shock, anaphylactic shock; temp, reduced body temperature; Th2, T Helper-2 cells; IgE, Immune Globulin E; SCFA, Short Chain Fatty Acids.

treatment of milk may be a strategy to increase its tolerability in children with cow's milk allergies.

Further, experimental studies have shown that raw milk ameliorates the allergic reaction. Mice sensitized to house dust mite in an asthma model showed reduced airway responsiveness and eosinophil infiltration when fed raw milk, while heated raw milk did not have this effect (15). Furthermore, raw milk also down-regulated allergic symptoms to an unrelated allergen (ovalbumin) in a food allergy model (13). This implies that heat sensitive whey proteins play a role in tolerance development. Furthermore, the same group of researchers showed that differences in immunogenicity appeared already after heating of the milk to just above 60°C for 30 min, which can be considered a milder treatment than the standard pasteurization procedure. Proteomic studies of heat-treated milk show decreased concentrations of several whey proteins which related to reduced tolerance in the mice model (13).

REDUCING RAW MILK RISKS

Naturally, there may be a risk that raw milk contains pathogens that may cause foodborne disease. Thanks to modern technologies and disease eradication and control programs in food producing animals, raw milk production can be carried out safely with closed milking systems and the maintenance of the cold chains. A review of outbreak data in USA associated with raw milk, reported by the Centers of Disease Control, indicates that such outbreaks have been reduced during the last decade, likely due to improved hygiene (18). Certain farmers specializing in producing raw milk for direct consumption may provide microbiologically safe raw milk by employing high hygienic standards. Berge and Baars (19) have provided evidence that various raw milk production systems exist that provide equal microbial safety as pasteurized milk. A sub-study revealed that the raw milk collected in the farms of the PASTURE study participants had an endotoxin content lower than pasteurized and ultra-high temperature processed milk kept in non-farmer family fridges (20). Further, risk—benefit analyses are necessary for raw milk and raw milk products for different types of vulnerable consumers, based on the latest information of safe raw milk production.

DISCUSSION

Thus, as emphasized by Carrucci et al. (21), the costs for asthma and allergy are increasing. Evidence is accumulating that consumption of raw cow's milk might be an effective preventive strategy that should be further explored. Microbiological safety of such milk must be high priority and strictly controlled. In Western countries this commodity can be produced hygienically and delivered to consumers with existing cold chains to assure that the risk of infectious disease due to its consumption is very low (19). Future research is a must to unravel how safe milk derived products may be produced without damaging important immune modulators. Heating to kill pathogens should not be the only solution to produce microbiologically safe milk. New technological solutions for more gentle processing of milk and training raw milk producers in biosecurity and food hygienic procedures, may minimize the risk of contamination of raw milk by pathogens. In addition, raw milk intended for use in small children may easily be checked for the absence of significant pathogens by rapid PCR procedures before sale of the milk.

AUTHOR CONTRIBUTIONS

TB and AB designed and structured the article, wrote, and read the manuscript. All authors listed have made a substantial and intellectual contribution to the work and approved it for publication.

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Conflict of Interest: AB was employed by the company Berge Veterinary Consulting BV, as independent consultants AB and TB perform limited paid consulting on dairy farms and presentations at workshops for raw milk producers. JG is partly employed at Danone Nutricia Research.

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 Pediatrics

BRIEF RESEARCH REPORT
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Macrophage Receptor With Collagenous Structure Polymorphism and Recurrent Respiratory Infections and Wheezing During Infancy: A 5-Years Follow-Up Study

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Background: Recurrent wheezing is a common clinical manifestation in childhood, and respiratory syncytial virus infection is a well-known risk factor. However, the genetic background favoring the development of recurrent wheezing is not fully understood. A possible role of macrophage receptor with collagenous gene (MARCO) polymorphism has been recently proposed.

Objective: To investigate a correlation between MARCO rs1318645 polymorphisms and susceptibility to recurrent wheezing during childhood.

Methods: We prospectively recruited 116 infants, of which 58 with respiratory syncytial virus bronchiolitis and 58 controls hospitalized at Regina Margherita Children's Hospital, Turin, Italy, between November 2014 and April 2015. All subjects were investigated for MARCO rs1318645 polymorphisms in the first period of life. Genotyping of rs1318645 was carried out by TaqMan mismatch amplification mutation assay real-time polymerase chain reaction procedure. Subjects were then enrolled in a 5-year follow-up study to monitor the occurrence of wheezing and respiratory infections.

Results: The analysis of MARCO rs1318645 of allelic frequencies shows an increasingly significant risk to develop recurrent infection ($p = 0.00065$) and recurrent wheezing ($p = 0.000084$) with a wild-type C allele compared with a G allele. No correlation was found between wheezing and past respiratory syncytial virus infection ($p = 0.057$) and for a history of atopy in the family ($p = 0.859$).

Conclusion: Our finding showed that subjects with C allelic MARCO rs1318645 polymorphism are at higher risk for recurrent infection and wheezing episodes during the first 5 years of life. Future studies of genetic associations should also consider other types of polymorphisms.

Keywords: atopy, MARCO polymorphisms, SNPs, rs1318645, recurrent wheezing

INTRODUCTION

Recurrent wheezing and consequently associated asthma are common respiratory disorders during childhood. A rise in prevalence and severity has been observed in recent years, contributing to increased health system costs (1). Historically, respiratory syncytial virus (RSV) has long been considered the most common trigger of recurrent wheezing (2), particularly in preterm infants (3). A recent meta-analysis performed by Liu et al. (4) also suggests an association between early life rhinovirus infections and the subsequent development of wheezing and asthma.

The variation in response to RSV infection suggests that host intrinsic factors influence susceptibility mechanisms and severity of symptoms. Recently, candidate gene single-nucleotide polymorphisms (SNPs) have been associated with RSV disease severity in infants (5).

We recognized macrophage receptor with collagenous structure (MARCO), an innate immunity scavenger receptor, as a possible gene candidate to RSV disease susceptibility. In the MARCO promoter, we identify a polymorphic region, the SNP rs1318645, −156 C/G (C is the wild type), which influences the transcriptional activity of the gene.

MARCO is a class A scavenger receptor, expressed on macrophages membrane, where it recognizes pathogen-associated molecular patterns and environmental or un-opsonized particles. Studies have demonstrated that MARCO and other scavenger receptors decrease the proinflammatory environment in infections, mediating the clearance of lung pathogens (6).

MARCO promoter contains a polymorphic region that, when deleted, reduced transcriptional activity. In enrolled children, we study SNPs rs1318645. The mutation from C (wild type) to G at the −156 sites has been associated with a deletion of an antioxidant response element of the MARCO promoter. That is a binding site for a transcription factor named "nuclear factor erythroid-derived 2-like 2," also known as NRF2.

TaqMan mismatch amplification mutation assay (TaqMAMA) combines the quantitative strengths of TaqMan with the allele-specific polymerase chain reaction (PCR) of mismatch amplification mutation assay (MAMA). This technique is used for screening human DNA samples for known genetic polymorphisms and may be suitable for broad and cost-effective genotyping applications in all types of laboratories.

Using the MARCO rs1318645 TaqMAMA-specific real-time PCR (7), the outcome of this study is to determine a correlation between MARCO rs1318645 SNP and incidence of bronchiolitis, RSV susceptibility. Consequently, as respiratory infections during infancy, especially by RSV, constitute a significant risk factor for the development of recurrent wheezing and asthma in childhood (8), with a 5-year follow-up, we tried to verify the potential correlation of MARCO rs1318645 SNP and the onset of recurrent infection and wheezing during childhood.

MATERIALS AND METHODS

Population and Enrollment

This case–control study was conducted in Turin Children Hospital Regina Margherita, Italy, between November 2014, April 2015, and December 2020. Study participants were full-term infants with age younger than 12 months. In the control group, we enrolled at term infants, adequate for gestational age (birth weight: 2,500–4,000 g), exclusively or predominant breast milk feeding, who were visited during outpatient control (follow-up visit and routine check) in our Department of Pediatrics at Regina Margherita Children Hospital, Turin, Italy. In the case group, we enrolled infants with a diagnosis of bronchiolitis made by trained pediatricians, hospitalized, and clinically monitored every day.

The parents of the enrolled infants were informed about the purpose, benefits, and possible risks of the study, and written informed consent was obtained. Exclusion criteria included known or suspected impairment of immunological function, major known congenital airway malformations, chronic lung disease, cardiac disease, prematurity (gestational age younger than 37 weeks), neuromuscular disorders affecting swallowing, and known or suspected coagulation disorders or bleeding tendency. Baseline population characteristics (age, sex, breastfeeding history, smoke exposition, family history of atopy, like atopic dermatitis, recurrent wheezing, asthma, and food allergy) reported by parents were collected by investigators. A pediatrician investigator monitored the clinical evolution of the participants by visiting them 5 years after enrollment.

The parents of the enrolled infants were informed about the purpose of the study to obtain written consent, and a standardized questionnaire was administered to investigate the onset of symptoms associated with recurrent wheezing and history of recurrent infections. The pediatrician investigators also asked for information about the development of allergic symptoms, discovering of food allergy, or execution of allergy test (e.g., Patch test or Prick test). The data reported by parents were collected and classified in the previous database.

The study was conducted in accordance with the International Council for Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines protocol, applicable regulations and guidelines governing clinical study conduct, and the ethical principles that have their origin in the Declaration of Helsinki[1].

Genetic Marker Selection and Samples Preparation

A MARCO rs1318645 SNP (GenBank access number NT_005403.18)[2] on chromosome 2 showing a C/G transition in position 24445994 was chosen.

Infants were subjected to a venous blood sample from 7:30 to 8:30 AM during the routine clinical blood sampling to reduce the stress of infants.

[1] Available online at: https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/

[2] Available online at: https://ftp.ncbi.nlm.nih.gov/genbank/

Genomic DNA was extracted from blood using the Maxwell16 LEV Blood DNA kit with automatic extractor Maxwell16 System (Promega, Madison, Wisconsin, USA) following the manufacturer's instructions.

Genotyping of rs1318645 was carried out by TaqMAMA real-time PCR procedure (MARCO rs1318645 cod. PP-036) (BioMole, Turin, Italy) (7).

The work described was carried out in accordance with the code of ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans[1].

TaqMan Mismatch Amplification Mutation Assay rs1318645 Real-Time Polymerase Chain Reaction Assays

MAMA allele-specific forward primers were designed. The last nucleotide at 3′ region gives the specificity of the primers. An additional deliberate mismatch in the third last from the 3′ end improves the specificity of MAMA primers. The established assays use the same probe and reverse primer and probe mix PP-036 (BioMole, Turin, Italy). The Probe is a TaqMan labeled at the 5′ end with 6-carboxyfluorescein and at the 3′ with 5-carboxytetramethylrhodamine.

PCR was set up in a volume of 20 μl, containing 5 μl of DNA and 15 μl of reaction mix (PP-036, BioMole, Turin, Italy). Assays were performed with 7500 Real-Time PCR System (Thermo Fisher Scientific, Waltham, Massachusetts, USA), following the standard Life Technologies profiles and subsequently analyzed using ABI Prism 7500 SDS software version 2.0 (Life Technologies, Thermo Fisher Scientific, Waltham, Massachusetts, USA).

Statistical Analysis

For statistical analysis, the chi-square test was performed using commercial software (GraphPad Prism 5 San Diego, California, USA); a $p < 0.05$ was considered statistically significant. The *P*-value was calculated for each allele in the control group and in patients with recurrent infections and wheezing. A *P*-value of <0.05 was considered significant with 95% confidence intervals.

RESULTS

Population

A total of 116 infants were enrolled. According to the criteria described in section Materials and Methods, 58 infants with bronchiolitis were identified in the case group and tested for

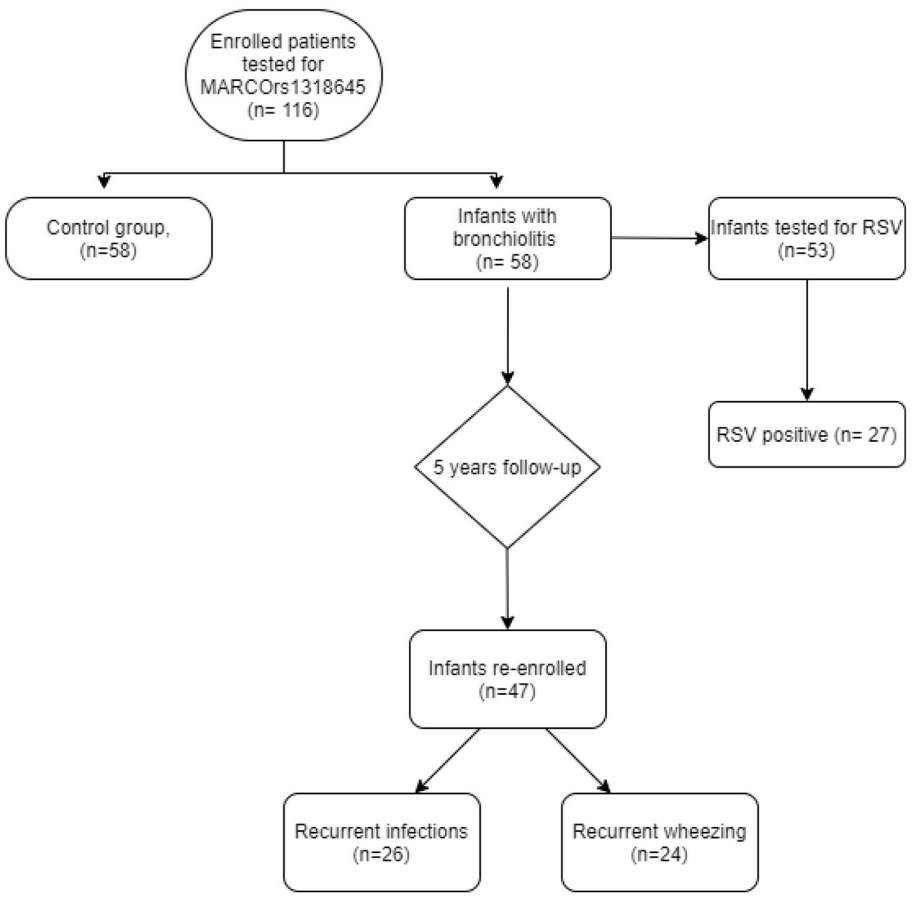

FIGURE 1 | Enrollment process and follow-up.

MARCO. Bronchiolitis group includes 29 (50%) male patients and 29 (50%) female patients as shown in **Figure 1**.

Fifty-eight children were enrolled in the control group, including 30 (51.7%) male patients and 28 (48.3%) female patients, at term, adequate for gestational age (birth weight: 2,500–4,000 g), exclusively or predominant breast milk feeding.

As shown in **Table 1**, both groups were similar ($p > 0.05$ indicates that no statistical effect was observed) for age at enrollment, sex, and type of feeding (breastfeeding). The mean duration of hospitalization in the bronchiolitis group was 6.87 days (range 5–21).

Respiratory Syncytial Virus Test Results

In the bronchiolitis group, we executed nasopharyngeal swabs for respiratory viruses. We study RSV susceptibility in 53 (91.4%) infants with bronchiolitis. A total of 27 (51%) tested positive for RSV. Five of 27 were also positive in nasopharyngeal pathogen detection for other viruses (rhinovirus and coronavirus NL63).

Infants with RSV-positive bronchiolitis (51%) and RSV-negative bronchiolitis (49%) have shown the same allelic and genotype frequencies: χ^2 analysis of genotypes ($p = 0.373$) and allelic ($p = 0.250$); frequencies showed no association between MARCO rs1318645 and RSV susceptibility.

Five-Year Follow-Up

In bronchiolitis cohort follow-up, we contacted families of bronchiolitis-group infants to organize a visit to our hospital. A team of pediatricians performed a medical examination of enrolled children and interviewed parents using a structured questionnaire.

The follow-up aimed to verify the possible association between MARCO rs1318645 SNP and the onset of recurrent respiratory infections, defined as one or more admissions to the pediatric emergency department for acute respiratory symptoms or three or more visits to general pediatricians for clinical respiratory infections and/or the development of recurrent wheezing [three or more episodes, which requires one or more accesses to the pediatric emergency department or pneumology or allergology specialistic visit and leading to bronchodilator drug (β2 agonist) or inhaled corticosteroids prescription].

Families who referred a history of recurrent wheezing focused on the necessity to access to the emergency department or consult an allergology or pneumology specialist and leading to bronchodilator drug (β2 agonist) or inhaled corticosteroids prescription.

We asked parents about smoke exposition and the development of airway malformations. We excluded two infants who developed airway complex chirurgical conditions. We correctly reenrolled 47 patients, in which we researched family history of atopy (atopic dermatitis, recurrent wheezing, history of asthma, and food allergy in parents).

A total of 19 (40.4%) infants were exposed to passive smoke during infancy. Eleven (57.9%) of these smoke-exposed children developed recurrent airway infections, but also 15 (54%) of 28 nonexposed children developed recurrent respiratory infections. No correlation between cigarette smoke exposition and recurrent infections resulted.

Nine (47.4%) of smoke-exposed children and 15 (54%) of nonexposed children developed recurrent wheezing. No correlation between cigarette smoke exposition and recurrent infections (p-value 0.770) or wheezing (p-value 0.676) resulted.

Nineteen children (40.4%) referred a history of atopy in their family (e.g., atopic dermatitis, recurrent wheezing, history of asthma, and food allergy). A total of 12 (63.2%) of these presented recurrent infections (p-value 0.373) and 10 (52.6%) referred recurrent wheezing (p-value 0.859).

Twenty-four children with past RSV-positive tampons were correctly enrolled in the follow-up. A total of 15 (62.5%) children referred recurrent airway infection, and the same number referred recurrent wheezing. No correlation between RSV infection during infancy and recurrent infections (p-value 0.191) or wheezing (p-value 0.057) resulted.

Role of Macrophage Receptor With Collagenous rs1318645 in Recurrent Airway Infections During Childhood

We defined recurrent infections as one or more admissions to the pediatric emergency department for acute respiratory symptoms or three or more visits to general pediatricians for the same symptoms. Allelic and genotype frequencies in an infant

TABLE 1 | Epidemiological characteristics of enrolled subjects.

	Infants with bronchiolitis ($n = 58$)	Healthy subjects ($n = 58$)	*P*-value
Age at enrollment in weeks (mean range)	14.6 (2–24)	13.0 (2–31)	0.830*
Gender			
Female, *n* (%)	29 (50.0)	28 (48.2)	0.853
Male, *n* (%)	29 (50.0)	30 (51.7)	
Breastfeeding *n* (%)	24 (41.3)	32 (55.2)	0.137
Nationality			
Italian, *n* (%)	26 (44.9)	30 (51.7)	0.552
Foreign, *n* (%)	32 (55.1)	28 (48.3)	

*Mann–Whitney test.

TABLE 2 | MARCO rs1318645 and recurrent airway infections susceptibility.

Target	SNP	Alleles/ genotypes	Recurrent infections ($n = 26$)	No recurrent infections ($n = 21$)	*p*-value
MARCO	rs1318645	C	38 (73.1%)	16 (38.1%)	0.001*
		G	14 (26.9%)	26 (61.9%)	
MARCO	rs1318645	CC	13 (50.0%)	5 (23.8%)	0.002#
		CG	12 (46.2%)	6 (28.6%)	
		GG	12 (46.2%)	10 (47.6%)	

*Allelic frequencies; #genotype sequences.

TABLE 3 | MARCO rs1318645 and recurrent wheezing during childhood.

Target	SNP	Alleles/ genotypes	Wheezing ($n = 24$)	Not wheezing ($n = 23$)	*p*-value
MARCO	rs1318645	C	37 (77.1%)	17 (37.0%)	0.001*
		G	11 (22.9%)	29 (93.0%)	
MARCO	rs1318645	CC	14 (58.3%)	4 (17.4%)	0.002#
		CG	9 (37.5%)	9 (39.1%)	
		GG	1 (4.2%)	10 (43.5%)	

*Allelic frequencies; #genotype frequencies.

with recurrent infections are shown in **Table 2**. The analysis of allelic frequencies shows a significant risk of C allele to develop recurrent infection compared with G (p-value = 0.001).

Role of Macrophage Receptor With Collagenous rs1318645 in Recurrent Wheezing

We define recurrent wheezing as three or more episodes that require one or more access to the pediatric emergency department or pneumology or allergology specialistic visit and leading to bronchodilator drug (β2 agonist) or inhaled corticosteroids prescription. Allelic and genotype frequencies in an infant with recurrent wheezing are shown in **Table 3**. The analysis of allelic frequencies shows a significant risk of C allele to develop recurrent wheezing episodes compared with G (p-value = 0.001). χ^2 analysis of genotypes frequencies are significant (p-value = 0.002).

DISCUSSION

Respiratory viruses are one of the causes of early life wheezing that may contribute to the development of childhood asthma. Several epidemiological studies reported that respiratory virus-associated wheezing during early life, particularly during infancy, contributes to the development of asthma during childhood (9–12).

Growing data have associated specific SNPs in several immune-related genes with altered susceptibility to infectious diseases, but despite their potential role, there are few data about SNPs associated with RSV and other respiratory infections; a systematic literature review has been performed by Drysdale et al. (13).

SNPs in genes coding for interleukin (IL)-8, IL-19, IL-20, IL-13 mannose-binding lectin, interferon-gamma, and a regulated on activation, normal T cell expressed and secreted polymorphism have been associated with subsequent wheeze after RSV lower respiratory tract infection in term-born infants (14). Utilizing the inpatient database of severe RSV infection or without, we compared MARCO rs1318645 [GenBank access number NT_005403.18[2]] polymorphism and risk of recurrent wheezing or recurrent airway infections during childhood. Our finding allowed us to identify a biologically plausible bronchiolitis susceptibility gene candidate: MARCO. Unexpectedly, we identified the wild-type C allele frequency as an increased risk of recurrent infection and wheezing incidence in an infant. We did not show genotypic or allelic different susceptibility in bronchiolitis RSV positive vs. RSV negative, and, in contrast to what High et al. (15) reported, this research did not find evidence supporting a causative role of C/C genotype o G allele for RSV disease severity. In fact, they determined that G allele and G/G genotypes were more likely to have severe RSV disease than children who were heterozygous or wild type for the C allele, showing a missense loss of function polymorphism in the human MARCO homolog associated with severe RSV disease in two populations of infected infants (15). Demonstrating a correlation between the wild-type C allele and increased susceptibility to bronchiolitis, we can speculate an important role of MARCO in the pathogenesis of airway infection, but our results about RSV disease severity differ from those of High et al. suggesting that the C allele increases the risk of bronchiolitis but in a mild presentation, whereas G allele is associated with severe clinical form of the disease. The precise role of MARCO is still not clear in our study, as well as the utility of more studies for understanding human RSV disease.

Although RSV infection is very common, a minority of infected children develop wheezing during childhood. The genetic factors that increase the risk of wheezing have not been clarified yet.

Respiratory viruses replicate in cells, damaging airway epithelium that is a chemical, physical, and immunological barrier. They cause loss of cilia, decreasing mucociliary clearance and promoting the sensibilization to allergens and environmental irritants. Causing sustained airway inflammation, recurrent infections of the respiratory tract play a major role in the pathogenesis of wheezing (16), and one wheezing episode or more during the first years of life are confirmed risk factors for the development of asthma (2, 17, 18).

Also, the atopic clinical history and the early-life wheezing episodes synergistically contribute to asthma pathogenesis (19, 20).

We hypothesize the immune system impairment on the epithelial barrier, in which MARCO is involved, predisposing to respiratory tract infections and recurrent wheezing in the first years of life. For this reason, we established a follow-up of children affected by bronchiolitis to study wheezing development as a risk of future asthma. As shown in our results, we identified the wild-type C allele frequency and C/C genotypes as increased risk of recurrent infections and wheezing 5 years after the bronchiolitis episode rather than the G allele. This confirms that, unexpectedly, the wild-type allele in SNP rs1318645 is correlated with an increased susceptibility to respiratory sequelae of bronchiolitis. We also observed a trend in our results that describe a major susceptibility to recurrent wheezing in children with previous RSV + bronchiolitis. This is confirmed by literature from years (21), although the link between RSV infection and the development of wheezing is still partially unclear. At the basis of this phenomenon, it would seem to be involved not only in the remodeling of the respiratory tract after the inflammatory process. For this reason, researchers are focusing on SNPs, speculating about their genetic predisposition to atopy, wheezing, and asthma. For example,

SNPs s8076131, rs12603332, and rs3744246 of gene ORMDL3, which has been reported to be involved in the lung epithelial defense pathway, have been associated with the development of asthma such as GSDMB SNPs rs7216389 (22, 23). Also, the SNPs rs1889570 (C/T), IL-17A rs4711998 (A/G), and IL-17A rs3819024 (A/G) of THE IL17F gene have recently emerged as associated with more post- bronchiolitis asthma development (24). ILs are also associated with allergic sensibilization, such as polymorphisms of the genes that code for IL-4 and IL-13 (25–27), such as a correlation between toll-like receptor 9 rs187084 gene polymorphism and wheezing has been described (28). From our study, apparently, the genetic predisposition seems to affect the airway infection susceptibility and the recurrent wheezing more than the previous RSV infection. As we can see, candidate gene SNPs are the vanguard for the future study of genetic contributions to complex, multifactorial diseases, specifically examining common variants and eventually making it possible to identify determinants of disease.

We admit that there are some limitations to our study. First, our research is focused on a single SNP (MARCO rs1318645 polymorphism), although nowadays, it is possible to investigate more polymorphisms, such as IL-4 rs2243250, ADRB2 rs1042713, and FCER1B rs569108 and IL-13 rs20541 (29, 30).

Besides, our methods of data collection do not include a respiratory function test, such as spirometry, to define the grade of bronchoconstriction in patients with wheezing.

A small number of subjects have been enrolled, and for this reason, it is necessary to explore the effects of other variables that might result in misleading interpretations of causality.

Nevertheless, our work remains relevant, as our findings contribute to the emerging global picture of recurrent wheezing and childhood-onset asthma and because it outlines the roots for future, more integrated studies.

In summary, we describe an association between MARCO rs1318645 polymorphisms and susceptibility to recurrent airway infection and recurrent wheezing in children, but mechanisms through which MARCO modulates the inflammatory response to RSV and the disease severity are not completely understood. As innate immunity receptors seem to play a critical role in host defense, as well as allergy, nutritional factors, and asthma, the genetic risk factors involved in susceptibility to RSV infections are needed to eventually prevent RSV disease and associated sequelae in children.

Future studies of genetic associations should consider the different wheezing phenotypes in infancy. In addition, stratified genetic analyses for subjects with atopy can be useful for elucidating the background mechanisms of recurrent wheezing.

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/s.

ETHICS STATEMENT

Ethical approval was not provided for this study on human participants because the work described was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans, http://www.wma.net/en/20activities/10ethics/10helsinki/index.html. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

The guarantors of the study are FS and MB, from conception and design to conduct of the study and acquisition of data, data analysis, and interpretation of data. FS, FP, and MB have written the first draft of the manuscript. FP has performed follow-up, tables, and searched references. VD, CC, CA, PM, and IG have performed genetic investigations. All co-authors have provided important intellectual input and contributed considerably to the analyses and interpretation of the data. All authors guarantee that the accuracy and integrity of any part of the work have been appropriately investigated and resolved, and all have read, edited, and approved the final version of the manuscript. The corresponding author had full access to the data and had final responsibility for the decision to submit for publication. No honorarium or other forms of payment was given to any of the authors to produce this manuscript.

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Increased Frequency of CTLA-4 and PD-1 Expressing Regulatory T Cells and Basophils With an Activating Profile in Infants With Moderate-to-Severe Atopic Dermatitis Hypersensitized to Food Allergens

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Background: Infants with severe atopic dermatitis (AD) may be sensitized to foods that have not been introduced into their diet, posing a risk for developing an immediate hypersensitivity reaction on the first exposure to the food to which they are sensitized. The aim of this work was to perform an analysis of the sensitization profile in infants with moderate-to-severe AD and to identify cellular and molecular markers for food allergy (FA).

Methods: Blood samples from healthy donors and children with moderate-to-severe AD were studied. Specific IgE to several allergens were determined using ImmunoCAP FEIA system and ISAC technology. Furthermore, using flow cytometry-based studies, basophils and regulatory T (Treg) cells were phenotypically characterized.

Results: 90% of children with AD were sensitized to food antigens before introducing them into the diet, and 100% developed FA. Phenotypic analysis showed a significantly higher percentage of CTLA-4 and PD-1 expressing Treg cells in AD patients than in healthy controls. Basophils from patients exhibited a marked reduction in the expression of CD300a, higher expression of FcεRI and CXCR4, and to some extent higher expression of CD63 and CD300c.

Conclusions: Infants with moderate-to-severe AD are at high risk of being sensitized to food allergens. Therefore, to avoid allergic reactions, broad-spectrum sensitization studies are necessary before introducing complementary diet. Increased expression of CTLA-4 and PD-1 suggests greater suppressive potential of Treg cells in infants with AD than healthy controls. Furthermore, our results suggest a role for CD300 molecules on circulating basophils as possible biomarkers for FA susceptibility.

Keywords: atopic dermatitis, food sensitization, specific IgE, regulatory T cells, basophils, CD300 receptors, inhibitory receptors

INTRODUCTION

Atopic dermatitis (AD) is a common inflammatory skin disease of increasing prevalence that affects 7–14% of adults and 10–20% of children globally (1). The onset of AD occurs during the first year of life in ∼60% of individuals and only the 10% of affected children start the disease after the age of 7 (2). It causes generalized skin dryness and eczematous lesions constituted by spongiosis, edema, and microvesicles, which give rise to itching, skin irritation, scratching, and symmetrically distributed inflammatory lesions (2, 3). Although the severity of the disease is variable, it is moderate-to-severe in up to one third of patients, many of whom require systemic treatment.

Several epidemiological studies have established that AD is often accompanied by allergic rhinitis, asthma and food allergy (FA) (the "atopic march"), as well as conjunctivitis (4–7). Additionally, although the mechanisms are still unknown, it has been shown that infants with severe AD may be sensitized to foods that have not yet been introduced into their diet (8–11), turning these children into high-risk patients when introducing new foods. In this sense, Palmer et al. observed in 2013 that a high proportion (36%) of children with moderate-to-severe AD were already sensitized to egg at 4 months of age before introducing solid foods in their diet (8). Moreover, there are studies showing that the early introduction of solid food at 4–6 months of age reduces the risk of developing FA in patients with severe AD not yet sensitized (12–14), which is changing the pattern of complementary feeding in children with AD. Despite these studies, in the everyday practice of hospitals in our geographic area only milk and egg specific immunoglobulin E (sIgE) are determined in infants with AD. As sensitization can affect other foods in addition to eggs and milk, we considered very relevant to extend the determination of sIgEs to other foods before starting complementary feeding.

Allergy is the result of a dysregulation of immune tolerance, that is, the immune system recognizes as dangerous a substance that is harmless to the body. One of the most important cells in the regulation of the immune system are regulatory T (Treg) cells, which play a major role in the regulation of allergic reactions by inducing and maintaining immune tolerance to allergens (15–18). Treg cells play an important role in various immune pathologies (15, 17, 19–22). Thus, it has been observed that the frequency of Treg cells is altered both in allergic patients and in patients with AD, compared to healthy individuals (23–28). Although others have found no significant differences in the frequency of blood Treg cells between patients and healthy controls (29–33).

The immune response is also regulated, among others, by activating and inhibitory receptors expressed on the surface of immune cells. In this sense, the involvement of CD300 surface receptors in the regulation of allergic responses has been demonstrated (34–39). It is known that CD300a expressed on basophils suppresses the basophil anaphylactic degranulation by its interaction with phosphatidylserine and phosphatidylethanolamine exposed on apoptotic cells (34, 35), whereas CD300c works as a costimulatory molecule during immunoglobulin E (IgE)-mediated basophils activation (37). Moreover, in basophils from allergic individuals, a lower expression of CD300a inhibitory receptor (34) and a higher expression of CD300c activating receptor (37, 38) have been described. In AD patients a significant increase in CD300a total expression was observed in biopsies from lesional skin compared to normal skin, specifically on eosinophils, macrophages, and mast cells (40). However, CD300 expression in basophils from AD patients has not been studied to date.

In this pilot study, we have done a comprehensive analysis of the sensitization profile of infants with moderate-to-severe AD before the introduction of complementary feeding. Moreover, we have performed immune profiling analysis and conducted surveys about the eating habits of families with the aim to understand possible mechanisms by which these children become sensitized at such an early age, and finally we have determined the development of FA by the age of two.

METHODS

Donors and Samples

Blood samples from healthy donors and infants with moderate-to-severe AD were collected prospectively through the Basque Biobank (http://www.biobancovasco.org), which complies with the quality management, traceability, and biosecurity set out in the Spanish Law 14/2007 of Biomedical Research and the Royal Decree 1716/2011. Parents of all subjects provided written and signed informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Ethics Committee for Clinical Research of Cruces University Hospital (CEIC E18/26).

As healthy controls ($n = 9$), children under 2 years of age who had not presented episodes of AD during their lifetime, did not have a diagnosis of allergy, and had not presented allergic symptoms to potentially allergenic foods were recruited. AD patients ($n = 10$) were under 12 months of age when they were included in the study and they were not being treated with corticosteroids at the time of blood sampling. The inclusion criterion was a diagnosis of AD in the first 6 months of life, either for lesions on the face and/or large areas that had required topical corticosteroids for more than 7 days a month (moderate AD) or generalized lesions that had been treated with a course of systemic corticosteroids (severe AD) (more details about inclusion criteria and clinical features are detailed in **Supplementary Material**). Potentially allergenic foods such as milk, egg, hake, cod, tuna, salmon, shrimp, mackerel, rooster, peanut, hazelnut, almond, nut, pistachio, cashew, wheat, soy, rice, potato, lentil, white bean, chickpea, kiwi, or peach had not been introduced into their diet when they were recruited for the study.

Food sensitization was diagnosed by *in vitro* sIgEs quantification that was made using the ImmunoCAP FEIA system, as specified below. sIgE values > 0.35 KU/L were considered positive. Regarding FA development, the evolution of the children was followed for 2 years approximately (**Supplementary Table 1**). During the monitoring, *in vitro* sIgEs quantifications and/or skin prick test in response to specific allergens were carried out. Skin tests were regarded as positive if the wheal diameter was >3 mm, and food has been introduced

in case of prick test < 3 mm. A positive control of histamine 10 mg/mL and a negative control of saline 0.9% were included. At 2 years of age, children with an exclusion diet were considered allergic to food. Food provocation was not justified, given the high predictive value of their degree of sensitization assessed by prick test and serum sIgE values (41–44) (more details in **Supplementary Material**).

Specific IgEs Quantification

sIgEs to cow's milk, egg white and yolk, hake, cod, tuna, salmon, shrimp, mackerel, rooster, peanut, hazelnut, almond, nut, pistachio, cashew, wheat, soy, rice, potato, lentil, white bean, chickpea, kiwi, peach and to specific allergens alfalactalbumin (ALA), betalactoglobulin (BLG), casein, ovalbumin (OA), ovomucoid (OM), Ara h1, Ara h2, Ara h3, Ara h 8, Ara h 9, Cor a1, Cor a8, rJug r1, rJug r3, rPru p1, rPru p4, rPru p3, rAct d8, rGad c1, nGly m4, nGly m5, and nGly m6 were determined with the ImmunoCAP FEIA system (Thermo Fisher Scientific) according to the manufacturer's instructions. Values higher than 0.35 kU/L were considered positive.

Immuno Solid-Phase Allergen Chip

IgE sensitization was also analyzed with a customized allergen chip based on the Immuno Solid-phase Allergen Chip (ISAC) technology (Thermo Fisher Scientific). Healthy donors and patients were tested for a panel of 112 allergen components (**Supplementary Table 2**), according to the manufacturer's instructions. Briefly, allergen components (triplicates) were immobilized on a glass slide. The assay includes a two-step reaction: (1) 30 μl of serum sample were applied onto the microarray reaction site; and (2) after incubation and washing, fluorescence-labeled anti-human IgE detection antibody was applied. After incubation, washing and drying, the microarrays were analyzed using a confocal laser scanner (LuxScan 10K Microarray Scanner) and evaluated by Phadia MIA Microarray Image Analyzer version 1.4.3 software (Thermo Fisher Scientific). For calibration and detection of background signals, a calibrator serum and sample diluent from Thermo Fisher Scientific were included in each analysis. The results were expressed as ISAC standardized units (ISU), and values ≥ 0.1 ISU were considered positive.

Flow Cytometry Analyses

Whole blood samples from healthy donors and AD patients were collected in sodium citrate containing tubes and were used for phenotypical characterization of basophils and Treg cells. For the characterization of basophils, 100 μl of whole blood was stained with fluorochrome conjugated mAbs for 20 min at room temperature (RT). Next, red blood cells were lysed using 1X BD FACS Lysing buffer for 15 min at RT. Then, cells were washed with phosphate buffered saline (PBS) to remove unbound mAbs and further acquired in a MACSQuant® Analyzer 10 Flow Cytometer (Miltenyi Biotec). As a negative control, we used fluorescence minus one (FMO) and unstained controls. FMO control contains all the fluorochrome conjugated mAbs with the exception of the marker of interest and it is used to identify and gate cells in the context of data spread due to the multiple fluorochromes in a given panel.

For the characterization of Treg cells, first peripheral blood mononuclear cells (PBMCs) were freshly isolated from donors' whole blood by Ficoll Paque Plus (GE Healthcare) density gradient centrifugation. PBMCs were incubated with LIVE/DEAD Fixable Near-IR Dead Cell Stain reagent (Invitrogen) before the extracellular staining in order to detect dead cells following the manufacturer's protocol. For extracellular staining, PBMCs were incubated with the specific mAbs for 30 min at 4°C in the dark. Cells were washed with PBS containing 2.5% of bovine serum albumin (BSA) (Sigma-Aldrich) and were permeabilized and fixed using Foxp3/Transcription Factor Staining Buffer Set (eBioscience) following manufacturer's instructions. Then, they were incubated with APC anti-Foxp3 and PerCP-Cy5.5 anti-Helios during 30 min at RT in the dark. Lastly, cells were washed, resuspended in 200 μl of PBS and acquired in a MACSQuant® Analyzer 10 Flow Cytometer. Flow cytometry data were analyzed using FlowJo [version 10.0.7 (TreeStar)] and FlowLogic (version 7.3) software. The flow cytometry panels used in this study are shown in **Supplementary Table 3**.

Statistical Analysis and Graphical Representation

GraphPad Prism software (version 9) was used for graphical representation and statistical analysis. Data were represented in scatter plots with bars showing the means ± standard error of the mean (SEM). For comparison between healthy and DA patients, the non-parametric, unpaired Mann–Whitney rank test was used. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$.

The detailed list of antibodies, allergens of the Immuno Solid-phase Allergen Chip (ISAC) and flow cytometry analyses are detailed in the **Supplementary Material** section.

RESULTS

Children With Moderate-to-Severe AD Are Very Likely to Be Hypersensitized to Food Antigens

With the aim of knowing the sensitization profile of infants with moderate-to severe AD before starting with complementary diet, we determined sIgEs to several food allergens using ImmunoCAP FEIA system. In addition, determination of sIgE for 112 allergens from 50 allergenic sources (43 foods) was also performed by ISAC. Nine out of 10 (90%) children with moderate-to-severe AD showed sIgE against some of the tested antigens (**Table 1**), while around 33% of healthy control children were sensitized to food antigens, although to a significantly lower number of allergens (**Table 2**).

Sensitization does not always equate to clinical allergy. In our study cohort, based on their clinical symptoms history, serum sIgE levels, and/or skin prick test results, while none of the healthy control children were diagnosed of FA, all 10 children that had been diagnosed with moderate-to-severe AD were found to be allergic to some food by the age of 2, and 70% of them were

TABLE 1 | sIgEs determined by ImmunoCAP FEIA and ISAC in infants with moderate-to-severe AD.

Patient	ImmunoCap (>0.35 KU/L)	ISAC >0.3 ISU-E	Age
DA001	Negative	Negative	3 months
DA003	Egg white (2.6); ovomucoid (0.37); ovalbumin (2.19); peanut (1.07); rArah1 (0.86)	Negative	11 months
DA004	Egg white (0.53); ovalbumin (0.46)	nd*	4 months
DA005	Egg white (5.78); ovomucoid (7.19); ovalbumin (0.69); peanut (6.05); rAra h2 (5.95); almond (0.72); hake (8.8)	Ovomucoid (1.3); rAra h2 (1.8)	11 months
DA006	Egg (55.50); egg white (66.40); ovomucoid (>100); ovalbumin (23.50); cow milk (8.42); α-lactoalbumin (0.47); β-lactoglobulin (1.25); casein (9.64); peanut (25.20); rAra h1 (10.90); rAra h2 (1.42); rAra h3 (2.64); hazelnut (16.60); nCor a9 (15.80); almond (8.38); potato (50.20); wheat (6.77)	Ovomucoid (12); ovalbumin (6.9); casein (1.5); rAra h1 (3.2); rAra h2 (7.2)	6 months
DA007	Egg (11.70); egg white (10.20); ovomucoid (9.73); ovalbumin (4.69); cow milk (3.84); casein (0.83); peanut (0.87); rAra h1 (1.46); rAra h9 (0.66); almond (1.68); oat (2.71); wheat (6.50)	Ovomucoid (0.7)	3 months
DA008	Cow milk (2.70); casein (1.65); ovalbumin (1.7); ovomucoid (27.70); potato (3.52); kiwi (8.09)	Ovomucoid (8.5); α-lactoalbumin (1.4); casein (1.3)	6 months
DA009	Ovalbumin (1.25); hake (0.71); rGad c1 Cod (0.96)	Negative	9 months
DA010	Ovalbumin (6.22); almond (0.43); wheat (1.02)	Ovalbumin (2)	2 months
DA011	Cow milk (1.97); casein (1.32); ovalbumin (1.90); potato (0.54)	Ovalbumin (0.4); β-lactoglobulin (1.3); casein (1)	4 months

**nd, no data available.*

TABLE 2 | sIgEs determined by ImmunoCAP FEIA and ISAC in healthy control children.

Patient	ImmunoCap (>0.35 KU/L)	ISAC > 0.3 ISU-E	Age
CDA001	Negative	Negative	1 year
CDA002	Egg white (0.66); ovalbumin (0.9)	Egg white (1.5)	7 months
CDA003	Negative	Negative	2 years
CDA006	Cow milk(1.38); ovalbumin (0.66); ovomucoid (0.61); casein (0.76)	Ovomucoid (1.1); α-lactoalbumin (0.5); casein (0.5)	9 months
CDA007	Negative	Negative	1 year
CDA008	Negative	Negative	1 year
CDA010	Negative	Negative	2 months
CDA012	Negative	Negative	3 months
CDA013	Egg white (1.21)	Negative	7 months

allergic to two or more foods. Although, the vast majority were allergic to eggs and/or milk, it is very important to point out that five of them (50%) were allergic to nuts, two (20%) were allergic to fish and one was allergic to kiwi (**Supplementary Table 1**). These results indicate that, in moderate-to-severe AD children, the determination of only sIgE to milk and egg before starting with the complementary diet is not enough to prevent allergic reactions that can become very serious.

Families Eating Habits Are Associated With the Sensitization Profile of Children With Moderate-to-Severe AD

In order to elucidate the possible mechanisms by which children with moderate-to-severe AD could be sensitized, we first analyzed whether the mothers had the same sIgEs as their children that could suggest a transmission either during pregnancy and/or through breastfeeding (45, 46). We could only obtain samples from four mothers, but only one had dust mite sIgEs, while her child did not (data not shown). We also studied the eating habits of people living with AD children to determine whether the sensitization profile correlated with food allergens that may be present in the home environment. Surveys of families revealed that sensitization to specific antigens in children was associated to foods commonly consumed not only by the mother but also by other members of the family unit.

Cellular and Molecular Markers in AD Children

Regulatory T Cells From Moderate-to-Severe AD Children

Peripheral blood circulating Treg cells can be identified as CD4+CD25highFoxP3+ cells or as CD4+CD25highCD127- cells (**Supplementary Figures 1A,B**). Compared to healthy controls, the analysis did not reveal significant differences in the frequency of total Treg cells in patients with moderate-to-severe AD (**Figure 1A** and data not shown). To further explore the differences, we performed analyses of Treg cell subsets. Based on the expression of Foxp3 and CD45RO, Treg cells were classified into activated Treg cells (aTregs) (CD45RO+Foxp3high), resting Treg cells (rTregs) (CD45RO-Foxp3low) and cytokine-secreting Treg cells (sTregs) (CD45RO+Foxp3low) (**Figure 1B**). Moreover, in order to differentiate peripherally derived Treg cells (Helios-) from thymic derived Treg cells (Helios+) the expression of the transcription factor Helios was analyzed (**Figure 1C**). We did not observe significant differences in the frequency of each subpopulation between moderate-to-severe AD and healthy control groups (**Figures 1B,C**).

Human Treg cells are characterized by the expression of inhibitory molecules such as programed cell death-1 (PD-1) and cytotoxic T-lymphocyte antigen-4 (CTLA-4) (21, 22, 47–50). We

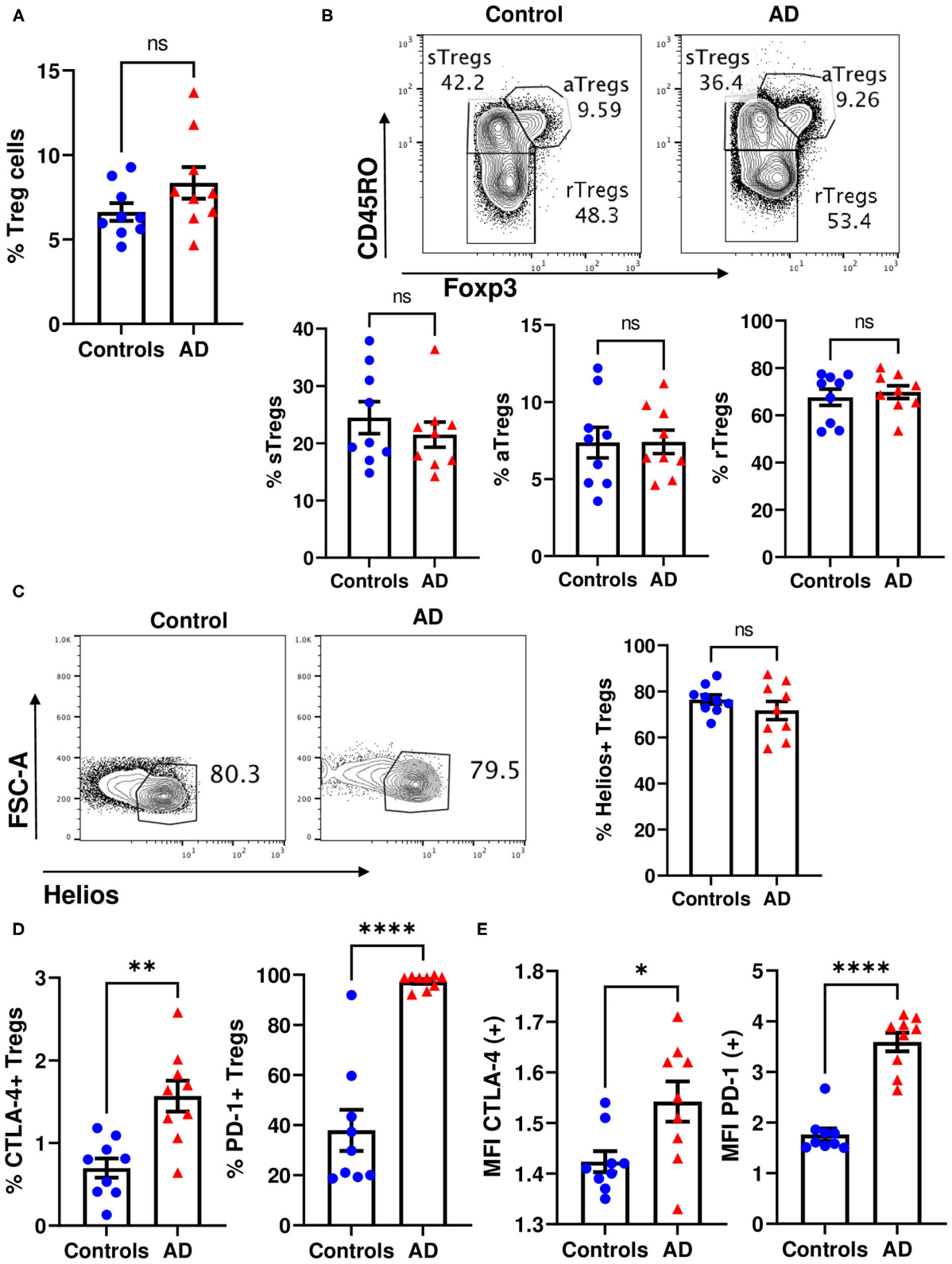

FIGURE 1 | Phenotypic characterization of Treg cells from moderate-to-severe AD children. **(A)** Dot-bar graphs showing the frequency of total Treg cells identified as CD4+CD25highFoxP3+ cells in healthy control (blue dots) and moderate-to-severe AD (red triangles) children. **(B)** Representative contour plots showing the

(Continued)

FIGURE 1 | percentages of activated Treg cells (aTregs) ($CD45RO^+Foxp3^{high}$), resting Treg cells (rTregs) ($CD45RO^-Foxp3^{low}$), and cytokine-secreting Treg cells (sTregs) ($CD45RO^+Foxp3^{low}$) in healthy control (left) and moderate-to-severe AD (right) children (upper line) and dot-bar graphs indicating the frequency of different Treg cells subsets (lower line). **(C)** Representative contour plots showing the percentages of Helios+ Treg cells in healthy control and moderate-to-severe AD children and dot-bar graph indicating the frequency of Helios+ Treg cells in healthy control (blue dots) and moderate-to-severe AD (red triangles) children. **(D)** Dot-bar graphs representing the percentage of CTLA-4 expressing Treg cells and of PD-1 expressing Treg cells in healthy control (blue dots) and moderate-to-severe AD (red triangles) children. **(E)** Dot-bar graphs showing the MFI of CTLA-4 and PD-1 in positive cells for each receptor. Each dot represents a donor, means ± SEMs are shown. Mann–Whitney test. ns, no significant, $^*p < 0.05$, $^{**}p < 0.01$, and $^{****}p < 0.0001$.

observed a statistically significant increase in the percentage of CTLA-4 and PD-1 expressing cells in moderate-to-severe AD patients compared to healthy controls (**Figure 1D**). Moreover, in CTLA-4 positive cells and in PD-1 positive cells the median fluorescence intensity (MFI) of the expression of each receptor was significantly higher in AD patients than in healthy controls (**Figure 1E**).

Phenotypic Characterization of Basophils From Moderate-to-Severe AD Children

Next, we performed an analysis of blood circulating basophils. We did not observe significant differences in the number of blood basophils between patients with moderate-to-severe AD and healthy controls (**Figure 2A**). Our results showed that, compared to healthy individuals, basophils from moderate-to-severe AD children expressed significantly higher expression levels of FcεRI, and that the expression of the basophil activation marker CD63 also tended to be higher (**Figure 2B**), in accordance with previously reported results in basophils from allergic individuals (38). Human basophils express, among others, the chemokine receptors CCR2, which positively regulates basophil degranulation in response to monocyte chemoattractant protein-1 (MCP-1), and CXCR4, important for basophil activation and recruitment to inflammatory sites (51, 52). We observed that the expression of CXCR4 was significantly increased in AD patients compared to healthy children (**Figure 2C**). Lastly, we analyzed the expression of CD300 molecules, surface receptors known to regulate the activation threshold of human basophils in response to IgE (39). As it has been previously described in allergic individuals (34, 37, 38), the surface expression of CD300a was significantly lower in moderate-to-severe AD patients than in control children while the expression of CD300c tended to be higher (**Figure 2D**). Taking together, although our analysis did not reveal significant differences in the number of blood basophils, patients with moderate-to-severe AD exhibited basophils with a more activating profile, reminiscent of basophils from allergic individuals.

DISCUSSION

In this study we have observed that children with moderate-to-severe AD had sIgEs against multiple food antigens before introducing them into their diet, which emphasizes the need for caution when first initiating complementary diet to these children.

It has been recently described that murine fetal mast cells mediate postnatal allergic responses dependent on maternal IgE (53). Moreover, it has been also demonstrated that allergen sIgEs are transmitted from maternal blood to breast milk, which suggests that the transmission of sIgEs from mothers to offspring *via* milk may affect the development of allergy in infants (45). Although we compared the sensitization profile of the children with that of the mothers, we did not find similarities in any of the studied cases, indicating that, in our cohort, sensitization was not dependent on maternal IgE. Nevertheless, more studies are required to rule out this possibility.

In recent years, increasing evidence regarding the importance of allergic sensitization through the skin are accumulating (4, 9, 12, 54–56). In this sense, we have observed that sensitization to specific antigens in children with moderate-to-severe AD was associated to foods commonly consumed not only by the mother, but also by other members of the family unit, suggesting that the skin could be one of the routes of sensitization. It has been well-established in animal studies that sensitization to food allergens can occur through the skin, giving rise to IgE-mediated hypersensitivity responses (4, 57–60). Not only in animal models, there is also clinical data supporting the dual allergen exposure hypothesis. The Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort study demonstrated that infants with peanut allergy by the age of 5 years were more likely to have had severe AD in the first 6 months of life and to have been treated with peanut oil for dry skin (9). Based on evidences from mouse models and clinical studies, it has been suggested that, if the skin barrier can be improved and/or the inflammation of AD can be proactively prevented, in combination with early introduction of food antigens, the incidence of FA and possibly other forms of allergic diseases might be decreased (55, 56, 60–62).

Regulatory mechanisms are necessary for the immune system to maintain peripheral tolerance and Treg cells are the most important cells involved in the immune system regulation. Tregs achieve their immunosuppressive function both through direct cell-to-cell contact (by CTLA-4, LAG-3, PD-1, and FasL) and through the secretion of immunoregulatory cytokines such as IL-10 and TGF-β. Some of the known functions of Treg cells are to inhibit the activation of Th2 cells by suppressing the production of IL-2, IL-5, IL-9, and IL-13, to block the migration of effector T cells into inflamed tissues, to suppress the production of IgE, to induce the production of IgG4 and to limit Th17-mediated inflammation (16, 21, 28, 63, 64). Whether Treg cells frequencies are altered in AD patients has been addressed by several studies. While some revealed an increase in the frequency of circulating Treg cells in AD patients compared to healthy controls, in others similar expansion has been reported (25, 26, 28, 31, 33). Also in allergy, some authors described a reduction on the frequency of Treg cells in blood (23, 24,

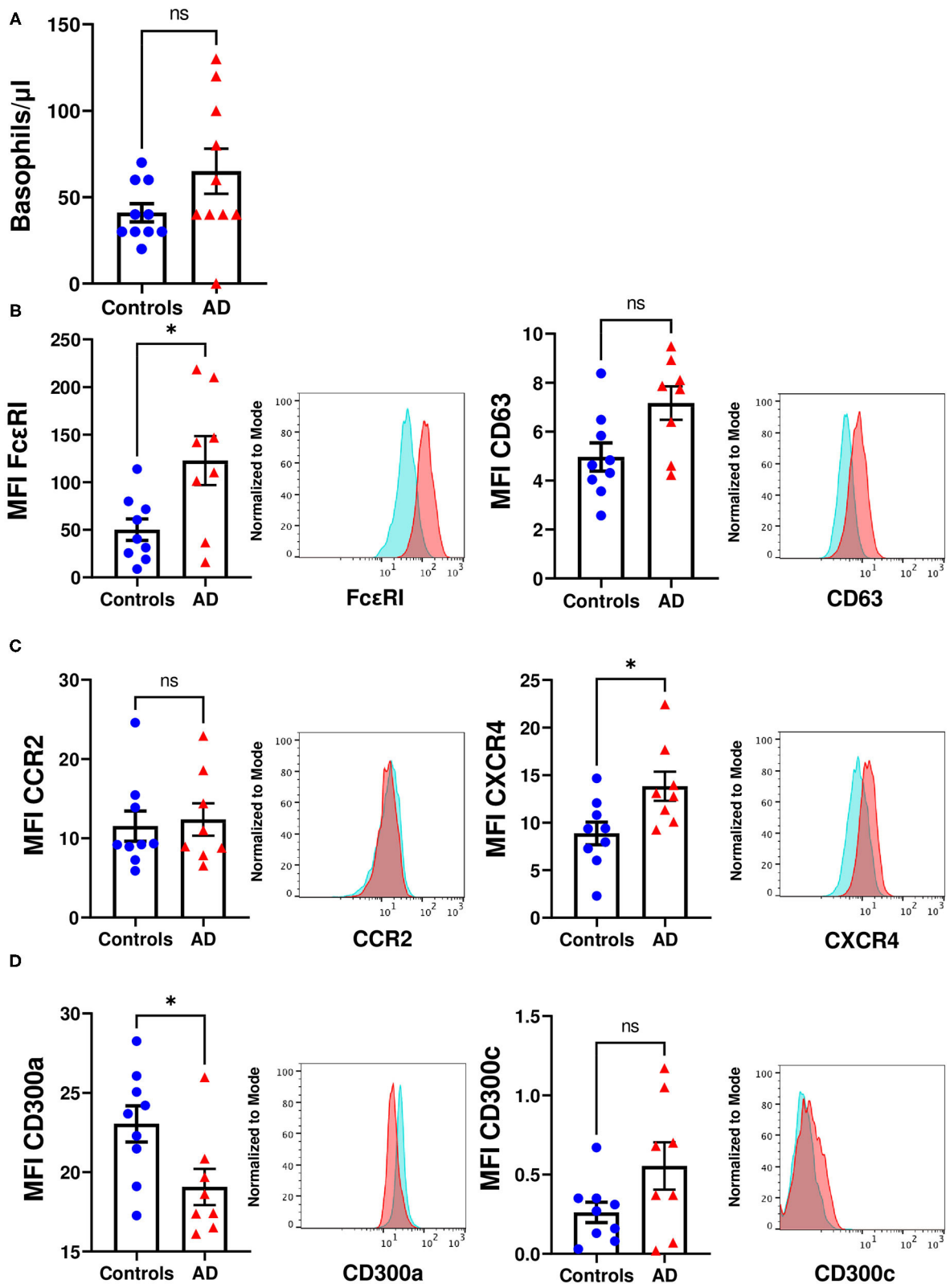

FIGURE 2 | Phenotypic characterization of basophils from moderate-to-severe AD children. **(A)** Dot-bar graphs showing the number of basophils/ul in healthy control (blue dots) and moderate-to-severe AD (red triangles) children's whole blood. **(B–D)** Dot-bar graphs showing MFI of FcεRI, CD63, CCR2, CXCR4, CD300a, and CD300c on circulating basophils from healthy control (blue dots) and moderate-to-severe AD (red triangles) children and histograms of representative individuals (healthy control in blue and AD patients in red). Each dot represents a donor, means ± SEMs are shown. Mann–Whitney test. ns, no significant, $^*p < 0.05$.

27) and others state that the frequency is similar in allergic and non-allergic individuals (29, 30, 32). We did not observe significant differences between moderate-to-severe AD infants and healthy controls in the percentage of Treg cells and neither in the frequency of the different subpopulations. However, the phenotypic analysis of Treg cells showed a significantly higher expression of the inhibitory molecules CTLA-4 and PD-1 in AD patients. A higher expression of CTLA-4 has been previously demonstrated in Treg cells from severe AD patients (28). PD-1 and CTLA-4 are two Treg cells functional markers involved in cell-to-cell communication between Treg and target cells. It has been suggested that CTLA-4 expression levels may indicate the suppressive potency of Treg cells (47, 48), while the expression of PD-1 has been associated not only with the suppressive capacity (21, 49, 65) but it has also been considered an activation marker of Treg cells (22, 50). Therefore, the increased expression of CTLA-4 and PD-1 in Treg cells from moderate-to-severe AD infants suggests that they are more activated and have a higher suppressive potency.

Multiple studies in the last few years have highlighted the importance of CD300 molecules in several pathological conditions (39, 66–68). Hence, it has been described previously that the expression of the inhibitory receptor CD300a is modulated in AD patients and that it could influence the inflammatory response (40). In fact, a significant increase in CD300a total expression was observed in AD biopsies from lesional skin when compared to normal skin (40). In the same line, several studies in allergic patients have suggested a key role of CD300 molecules in the modulation of the activation threshold of basophils, eosinophils, and mast cells, during allergic reactions (39). In fact, based on previously published evidences, we have recently suggested a model describing how CD300 activating and inhibitory receptors regulate IgE-dependent basophils activation (37, 39). Here, we have observed that basophils from patients with moderate-to-severe AD infants exhibited a marked reduction in the expression of CD300a and a slightly higher expression of CD300c than healthy controls, suggesting that these basophils have a more activating profile, reminiscent of basophils from allergic individuals. In fact, when we analyzed allergy incidence at 2 years of age we observed that all children included in the study had developed food allergy, being 70% allergic to two or more foods, suggesting that the expression levels of CD300a and CD300c on basophils may indicate an allergic predisposition in moderate-to-severe AD infants.

Finally, the CXCR4/CXCL12 signaling axis plays a pivotal role in numerous biological processes and an overexpression of CXCR4 has been associated with several diseases including skin inflammatory diseases such as psoriasis and AD (69, 70). In this sense, it has been recently demonstrated that an endogenous CXCR4 antagonist reduced skin inflammation in an AD mouse model, demonstrating a role for the CXCR4/CXCL12 axis in AD, as well as showing a potential role for CXCR4-antagonizing agents as therapeutic options in skin inflammatory diseases (70). In addition, higher serum levels of CXCL12 were found in AD patients compared aged-matched healthy controls (71). Here, we have described for the first time an increased expression of CXCR4 on basophils from infants with moderate-to-severe AD compared to healthy controls, which could suggest a greater recruitment potential of basophils to the site of inflammation. However, further studies are needed in order to understand the functional significance and clinical relevance of the increased expression of CXCR4 on circulating basophils from infants with moderate-to-severe AD and its relationship with allergic susceptibility.

Altogether, our results show that infants with moderate-to-severe AD are at very high risk of being sensitized to food allergens and highlight the need for broad-spectrum food sensitization studies in order to personalize the introduction of complementary foods and thus avoid allergic reactions that could become serious. We have observed an increment in the expression of CTLA-4 and PD-1 in Treg cells from AD patients, suggesting a higher suppressive activity. Moreover, our results suggest a role for CD300 molecules on circulating basophils as biomarkers for FA susceptibility.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, upon reasonable requests.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee for Clinical Research of Cruces University Hospital (CEIC E18/26). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

OZ and AB conceived the project. OZ and FB designed experiments. AB, RP-G, and IR obtained the clinical samples and clinical data from AD patients. AB and RP-G obtained samples and clinical data from healthy controls. AI interviewed families on eating habits. OZ performed experiments, data analysis, compiled figures, and wrote the manuscript. FB, IT, AO, and GA-P provided intellectual input. All authors critically reviewed the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2021.734645/full#supplementary-material

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