TH2-ASSOCIATED IMMUNITY IN THE PATHOGENESIS OF SYSTEMIC LUPUS ERYTHEMATOSUS AND RHEUMATOID ARTHRITIS

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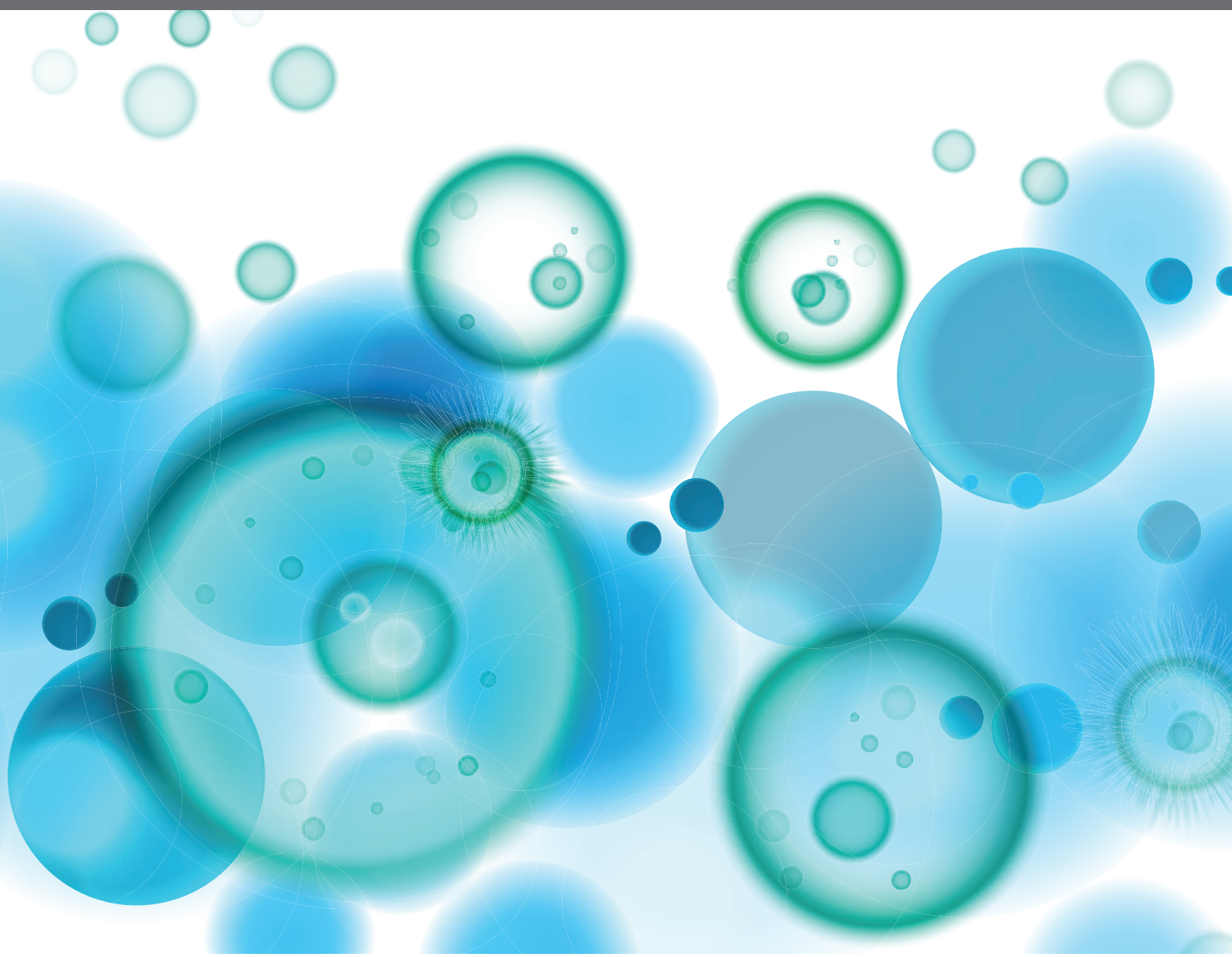

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TH2-ASSOCIATED IMMUNITY IN THE PATHOGENESIS OF SYSTEMIC LUPUS ERYTHEMATOSUS AND RHEUMATOID ARTHRITIS

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Editorial: Th2-associated immunity in the pathogenesis of systemic lupus erythematosus and rheumatoid arthritis

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Editorial on the Research Topic

Th2-Associated Immunity in The Pathogenesis of Systemic Lupus Erythematosus and Rheumatoid Arthritis

CD4$^+$ T helper (Th) cells play a vital role in coordinating immune responses by promoting the activation and maturation of other immune cells (such as macrophages, dendritic cells, and B cells) (1). Th subsets (such as Th1 and Th2 cells) are characterized by the cytokines they secrete and their subsequent effector functions (1). Th2 cells activate and maintain humoral or antibody-mediated immune responses by producing cytokines (such as interleukin [IL]-4, IL-5, IL-6, IL-9, IL-10, IL-13, and IL-25), extracellular vesicles (EVs), and/or direct contact with target cells (2). Furthermore, Th2-associated immunity also includes other factors, such as basophils, mast cells, IgE, IgG4, Th2-related transcriptional factors (including Pparγ and Gata3), and pathways (such as Janus kinase [JAK]-signal transducer and activator of transcription [STAT] signaling and basic leucine zipper ATF-like transcription factor [Batf]/interferon regulatory factor 4 [Irf4] pathway) (2).

Increasing evidence has recently demonstrated that Th2-associated immunity targets helminths and immune responses that promote tissue repair (2) as well as plays a crucial role in autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), potentially contributing to disease diagnosis and prognosis (such as biomarkers), as well as targeted therapy (3–5). However, the detailed molecular regulatory mechanism of Th2-related immunity in the pathogenesis of SLE and RA and its clinical applications require further study and validation. To this end, we collected several manuscripts that analyzed the role of Th2-associated immunity in the pathogenesis of autoimmune diseases and discussed interventions targeting the relevant mechanisms.

The role of Th2-associated immunity in SLE pathogenesis is the focus of the review by Ko et al. This review summarizes lupus patients and mouse model studies on Th2-related immunity and outlines the influencing factors in the SLE microenvironment. First, IL-33 and auto-IgE activate plasmacytoid dendritic cells (pDCs) and basophils in patients with SLE. Subsequently, these activated basophils migrate to secondary lymphoid organs (SLO) and promote T cell differentiation into Th2 and Tfh2 cells. Tfh2 cells further induce B cells to differentiate into plasma cells, which produce IgE autoantibodies that consequently activate pDCs and basophils. Simultaneously, IgE autoantibodies in circulation also led to an immune complex deposition in the kidney, leading to lupus nephritis.

Notably, a study by Pellefigues et al. demonstrated that AMG853 (a bi-specific antagonist of prostaglandin D2 receptor [PTGDR]-1 and PTGDR-2) administration ameliorated lupus in Lyn-deficient female mice, whereas inhibition of PTGDR-1 or PTGDR-2 alone was ineffective. Mechanistically, AMG853 may improve lupus by inhibiting basophil activation and their subsequent recruitment to secondary lymphoid organs (SLOs), inhibiting plasmablast proliferation and autoantibody formation. However, the efficacy of AMG853 in other lupus-prone mouse models still need to be further investigated.

Sylvester et al. reviewed the concepts of autoinflammation and type 2 immunity as well as their interactions. Additionally, the authors discussed the epidemiology of a few monogenic and complex autoinflammatory diseases and the mechanisms of the interaction between autoinflammation and type 2 immunity. Delineating these mechanisms could help treat patients with various autoimmune and allergic diseases.

Recently, a study by Haddadi et al. reported that cutaneous lesions in mouse models of cutaneous lupus erythematosus (CLE) were triggered by Th2 cells, which converted to a Th1-like phenotype in response to a TLR7-driven immune environment. In this model, persistent self-reactive T-resident memory cells could serve as potential therapeutic targets.

In another study, Schubert et al. collected urine samples (56 days, 12 h intervals) from a middle-aged woman with mild SLE disease activity and measured urinary IL-6, creatinine, and protein/creatinine levels. They observed that an increase in urinary IL-6 concentration preceded an increase in urinary protein levels, which coincided with an increase in oral ulcers. While this study points to real-world clinical feedback between cytokine production and SLE symptoms, the mechanism remains unclear. In addition, owing to the heterogeneity of SLE, the delayed effect between cytokine production and SLE symptoms may not be generalized.

A review by Deng et al. focused on the dual immunomodulatory role of IL-17E (IL-25) during the progression of various autoimmune diseases. IL-25 may act as an inflammatory cytokine that promotes the production of Th2-type cytokines, including IL-4, IL-5, and IL-13, thus exacerbating allergic inflammation. IL-25 also aggravates psoriasis and Sjögren syndrome by activating innate immune cells and producing other inflammatory cytokines. In contrast, IL-25 can produce Th2-type cytokines to inhibit Th1 or Th17 differentiation, hence playing a role in RA, multiple sclerosis, and SLE. Since IL-25 plays a role in different diseases and inflammation, defining the function of IL-25 will help in its targeting for the treatment of inflammatory diseases in the future.

A review by Qin et al. showed that regulatory eosinophils (rEos) have a pro-inflammatory resolving role in RA. rEos continue to persist in the synovium of RA patients in remission and proliferate in response to innate lymphocyte (ILC)-derived IL-5 stimulation. rEos ameliorate arthritis by secreting resolvins and promoting the switch of synovial macrophages to the anti-inflammatory M2 phenotype. The authors suggest that these pro-inflammatory resolving effects of rEos could contribute to developing new therapeutic options for RA.

The role of long non-coding RNAs (lncRNAs) in SLE and RA is summarized in a review by Wu et al. In SLE, lncRNAs such as nuclear paraspeckle assembly transcript 1 (NEAT1) and growth arrest-specific 5 (GAS5) are dysregulated and hence, may be used as novel biomarkers and therapeutic targets. In RA, many validated lncRNAs, such as HOX antisense intergenic RNA (HOTAIR) and GAS5, have been identified as promising novel biomarkers for diagnosis and treatment. LncRNAs shared by SLE and RA, such as GAS5, may play critical roles in pathogenesis through diverse protein kinase pathways.

In the past decade, many studies have shown that mesenchymal stem cell (MSC) transplantation, a promising treatment option for SLE, can effectively ameliorate disease in patients with active and refractory SLE (6, 7). However, few studies have demonstrated that MSC therapy is ineffective. A review by Li et al. summarized the potential reasons for the poor effect of MSC treatment, including defects in bone marrow (BM)-MSCs in patients with SLE, factors influencing MSC proliferation *in vitro*, and the complex microenvironment of patients with SLE. The authors also proposed various MSC modification methods that may be beneficial for enhancing the immunosuppression of MSC in SLE. However, the therapeutic effects and potential adverse reactions of MSC modification in patients with SLE must be confirmed by further experimental and clinical evidence.

Further, gut microbiota dysregulation reportedly plays a vital role in the pathogenesis of SLE (8). Pan et al. elaborated on gut microbiota dysregulation in patients with lupus and mice. The authors also analyzed the mechanisms of gut microbiota dysregulation in SLE from multiple perspectives, such as molecular mimicry, gut-specific pathogenic bacterial infection, gender bias, and intestinal epithelial cell autophagy. The authors additionally proposed treatment options that may be applied to

target gut microbiota dysregulation, such as oral antibiotic therapy, fecal microbiota transplantation, regulation of intestinal epithelial cell autophagy, MSC therapy, and vaccination. Thus, targeting intestinal bacteria may also be a promising strategy for SLE treatment.

Finally, a review by Chen et al. summarized mouse models of the humanized immune system based on immunodeficient mice, which better mimic the onset and progression of human disease compared to ordinary animal models. Furthermore, the authors discuss the hurdles that need to be overcome in humanized mouse models of SLE, including the short life span of mice, resulting in an insufficient observation period.

Conclusions

This Research Topic highlights the vital role of Th2-related immunity in the pathogenesis of autoimmune diseases, such as SLE and RA. This knowledge will create the foundation for developing new therapeutic insights for Th2-related autoimmune diseases.

Author contributions

Authors on the list have contributed a substantial amount, directly and intellectually to the work, and given their consent to publish.

Conflict of interest

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

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Roles of IL-25 in Type 2 Inflammation and Autoimmune Pathogenesis

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Interleukin-17E (IL-25) is a member of the IL-17 cytokine family that includes IL-17A to IL-17F. IL-17 family cytokines play a key role in host defense responses and inflammatory diseases. Compared with other IL-17 cytokine family members, IL-25 has relatively low sequence similarity to IL-17A and exhibits a distinct function from other IL-17 cytokines. IL-25 binds to its receptor composed of IL-17 receptor A (IL-17RA) and IL-17 receptor B (IL-17RB) for signal transduction. IL-25 has been implicated as a type 2 cytokine and can induce the production of IL-4, IL-5 and IL-13, which in turn inhibits the differentiation of T helper (Th) 17. In addition to its anti-inflammatory properties, IL-25 also exhibits a pro-inflammatory effect in the pathogenesis of Th17-dominated diseases. Here, we review recent advances in the roles of IL-25 in the pathogenesis of inflammation and autoimmune diseases.

Keywords: IL-25, IL-25 signal transduction, type 2 inflammation, systemic erythematosus lupus, rheumatoid arthritis

INTRODUCTION

The interleukin-17 (IL-17) family belongs to a group of cytokines that play a crucial role in host defense against extracellular pathogens and inflammatory response during autoimmune pathogenesis (1). As the first cytokine identified in IL-17 family, IL-17A, firstly named as cytotoxic T lymphocyte-associated antigen-8 (CTLA-8), encodes a protein with the same homology as the putative protein encoded by the ORF13 gene of herpesvirus Saimiri (2, 3). Based on the sequence of IL-17A, other IL-17 family members are identified, including IL-17B, IL-17C, IL-17D, IL-17E (also known as IL-25) and IL-17F. IL-17 family cytokines exhibit functional activity by covalently binding to form heterodimers or homodimers. IL-17A and IL-17F can form both homodimer and heterodimer, while IL-17B, IL-17C, IL-17D and IL-25 form homodimers to bind receptors (4, 5).

IL-17 family cytokines play an essential role in host defense against pathogens as well as in various diseases including cancers and autoimmune disorders (1, 6). Recent studies have demonstrated that IL-17A and IL-17F act as pro-inflammatory cytokines in the pathogenesis of Sjögren's syndrome (SS) (7, 8). In addition, IL-17A can sustain plasma cell response and exacerbate the development of systemic lupus erythematosus (SLE) (9). IL-17F has also been shown to drive

renal tissue injury in lupus mice, suggesting the pathogenic functions of IL-17A and IL-17F in lupus pathogenesis (10, 11). Moreover, increased levels of IL-17A and IL-17F expression are detected in the inflamed guts of patients with inflammatory bowel disease (IBD) (12, 13). Furthermore, elevated serum IL-17A and increased islet antigen-specific IL-17A-producing $CD4^+$ T helper (Th17) cells are detected in patients with type 1 diabetes (T1D) while adoptive transfer of Th17 cells into non-obese diabetic (NOD) mice promotes pancreatic inflammation (14, 15). In multiple sclerosis (MS) patients, IL-17A is found to impair the neural cell function in central nervous system (CNS) and causes tissue destruction (16). Extensive evidence indicates that IL-17A plays a key role in the pathogenesis of psoriasis. IL-17A can induce keratinocytes to produce various chemokines that recruit immune cells and promote the proliferation of endothelial cells, leading to angiogenesis (17). IL-17A is critically involved in the pathogenesis of collagen-induced arthritis (CIA) in mice and rheumatoid arthritis (RA) in patients (18). IL-17A stimulates the synoviocytes to produce vascular endothelial growth factor (VEGF) and induces stromal cells to produce pro-inflammatory cytokines and hematopoietic cytokines (19, 20). As a pro-inflammatory cytokine, IL-17B can recruit neutrophils in immune reactions (21). Elevated levels of IL-17B expression have been found in synovial tissue of CIA mice and RA patients while further blockade of IL-17B with neutralizing antibodies ameliorates disease progression, indicating a pathogenic role of IL-17B in autoimmune diseases (22, 23). Unlike IL-17A, IL-17C is mainly expressed by epithelial cells and can regulate epithelial immune response in an autocrine manner (24, 25). In a dextran sulfate sodium (DSS)-induced colitis mouse model for IBD, IL-17C exhibits a protective role in colitis development (24, 26). However, in mice with imiquimod-induced psoriasis, IL-17C elicits a pathogenic effect and exacerbates psoriatic inflammation, in which intradermal injection of IL-17C triggers leukocyte infiltration and epidermal thickening (24). Thus, IL-17C exerts diverse functions in the development of various autoimmune diseases. Among IL-17 family cytokines, IL-17D is a less studied cytokine, which has been found to induce the expression of pro-inflammatory cytokines including IL-6 and IL-8 in endothelial cells (27). A recent study has identified CD93 as the IL-17D receptor expressed in group 3 innate lymphoid cells (ILC3s) whereas IL-17D exerts anti-inflammatory effects in DSS-induced colitis through inducing IL-22 production by ILC3s (28).

IL-25 was first identified by sequence alignment from human genomic DNA sequence information and considered as a novel proinflammatory cytokine *via* activation through the nuclear factor-κB (NF-κB) (29). Subsequently, IL-25 was defined as a type 2 cytokine produced by Th2 cells, which was capable of inducing IL-4, IL-5 and IL-13 gene expression and further amplifying allergic inflammatory response in the lung and the digestive tract (30). The functions of IL-25 as a "barrier surface" cytokine in epithelial immunology and airway diseases have been recently reviewed (31, 32). Here, we summarize research advances in understanding the roles of IL-25 in inflammation with an emphasis on autoimmune pathogenesis.

IL-25 AND ITS SIGNAL TRANSDUCTION

The IL-17 cytokine family binds to its receptors for signal transduction, which include five receptor subunits, IL-17RA, IL-17RB, IL-17RC, IL-17RD and IL-17RE (33). Each IL-17R subunit is a single transmembrane domain-containing protein with several conserved motifs, including extracellular fibronectin III-like motifs, transmembrane regions and cytoplasmic SEF/IL-17R (SEFIR) domains (34). In addition to the SEFIR domain expressed by all IL-17R subunits, IL-17RA also expresses Toll/IL-1R-like loop (TIR-like loop, TILL) domain and C/EBPβ-activation domain (CBAD) (34, 35). IL-17R subunits from both mouse and human range in size from 272 to 866 amino acids and contain full-length forms and smaller alternatively spliced isoforms (36). Since IL-17RA contains most of the cytoplasmic domains, it is the largest member of the IL-17R family and is the key component used at least by IL-17A/IL-17F, IL-17B and IL-25 (37–39). Dimeric IL-17A and IL-17F can bind to receptors consisting of IL-17RA/IL-17RC, IL-17RA/IL-17RD or IL-17RC/IL-17RC (38, 40, 41). In addition, IL-17C uses IL-17RA and IL-17RE to transduce signal (42). Recently, CD93 has been identified as a functional receptor that recognizes IL-17D, but whether CD93 pairs with other receptors to transduce signals from IL-17D requires further investigation (28). Both IL-17B and IL-25 signal through a heterodimeric receptor of IL-17RA and IL-17RB (37, 39). IL-25 shows low affinity for IL-17RA but high affinity for IL-17RB. However, IL-25 can also bind to IL-17RA after it is captured by IL-17RB (43, 44) (**Table 1**).

The SEFIR domain is expressed by all IL-17R family members, whereas the TILL domain and CBAD are expressed only by IL-17RA, indicating that IL-17RA might be responsible for more complex signaling process than other IL-17R subunits (34). The SEFIR domain was identified as a conserved segment similar to TIR domain which is known to mediate homotypic interactions (51). Multiple sequence alignments showed that box 1 and box 2 motif in TIR domain are conserved in SEFIR domain, indicating that SEFIR domain-containing protein can interact homotypically with other SEFIR domain-containing proteins (51). A SEFIR domain-containing protein involved in IL-17 cytokine family signaling is activator 1 (Act1), which is an NF-κB activator (52). Act1 can be recruited to IL-17R upon cytokine engagement through SEFIR-SEFIR domain binding (53, 54). Two tumor necrosis factor (TNF) receptor-associated factor-binding (TRAF-binding) sits are shown at the N terminus of Act1, therefore TRAF-containing proteins including TRAF3, TRAF6 and transforming growth factor β-activated kinase 1 (TGFβ-activated kinase 1, TAK1) bind to IL-17R upon engagement (54). TILL domain resembles box 3 motif of TIR domain and are unique in IL-17RA subunit. Mutation of the TILL domain renders mice insufficient response to LPS (34). Another C-terminal domain, CBAD is also unique in IL-17RA subunit, which is required for activation of C/EBPβ and induction of IL-17 target gene expression (34). Signal transduction *via* IL-25 requires heterodimer of IL-17RA and IL-17RB subunits, therefore SEFIR domain, TILL domain and CBAD of IL-17RA as well as SEFIR domain of IL-17RB serve as functional motifs responsible for activation of IL-25 signal (34).

TABLE 1 | IL-17 family cytokines, receptors and functions in autoimmune diseases.

Cytokine	Structure	Receptors	Functions	Ref
IL-17A	IL-17A/IL-17A	IL-17RA/IL-17RC	Pathogenic in psoriasis, SLE, SS, T1D, RA, MS and IBD	(7, 9, 12–14, 16, 17, 19, 38, 40, 41)
IL-17F		IL-17RA/IL-17RD		
	IL-17A/IL-17F	IL-17RA/IL-17RC		
	IL-17F/IL-17F	IL-17RC/IL-17RC		
IL-17B	IL-17B/IL-17B	IL-17RA/IL-17RB	Pathogenic in RA and SLE	(22, 23, 37)
IL-17C	IL-17C/IL-17C	IL-17RA/IL-17RE	Pathogenic in IMQ-induced psoriasis Protective in DSS-induced colitis	(24, 42)
IL-17D	IL-17D/IL-17D	CD93	Protective in DSS-induced colitis	(28)
IL-17E (IL-25)	IL-25/IL-25	IL-17RA/IL-17RB	Pathogenic in psoriasis, SS and type 2 inflammation Protective in IBD, T1D, MS and SLE	(39, 45–50)

SLE, systemic lupus erythematosus; SS, Sjögren's syndrome; T1D, type 1 diabetes; RA, rheumatoid arthritis; MS, multiple sclerosis; IBD, inflammatory bowel disease; IMQ, imiquimod; DSS, dextran sulfate sodium.

Unlike IL-17RA requires Act1 for association, it is reported that IL-17RB can bind TRAF6 directly for the activation of NF-κB (53, 55). However, the activation of mitogen-activated protein kinase (MAPK) including extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 downstream of IL-25 is independent of TRAF6 (55).

IL-25 IN TYPE 2 INFLAMMATION AND AUTOIMMUNE PATHOGENESIS

Type 2 Inflammation and Allergic Response

Type 2 inflammation in respiratory system is the hallmark of diseases such as asthma and allergy (56). IL-25, originally identified as a type 2 cytokine produced by Th2 cells, promotes the production of IL-4, IL-5 and IL-13, leading to inflammation in the respiratory tract (30). In addition to Th2 cells as the cellular source, IL-25 may also be derived from group 2 innate lymphoid cells (ILC2s), macrophages, eosinophils, basophils and pulmonary epithelial cells (57). It has been reported that transgenic mice with IL-25 overexpression in pulmonary epithelial cells spontaneously develop asthma-like symptoms, including mucus production and airway infiltration by macrophages and eosinophils (45). Moreover, IL-25 produced by Th2 memory T cells can induce angiogenesis in asthmatic bronchial mucosa (58). Further, blockade of IL-25 significantly reduced antigen-induced infiltration of eosinophils and $CD4^+$ T cells in the airways (59). Notably, combined blockade of type 2 cytokine IL-13 and IL-25 was even more effective than blockade alone in reducing infiltration of inflammatory cells in the airways with attenuated airway hyperresponsiveness and tissue remodeling (60). In a mouse model of asthma, natural killer T cells (NKT) with a phenotype of $CD4^+IL\text{-}17RB^+$ are able to produce IL-13 and Th2 chemokines in response to IL-25 stimulation and therefore promote airway hyperresponsiveness (61). Recent studies have demonstrated that IL-25 drives the expression of the transcription factor GATA-3 in naïve T cells by potentiating the induction of NFATc1 and JunB (45). Moreover, IL-25 can increase the expression of vascular endothelial growth factor (VEGF) and VEGF receptor *via* activating phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) and ERK/MAPK pathways in endothelial cells (58). As an adaptor protein in the downstream of IL-17 cytokine family, Act1 controls the allergic asthma-like inflammation initiated by IL-25 while depletion of Act1 abolishes the asthma symptom in mice (62). In addition, IL-25 promotes eosinophils to produce monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1α (MIP-1α), IL-6 and IL-8 *via* the activation of JNK, p38 MAPK and NF-κB pathways (63). Together, available evidence indicates that IL-25 is critically involved in the development of type 2 inflammation. In a preclinical study, ABM125, an anti-IL-25 monoclonal antibody that neutralize human and mouse IL-25, has shown therapeutic effects in treating virus-induced allergic airway disease (64). Thus, targeting IL-25 or $IL\text{-}17RB^+$ immune cells may represent a promising strategy for the treatment of allergic inflammation.

Skin Inflammation

Psoriasis is a typical autoimmune disease of skin inflammation characterized by epidermal hyperplasia, increased angiogenesis and dermal inflammation (65). Although the exact pathogenesis of psoriasis is not clear, it has been suggested that systemic Th1/Th2 imbalance and the involvement of Th17 cells contribute to the initiation and exacerbation of this disease (66). Studies by Senra et al. have demonstrated that IL-25 derived from keratinocytes can directly induce skin inflammation *in vivo* by recruiting neutrophils and activating macrophages (67, 68). IL-25 promotes recruitment of human primary neutrophils by activating human primary macrophages. Moreover, IL-25 stimulates human primary macrophages *via* activation of p38 and NF-κB (67). IL-25 is highly expressed in the skin lesion of patients with psoriasis and in a mouse model of psoriasis. IL-25 is found to promote proliferation of $IL\text{-}17RB^+$ keratinocytes and exacerbation of psoriasis (46). As the major IL-17RB-expressing cells in psoriasis, keratinocytes can be activated by IL-25 *via* activation of STAT3 transcription factor (46). Notably, blockade of IL-17RA using Brodalumab, a co-receptor for IL-17A, IL-17F and IL-25, has shown high efficacy in the treatment of

psoriasis (69). Thus, blockade of IL-25 may represent a promising strategy for targeting skin inflammation.

Inflammatory Bowel Disease

As a chronic inflammatory disorder of gastrointestinal tract, inflammatory bowel disease (IBD) contains two major idiopathic forms: ulcerative colitis and Crohn's disease (CD). It has been recognized that dysfunctions of mucosal immune response to commensal bacterial flora, as well as genetic and environmental factors, contribute to the pathogenesis of IBD (70). Using *Campylobacter jejuni* infection and dextran sulfate sodium (DSS) treatment to induce colitis in mice, Jennifer R. O'Hara et al. showed a significant decrease in both IL-25 and IL-17A in mouse colonic homogenates, as well as disrupted Toll-like receptor 9 (TLR9) signaling in apical epithelium, which is responsible for maintaining colonic homeostasis (71). Furthermore, IL-25 production by intestinal epithelial cells inhibits Th17 expansion by suppressing macrophage-derived IL-23 production (72). In addition, IL-25 has been shown to suppress intestinal mucosa $CD14^+$ cell-derived IL-12 production (73). In patients with active IBD, IL-25 is significantly decreased in serum and inflamed mucosa. Moreover, *in vitro* studies show that TNF, IFNγ and IL-17A production in IBD $CD4^+$ T cells is inhibited by IL-25, which also has an inhibitory function in Th1 and Th17 differentiation (47). Similarly, levels of IL-25 are significantly lower in the intestine of IBD patients than those in normal controls. Consistently, stimulation of normal colonic explants with TNF-α reduced IL-25 synthesis (74). However, treatment with TGF-β1 induces IL-25 production in normal colonic explants (74). Interestingly, IL-25-deficient mice display resistance to DSS-induced colitis while IL-25 upregulates IL-33, IL-6 and TNFα expression in colonic epithelial cells, indicating that IL-25 may contribute to the pathogenesis of IBD (75). Currently, it is unclear how IL-25 exerts dual functions in different cell types or disease stages of IBD. Therefore, further clinical investigations await to validate IL-25 as a therapeutic target for the treatment of patients with IBD.

Type 1 Diabetes

Type 1 diabetes (T1D) is featured with immune dysregulations including pancreatic β-cell destruction triggered by T cells such as Th1 cells and Th17 cells (76, 77). However, IL-25, as an IL-17 cytokine family member, exhibits an inhibitory effect on the pathogenesis of type 1 diabetes. Studies by Emamaullee et al. have reported that IL-25 administration in non-obese diabetic (NOD) mice with spontaneous T1D onset significantly reduces T cell infiltration in the pancreas and decreases serum autoantibodies with similar effects to anti-IL-17A administration, suggesting a protective role of IL-25 in the pathogenesis of T1D (78). Intriguingly, peripheral blood mononuclear cells (PBMC) from T1D patients display significantly increased IL-25 expression together with enhanced production of IL-17A and IL-6 when compared with healthy donors (79). Thus, further studies are needed to address possible dual functions of IL-25 in mediating inflammatory responses in T1D, which may provide a rationale in therapeutic design of IL-25 blockade for treating T1D patients at different disease stages.

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by inflammation in synovium, cartilage damage and bone erosion, which further leads to joint destruction. It has been shown that IL-25 is overproduced by RA synovial fibroblasts as a pro-inflammatory cytokine during disease pathogenesis (80). However, IL-25 can also act as a receptor antagonist of IL-17A function, resulting in suppressed Th17 response. Moreover, IL-25 can inhibit IL-22-induced osteoclastogenesis *via* activation of signal transducer and activator of transcription 3 (STAT3) and p38 MAPK pathway (81, 82). Lavocat et al. reported that RA synoviocytes express IL-17RB and also secrete IL-25 while TNFα treatment increases IL-17RB expression (81). IL-25 treatment of fibroblast-like synoviocytes (FLS) from RA patients inhibits p38 phosphorylation whereas IL-25 pretreatment downregulates the phosphorylation of STAT3, p38 and IκB-α triggered by IL-22 stimulation in FLS from RA patients (82). In mice with collagen II-induced arthritis (CIA), IL-25 is significantly increased at the late stage of CIA while IL-17 is increased at the early stage, suggesting that IL-25 and IL-17 may be involved in arthritic progression at different stages of inflammatory responses (83).

Multiple Sclerosis

Multiple sclerosis (MS) is a chronic autoimmune neurological disease of the central nervous system (CNS), which attacks the myelinated axons and destroys the myelin and axons to varying degrees (84). Th17 cells have been characterized as a major $CD4^+$ T cell subpopulation mediating the pathogenesis of MS. Recent studies show that IL-25-deficient ($Il25^{-/-}$) mice are highly susceptible to experimental autoimmune encephalomyelitis (EAE), a mouse model for human MS, while neutralization of IL-17A prevents EAE in IL-25-deficient mice, indicating a role of IL-25 in attenuating inflammation by inhibiting Th17 function (48). In addition, IL-25 inhibits T cell-triggered neuronal injury and cell death by reducing expression of lymphocyte function-associated antigen-1 (LFA-1) (85). Moreover, Sonobe Y et al. reported that in TNF-α-induced impairment of blood-brain barrier (BBB) permeability, IL-25 treatment downregulates expression of junction adhesion molecule claudin-5, *via* phosphorylation of protein kinase C epsilon (PKCε), suggesting that IL-25 produced by brain capillary endothelial cells can maintain BBB integrity (86). Together, available evidence indicates a protective role of IL-25 in the development of MS.

Systemic Lupus Erythematosus

Systemic Lupus Erythematosus (SLE) is a systemic autoimmune disease involving multiple organs including kidney and brain, characterized by anti-nuclear autoantibody (ANA) and immune complex deposition in kidney, which further causes immune-complex glomerulonephritis (87, 88). Several studies show that IL-25, together with other Th2-related cytokines, is significantly increased in SLE patients, especially in those with lupus nephritis, contributing to the pathogenesis of SLE (89, 90). Although IL-25 is upregulated in SLE patients, IL-25 can ameliorate lupus pathogenesis in mice by inhibiting

inflammatory cytokines (49). We have recently identified a critical role of IL-17 in maintaining plasma cell survival and autoantibody production in both SLE patients and murine lupus (9). Currently, it is unclear whether IL-25 modulates the multiple functions of various B cell subsets in autoimmune pathogenesis (91). Thus, further investigation is needed to determine whether IL-25 plays a pro-inflammatory or anti-inflammatory role during the development of SLE.

Sjögren's Syndrome

Primary Sjögren's syndrome (pSS) is characterized as a systemic autoimmune disease with progressive inflammation of salivary glands (SG) and lacrimal glands, which leads to dry mouth and dry eyes (92). Our previous studies have demonstrated that Th17 cells are important in initiating the pathogenesis of SS, indicating a key role for IL-17A in SS (7). Recently, we observed significantly increased expression of IL-25 in SG and peripheral blood from pSS patients compared with healthy controls (50). In culture, IL-25 significantly increases the number of IL-17RB^{+} inflammatory ILC2s (iILC2s) from SG and peripheral blood (50). Furthermore, blockade of IL-25 using a neutralizing antibody markedly improves saliva flow rate and ameliorates SG tissue damage in mice with experimental SS (ESS), accompanied with decreased ILC2 infiltration in SG of ESS mice. In SGs of pSS patients, significant upregulation of TRAF6 in CD3^{+} T cells and ILC2s suggests that IL-25 signal is functional *via* coordinating activation of ERK1/2 and relative transcription factors (50). Recent studies show that ILC2

TABLE 2 | IL-25 in inflammatory and autoimmune disorders.

Disease	Effect	Change	Signaling Pathways	Ref
Allergies	Pro-inflammatory	Increase	NFATc1/JunB-GATA3; PI3K/AKT; ERK; JNK; p38; NF-κB	(45, 58, 63)
Psoriasis	Pro-inflammatory	Increase	JAK/STAT3; p38; NF-κB	(46, 67)
SS	Pro-inflammatory	Increase	ERK	(50)
IBD	Pro-/Anti-inflammatory	Decrease	N/A	(47)
T1D	Anti-inflammatory	Increase	PI3K/AKT; p38; ERK	(94)
MS	Anti-inflammatory	Increase	PKC-claudin-5	(86)
RA	Pro-/Anti-inflammatory	Increase	JAK/STAT3; p38	(82)
SLE	Anti-inflammatory	Increase	N/A	(49)

SS, Sjögren's syndrome; IBD, inflammatory bowel disease; T1D, type 1 diabetes; MS, multiple sclerosis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

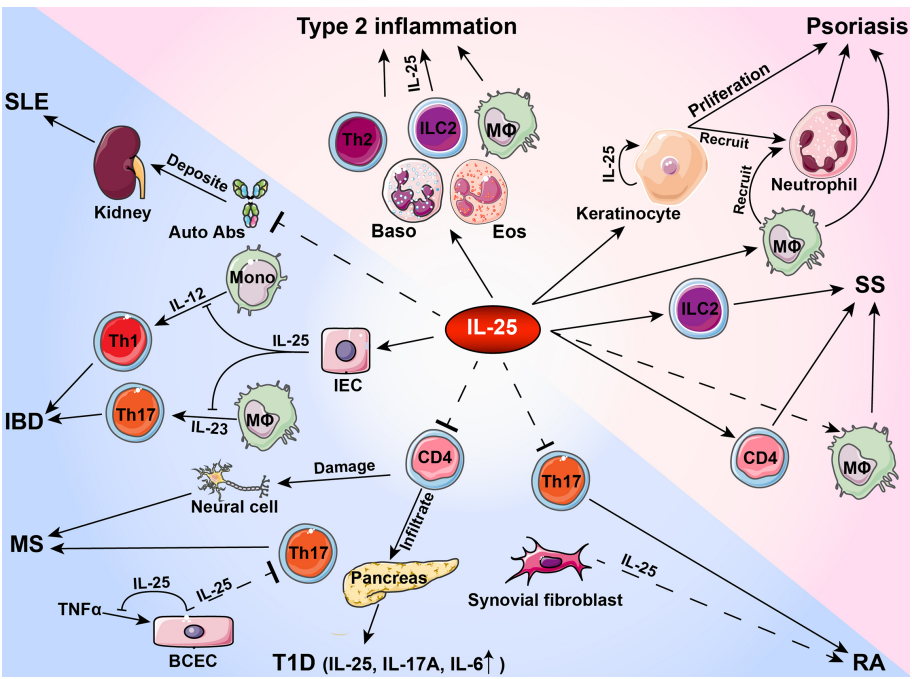

FIGURE 1 | Roles of IL-25 in type 2 inflammation and autoimmune pathogenesis. During the development of autoimmune diseases, IL-25 plays a pro-inflammatory or anti-inflammatory role in activating or inhibiting immune cells and tissue cells. Auto Abs, autoantibodies; Baso, basophil; BCEC, brain capillary epithelial cell; Eos, eosinophil; IBD, inflammatory bowel disease; IEC, intestinal endothelial cell; ILC2, group 2 innate lymphoid cells; SS, Sjögren's syndrome; MΦ, macrophage; Mono, monocyte; MS, multiple sclerosis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; T1D, type 1 diabetes.

provokes inflammation in airways causing persistent asthma symptoms, which can be activated by IL-25. Therefore, available studies have indicated that IL-25 plays a pathogenic role during the development of SS (50, 93).

CONCLUSION AND PERSPECTIVE

As a member of IL-17 cytokine family, IL-25 acts primarily as a type 2 cytokine and is functionally distinct from other IL-17 cytokines. In inflammation and autoimmune pathogenesis, IL-25 binds to receptor subunit IL-17RB expression in immune cells and tissue cells whereas IL-25 levels increase in peripheral blood and inflammatory microenvironment. Current studies suggest that IL-25 has a dual role in regulating immune responses during the development of autoimmune diseases. As a pro-inflammatory cytokine, IL-25 exacerbates allergic inflammation by promoting the production of type 2 cytokines including IL-4, IL-5 and IL-13 by Th2 cells. Moreover, IL-25 activates innate immune cells and induces proliferation, production of other pro-inflammatory cytokines and recruitment of immune cells in psoriasis and SS. In contrast, IL-25 exerts anti-inflammatory effects by inhibiting Th1 or Th17 differentiation *via* production of Th2 cytokines in IBD, T1D, RA, MS and SLE (**Table 2** and **Figure 1**). Given that IL-25 exerts dual functions in various autoimmune diseases, further investigations are needed to determine the exact roles played by IL-25 at different stages of inflammatory responses and autoimmune diseases. Increasing evidence indicates the functional diversities of both B cells and T cells in autoimmune pathogenesis. Future studies on the roles of IL-25 in regulating immune responses may contribute to the design of new therapeutic interventions by targeting IL-25 for the treatment of inflammatory disorders.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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REVIEW
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Mesenchymal Stem Cell Therapy: Hope for Patients With Systemic Lupus Erythematosus

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Systemic lupus erythematosus (SLE) is a chronic autoimmune disease. Although previous studies have demonstrated that SLE is related to the imbalance of cells in the immune system, including B cells, T cells, and dendritic cells, etc., the mechanisms underlying SLE pathogenesis remain unclear. Therefore, effective and low side-effect therapies for SLE are lacking. Recently, mesenchymal stem cell (MSC) therapy for autoimmune diseases, particularly SLE, has gained increasing attention. This therapy can improve the signs and symptoms of refractory SLE by promoting the proliferation of Th2 and Treg cells and inhibiting the activity of Th1, Th17, and B cells, etc. However, MSC therapy is also reported ineffective in some patients with SLE, which may be related to MSC- or patient-derived factors. Therefore, the therapeutic effects of MSCs should be further confirmed. This review summarizes the status of MSC therapy in refractory SLE treatment and potential reasons for the ineffectiveness of MSC therapy from three perspectives. We propose various MSC modification methods that may be beneficial in enhancing the immunosuppression of MSCs in SLE. However, their safety and protective effects in patients with SLE still need to be confirmed by further experimental and clinical evidence.

Keywords: systemic lupus erythematosus, mesenchymal stem cells, immunomodulation, transplantation, inefficacy, modification

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SLE TREATMENT HAS A LONG WAY TO GO

Systemic lupus erythematosus (SLE) is an autoimmune disease that exhibits high population heterogeneity, with women of childbearing age being the most highly affected population. The pathogenesis of SLE remains unclear. Previous studies showed that abnormal activation of immune cells, such as B cells (1), T cells (2), macrophages (3), basophils (4) and dendritic cells (DCs) (5), etc., played a crucial role in SLE. These activated immune cells also contributed to the production of pro-inflammatory factors and pathogenic autoantibodies, causing the deposition of immune complexes in tissues and inducing multiple organ damage. SLE is difficult to diagnose in the early stages because the symptoms and signs are not typical. Currently, the classic methods for SLE treatment are corticosteroids and immunosuppressors, which chronically prolong the disease course and mostly exhibit chronic remission-relapse, whereas a few patients achieve long-term remission (6). Importantly, immunosuppressive therapies fail to prevent disease relapse in more than half of the

patients, and high-dose treatment may even increase the risk of severe infection and death (7). Additionally, most patients exhibit damage to the kidneys or other organs, partly limiting the application of immunosuppressive therapy. Hence, the development of new drugs and therapies is urgently needed (8) and especially the biological agents have gained the attention of researchers. In the past 60 years, belimumab has been the only biological agent approved by the US FDA for SLE treatment; however, this agent utilizes a single target and cannot inhibit plasma cells and switched memory B cells (9). Also, other biological agents, such as tabalumab, do not significantly improve the disease conditions and even have adverse side effects in patients with SLE (10).

Lymphopenia or leukopenia has been reported in patients with autoimmune diseases, such as SLE (11). Therefore, autologous hematopoietic stem cell (HSC) transplantation performed for SLE treatment (12). However, it was demonstrated that this therapy had high transplant-related mortality and relapse (13), possibly because of defects in the bone marrow stem cells and abnormal immune function in patients with SLE (14). Later, it was revealed that both genetic and inflammatory factors altered the number and function of HSCs in a murine lupus model (15). Also, it has been reported that allogeneic HSC transplantation caused relapses and opportunistic infections after seven months, which did not significantly differ from the adverse effects of autologous transplantation (16). Therefore, SLE treatment remains challenging.

In the 1960s and 1970s, Friedenstein discovered a cell that could differentiate and adhere to plastic under culture conditions (17). In 1991, Caplan named these cells mesenchymal stem cells (MSCs) (18). Then, bone marrow-derived MSCs (BM-MSCs) were isolated and cultured *in vitro* and transferred back into patients with hematologic malignancies (19). No transplantation-related adverse reactions were observed in this report. Autologous BM-MSCs are more accessible to obtain than allogeneic BM-MSCs, and they do not induce immune rejection; thus, they were used for disease treatment (20). However, a clinical study of autologous MSC transplantation for SLE treatment revealed that autologous MSCs increased Treg cells but had no effect on disease activity and could not reduce the patient's clinical symptoms (21). Allogeneic MSC transplantation has a more extensive therapeutic range and therapeutic potential than autologous transplantation. This approach is widely used to treat various diseases, including graft versus host disease (GVHD), osteoarthritis, and asthma, and in the regeneration and repair of damaged tissues (22). Therefore, research on MSCs has shifted from basic research to clinical applications, particularly in SLE, as shown in **Figure 1**.

MSCs can also be successfully isolated from the umbilical cord tissues and placenta, and the properties and functions of these MSCs were similar to BM-MSCs; however, compared with BM-MSCs, these cells exhibited lower immunogenicity and more vigorous proliferation and differentiation abilities (23, 24). Subsequently, several allogeneic MSC transplantation for patients with active and refractory SLE were carried out (25–27). A series of results have been obtained that the doses of immunosuppressive drugs used in patients with SLE reduced, and the mortality rate significantly decreased. However, MSC therapy for SLE is currently in the clinical stage. Although most clinical studies have confirmed that MSCs are effective for SLE treatment, many challenges remain to overcome before clinical application.

MOLECULAR MECHANISMS OF MSCS IN SLE

MSCs Regulate Adaptive Immune Cells

The immunosuppressive effect of MSCs is essential for MSC therapy. MSCs can express prostaglandin E2 (PGE2) (28), transforming growth factor-beta (TGF-β) (29), nitric oxide (NO) (30), C–C motif chemokine ligand 2 (CCL2) (31), indoleamine-pyrrole 2,3-dioxygenase (IDO) (32), interleukin-10 (IL-10) (29, 33), and programmed cell death-1 ligands (PD-L1 and PD-L2) (34). Transplanted MSCs can act on tissues or organs through cell–to–cell contact, secrete cytokines and extracellular vesicles (EVs), which further inhibit the production of pro-inflammatory cytokines, and exert immunosuppressive effects, as shown in **Figure 2** (35–37).

Abnormally activated B cells in patients with SLE exert multiple functions, such as producing large quantities of autoantibody (*e.g.*, anti-dsDNA and ANA), secreting pro-inflammatory cytokines (*e.g.*, IL-6 and IFN-γ), and anti-inflammatory cytokines (*e.g.*, IL-10 and TGF-β) (1). MSCs could inhibit B cells differentiation into plasma cells and antibody production *via* soluble factors and cell–to–cell contact involving the PD-1/PD ligand pathway (35, 36). Regulatory B cells (Bregs) exert immunosuppressive functions at least partly through the production of IL-10 and TGF-β in SLE (38, 39). MSCs can induce the expansion of Bregs and inhibit excessive inflammatory responses in a murine lupus model (33). Currently, whether MSCs could affect the expression of B cell co-stimulatory molecules and cytokine production is unknown.

Abnormal activation of T cells, imbalance of Th1/Th2, and other cell subsets are generally involved in the pathogenesis of SLE. The serum IL-17 from patients with SLE were significantly higher than healthy controls, which positively correlated with the SLEDAI score (40). It has been widely reported that T follicular helper (Tfh) cells could help B cells produce autoantibodies and form immune complexes, which caused tissue and organ damage, and eventually aggravated the condition of SLE patients (2). MSCs inhibited the differentiation of naïve $CD4^+$ T cells into Tfh cells through cell–to–cell contact and the activation of iNOS, decreased the production of IL-21, alleviated lupus nephritis, and prolonged the survival rate of lupus-prone mice (30, 41). Similarly, Th1/Th2 subgroups in patients with SLE were unbalanced (biased toward Th1) and released pro-inflammatory cytokines; they have been considered important for disease and promote SLE progression (42). Additionally, studies have shown that MSCs could inhibit T cell activation in a dose-dependent manner; inhibit the differentiation of $CD4^+$ T cells into Th1, Th17, and Tfh cells; promote Treg proliferation and secretion of IL-10, reduce the ratio of Th1/Th2; and restore the proportion of

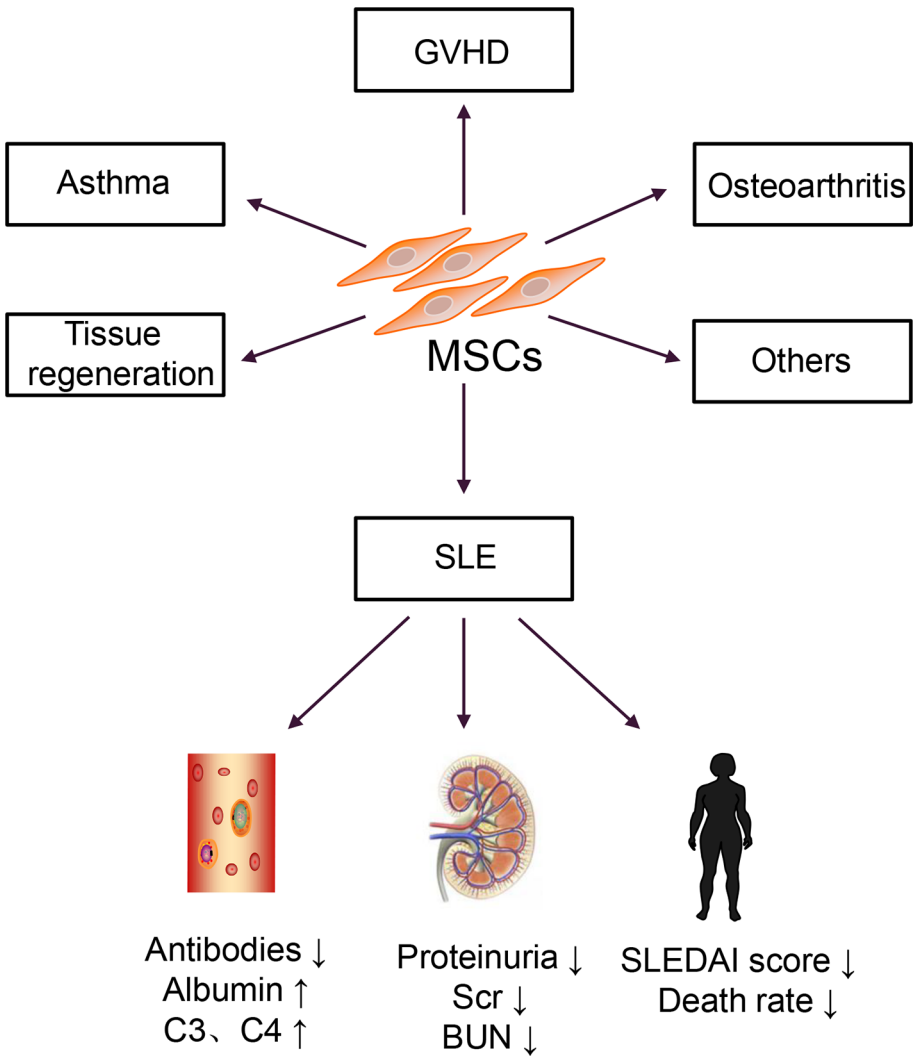

FIGURE 1 | Clinical applications of MSCs.

Treg/Tfh cells, thereby correcting the abnormally activated T cells and cell subsets in patients with SLE (41, 43). In addition, it is reported that after treating thirty refractory SLE patients with human umbilical cord-MSCs for three months, the Treg subgroups and the levels of TGF-β in the peripheral blood were increased (40). In contrast, the expression levels of Th17 cells and IL-17, tumor necrosis factor (TNF-α), and other pro-inflammatory factors were significantly decreased (40). They further co-cultured human umbilical cord-MSCs with peripheral blood mononuclear cells from SLE patients and found that MSCs could upregulate the expression of TGF-β and Treg cells in a dose-dependent manner *in vitro* (40). Researchers also employed a combination of MSCs with five SLE clinical drugs, *viz.*, prednisone, dexamethasone, cyclosporin A, mycophenolate mofetil, and rapamycin, in animal experiments. These drugs could improve the therapeutic effect of MSCs, thereby enhancing the functions of Treg and alleviating the drug cytotoxicity (44).

MSCs Regulate Innate Immune Cells

The innate immune response is the first line of defense against viral invasion *in vivo*. Recent studies have revealed that the innate immune response plays a vital role in SLE progression by initiating and maintaining an adaptive immune response.

Macrophages have two functional states, often exhibited pro- (M1) as well as anti-inflammatory (M2) properties (45). In SLE, macrophages have the defective phagocytic ability and are abnormally activated, promoting disease progression (3). When co-cultured with macrophages, MSCs exerted an immunomodulatory effect by upregulating anti-inflammatory and downregulating pro-inflammatory molecules of macrophages in a murine lupus model (46). Except for regulating macrophage

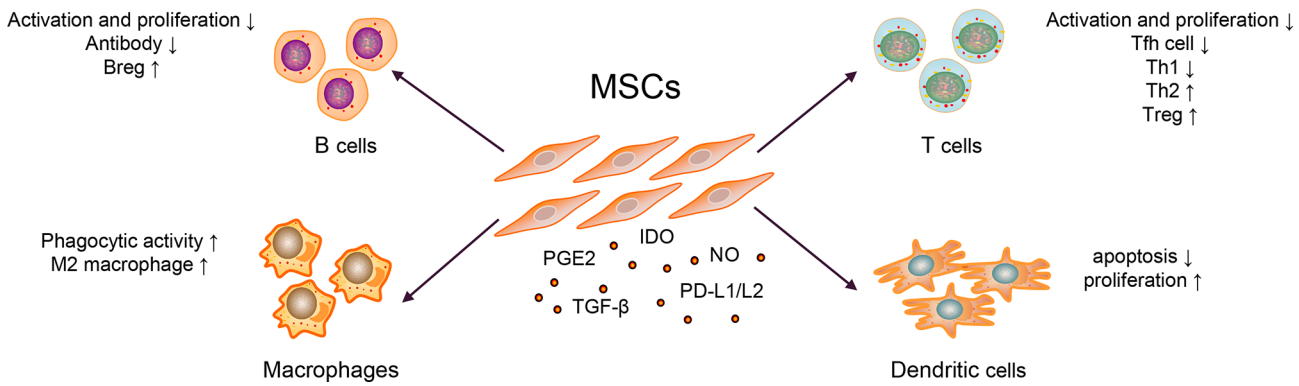

FIGURE 2 | Mechanisms of MSC Therapy in SLE.

polarization, the study also revealed that MSCs could enhance the phagocytic activity of macrophages, thereby alleviating disease activity in a murine model (46, 47). In other diseases, such as leukemia, MSCs could also help host macrophages to repair the damaged bone marrow microenvironment by reprogramming macrophages (48).

DCs play a critical role in activating T cells and B cells (5, 49). Two types of DC subsets have been identified in *Homo sapiens*: myeloid DCs and plasmacytoid DCs (50). In the pathogenesis of SLE, plasmacytoid DCs were considered the primary source of type I interferon (IFN), which promoted the activation of T and B cells (51). Similarly, co-stimulatory molecules overexpressed by myeloid DCs could accelerate T cell maturation, promote T cells differentiation into pro-inflammatory cells and lead to organ damage *in vitro* (5). It confirmed that MSCs could inhibit the maturation and function of DCs and reduced the expression of presentation molecules, such as human leukocyte antigen-DR and co-stimulatory molecules, such as CD80 and CD86 (52). MSCs could also induce the production of regulatory DC (DCregs) and escaped apoptosis, further enhancing phagocytosis's ability and inhibiting T cells' activation and proliferation (53, 54). In addition, MSCs inhibited the secretion of TNF-α by DCs and upregulated the secretion of IL-10 (55). But these mechanisms are known little in SLE. As DCs is essential for the pathogenesis of SLE, these functions of MSCs need to be confirmed in murine model or patients with SLE. Later, it was revealed that the numbers of tolerogenic $CD1c^+$ DCs in the peripheral blood and the levels of serum FLT3L in patients with SLE significantly decreased (56). After transplanting of umbilical cord-MSCs, the significantly upregulated levels of FLT3L promoted the proliferation and inhibited the apoptosis of tolerogenic $CD1c^+$ DCs, thereby improving the condition of lupus (56). In SLE, the activity of myeloid-derived suppressor cells impaired in both murine and humans (57) could promote Th17 cells and Treg differentiation and shift the ratio of Th17/Treg, thereby promoting the progression of SLE (58). MSC infusion could restore the activity of myeloid-derived suppressor cells in inflammatory and autoimmune diseases, such as Sjögren syndrome (28, 59); however, it remains unclear in SLE.

CURRENT STATUS AND CHALLENGES OF THE APPLICATION OF MSCS IN SLE

There are currently thirteen clinical studies on the treatment of SLE with MSCs registered at www.clinicaltrials.gov, among which nine have completed, and four are in progress. Many studies suggested that MSC transplantation could effectively improve the clinical symptoms of active and refractory SLE patients; reduce the SLEDAI score; decrease the levels of proteinuria, autoantibodies, and complements; and reverse multiple organ damage (26, 60).

A meta-analysis on MSC therapy in a murine lupus nephritis model was conducted from October 2009 to October 2020 and revealed that MSC therapy increased the levels of serum albumin and reduced the levels of dsDNA and proteinuria (61). Moreover, MSCs could reduce the levels of IL-2, IL-12, IL-17, IFN-γ and improve the renal sclerosis score (61). Also, allogeneic MSC transplantation was used for fifteen patients with active and refractory SLE and followed up for 17.2 ± 9.5 months (25). No severe toxicities or adverse events were reported (25). All patients attained disease remission, and the SLEDAI score, anti-dsDNA levels, and 24h proteinuria levels markedly decreased within one year. One year later, two patients experienced a relapse of proteinuria (25). This approach was a good starting point for MSC therapy of SLE. Subsequently, a six-year follow-up observation of allogeneic MSC transplantation for refractory SLE found that all patients tolerated the treatment well, with no increase in the risk of tumor formation or infection (27). Furthermore, MSC therapy decreased the SLEDAI score, the levels of autoantibodies, proteinuria and increased serum albumin levels (62). The latest long-term retrospective study reported that MSC transplantation-related mortality was only 0.2%, confirming the effectiveness and safety of MSC transplantation (63). To date, MSC therapy was the most promising treatment for SLE, particularly for patients who do not respond well to traditional therapies.

Although most studies indicated that MSC transplantation could improve the disease condition of patients with SLE, a few have shown that MSC therapy was ineffective. It was worth

noting that no severe adverse effects were observed after autologous BM-MSC transplantation. Although BM-MSCs increased the numbers of $CD4^+CD25^+FoxP3^+$ cells, they did not improve disease conditions, even in young patients (21, 64). Murine BM-MSCs inhibited the deposition of immune complex in the glomerulus and restrained lymphocytic infiltration and glomerular proliferation in lupus animal models (36). However, they did not affect the production of anti-dsDNA or proteinuria (36). In addition, based on standard immunosuppressive therapy, twenty-five patients with SLE were recruited to treat with human umbilical cord MSCs (dose 2×10^8 cells/person) in 2012 (NCT01539902) (65). However, the clinical study was terminated after treating eighteen patients and revealed that the levels of proteinuria, serum albumin, complement, SLEDAI score, and renal function in the MSC therapy group had no significant difference compared to the placebo group (65). However, the authors did not provide specific reasons for this failure. Hence, the therapeutic effects of MSCs must be confirmed in large-scale clinical studies.

Notably, intravenous infusion of MSCs is complicated and may have serious side effects, such as vascular occlusion and the induction of tumor formation. Considering the safety of MSC transplantation, many investigators have focused on EVs derived from MSCs. Compared with MSCs, EVs derived from MSCs exhibited similar immunosuppressive functions in several studies *in vitro* as cell-free therapy and showed high safety (66, 67). However, there is currently no standard and high-efficiency extraction method for EVs, which leads to low production yields and increased heterogeneity of MSC EVs (68). Notably, EVs derived from MSCs have not been used in clinical studies of SLE.

POTENTIAL CAUSES OF MSC THERAPY INEFFICACY IN SLE TREATMENT

In the past few decades, there have been successes and failures in using MSCs to treat SLE. There is no evidence that MSCs are unsafe or promote the progression of SLE. However, reports showed that MSCs could secrete cytokines with strong pro-inflammatory effects, such as IL-6 (69, 70), which might be related to some controversy in their application. Also, MSCs are susceptible to aging due to the influence of the surrounding environment (71). The etiology and pathogenesis of SLE are still unclear. The microenvironment of patients with SLE is complicated, causing the therapeutic effects of intravenously infused MSCs can be influenced by many factors. We summarize the potential causes of MSC therapy inefficacy in SLE from the following aspects: the defective BM-MSCs in patients with SLE, the expansion of MSCs *in vitro*, and the complex microenvironment in patients with SLE. It is worth noting that studies in many other diseases have confirmed that most intravenously infused MSCs could be trapped and cleared in the lung (72–74), which may be one of the reasons for MSC therapy inefficacy.

Defective BM-MSCs in Patients With SLE

SLE is an autoimmune disease that is genetically inherited, with patients showing disease-related susceptibility genes (75, 76). It is well known that long-term use of high doses of immunosuppressive agents could increase the risk of bone marrow suppression in patients, which aggravated complications such as infection and anemia. MSCs from the bone marrow of patients with SLE were defective (14). Hence, SLE is considered a type of stem cell-mediated disease, resulting in weakened HSCs growth and differentiation (14, 77). The morphology of BM-MSCs in patients with SLE was similar to that of healthy controls, and both exhibited the typical immunophenotype, positive for CD44, CD73, and CD105, and negative for CD34, CD19, CD45, and other hematopoietic cell indicators; however, BM-MSCs from patients with SLE exhibited proliferation, differentiation, migration, and homing ability defects and are more prone to senescence and apoptosis (14). Moreover, their ability to secrete cytokines is weakened, causing a decrease in the inhibitory effect on T and B cells and other immune cells, thereby promoting the progression of SLE (71, 77). In addition, BM-MSCs are affected by age. As donors grow older, BM-MSCs tend to senescence, and their functions gradually weaken, resulting in poor effects after transplantation (77).

If combined transplantation of MSCs and HSCs, MSCs could promote the transplantation of HSCs, enhance hematopoietic function, and improved GVDH condition *in vivo* (78). This finding indicates that MSCs have potent roles in promoting body repair while exerting immunosuppressive effects. However, it is unknown whether MSCs transplanted into the body could promote the recovery of BM-MSC function in SLE. Additional clinical data are required to confirm these findings. If appropriate methods are used to modify the BM-MSCs of patients with SLE *in vitro* and then re-inject them into the body, the function of autologous defective MSCs in these patients is expected to be restored.

Effect of *In Vitro* Expansion of MSCs

A murine model showed when the generation of MSCs expanded *in vitro* is low, the cells possess a stronger ability to home to damaged tissues (79). However, the morphology and function of younger MSCs are unstable, resulting in unknown effects. When the number of MSCs is lower, fewer cells can be used for transplantation. And a low number of MSCs could promote lymphocyte proliferation while a larger dose always has an inhibitory effect on lymphocyte proliferation (80). Therefore, to achieve the best therapeutic effect, MSCs must be cultured *in vitro* to obtain sufficient cells.

However, there is currently no standardized system for the isolation, culture, and expansion of MSCs. When MSCs are cultured and expanded *in vitro*, gene mutations may occur because of the culture system and conditions used, resulting in expansion-related senescence, weakened proliferation and differentiation ability, reduced adhesion, and homing ability (81). Also, if MSCs are expanded *in vitro* for a long time, they could gradually lose the ability to recognize endogenous tissues and exhibit weakened genetic stability (82); therefore, when these

cells are transplanted into the body, their therapeutic effect may decrease, or they may pose a safety risk.

Complex Microenvironment in Patients With SLE

MSCs were mostly trapped in the lungs when injected intravenously into the body (83) and could not be detected after 7–14 days [65]. However, due to the different microenvironments of patients with SLE, the residence time and efficacy of MSCs differ; when harmful factors damaged local tissues, the residence time of MSCs *in vivo* could be extended, promoting the repair of damaged tissues (84). In addition, high-level inflammatory factors could enhance the immunosuppressive effect of MSCs on immune cells by simulating the inflammatory microenvironment of patients *in vitro* (85).

Evidence showed an imbalance in Th1/Th2 and other cell subpopulations in patients with SLE, which significantly increased the levels of the pro-inflammatory cytokines IL-6, TNF-α, and IL-1β (86). IL-6 and IL-1β were known to drive Th17 differentiation and promote the levels of serum IL-21 and IL-17 which correlated with disease activity (87, 88). When MSCs exposed to IL-6, the stemness of MSCs was enhanced through an ERK1/2-dependent mechanism (89); however, whether IL-6 could reduce the immunosuppressive function of MSCs still unclear. Therefore, it needs to further investigate the specific effect of IL-6 on MSCs. In patients with SLE, high concentrations of serum TNF-α could significantly inhibit the migration and homing capacity of SLE BM-MSCs *via* TNF receptor I (90). Also, anti-TNF therapies for rheumatic diseases led to the formation of anti-dsDNA and drug-induced lupus (91). In addition, the upregulation of renal TNF-α was considered to play a vital role in the activation of local inflammation and formation of tissue damage (92); however, in collagen-induced arthritis, when TNF-α was present in large quantities, increasing the number of MSCs does not relieve the clinical symptoms (93). When co-stimulated with TNF-α and IL-1β, MSCs exhibited pro-inflammatory effects and promoted T cell proliferation and differentiation (94). In summary, the inflammatory environment may induce MSCs to exert pro-inflammatory effects, leading to the failure of MSC therapy in autoimmune diseases, including SLE.

MSCs can secrete anti-inflammatory cytokines such as indoleamine-pyrrole 2,3-dioxygenase and prostaglandin E2, as well as pro-inflammatory cytokines such as IL-6 and TNF-α (69), which may accelerate disease progression. Another study revealed that IL-6 silencing could weaken the inhibition of the proliferation of activated T cells (95). Therefore, MSCs may have dual effects on the disease.

NOVEL MECHANISMS AND DIRECTIONS OF MSCS IN SLE TREATMENT

MSCs have strong immunomodulatory plasticity and could be easily influenced by the microenvironment, which is among the reasons why MSC therapy is ineffective. Thus, MSC modifications, such as genetic and preconditioning modifications, could avoid the influence of the environment. The former alters MSCs by inserting a gene, whereas the latter alters MSCs using chemical and/or physical factors *in vivo*, thereby overexpressing specific genes and improving the efficacy of disease treatment. Modified MSCs are now widely used to treat tumors, cardiovascular diseases, neurological diseases, bone, and joint diseases, and so on (96).

Pro-inflammatory cytokines contribute to the pathogenesis of SLE; however, for MSCs, strong inflammatory cytokines were effective attractors that could activate the immune-suppressive function, whereas low levels of inflammatory factors could reduce the immune-suppressive role or even trigger the immune system (97, 98). In a murine model, transplanted IL-37 overexpressed MSCs inhibited the inflammatory microenvironment *in vivo*, prolonged survival, and reduced SLE-like symptoms (99). Similarly, pretreated MSCs with media containing pro- or anti-inflammatory cytokines or related molecules such as poly (I:C) and glucocorticoids could enhance the immunosuppression of MSCs (100–102). Pretreated MSCs with IFN-γ could increase IDO (32) and significantly inhibited splenic B cells' proliferation and the production of antibodies (103). The pretreatment of MSCs with IL-1β significantly increased the number of Treg and Th2 cells and decreased Th1 and Th17 cells (104). Besides, if IFN-γ co-cultured with any of the three other pro-inflammatory cytokines, *viz*., TNF-α, IL-1α, and IL-1β, the adhesion, migration, and homing abilities of MSCs could be enhanced (105). Modified MSCs with IL-10 could inhibit tumor growth by reducing the production of IL-6 (106). These results indicate that MSC modification could enhance the immunosuppression of MSCs, providing a new and feasible direction for SLE therapy.

The aging phenotype of MSCs could be wholly or partially reversed by inhibiting MSC senescence-related genes, which improves the immune regulation function *in vitro* (107, 108). Recently, several studies demonstrated that the pretreatment of MSCs with rapamycin and Dickkopf-1 reversed the senescence of MSCs and improved the immune regulation of MSCs (108–110). There are also other ways to modify MSCs and reverse senescence phenotype, such as pretreated MSCs with hypoxia or by upregulating the expression of CBX4 or Erb-B2 receptor tyrosine kinase 4 (ERBB4), which could change the senescence phenotype, reduce the expression of senescence-associated β-gal, and maintain the stemness of MSCs (107, 111–113). In contrast, upregulated CD146, CD264, SIRT3, and TLR3 expression levels in MSCs increase senescence (114–117). MSC senescence is unavoidable in SLE treatment. If the senescence phenotype of MSCs is modified by various methods, the function of MSCs can be improved, thereby enhancing their therapeutic ability.

Several studies have revealed that increased the expression of homing molecules and cell surface receptors, such as CC chemokine receptors 1 (CCR1) (118), C–C motif chemokine

ligand 2 (CCL2) (31), C–X–C motif chemokine receptors 2 (CXCR2) (119), CXCR4 (120) by modifying MSCs could promote the therapeutics of MSCs.

However, it is controversial whether the migration and homing of MSCs to damaged tissues are required for their immunomodulation effects. For the local immune response of MSCs, the therapeutics of MSCs may be associated with its migration and homing abilities. It has been reported that the overexpression of CCR1, CXCR2, and CXCR4 in MSCs or modified MSCs with biomimetic extracellular matrices and poly (dimethylsiloxane) could stimulate more MSCs to migrate to the lesion sites, secrete more anti-inflammatory cytokines, and accelerate tissue healing (118–122). The pretreatment of MSCs with miR-9-5p or TNF-α could also improve the migration ability of MSCs, whereas the inhibition of miR-9-5p reduced MSC migration (123, 124). For the systemic immune responses of MSCs, many studies observed the phenomenon that most of the MSCs were trapped in the lung after IV infusion in murine models (72–74). For the mechanisms, the MSCs trapped in the lung after IV infusion may secret bioactive molecules and EVs into the blood and efficiently regulate systemic immune responses (125, 126). For example, MSCs trapped in the lung with higher expression of the gene for a multifunctional anti-inflammatory protein tumor necrosis factor-α stimulated gene/protein 6 (TSG-6) could efficiently regulate systemic immune responses in lung injury mice (125). Meanwhile, the overexpression of CCL2 in MSCs from patients with SLE could improve MSC immunoregulatory abilities both *in vitro* and *in vivo*, whereas the knockdown of CCL2 from normal MSCs led to a weakened immunoregulatory power (31). This may also correlate to the bioactive molecules secreted by MSCs. However, the specific molecular mechanisms of these bioactive molecules and EVs regulating the systemic immune responses need further studies.

Studies showed the immunomodulatory of MSCs is partly the result of EVs, which play an increasingly important role in MSC therapy (127, 128). As a novel cell-free therapy, EVs could deliver specific molecules to target tissues or organs and exhibit nearly the same immunomodulation ability as MSCs (128, 129). EVs derived from modified MSCs are widely used in several diseases *in vitro* (37, 127–129). Exosomes derived from miR-122-modified MSCs could improve the sensitivity of tumor cells to drugs and increase the effect of drugs on cancer treatment (127). In the rat models of spinal cord injury, exosomes derived from miR-126-modified MSCs could promote the recovery of injury volume and trigger the regeneration of axons (128). Moreover, exosomes from Akt-modified MSCs in the acute myocardial infarction rat models could reduce myocardial cell apoptosis, increase cardiac regeneration, and improve cardiac function (129). In rheumatoid arthritis (RA) murine model, exosomes derived from miR-150-5p modified MSCs could decrease the regeneration of synoviocytes and reduce joint destruction, thereby being the potential treatment for RA (37). However, EVs derived from MSCs remains limited in murine models and patients with SLE.

FUTURE PERSPECTIVES

MSCs are currently used to treat patients with active and refractory SLE, and a series of promising results have been obtained. However, MSCs do not always exhibit strong immunosuppressive function and may lose their therapeutic effect under the influence of many factors. This may occur for the following reasons: defects of BM-MSCs in patients with SLE, the impact of MSCs culture *in vitro*, and the complex microenvironment of patients with SLE. To maximize the therapeutic effects of MSCs or EVs derived from MSCs *in vivo*, MSCs need to be pretreated by various means, including pro- and anti-inflammatory cytokines, improving their senescence and enhancing their migration and homing ability.

However, MSC modification must also be confirmed to determine whether MSC gene mutation will occur or if the transplantation of MSCs will harm the body in the long term, including serious problems such as tumorigenesis and teratogenesis. No studies have focused on whether the modification of MSCs alters their safety. In the long term, MSC modification may improve the therapeutic effects of MSCs in autoimmune diseases, particularly in SLE.

MSCs are in the early stages of clinical application and are typically combined with hormonotherapy. Whether hormone therapy can be discontinued using modified MSCs should be examined. In addition, MSC therapy improves but does not completely cure SLE. Thus, whether modified MSCs can cure SLE requires further analysis. Limited by their high cost, safety concerns, and lower SLEDAI scores of disease conditions, MSCs are rarely used in patients with mild SLE. However, comprehensive studies of MSCs and improvements in their preparation process can reduce costs and significantly expand the application of MSCs. MSCs may be more effective in patients with mild SLE or preempted for those with a genetic background of SLE, which may relieve these patients' conditions.

AUTHOR CONTRIBUTIONS

AL, FG and QRP wrote the manuscript and designed the figures. SC, JC, and H-FL revised the manuscript. All authors contributed to the article and approved the submitted version.

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Emerging Role of Eosinophils in Resolution of Arthritis

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Eosinophils are a minor component of circulating granulocytes, which are classically viewed as end-stage effector cells in host defense against helminth infection and promoting allergic responses. However, a growing body of evidence has emerged showing that eosinophils are versatile leukocytes acting as an orchestrator in the resolution of inflammation. Rheumatoid arthritis (RA) is the most common chronic inflammatory disease characterized by persistent synovitis that hardly resolves spontaneously. Noteworthy, a specific population of eosinophils, that is, regulatory eosinophils (rEos), was identified in the synovium of RA patients, especially in disease remission. Mechanistically, the rEos in the synovium display a unique pro-resolving signature that is distinct from their counterpart in the lung. Herein, we summarize the latest understanding of eosinophils and their emerging role in promoting the resolution of arthritis. This knowledge is crucial to the design of new approaches to rebalancing immune homeostasis in RA, considering that current therapies are centered on inhibiting pro-inflammatory cytokines and mediators rather than fostering the resolution of inflammation.

Keywords: eosinophil, rheumatoid arthritis, resolution, innate lymphoid cells, alternatively activated macrophages

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INTRODUCTION

Eosinophils are leukocytes that normally amount to less than 5% of white blood cells in the peripheral blood. In certain pathological settings, eosinophils significantly expand and count over 1,500 cells/μl blood, which is defined as hypereosinophilia (1). Although previously considered as the end-stage effector cells involved in helminth infection and allergic diseases like asthma, increasing evidence shows that eosinophils are multifunctional granulocytes involved in regulating adaptive immune responses, especially in inflammatory and autoimmune disorders (2).

Rheumatoid arthritis (RA) is the most common chronic immune-mediated inflammatory disease characterized by persistent synovitis that lacks self-remission (3). Although the pathogenesis of RA remains incompletely understood, the general consensus is that self-tolerance breakdown triggers autoantibody production in genetically predisposed individuals before progressing into clinically apparent RA (3). During the transition from asymptomatic autoimmunity to synovial inflammation, a diverse range of pro-inflammatory cytokines produced by immune cells such as $CD4^+$ T cells, macrophages, and fibroblast-like synoviocytes emerge quickly, which eventually contribute to cartilage damage and bone erosion in the joint (4).

Notably, once the joint inflammation is established, it tends to be chronic, as evidenced by the insufficiency of regulatory factors that counteract or rebalance aberrant immune responses (4). Hence, ineffective resolution of RA remains a major clinical challenge, although novel anti-inflammatory biological agents have been increasingly introduced (5, 6).

In contrast with the pro-inflammatory properties of eosinophils in asthma that cause structural remodeling of the airways, recent studies by us and collaborators have suggested that, as a crucial component of Th2 immune responses, eosinophils have previously undifferentiated pro-resolving signature in RA (7–10). In this review, the emerging role of eosinophils in promoting the resolution of arthritis is summarized. The potential underlying mechanisms that allow eosinophils to exert anti-inflammatory properties and therapeutic implications of eosinophils in arthritis are also discussed.

EOSINOPHIL DEVELOPMENT AND BIOLOGY

Eosinophils are generated in the bone marrow from multipotent hematopoietic stem cells, which give rise to eosinophil-committed progenitors (EoPs). These will eventually differentiate into mature eosinophils in response to several cytokines such as IL-5, IL-3, granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-33 (11–13). Eosinophilopoiesis is governed by at least three transcription factors, including GATA-1 (a zinc family finger member), PU.1 (an ETS family member), and C/EBP members (CCAAT/enhancer-binding protein family) (14, 15). Notably, GATA-1 is essential for eosinophil differentiation, since the deletion of a GATA-binding enhancer site in the GATA-1 gene generated a specific eosinophil-deficient ΔdblGATA mouse with no influence on other cell lineages (16). In addition, some microRNAs and long non-coding RNAs have been reported to be involved in eosinophilopoiesis (17–19).

Once eosinophils mature in the bone marrow, they are released into circulation and migrate into peripheral tissues under stimulation of IL-5 and eotaxin-1 (CCL11) (2). In homeostatic conditions, eosinophils are distributed in the spleen, gastrointestinal tract, thymus, adipose tissue, and uterus, indicating that they are likely to be responsible for maintaining homeostasis in different tissues (20). It is well established that eosinophils synthesize a broad range of mediators stored in granules throughout the cytoplasm, including cytotoxic granule proteins such as major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil peroxidase (EPX), and eosinophil-derived neurotoxin (EDN) (21). When encountering the stimulus present in the tissue, eosinophils release granule contents rapidly, which is termed degranulation, to exert host immune defense against pathogens (2). Hence, historically it is considered that the primary effector function of eosinophils was in anti-pathogen responses, especially those involving parasites. Nonetheless, eosinophil granules also contain numerous cytokines, particularly type 2 cytokines, as well as growth factors and resolvins, suggesting their ability to be involved in tissue repair and a wide range of immunological disorders such as allergy and asthma (22, 23).

REGULATION OF TH2 RESPONSES BY EOSINOPHILS

Although they represent a minor component of innate immune cells, eosinophils are well known to be an important innate immune regulator in pathogen clearance by releasing cytokines and chemokines or by interacting with other innate immune cells (24–28). Meanwhile, increasing evidence has extended understanding that eosinophils are versatile leukocytes capable of modulating adaptive immune responses as well. For example, murine eosinophils can present antigen *via* MHC class II and promote IL-4, IL-5, and IL-13 production from antigen-specific $CD4^+$ T cells in the context of helminth infection or asthma (23, 29, 30).

Other than behaving as antigen-presenting cells, eosinophils are thought to regulate Th2 immune responses in multiple ways. A Notch ligand Jagged1, which constitutes an instructive signal for Th2 differentiation, has been found to express on human eosinophils constitutively, indicating the capability of eosinophils to provide a polarization signal to naïve $CD4^+$ T cells (31, 32). Studies on helminth infection models revealed that eosinophils precede lymphocyte recruitment into inflammatory sites (33, 34). In eosinophil-deficient ΔdblGATA mice infected with *Trichinella spiralis*, infiltration of Th2 cells into the muscles was highly decreased (35). In another study using IL-5/eotaxin double-knockout mice in which eosinophil counts are severely reduced, significantly decreased IL-13 production by Th2 cells in response to the OVA challenge was observed (36). Notably, this defect can be rescued by the adoptive transfer of eosinophils, suggesting the role of eosinophils in the regulation of Th2 immune responses (36). In addition to the secretion of Th2-related cytokines, eosinophils can also promote Th2 responses through the synthesis of indoleamine 2,3-dioxygenase (IDO), an enzyme that catalyzes the oxidative catabolism of tryptophan to kynurenines (37).

EOSINOPHILS PROMOTE THE RESOLUTION OF INFLAMMATION

Inflammation is an evolutionary defensive host response to injury, characterized by the recruitment of leukocytes and cytokines from the circulation to the inflamed tissue. Generally, acute inflammation in healthy individuals is self-limited and resolves timely, thus preventing to progress to chronic inflammation (38). During the course of acute inflammation, the migration of polymorphonuclear neutrophils (PMNs) into tissues is the early event, followed by the recruitment of monocytes that will further differentiate into tissue macrophages. It is known that a variety of classic proinflammatory mediators such as prostaglandins (PGs) and leukotrienes (LTs) coordinate these initial events of acute inflammation by regulating vascular permeability and leukocyte infiltration (39). Once the malicious components are removed by

phagocytosis, the inflammatory response must be promptly resolved to prevent excessive tissue damage and return to homeostasis. However, uncontrolled or long-lasting inflammation is believed to exist in the pathogenesis of many human autoimmune and inflammatory diseases including RA (40).

In recent years, a growing body of evidence has emerged that shows that resolution of acute inflammation is not a passive but an active process controlled by endogenous resolving mediators, termed specialized pro-resolving mediators (SPMs) such as protectins, resolvins, and maresins, which belong to families of lipid mediators (41). Blocking lipid mediator biosynthesis by either cyclooxygenase (COX)-2 or lipoxygenase (LOX) inhibitors resulted in resolution defect, which is characterized by sustained leukocyte infiltration in inflamed sites and impaired removal of phagocytes to the draining lymph nodes (42), suggesting the critical role of these lipid mediators in regulating the timely resolution of acute inflammation.

Interestingly, eosinophils are an orchestrator in the resolution of inflammation. In a murine zymosan-induced peritonitis model, eosinophils were recruited to the inflamed site during the resolution phase of acute peritonitis (43). Liquid chromatography–tandem mass spectrometry (LC-MS/MS)-based lipidomics analyses revealed that pro-resolving mediators such as protectin D1 (PD1) were increased during the resolution phase in a 12/15-LOX dependent manner (43). PD1 promotes the resolution process by inhibiting PMN influx and stimulating macrophage ingestion of apoptotic PMNs, as well as increasing phagocyte clearance into draining lymph nodes (42). Importantly, the researchers revealed that eosinophils were the main PD1-producing cells in the resolution phase of zymosan-induced peritonitis (43). Depletion of eosinophils or CXCL13 *in vivo* caused a resolution defect, characterized by impaired lymphatic drainage of inflammatory phagocytes carrying engulfed zymosan in the draining lymph node, and delayed removal of PMNs in the inflamed tissues (44). Notably, administration of PD1, CXCL13, or adoptive transfer of eosinophils from wild-type but not from 12/15-LOX deficient mice reversed the defective phenotype of the resolution process, suggesting that eosinophils promote the resolution of inflammation through pro-resolving mediators and CXCL13 pathway (43, 44). In another experimental colitis model, more severe colitis was observed in eosinophil-deficient mice compared with wild-type controls, accompanied with decreased level of PD1 in the colon (45). Furthermore, administration of exogenous PD1 alleviated the severity of colitis and reduced neutrophil infiltration. All these findings indicated that eosinophils contribute to the resolution of inflammation by producing pro-resolving lipid mediators such as PD1 *via* the 12/15-LOX-mediated biosynthetic pathway.

REGULATORY EOSINOPHILS IN RA

Although eosinophils act as a counter regulator in several inflammatory disorders, the association between eosinophils and the development of RA was largely undetermined, probably due to the uncommon clinical manifestation of eosinophilia in RA. Indirect evidence from previous studies reported that RA patients, especially those with high activity and short disease duration, have increased serum levels of ECP, supporting the notion that eosinophils were involved in the inflammatory responses of RA (46, 47). In a prospective multi-center cohort study, the prevalence of eosinophilia in patients with new-onset arthritis (disease duration ranges from 6 weeks to 6 months) is only 3.2% (48). Notably, after 3 years, patients with eosinophilia presented with signs of higher disease activity compared with those without eosinophilia at baseline, suggesting that baseline eosinophilia might be a poor prognosis marker in early arthritis patients (48). A more recent prospective observational study investigated clinical characteristics of RA patients with persistent eosinophilia and compared with the patients without eosinophilia (49). After excluding secondary causes of eosinophilia such as concomitant allergic diseases and intestinal helminth infection, the authors did not find differences in clinical features between RA patients with or without eosinophilia (49). The discrepancies among studies might reflect the complex heterogeneity of eosinophil phenotype or function in inflammatory arthritis.

Recently, we have shown that the expression of synovium EPX was elevated in RA patients compared with osteoarthritis (OA) patients (7). Consistently, serum EPX level was higher in RA patients than pre-RA and healthy controls (7). In the K/BxN serum-induced arthritis model, IL-5 transgenic (IL-5tg) mice that have extraordinary hypereosinophilia showed a significant reduction of arthritis score, whereas eosinophil-deficient ΔdblGATA mice presented with higher disease activity (7). In addition, the adoptive transfer of eosinophils into collagen-induced arthritic mice led to the alleviation of arthritis, accompanied by reduced joint inflammation and bone erosion in histology evaluation (9). Altogether, these data suggested that eosinophils have previous unknown pro-resolving properties in promoting the resolution of inflammatory arthritis.

Considering the dual nature of eosinophils as pro-inflammatory and pro-resolving cells, it is reasonable to speculate that eosinophils have different subsets responsible for different biological functions. Indeed, this is supported by the research that revealed two distinct eosinophil subsets, lung resident eosinophils and recruited inflammatory eosinophils, in asthmatic lungs (50). Remarkably, a very recent study described a specific population of eosinophils, named regulatory eosinophils (rEos), was present in the joints of arthritic mice as well as synovium of RA patients (8). OVA allergen challenge triggered an earlier resolution of K/BxN serum-induced arthritis, accompanied with increased eosinophils in the arthritic joints. Strikingly, this protective manifestation was only observed in wild-type but not eosinophil-deficient ΔdblGATA mice, indicating the essential role of eosinophils in the asthma-induced resolution of joint inflammation (8). In further analyses, both single-cell RNA sequencing and proteome profile analyses confirmed that the rEos in the joint display a unique pro-resolving characteristic, which is distinct from their counterpart in the lung. For example, joint rEos have strongly upregulated expression of 5-LOX and 12/15-LOX (8), which could explain the anti-inflammatory and pro-resolving effector function of rEos, since the deletion of 12/15-LOX was linked to

uncontrolled inflammation and tissue damage in chronic arthritis (51). Moreover, rEos has a specific secretion pattern compared with its inflammatory counterpart in the lung, characterized by the production of MMP-3, osteopontin, and serpin E1, suggesting that rEos might also foster synovial tissue recovery besides ceasing inflammation (8). Interestingly, rEos was infiltrated more frequently in RA patients in remission than patients in the active stage. Inactive RA patients with concomitant asthma developed a flare of disease after anti-IL-5 monoclonal antibody treatment, which might be explained reasonably by the depletion of rEos (8).

ILC2–EOSINOPHIL–M2 MACROPHAGE AXIS COUNTERACTS JOINT INFLAMMATION

As a messenger between the innate and adaptive immune systems, innate lymphoid cells (ILCs) have been realized to be intensely involved in the pathogenesis of inflammatory arthritis (52, 53). In particular, ILC2 acts as a crucial regulator in damping joint inflammation in contrast to proinflammatory ILC1/ILC3. Circulating ILC2 count was inversely correlated with disease activity index in RA patients and increased after receiving anti-rheumatic treatment (54). In line with these human data, genetic deletion of ILC2 in mice aggravated K/BxN serum-induced arthritis whereas expansion of ILC2 by IL-25/IL-33 mini-circles or adoptive transfer of ILC2 from wild-type but not IL-$4^{-/-}$IL-$13^{-/-}$ mice attenuated arthritis (55). Furthermore, ILC2 was found to inhibit osteoclast differentiation and bone loss independently of inflammation (56).

Interestingly, it has been established that tissue-resident ILC2 regulates eosinophil homeostasis and accumulation into tissues through constitutive secretion of IL-5 (57). In asthmatic lung, ILC2 was the main producer of IL-5, which consequently drives the expansion and infiltration of rEos into the arthritic joints (8). Another recent work by us showed that activation of ILC2 by a small neuropeptide significantly suppressed the development of collagen-induced arthritis, accompanied by the expansion of eosinophils in the arthritic joints (10). In addition, induction of ILC2 by administration of IL-25/IL-33 accelerated the resolution of K/BxN serum-induced arthritis in wild-type but not eosinophil-deficient ΔdblGATA mice (8). On the contrary, neutralization of IL-5 by a monoclonal antibody blocked asthma-induced resolution of arthritis, with a reduced expansion of rEos in the joints (8). All these results supported the perspective that eosinophils are indispensable for ILC2-mediated resolution of arthritis (8).

Besides secreting a variety of pro-resolving lipid mediators that are crucial for the resolution of inflammation, the role of eosinophils in the suppression of arthritis could also include switching macrophages from pro-inflammatory M1 to anti-inflammatory M2 phenotype. As is well known, synovial macrophages act as central effector cells in the development of synovitis (3). The abundant presence of pro-inflammatory cytokines such as TNFα, IL-6, and IL-1β in the inflamed synovium suggests a predominant M1 macrophage phenotype in RA. However, the phenotype of synovial macrophages *in vivo* is highly complex and often exhibit a mixed polarization state (58). Previously it has been reported that even the same M1 macrophages recruited during the initial phase of arthritis can switch their phenotype toward M2 macrophages (59). In contrast to M1 macrophages that facilitate the inflammatory cascade in the synovium, M2 macrophages halt joint inflammation by removing dead cells (efferocytosis) and producing pro-resolving lipid mediators (58). It has been demonstrated that eosinophils induce polarization of macrophages toward M2 phenotype through secretion of IL-4, IL-13, and 12/15-LOX-derived lipid mediators (43). Eosinophil deficiency was associated with the impaired distribution of anti-inflammatory MHC-II^{-} macrophages in the steady state as well as in arthritis (7). Both *in vitro* and *in vivo* studies showed that eosinophils foster the polarization of M1 to M2 macrophages in the synovial tissue, partly *via* the IκB/P38 MAPK signaling pathway (8, 9). This is consistent with previous studies that reported that eosinophils in adipose tissue mediate macrophage differentiation into M2 phenotype, which are required for glucose homeostasis (60, 61). Taken together, the ILC2–eosinophil–M2 macrophage axis represents a novel and important immunological pathway counteracting joint inflammation and eliciting resolution of arthritis (**Figure 1**).

FUTURE PERSPECTIVE IN THERAPY

With accumulating evidence and advancing technologies, the classical view of eosinophils has changed from a pro-inflammatory cell in helminth infection and allergy to a cell type aggressively involved in anti-inflammatory responses in the resolution of chronic inflammation. The existence of rEos in the synovium of RA patients extended previous understanding that eosinophils are critical in counteracting joint inflammation and facilitating the resolution of disease. These findings are crucial for designing new approaches to rebalancing immune homeostasis in inflammatory arthritis, considering that current therapies are centered on inhibiting pro-inflammatory cytokines and mediators rather than fostering the resolution of inflammation. Hence, understanding how rEos are activated and expanded will offer a novel strategy for the development of safe and effective treatment for arthritis.

Indeed, it is well known that the major extrinsic driver of eosinophil expansion was helminth infection. Numerous previous studies in experimental mouse models have demonstrated clinical improvement of inflammatory activity in a variety of autoimmune and inflammatory diseases including RA (7, 62–67). These observations led to the proposal that harness helminth and their secreted products would represent a promising interventional approach for the treatment of RA, as supported by the findings that a filarial nematode-derived glycoprotein, ES-62, has been proved to exert an anti-inflammatory and anti-osteoclastogenic effect in mouse arthritis models (68–71). In addition, several clinical trials have been performed to evaluate the immune-regulatory effect of helminth on autoimmune diseases, especially in inflammatory bowel disease (72). The mechanisms by which

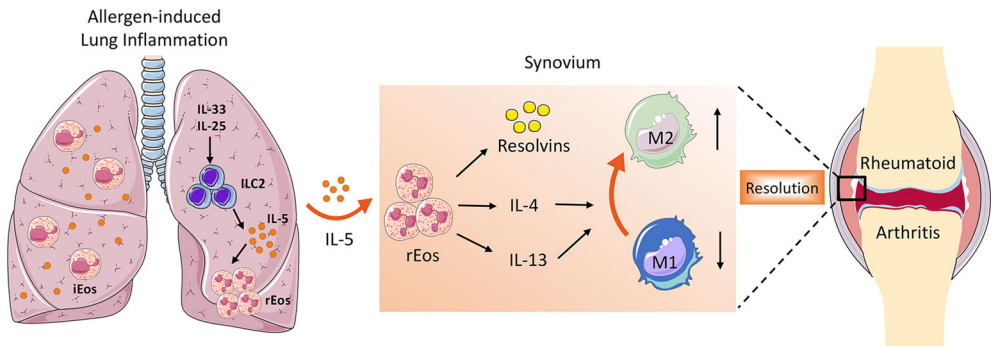

FIGURE 1 | The role of ILC2–eosinophil–M2 macrophage axis in promoting resolution of arthritis. In allergen-triggered lung inflammation, ILC2 was expanded and activated in response to stimulators such as IL-33 and IL-25. Systemically elevated IL-5 secreted by ILC2 drives proliferation and recruitment of rEos into the inflamed joints, where they produce a variety of lipid mediators and anti-inflammatory type 2 cytokines, which facilitate M2 macrophage priming and eventually contribute to the resolution of arthritis.

helminth and their derivatives modulate the host's immune system were attributed to shifting immune responses from Th1 to Th2, induction of regulatory T and B cell subsets, as well as downregulation of IFN-γ and IL-17 (73). This helminth-based immunotherapy is becoming of major interest since current conventional management of RA relies generally on nonspecific inhibition of the immune system, which often results in severe infections and malignancies. Consistent with this concept, one of our recent studies showed that a small neuropeptide named Neuromedin U successfully alleviated collagen-induced arthritis, with evidence of ILC2-eosinophil activation (10). On the other hand, the plasticity of eosinophils offers another strategy that induces differentiation of pro-inflammatory eosinophils into regulatory phenotypes.

CONCLUSION

In summary, emerging evidence has shown that eosinophils not only act as a pro-inflammatory effector cell but also display a pro-resolving feature in RA. They consistently reside in the synovium of RA patients in remission and proliferate under stimulation of ILC2-derived IL-5. Mechanistically, the rEos promote the resolution of arthritis through secreting resolvins in a 12/15-LOX-dependent manner and switching synovial macrophages into the M2 phenotype.

AUTHOR CONTRIBUTIONS

YQ, H-ZJ, and Y-JL drafted the manuscript. ZC revised the manuscript. All authors contributed to the discussion and approved the submitted version.

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REVIEW
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Gut Microbiota Dysbiosis in Systemic Lupus Erythematosus: Novel Insights into Mechanisms and Promising Therapeutic Strategies

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Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that was traditionally thought to be closely related to genetic and environmental risk factors. Although treatment options for SLE with hormones, immunosuppressants, and biologic drugs are now available, the rates of clinical response and functional remission of these drugs are still not satisfactory. Currently, emerging evidence suggests that gut microbiota dysbiosis may play crucial roles in the occurrence and development of SLE, and manipulation of targeting the gut microbiota holds great promises for the successful treatment of SLE. The possible mechanisms of gut microbiota dysbiosis in SLE have not yet been well identified to date, although they may include molecular mimicry, impaired intestinal barrier function and leaky gut, bacterial biofilms, intestinal specific pathogen infection, gender bias, intestinal epithelial cells autophagy, and extracellular vesicles and microRNAs. Potential therapies for modulating gut microbiota in SLE include oral antibiotic therapy, fecal microbiota transplantation, glucocorticoid therapy, regulation of intestinal epithelial cells autophagy, extracellular vesicle-derived miRNA therapy, mesenchymal stem cell therapy, and vaccination. This review summarizes novel insights into the mechanisms of microbiota dysbiosis in SLE and promising therapeutic strategies, which may help improve our understanding of the pathogenesis of SLE and provide novel therapies for SLE.

Keywords: systemic lupus erythematosus, autoimmune disease, gut microbiota dysbiosis, extracellular vesicle, miRNA, mesenchymal stem cell therapy

1 INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by the generation of autoantibodies and immune complexes, which can cause multiple organ damage to the skin, kidney, and central nervous system (1). The pathogenesis of SLE is very complex and is traditionally thought to be closely related to genetic and environmental risk factors (2). Infection is a significant cause of morbidity, disease exacerbation, and death in patients with SLE (3, 4). Recently, increasing evidence has shown that different degrees of intestinal-infection-related dysbacteriosis

exist in patients with SLE and SLE mice, which are closely related to the development of SLE (5, 6). Previous studies have shown that the mechanisms associating gut microbiota dysbiosis and SLE pathogenesis include immune system imbalance, molecular mimicry, impaired intestinal barrier function, biofilms, and sex hormones. Under normal circumstances, the special barrier functions of the intestine include physical, biochemical, and immune barriers, which can separate the host from the environment. Intestinal epithelial cells are joined by tight junction proteins to form the intestinal physical barrier (7). In patients with SLE, impaired intestinal barrier function leads to increased intestinal permeability, allowing pathogens, toxins, and bacteria to leak out of the gut lumen and translocate to other organs, which is called a "leaky gut" (8). In addition, the antigens of the translocated bacteria are similar to some of the host's structures, which cause cross-reactivity to produce autoantibodies and damage target organs in patients with SLE, a process called molecular mimicry (9, 10). Furthermore, curli amyloid in biofilms is associated with autoantibody production. Gut microbiota dysbiosis in SLE is sex-biased, which may be due to sex hormones (11).

The treatment of SLE mainly includes immune regulation and immunosuppression, with the aim of maintaining long-term remission or low disease activity, protecting organ function, and avoiding complications and adverse drug reactions (12, 13). Currently, although treatment options for SLE with hormones, immunosuppressants, and biologic drugs are now available, the rates of the clinical response and functional remission of these drugs are still not satisfactory, which may lead to serious side effects (14, 15). Therefore, there is an urgent need to develop treatment options that have good therapeutic effects in patients with few adverse effects. Emerging evidence has shown that intestinal dysbacteriosis may play an essential role in the pathogenesis of SLE and may be a novel therapeutic target for SLE. Previous studies have shown that interventions targeting the gut microbiota for SLE include dietary interventions, probiotics or prebiotics, antibiotic therapy, vaccination, and fecal microbiota transplantation (FMT). These treatments are currently only studied in lupus murine models, and further clinical trials are required to confirm their efficacy.

In this review, we summarize the gut microbiota dysbiosis in patients with SLE and mouse models, and first described the possible role of IECs autophagy, and extracellular vesicles (EVs) and miRNA, in the gut microbiota homeostasis of SLE, as shown in **Figure 1**. In addition, we propose several novel treatment strategies targeting gut microbiota, including regulation of IECs autophagy, EV-derived miRNA therapy, and mesenchymal stem cell therapy, which may have great value for SLE treatment in the future, as shown in **Figure 2**.

2 GUT MICROBIOTA DYSBIOSIS IN PATIENTS WITH SLE

Recently, many studies have attempted to determine the correlation between gut microbiota dysbiosis and SLE pathogenesis, as shown in **Table 1**. A study showed that compared with healthy controls, patients with SLE suffered from intestinal dysbiosis and had a significantly lower ratio of *Firmicutes*/*Bacteroidetes* (F/B) (22). This result was confirmed by subsequent studies (18, 19, 23, 24). Importantly, *Firmicutes* are inversely correlated with the SLE disease activity index (SLEDAI score) (20), indicating that *Firmicutes* can delay lupus progression. It follows that the reduced F/B ratio is an important manifestation of gut microbiota dysbiosis in patients with SLE. A recent study analyzed stool samples from 117 untreated patients with SLE and reported that the gut microbiota of patients with SLE showed a pro-inflammatory and autoimmune profile compared to healthy controls (6). Furthermore, patients with SLE mostly show decreased richness and diversity of intestinal microbiota compared to healthy controls (6, 8, 25), and this was particularly severe in patients with high SLEDAI scores (8).

Interestingly, the abundance of *Ruminococcus gnavus* (*R. gnavus*) was elevated 5-fold in the gut microbiota of 61 patients with SLE compared to that in healthy control and was strongly associated with SLE disease activity (8). And serum anti-*R. gnavus* antibodies were positively correlated with the SLEDAI score and anti-dsDNA levels (8).

Collectively, gut microbiota dysbiosis in patients with SLE typically displays a decreased F/B ratio, richness, and diversity. Meanwhile, impaired intestinal barrier function leads to microbiome translocation, which exacerbates disease progression in patients with lupus. Furthermore, proliferation of some specific microbiota such as *R. gnavus* may be significantly related to lupus progression.

3 GUT MICROBIOTA DYSBIOSIS IN LUPUS MOUSE MODEL

Recently, many studies have also revealed gut microbiota dysbiosis in lupus mouse models, as shown in **Table 2**. Zhang et al. (26) revealed that lactobacilli significantly reduced and *Lachnospiraceae* increased in the gut microbiota of MRL/lpr mice, which was more severe in female mice. Moreover, *Lachnospiraceae* is strongly associated with lupus progression in MRL/lpr mice. In contrast, the intestinal colonization of *Lactobacillaceae* was negatively correlated to the lupus activity in mice. Those results suggested that *Lactobacillaceae* may be a probiotic in the treatment of SLE. Another study reported similar results; antibiotic treatment can eliminate harmful microbiota *Lachnospiraceae* and enrich the probiotic *Lactobacillus Spp.*, thereby attenuating lupus (27). On the other hand, many studies have found that leaky gut occurs in lupus-prone mice with impaired intestinal barrier function, resulting in increased microbial translocation, endotoxemia, and lupus progression, which can be reversed after treatment of lupus-prone mice (11, 28, 29, 34). These results suggested that impaired gut barrier function significantly influenced lupus progression.

In addition, *Enterococcus gallinarum*, a specific pathogenic bacterium in (NZW × BXSB) F1 lupus mice, was shown to induce intestinal barrier impairments and translocate to the liver

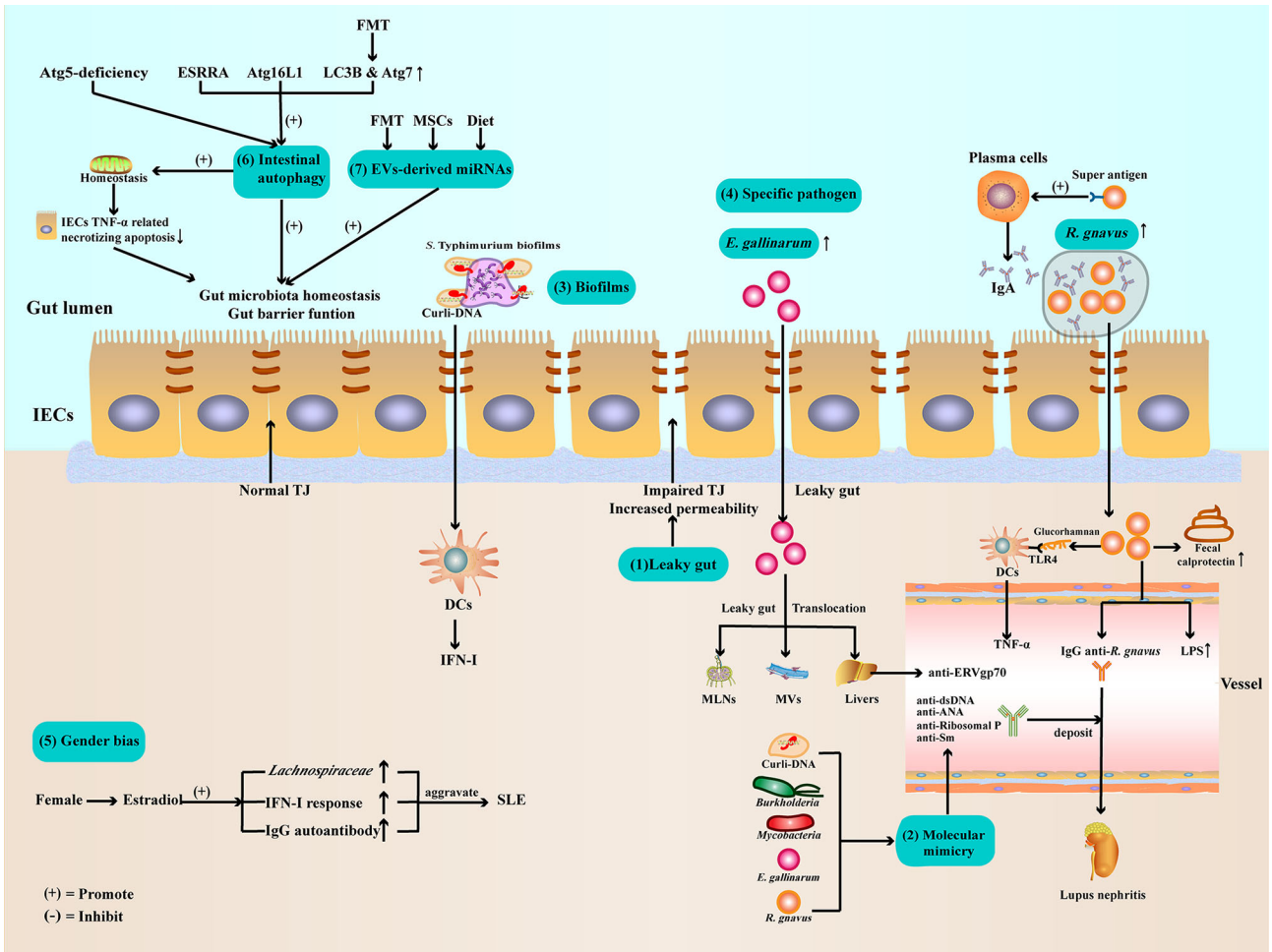

FIGURE 1 | Potential mechanisms of gut microbiota dysbiosis in SLE (1). Gut barrier function impaired and leaky gut allow pathogen leak out of the gut lumen and translocate to other organs (2). Gut microbiota and Curli-DNA of biofilms produce autoantibodies through molecular mimicry, which deposit in kidneys, leading to lupus nephritis (3). Curli-DNA of biofilms activated DCs to secrete pathogenic IFN-I (4). *E. gallinarum* can disrupt *intestinal barrier* function and translocate to MLNs, MVs, and livers. At the same time, *E. gallinarum* promoted systemic autoimmunity by inducing ERV gp70 overexpression in the liver. *R. gnavus* express a B-cell superantigen to stimulate IgA antibodies production and encapsulate itself to facilitate intestinal colonization. Furthermore, *R. gnavus* can produce a glucorhamnan inflammatory polysaccharide that promotes DCs to secrete the inflammatory factor TNF-α *via* TLR4. In addition, *R. gnavus* can disrupt intestinal barrier function, resulting in increased calprotectin levels in stool samples and LPS levels in sera. Subsequently, the impaired intestinal barrier function exposes the intestinal commensal *R. gnavus* antigen, leading to mimicry of the molecule to produce anti-dsDNA autoantibodies, aggravating lupus nephritis (5). Estradiol promotes pathogen like *Lachnospiraceae* colonization, IFN-I response, and IgG autoantibody production (6). Regulate ESRRA, Atg16L1, LC3B, and Atg7 can activate IECs autophagy to maintenance gut microbiota homeostasis and intestinal barrier function (7). Evs-derived miRNAs from FMT, MSCs therapy, or dietary improve gut microbiota balance and enhance intestinal barrier function. ATG, autophagy-related protein; DCs, dendritic cells; *E. gallinarum*, *Enterococcus gallinarum*; ESRRA, estrogen related receptor alpha; FMT, Fecal microbiota transplantation; IECs, intestinal epithelial cells; IFN-I, type I interferon; LC3B, microtubule-associated protein 1 light chain 3B; LPSs, lipopolysaccharides; MLNs, mesenteric lymph nodes; MVs, Mesenteric veins; MSCs, mesenchymal stem cells; *R. gnavus*, *Ruminococcus gnavus*; TJ, tight junction; TLR4, toll-like receptor4; TNF-α, Tumor necrosis factor-α.

to cause autoimmune hepatitis (28). More importantly, *E. gallinarum* was also found in the liver tissues of patients with SLE and autoimmune hepatitis, but not in healthy controls and non-autoimmune hepatitis patients.

Taken together, the above results indicate that intestinal microbiota dysbiosis in an SLE mouse model presents with decreased microbial diversity, increased colonization of harmful bacteria such as *E. gallinarum*, or decreased probiotics. At the same time, impaired intestinal barrier function plays a very important role, which can increase gut microbiota translocation and promote lupus progression.

4 MECHANISMS OF GUT MICROBIOTA DYSBIOSIS IN SLE

Currently, it is unclear whether gut microbiota dysbiosis is the cause or consequence of SLE. Genetic susceptibility is an

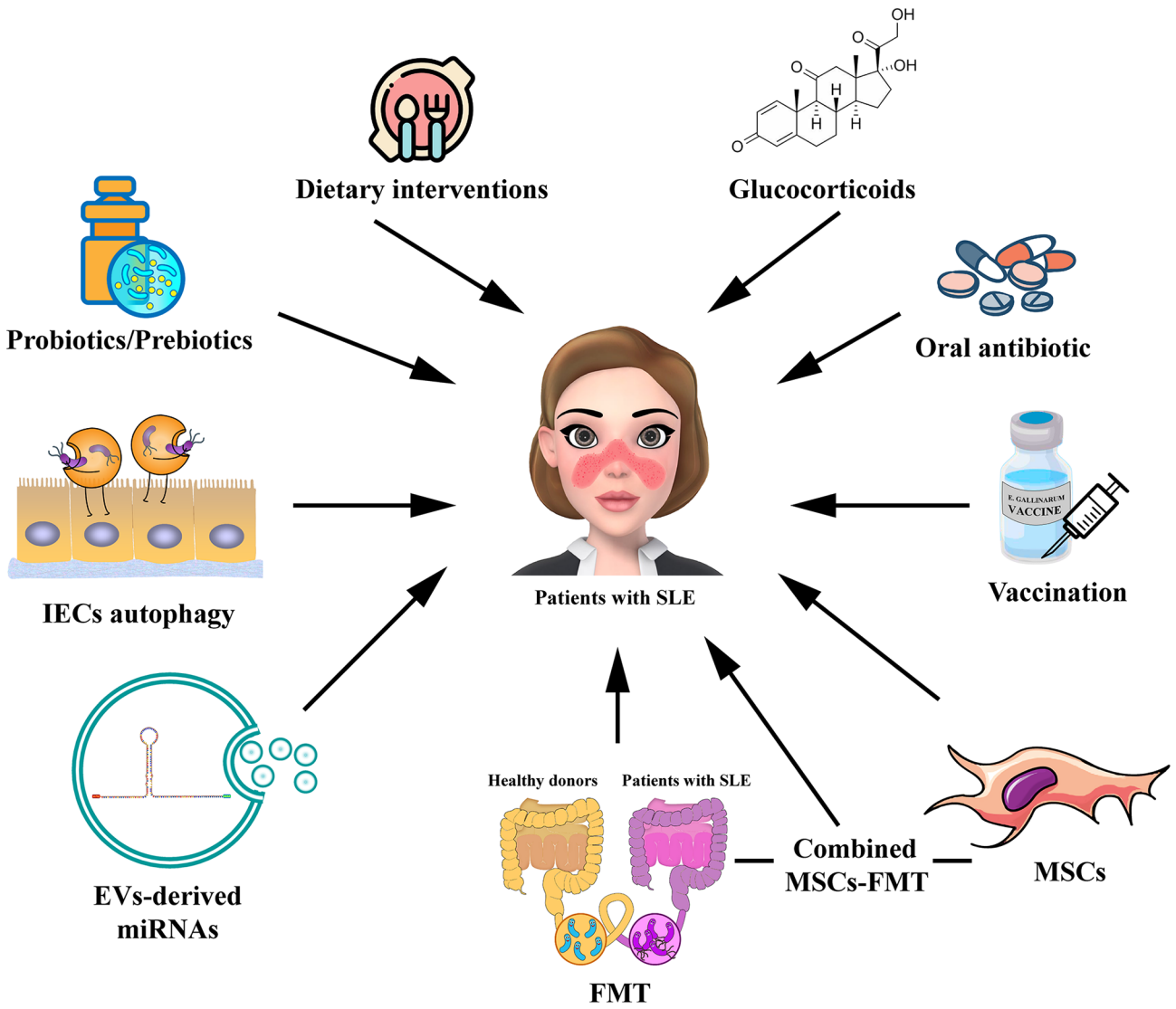

FIGURE 2 | Potential strategies for targeting gut microbiota in the treatment of patients with SLE. The potential therapies for modulating gut microbiota for SLE, including probiotic or prebiotic therapy, dietary interventions, oral antibiotic therapy, GC therapy, vaccination, FMT, regulation of IECs autophagy, EV-derived miRNA therapy, and MSC therapy. The combined MSC-FMT transplantation approach may have a better therapeutic effect for SLE. EV, extracellular vesicle; GC, glucocorticoid; FMT, Fecal microbiota transplantation; MSCs, mesenchymal stem cells.

important factor leading to gut microbiota dysbiosis and autoimmune disease progression in lupus-prone mice (35). However, the data from 1,046 healthy individuals suggested that environmental factors are more important than host genetics in shaping the human gut microbiota (36). In the past decades, the rising incidence of autoimmune diseases has been associated with environmental factors, including a high-salt diet (HSD) (37). Previous studies have shown that HSD could activate DCs and induce the production of pathogenic T Helper 17 (Th17) cells through the p38/MAPK-STAT1 signaling pathway, resulting in gut microbiota dysbiosis, hypertension and autoimmune progression (37–39). Moreover, gut microbiota dysbiosis may induced immune system imbalance and aggravates SLE (40). There are other potential mechanisms underlying for the role of gut microbiota dysbiosis in SLE, which we will elaborate on the following aspects.

4.1 Intestinal Barrier Function and Leaky Gut

At present, the pathogenesis of SLE is still not well known, but growing evidence suggests that the impaired intestinal barrier may be one of the essential factors (41). The intestinal mucosa needs the intestinal barrier function to defend against the invasion of foreign antigens, such as food antigens, bacteria, and toxins (7). As previously mentioned, a leaky gut was observed in patients with SLE and in mice. Calprotectin, a

TABLE 1 | Gut microbiota dysbiosis in patients with SLE.

Study (Year)	Subjects (n)	Gut microbiota in SLE	Role of microbiota	Reference
López, et al. (2016)	SLE (20) HC (20)	**Phyla**: *Firmicutes*, *Synergistetes*↓.	*Firmicutes* was a negative correlation with Th17 cells; *Synergistetes* was a negative correlation with anti-dsDNA antibodies;	(16)
Azzouz, et al. (2019)	SLE (61) HC (17)	**Genus:** *Ruminococcus gnavus*↑.	Anti-*R. gnavus* antibodies were positively correlated with SLEDAI score, anti-dsDNA antibodies and lupus nephritis.	(8)
Bellocchi, et al. (2019)	SLE (27) HC (27)	**Genus**: *Bifidobacterium*, *Ruminiclostridium, Streptococcus* and *Collinsella*↑; *Lachnoclostridium*, *Lachnospira*, and *Sutterella* ↓.	*Streptococcus* has been associated with inflammatory intestinal conditions.	(17)
Li et al. (2019)	SLE (40) HC (20)	**Phyla**: *Firmicutes/Bacteroidetes* ratio↓; **Family**: *Streptococcaceae*, *Lactobacillaceae*↑ **Genus**: *Faecalibacterium*, *Roseburia*↓; *Streptococcus*, *Lactobacillus* and *Megasphaera*↑; **Species**: *F. prausnitzii*↓; *S. anginosus, L. mucosae*↑	*Streptococcus*, *Campylobacter*, *V eillonella*, *anginosus* and *dispar* were positively correlated with lupus activity; *Bifidobacterium* was negatively associated with SLE disease activity.	(18)
Guo, et al. (2020)	SLE (20) HC (20)	**Phyla**: *Firmicutes/Bacteroidetes* ratio↓; *Bacteroidetes*↑; **Genus**: *Dialister*, *Gemmicer*↓	*Dialister and Gemmiger* were a negative association with inflammatory cytokines.	(19)
He, et al. (2020)	SLE (21) HC (10)	**Phyla**: *Bacteroidetes*↓, *Proteobacteria*↑; **Family**: *Ruminococcaceae*↓, *Enterococcaceae*↑; **Genus**: *Clostridia* and *Faecalibacterium*↓, *Escherichia_Shigella*↑.	Most of the bacteria that are negatively correlated with SLEDAI belong to *Firmicutes*.	(20)
Chen, et al. (2021)	SLE (117) HC (115)	**Species**: *Clostridium* sp. *ATCC BAA-442*, *Atopobium rimae*, *Shuttleworthia satelles*, *Actinomyces massiliensis*, *Bacteroides fragilis*, *Clostridium leptum*↑ and	These species were reduced after treatment.	(6)
Wen, et al. (2021)	SLE (33) HC (28)	**Phyla**: *Proteobacteria* **Order**: *Enterobacteriales* **Family**: *Ruminococcaceae*		(21)

TABLE 2 | Gut microbiota dysbiosis in lupus mouse models.

Study (Year)	Mouse model	Gut microbiota in SLE	Intervention	Reference
Zhang, et al. (2014)	MRL/lpr mice *vs.* MRL/MpJ mice; B6/lpr mice *vs.* C56BL/6 mice	**Family**: *Lactobacillaceae*↓; *Lachnospiraceae*↑ *Lachnospiraceae* was associated with lupus severity. *Lactobacillaceae* was negatively correlate with lupus activity.	Retinoic acid as a dietary intervention increased lactobacilli and relieved lupus severity.	(26)
Mu, et al. (2017)	MRL/lpr mice	**Family**: *Lactobacillaceae*↓; *Lachnospiraceae*↑	Oral antibiotics therapy ameliorated lupus in MRL/lpr mice by removing *Lachnospiraceae* and enriching *Lactobacillus spp*	(27)
Manfredo Vieira, et al. (2018)	(NZW x BXSB) F1 mice *vs.* C56BL/6 mice	**Species:** *Enterococcus gallinarum*↑ *E. gallinarum* can induce intestinal barrier impairments and lupus nephritis.	Vancomycin or vaccination therapy can remove *E. gallinarum* and thus ameliorate lupus.	(28)
Mu, et al. (2019)	MRL/lpr mice *vs.* MRL/MpJ mice;	**Species:** *Lactobacillus animalis*↑ (After vancomycin treatment)	Vancomycin treatment increased *L. animalis* and ameliorated lupus symptoms in common MRL/lpr mice, but aggravated lupus in pregnant and postpartum (PP) mice.	(29)
He, et al. (2019)	MRL/lpr mice	**Genus**: *Mucispirillum*, *Oscillospira*, *Bilophila and Rikenella*↓, *Anaerostipes*↑ (After prednisone treatment)	Prednisone treatment decrease *Mucispirillum* and increase *Anaerostipes*. Bromofuranone did not alleviate lupus but enhanced the efficacy of prednisone in the treatment of SLE.	(30)
Zhang, et al. (2020)	MRL/lpr mice *vs.* C57BL/6 mice	**Genus**: *Proteus*, *Klebsiella*, *Bilophila*, *Allobaculum*, *Bifidobacterium* and *Adlercreutzia*↓ (After prednisone treatment)	Short-term and early-stage antibiotic treatment aggravated SLE, while FMT treatment shown to be beneficial. However, short-term premorbid antibiotic treatment or FMT could inhibit the therapeutic effect of prednisone on lupus in MRL/lpr mice aged 9 to 13 weeks.	(31)
de la Visitación, et al. (2021)	NZBWF1 mice *vs.* NZW/LacJ mice	**Phyla**: *Verrucomicrobia*, *Proteobacteria*, *Bacteroidetes* and *Proteobacteria*↑; *Firmicutes*↓; **Genus**: *Parabacteroides*, *Pedobacter*, *Olivibacter* and *Clostridium*↑	Antibiotic treatments restored the composition of gut microbiota, and inhibited the increment of blood pressure, renal injury and disease activity in lupus-prone mice.	(32)
Wang, et al. (2021)	MRL/lpr mice	**Genus**: *Ruminococcus, Alistipes*↓; *Lactobacillus*↑ (After prednisone treatment)	The effects of prednisone on gut microbiota were dose-dependent in the treatment of MRL/lpr mice.	(33)

calcium-containing protein from neutrophils and macrophages, is a well-recognized biomarker of impaired intestinal barrier function (42). Calprotectin levels were significantly increased in stool samples from patients with SLE, indicating impaired intestinal barrier function (8, 28). At the same time, serum soluble CD14, α1-acid glycoprotein, and lipopolysaccharides (LPSs) levels were increased in patients with SLE, indicating the presence of intestinal bacterial translocation (8).

Interestingly, Thim-Uam et al. (43) used dextran sulfate solution to induce a leaky gut in FcgRIIb$^{-/-}$ lupus mice and pristane-induced lupus mice. They found that the leaky gut aggravated the progression and disease activity of these two murine models of lupus. Leaky gut increases the intestinal translocation of endotoxins or other organic molecules, thereby promoting apoptosis. Most notably, leaky gut promotes the production of anti-dsDNA autoantibodies and immune complex deposition, ultimately leading to lupus exacerbation. Recently, another study indicated that impaired intestinal barrier function is associated with intestinal oxidative stress in MRL/lpr lupus mice (44). This result further complements the mechanism involved in the development of the leaky gut in lupus mice. In addition, impaired gut barrier function and lupus were significantly ameliorated after treatment with antibiotics, probiotics, or dietary interventions in lupus mice (27, 34, 45).

Taken together, these results suggest that impaired intestinal barrier function is associated with SLE disease severity. Both patients with SLE and mice have varying degrees of impaired intestinal barrier function and a leaky gut. Mechanistically, impaired intestinal barrier function allows symbiotic bacteria or their contents to leak out of the intestinal lumen, which may be related to intestinal oxidative stress. Translocated gut bacteria or bacterial components can promote the production of autoantibodies through molecular mimicry. Finally, the deposition of immune complexes aggravates SLE progression.

4.2 Molecular Mimicry

Molecular mimicry is another critical condition that leads to the development of autoimmunity (6, 9, 46). Molecular mimicry means that certain structures of a microorganism are similar to the self-structures of the host, which causes an autoimmune response and tissue damage (47). Therefore, certain bacteria with epitope structures similar to self-antigens can stimulate patients with SLE to produce cross-reactive autoantibodies. Zhang et al. (9) found that *Burkholderia* bacterial partial purified antigen and transcriptional regulatory peptide RAGTDEGFG could bind to dsDNA antibodies in sera from patients with SLE (9). These results suggest that the production of anti-dsDNA antibodies in patients with SLE is associated with *Burkholderia* bacterial molecular mimicry. Interestingly, another study found that glycolipids of the mycobacterial cell wall can bind to anti-dsDNA autoantibodies derived from patients with SLE and mice (10). Thus, the production of autoantibodies can result from the molecular mimicry caused by different bacterial infections in SLE. Recently, it has been shown that peptides produced by *Odoribacter splanchnicus* and *Akkermansia muciniphila* bacteria are highly similar to Sm antigen and Fas antigen epitopes (6). More importantly, peptides from these bacteria can activate CD4$^+$ T cells or B cells to produce autoantibodies (6). However, these results were limited *in vitro* experiments, *in vivo* experiments need to be designed to confirm these standpoints. In another study, molecular mimicry of commensal or environmental microbes was shown to promote autoantibody production in SLE, which was driven by T cells and HLA-DR restriction (48).

Molecular mimicry has also been associated to the pathogenesis of other autoimmune diseases, such as antiphospholipid syndrome (APS) (49). APS is an autoimmune disease characterized by anti-β2-glycoprotein I (β2GPI) autoantibodies production (50), which can be secondary to SLE (51). On the other hand, the intestinal commensal *Roseburia intestinalis* (*R. int*) mimotope cross-react with β2GPI-reactive memory CD4$^+$ Th1 cells and produce anti-*R. int* autoantibodies in patients with APS (49). And oral gavage with *R. int* in BALB/c mice induced anti-human β2GPI autoantibodies and APS-associated autoimmune pathologies (49). Therefore, *R. int* promotes anti-β2GPI autoantibodies production and contributes to APS pathogenesis. In addition, aPL also targeted to β2GPI in SLE (52). Thus, the intestinal commensal *R. int* may be related to the pathogenesis of SLE, but further studies will be required.

In summary, bacterial molecular mimicry is an important factor in the pathogenesis of autoimmune diseases, including SLE and APS. Different bacteria can promote autoantibody production through molecular mimicry. T and B cells are involved in the bacterial molecular mimicry process; however, the precise mechanism remains unclear.

4.3 The Pathogenic Role of Bacterial Biofilms

Biofilms are considered to be a membrane in which the bacterial community produces an extracellular matrix and wraps itself (53), which can protect bacteria from the host immune response (54) and enable bacteria to develop drug resistance (55). The main structure of biofilms is amyloid protein rich in β-folding, which is associated with human autoimmune diseases (56–58). Curli fibrils in *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) amyloid could combined to the DNA in bacterial, and these complexes could promote biofilm formation, also contributing to SLE pathogenesis (57). In pre-lupus NZBxW/F1 mice, curli-DNA complexes activated the innate immune cells such as dendritic cells (DCs) to secrete pathogenic type I interferons (IFNs). NZBxW/F1 lupus-prone mice rapidly developed anti-dsDNA and ANA autoantibodies after intraperitoneal injection of curli-DNA complexes at six weeks of age, whereas injection of BSA did not show the same effect. Most importantly, normal control C57BL/6 mice also developed anti-dsDNA and ANA autoantibodies two weeks after intraperitoneal injection of curli-DNA complexes at six weeks of age. In addition, curli-DNA complexes promoted the proliferation of activated T cells, activated B cells, and inflammatory monocytes. Finally, infection with curli biofilm of *S.* Typhimurium promoted autoantibody production in lupus mice (57). These results suggest that curli-DNA complexes of bacterial biofilms not only promote the production of

autoantibodies in lupus-prone mice, but also disrupt self-tolerance in non-autoimmune mice, causing lupus pathogenesis. This has also been shown in another study that curli-bacterial DNA complexes of urinary tract infections in patients with SLE cross-reacted with lupus autoantigens such as dsDNA (59). The above two studies suggest that bacterial proteins that interact with DNA may cause loss of immune tolerance to autoantigens and induce the production of autoantibodies, leading to SLE pathogenesis. Interestingly, a recent study by Fu et al. (58) suggested that DNABII proteins interacting with DNA in biofilms may not directly contribute to anti-dsDNA production but other mechanisms may be involved. Sera from patients with SLE specifically recognize the DNAB II protein-derived HU1 peptide in bacterial biofilms. Anti-HU1 aggravates the progression of lupus nephritis (LN) in patients with SLE and a pristane-induced lupus murine model. Although anti-HU1 antibodies can inhibit biofilm formation by *Staphylococcus aureus*, it is accompanied by cross-reactivity with the autoantigen P4HB on the glomerular cell membrane to induce LN (58).

In conclusion, certain components in bacterial biofilms such as curli and curli-DNA complexes can cross-react with autoantigens and induce the production of autoantibodies, resulting in SLE pathogenesis or disease aggravation.

4.4 Intestinal Specific Pathogens Infection

Intestinal infections with specific pathogens have been reported to be associated with the onset and progression of SLE. It is of great significance to study the mechanism of action of these specific pathogens in SLE.

4.4.1 Enterococcus gallinarum

Enterococcus gallinarum (*E. gallinarum*) is a human intestinal commensal bacterium that can invade the blood to induce sepsis when the immunity of the organism is low (60). Interestingly, Vieira et al. (28) observed that *E. gallinarum* plays an important role in the pathogenesis of SLE. Pathogenic *E. gallinarum* disrupted intestinal barrier function and promoted Th17 and Tfh cell proliferation in (NZW × BXSB) F1 lupus mice. Subsequently, the damaged intestinal barrier promoted translocation of *E. gallinarum* to mesenteric lymph nodes, mesenteric veins, and liver. At the same time, *E. gallinarum* promoted systemic autoimmunity by inducing ERV gp70 overexpression in the liver (28). Thus, *E. gallinarum* is a pathogenic bacterium that is closely related to the pathogenesis of SLE in (NZW × BXSB) F1 lupus mice. Surprisingly, *E. gallinarum* was detected in liver biopsies from patients with SLE and autoimmune hepatitis, but not in healthy controls and non-autoimmune hepatitis patients. This suggests that *E. gallinarum* of lupus mice were also present in patients with SLE; most importantly, after inoculation with the *E. gallinarum* vaccine, serum autoantibody levels were reduced, the survival time was prolonged, and bacterial translocation was inhibited in (NZW × BXSB) F1 mice (28). Therefore, pathogen-specific therapy can suppress host autoimmune processes without the use of immunosuppressants. More recently, another study showed that *E. gallinarum* is associated with autoimmune responses to autoantibodies such as anti-Ribosomal P, anti-dsDNA, and anti-Sm in patients with SLE (61). This study further confirmed that *E. gallinarum* is a specific pathogen of SLE-susceptible individuals. However, this study did not prove that whether *E. gallinarum* acts as the same role in other lupus mouse models and patients with SLE.

Taken together, the pathogenic bacteria *E. gallinarum* can be translocated into systemic organs by disrupting the intestinal barrier, which leads to SLE pathogenesis. Translocated *E. gallinarum* promotes Th17 and Tfh cell proliferation and autoantibody production. At the same time, *E. gallinarum* may also directly induce autoantigens, ERV proteins, and other substances to promote autoimmune processes.

4.4.2 Ruminococcus gnavus

As previously mentioned, *Ruminococcus gnavus* (*R. gnavus*) plays an important role in SLE (6, 8). Studies have shown that *R. gnavus* expresses a B-cell superantigen that stimulates the gut of mice to produce large amounts of plasma cells that secrete IgA antibodies (62) These IgA antibodies recognize and highly encapsulate *R. gnavus*, which may be associated with intestinal colonization of *R. gnavus*. Furthermore, *R. gnavus* can produce a glucorhamnan inflammatory polysaccharide that promotes dendritic cells to secrete the inflammatory factor Tumor necrosis factor-α (TNF-α) *via* toll-like receptor4 (TLR4) (63). A recent study has shown that some isolated strains of *R. gnavus* could produce capsular polysaccharides that promote the immune tolerance of *R. gnavus*. However, *R. gnavus* isolates without capsular polysaccharide produced a strong pro-inflammatory response and increased intestinal inflammatory indicators in sterile mice (64).

Interestingly, Azzouz et al. found that sIgA-coated *R. gnavus* increased in stool samples from patients with SLE, and the proliferation of *R. gnavus* was proportional to SLE disease activity (8). Thus, aberrant superantigen expression of *R. gnavus* may facilitate intestinal colonization of *R. gnavus*, thereby aggravating SLE progression. In addition, *R. gnavus* can disrupt intestinal barrier function, resulting in increased levels of calprotectin in stool samples and lipopolysaccharides (LPSs) in sera. Subsequently, the impaired intestinal barrier function exposes the intestinal commensal *R. gnavus* antigen, leading to mimicry of the molecule to produce anti-dsDNA autoantibodies, aggravating lupus (8).

In summary, *R. gnavus* may affect disease progression in SLE, but the causal relationship remains unresolved.

4.5 Gender Bias

Generally, SLE shows a strong female bias with a male-to-female ratio of 9:1 (65). In fact, there was also a gender bias in the intestinal microbiota in SLE. For example, over-colonization of *Lachnospiraceae* in the intestinal tract of female MRL/lpr lupus mice was associated with early onset or exacerbation of lupus, but not in male mice (26). Another study showed an increase in *Lachnospiraceae* and exacerbation of lupus in the gut microbiota of MRL/lpr mice after administering a phytoestrogen-supplemented diet (66). These results suggest that estrogen may account for gender bias in gut microbiota dysbiosis in

SLE, but the underlying mechanism remains to be clarified. Moreover, estradiol exacerbates SLE disease severity by promoting type I interferon responses and IgG autoantibody production from B cells (67, 68). Abnormal modification of steroid receptors in T cells may alter the expression of estrogen receptor (ERα), thereby promoting the effect of estrogen (65). In contrast, testosterone is generally considered to be a beneficial sex hormone that inhibits B-cell activation and autoantibody production to alleviate LN (69). On the other hand, Mu et al. found that *Lactobacillus* treatment ameliorated lupus nephritis, increased IL-10, and decreased luteinizing hormone in female and emasculated male MRL/lpr mice, but not in intact male mice (11). These results suggest that *Lactobacillus* treatment ameliorates LN in MRL/lpr mice in a sex hormone-dependent manner. In addition, antibiotic treatment has been shown to inhibit SLE progression in lupus-prone (SWR × NZB) F1 female mice, but not in male mice. Orchiectomy alters the composition of the gut microbiota and promotes autoimmune progression in male mice (70).

In conclusion, estrogen can alter gut microbiota and promote type I interferon response and autoantibody production to aggravate SLE progression; conversely, androgen plays a protective role.

4.6 Intestinal Epithelial Cells Autophagy

At present, the relationship between autophagy and intestinal bacteria in SLE has not been reported. However, in another autoimmune disease, inflammatory bowel disease (IBD), autophagy is crucial for the homeostasis of intestinal bacteria and intestinal barrier function. On the one hand, autophagy may be beneficial to gut barrier function. The autophagic protein Atg16L1 prevents necrotizing apoptosis mediated by TNF-α in intestinal epithelial cells (IECs) by promoting mitochondrial homeostasis (71). Autophagy can also reduce epithelial permeability by inducing lysosomal degradation of the pore-forming tight junction protein claudin-2, thus enhancing intestinal barrier function (72). On the other hand, IECs autophagy plays a crucial role in regulating the diversity and composition of the gut microbiota. For example, the estrogen-associated receptor alpha (ESRRA) protects the host from mitochondrial dysfunction by activating autophagy and maintaining intestinal microbiota homeostasis, thereby attenuating intestinal inflammation (73). IECs-specific knockout of autophagy-associated gene 5 (Atg5) resulted in significant changes and decreased diversity of gut microbiota in mice. In Atg5-deficient mice, the abundance of inflammation-inhibiting *Akkermansia muciniphila* decreased, but the abundance of pro-inflammatory *Candidatus* Athromitus and potentially pathogenic *Pasteurellaceae* increased (74). In addition, fecal microbiota transplantation could increase the expression of LC3B and Atg7 to activate intestinal mucosal autophagy, thereby improving intestinal barrier function in piglets (75). These studies suggest that autophagy of host intestinal mucosal cells may affect the gut microbiota to ameliorate intestinal injury.

The previous discussion indicates that dysregulation of gut microbiota and impaired intestinal barrier function can lead to aggravated SLE progression. IECs autophagy contributes to the maintenance of gut microbiota homeostasis and intestinal barrier function. A review by Bhattacharya et al. (76) indicated that exploring the mechanism of the interaction between autophagy and gut microbiota is beneficial for the study of autoimmune diseases. Therefore, we hypothesized that IECs autophagy is closely related to the dysregulation of gut microbiota in SLE and affects the progression of SLE. However, further studies are required to confirm our findings.

4.7 Extracellular Vesicle and miRNA

Extracellular vesicles (EVs) are a group of membrane-enclosed nanoscale vesicles that carry various RNA, DNA, proteins, and lipids and transmit information between cells (77). Exosomes are EVs ranging in diameter from approximately 40 to 160 nm that carry miRNAs and other non-coding RNAs with the potential for diagnosis and treatment of diseases (78). miRNAs are single-stranded non-coding RNA molecules of approximately 22 nucleotides in length that play important roles in regulating gene expression and biological function (79). In recent years, studies have shown that EV-derived miRNA expression is related to gut microbiota and intestinal barrier function (80–82). Mice deficient in IECs miRNA showed intestinal dysbiosis and exacerbation of colitis, which ameliorated after transplantation with fecal EV-derived miRNA from wild-type mice (80). This study suggests that fecal EV-derived miRNAs can regulate the gut microbiota and ameliorate the progression of intestinal inflammation. Another study found that EV-derived miRNAs of dietary ginger can induce IL-22 production to improve intestinal barrier function and thus ameliorate intestinal inflammation (83). A recent study showed that exosome miR-181a derived from MSCs alleviated colitis by improving gut microbiota imbalance and intestinal barrier function and reducing pro-inflammatory factor secretion (82). Taken together, the above results suggest that some EV-derived miRNAs in the intestinal tract may inhibit the progression of SLE by improving gut microbiota homeostasis and intestinal barrier function. More studies are required to confirm this hypothesis.

5 POTENTIAL THERAPY FOR SLE: MODULATING GUT MICROBIOTA

At present, the study of intestinal bacteria intervention in the treatment of SLE is still in its infancy, but it can learn from other dysbacteriosis-associated diseases and predict future regimens in the treatment of SLE. As described in a recent review (40), probiotics/prebiotic therapy are currently practical approaches for ameliorating intestinal dysbacteriosis to treat SLE. Probiotics and prebiotics can induce differentiation of Treg cells, improve Th17/Th1 imbalance, and reduce the production of autoantibodies, thereby reducing the severity of lupus (40). Nevertheless, the efficacy of probiotics/prebiotic in the treatment of SLE remains unclear and has not been confirmed by clinical trials. There are differences in phenotypic

manifestations caused by gut microbiota in SLE. To illustrate, intestinal commensal *E. gallinarum* can translocation to the liver and cause autoimmune hepatitis in patients with SLE (28). *R. gnavus* could increase serum anti-dsDNA antibody and LPS levels (8). The curli-DNA complex of biofilms containing *S.* Typhimurium promoted lupus progression. These differences may influence the approaches to targeting gut microbiota for SLE. These differences may have an impact on choosing the most appropriate modulation method of gut microbiota. Next, we discuss several options for the intervention of gut microbiota in the treatment of SLE.

5.1 Dietary Intervention

Dietary intervention may regulate the imbalance of gut microbiota, and thus ameliorate SLE progression. The alteration of the pH value of drinking water could beneficially influence on gut microbiota composition and disease progression in SWR×NZB F1(SNF1) lupus mice (84). Dietary retinoic acids supplementation could upregulate lactobacilli and ameliorate lupus in MRL/lpr mice (26). However, the efficacy of dietary retinoic acids in SLE treatment remains controversial (45) and still needs further study.

Also, the high-salt diet plays an important role in the pathogenesis of gut microbiota dysbiosis in autoimmune diseases. Interestingly, a recent randomized controlled trial demonstrated that a low-salt diet increased circulating SCFAs and decreased blood pressures by affecting the gut microbiota in humans (85). Therefore, reducing dietary salt intake or targeting salt-sensitive associated protein may be a new therapeutic strategy for SLE treatment. But this strategy still needs to be confirmed by further studies.

In addition, celiac disease (CeD) is an autoimmune enteropathy that is proposed to be associated with SLE (86, 87). An analysis of 29,000 patients with biopsy-confirmed CeD found that patients with CeD had a three-fold increased risk of developing SLE compared with healthy controls (86). In contrast, a large case-control study involving 5018 patients with SLE reported a significantly higher prevalence of CeD in patients with SLE compared with matched controls (87). And gluten, the major protein of wheat grains, is one of the factors contributed to the coexistence of SLE and CeD (87). The gliadin polypeptide of gluten increased intestinal permeability and activated $CD4^+$ T cells resulted in CeD (88, 89). Therefore, gluten may be one of the causes of impaired intestinal barrier function in patients with SLE. Currently, the gold standard treatment for CeD is a strict and life-long gluten-free diet (GFD) (90). However, the implementation of GFD is limited by high cost, decreased quality of life of patients and complex pathogenesis (90). GFD may contribute to improve gut barrier function but still requires additional study.

Altogether, dietary intervention may be an important and new therapy in SLE.

5.2 Oral Antibiotic Therapy

In recent years, many studies have attempted to use antibiotics to treat lupus mice. For example, treatment with broad-spectrum antibiotics or vancomycin after onset in lupus MRL/lpr mice removes harmful bacteria from the gut, enriches probiotics, and restores gut barrier function, thereby ameliorating lupus (27). Moreover, antibiotic treatment alleviates Treg/Th17 imbalance in lupus mice (27) and inhibits the high blood pressure caused by Th17 cell infiltration (32). Vieira et al. (28) found that vancomycin treatment of NZB/WF1 lupus mice cleared *E. gallinarum*, a specific pathogen in the intestine, improved intestinal barrier function, and delayed lupus progression. However, another study showed that treatment with antibiotics has no significant effect on both the gut microbiota and SLE progression in NZB/WF1 lupus mice, the mechanism of which is unclear (91). Similarly, Zhang et al. showed that antibiotic treatment exacerbated the disease in MRL/lpr mice, possibly due to a short course and insufficient dose of antibiotics before lupus onset (31). Alternatively, another study found that vancomycin treatment ameliorated lupus symptoms in common MRL/lpr mice, but aggravated lupus in pregnant and postpartum (PP) mice. Mechanistically, vancomycin treatment aggravates LN in PP mice by downregulating the expression of Treg cells through inhibition of IDO and upregulation of IFN-γ (29).

In conclusion, the antibiotic therapy regimen for SLE is controversial. In general, antibiotic treatment decreases pathogenic bacteria, enriches probiotics, and ameliorates intestinal leakage in lupus mice, thereby inhibiting lupus progression. However, antibiotics may also exacerbate lupus severity in premorbid and pregnant or lactating mice. Moreover, there are some limitations in the routine use of antibiotics to treat patients with SLE. Because antibiotic treatment may inhibit the therapeutic effect of prednisone on lupus in MRL/lpr mice (31), while prednisone is a common drug for patients with SLE in clinical practice. Furthermore, antibiotic abuse may lead to drug-resistant bacterial infection (92), which is an important cause of death in patients with SLE (93). Therefore, the use of antibiotics in the treatment of SLE needs to be further studied to specifically remove pathogenic bacteria without causing gut microbiota disorders as much as possible.

5.3 Fecal Microbiota Transplantation

Fecal microbiota transplantation (FMT) is defined as the transplantation of bacteria from the feces of healthy donors into the patient's intestine to restore microecology homeostasis and thus treat diseases associated with gut microbiota imbalance (94). In 2013, FMT was included in the official therapeutic guidelines for *Clostridium difficile* infection (CDI) (95). In recent years, studies have shown that FMT is effective in the treatment of SLE mouse models (31, 33, 84). An acidic water diet can restore the balance of gut microbiota in lupus mice, and that this repaired gut microbiota can be used for FMT to treat control lupus mice [25703185]. In addition, short-term antibiotic treatment of early-stage MRL/lpr lupus mice promoted SLE progression, and the disease severity in these mice was reduced after FMT treatment in the following week. However, short-term premorbid antibiotic treatment or FMT could inhibit the therapeutic effect of prednisone on lupus in MRL/lpr mice aged 9 to 13 weeks (31). This study suggests that performing FMT early in the onset of lupus suppresses the progression of lupus, but, at the same time, affects the therapeutic effect of

glucocorticoid therapy. If patients with SLE are routinely treated with glucocorticoids, treatment with FMT should be carefully considered. More recently, a study found that untreated lupus MRL/lpr mice transplanted with fecal microbiota from prednisone-treated mice experienced lupus attenuated without the side effects of prednisone-treated mice (33). These results suggest that FMT may be an effective SLE therapy to avoid adverse glucocorticoid reactions. The effect of the interaction between FMT and glucocorticoid therapy on the progression of SLE requires further study. FMT clinical trials have been studied for other autoimmune diseases, such as ulcer colitis and type 1 diabetes, and some efficacy has been achieved (96, 97). Therefore, clinical trials of FMT in patients with SLE are promising, but further studies are needed.

A recent article reported that a patient succumbed to infection due to drug-resistant *E. coli* bacteria in donor stool samples (98). Therefore, donor screening must be improved to prevent transmission of microorganisms leading to infectious events. In conclusion, the benefits and risks of FMT in the treatment of SLE need to be assessed, and how to apply it in clinical practice still needs further study.

5.4 Glucocorticoid Therapy

Glucocorticoids (GCs) are steroids that can bind and activate the cytosolic glucocorticoid receptors (GRs) to exert an anti-inflammatory effect (99). GCs have become one of the main traditional drugs for SLE due to their rapid and potent anti-inflammatory effects, low cost, and easy availability. Moreover, long-term high-dose GC treatment regimens are accompanied by an increase in side effects and infections (100). Enhancing the efficacy of GCs and reducing their side effects in patients with SLE is a challenge. In recent years, studies have shown that the efficacy of GCs in the treatment of SLE is related to changes in the gut microbiota. For example, NZB/W F1 mice treated with dexamethasone had increased diversity of intestinal bacteria and a significant reduction in a certain *Lactobacillus* species associated with lupus progression (101). In another study, prednisone treatment caused alterations in the gut microbiota, including a decrease in *Mucispirillum* and an increase in *Anaerostipes*, which were inversely associated with disease activity in SLE. Bromofuranone did not alleviate lupus but enhanced the efficacy of prednisone in the treatment of SLE (30). As previously mentioned, Wang et al. demonstrated that prednisone ameliorates gut microbiota dysbiosis in SLE mouse models, and FMT treatment of SLE may prevent glucocorticoid adverse reactions (33). Moreover, GCs treatment restored the gut *Firmicutes*/*Bacteroidetes* ratio and increased the abundance of probiotics such as *Lactobacillus* and *Bifidobacterium* in patients with SLE (19).

All those studies suggest that intestinal dysbacteriosis may be a target for GCs in the treatment of SLE, but the mechanism remains unclear. A comparative study indicated that an increased levels of *Lactobacillus* in patients with SLE under GCs treatment (19). Moreover, *Lactobacillus* contributes to the alleviation of lupus severity by upregulating $Foxp3^+$ regulatory T (Treg) cells (102). Treg cells are indispensable GC target cells *in vivo* (103). And GCs directly act on GRs in Treg cells and regulate miR-342-3p dependent metabolic programming to exert therapeutic effects (103). Therefore, *Lactobacillus* may affect the therapeutic efficacy of GCs by promoting the proliferation of Treg cells.

Overall, intestinal dysbacteriosis is one of the targets of GCs in the treatment of SLE. Certain drugs such as bromofuranone are associated with enhancing the therapeutic effects of GCs, and FMT may be an effective treatment regimen to reduce the side effects of GCs. Certain intestinal bacteria such as *Lactobacillus* may affected the therapeutic effect of GCs by regulating Treg cells. However, the specific mechanism by which GCs modulate intestinal bacteria in the treatment of SLE needs to be elucidated in future studies.

5.5 Regulate IECs Autophagy and EV-Derived miRNA Therapy

Autophagy is crucial for maintaining the homeostasis of gut microbiota and intestinal barrier function (71, 72). Therefore, regulating IECs autophagy may help improve the gut microbiota balance for the treatment of SLE. First, some drugs can improve gut microbiota composition and intestinal barrier function by promoting IECs autophagy, thus reducing intestinal inflammation and inhibiting autoimmune (104–106). For example, rapamycin can inhibit the progression of multiple sclerosis by promoting IECs autophagy and restoring intestinal microbiota balance (107). Galangin increases the expression of autophagy-related proteins and promotes the formation of colonic autophagy, increases the richness of intestinal probiotics, and reduces intestinal inflammation (108). Second, FMT can increase the expression of intestinal mucosal autophagy-related proteins and reduce intestinal permeability in piglets (75).

In contrast, EV-derived miRNAs may treat SLE by modulating the gut microbiota. A recent review reported that food-derived miRNAs could regulate the composition of gut microbiota and enhance intestinal barrier function, which is beneficial to human health (109). For instance, dietary ginger-derived miRNAs can induce IL-22 production to improve intestinal barrier function and thus ameliorate intestinal inflammation (83). Furthermore, MSC-derived exosome miR-181a can alleviate colitis by improving gut microbiota imbalance and intestinal barrier function and reducing pro-inflammatory factor secretion (82). Mice deficient in IECs miRNA showed intestinal dysbiosis and exacerbation of colitis, which ameliorated after transplantation with fecal EV-derived miRNA from wild-type mice (80).

In summary, IECs autophagy and EV-derived miRNAs can restore gut microbiota balance and intestinal barrier function, thereby inhibiting autoimmune-related intestinal inflammation. Therefore, we believe that regulating autophagy and EV-derived miRNAs is a promising therapeutic option for SLE.

5.6 Mesenchymal Stem Cell Therapy

MSCs are stromal cells with self-renewal and multi-lineage differentiation potential that can be obtained from tissues such as bone marrow (110). With low immunogenicity and strong immunomodulatory effects (111), MSCs can be used to treat SLE (112, 113). Allogeneic MSC transplantation ameliorates clinical symptoms, decreases SLEDAI score, and ameliorates LN in patients with refractory SLE (113, 114). Moreover, studies have shown that MSCs can regulate gut microbiota, increase insulin-

like growth factor-1 (IGF-1), promote intestinal healing, and ameliorate the mouse model of IBD (115, 116). In contrast, miRNA-181a of MSC-derived exosomes can attenuate intestinal inflammation in mouse models of colitis by improving the composition of gut microbiota and restoring barrier function. These studies suggest that MSCs or MSC-derived exosomes can improve gut microbiota dysbiosis and intestinal barrier function and ameliorate intestinal inflammation (82).

Recently, it has been shown that human umbilical mesenchymal stem cells (hUC-MSCs) treat rheumatoid arthritis (RA) by regulating the interaction between gut microbiota and host immunity through the aryl hydrocarbon receptor (AhR) (117). Thus, we hypothesized that MSCs can inhibit SLE progression by ameliorating gut microbiota dysbiosis and intestinal barrier function. However, the specific mechanism of action of MSCs in the treatment of SLE is unknown, and the therapeutic effects of clinical trials remain controversial. In a clinical trial, hUC-MSCs did not have a positive therapeutic effect in patients with severe LN compared with placebo control (118). Meanwhile, a review suggested that the immunomodulatory effects of MSCs depend on the inflammation status, and that MSCs can both suppress and promote immune responses (119). Moreover, gut microbiota dysbiosis may inhibit the therapeutic effect of MSCs. For example, chronic hypoxia has been found to lead to intestinal dysbiosis and promote senescence of bone marrow MSCs (120). Another study demonstrated that intestinal dysbacteriosis might inhibit the therapeutic effect of MSCs in diabetic mice, while modulation of intestinal bacteria may help to enhance the therapeutic effect of MSC transplantation (121). These results suggest that intestinal bacteria can affect the immunomodulatory effects of MSCs, which may be one of the reasons for the poor efficacy of some MSCs in the treatment of SLE. In addition, Ocansey's review (122) suggested that there would be a higher clinical remission rate in patients with IBD treated with the combined MSC-FMT transplantation approach compared with MSC transplantation alone or FMT transplantation. Similarly, we believe that the combined MSC-FMT transplantation approach will have a better therapeutic effect in the treatment of SLE.

Taken together, the study of MSCs in the treatment of SLE has fallen into a bottleneck, and gut microbiota will be a very promising direction for future research.

5.7 Vaccination

To prevent infection, the EULAR guidelines recommend vaccinations such as pneumococcal vaccines (PCV13) for patients with SLE during inactive periods (123). Vaccination of MRL/lpr mice with PCV13 ameliorated lupus severity (124). As previously described, SLE mouse models may suffer from infection with specific intestinal pathogens, such as *E. gallinarum* and *R. gnavus* (8, 28). The development of vaccines against these specific pathogenic bacteria could contribute to the treatment of SLE. Accordingly, Vieira et al. (28) demonstrated that after inoculation of *E. gallinarum* vaccine in (NZW × BXSB) F1 lupus mice, intestinal barrier function was restored and SLE was alleviated. This study shows that specific targeted therapy for intestinal pathogens can inhibit host autoimmune progression independent of other drugs. Importantly, *E. gallinarum* was also detected in the gut and liver of patients with SLE (28). This illustrates that *the E. gallinarum* vaccine is very promising for the treatment of patients with SLE, but further studies are still needed.

At present, research on intestinal microbiota vaccines is still in the preliminary stage, but there is no doubt that targeted vaccine therapy of specific intestinal pathogens is a very promising treatment for SLE.

6 FUTURE PERSPECTIVES

Gut microbiota dysbiosis is closely related to the occurrence and development of SLE. The interaction of factors such as impaired intestinal barrier function, molecular mimicry, biofilms, specific pathogens, and sex hormones can disrupt gut microbiota balance and aggravate SLE. In addition, we suggest that IECs autophagy and EV-derived miRNAs may also affect progression in SLE by regulating the gut microbiota.

Traditionally, it is difficult to treat SLE due to its heterogeneity and complex pathogenesis, while targeting intestinal bacteria may be a breakthrough. In previous studies, probiotics or prebiotic modulation of intestinal bacteria have shown some efficacy in the treatment of SLE, but it is still controversial and has not been confirmed in clinical trials. Dietary interventions such as oral retinoic acids, low-salt diets and gluten-free diets may be beneficial in the treatment of SLE, but further research is needed. Oral antibiotics have some efficacy but may lead to more severe intestinal dysbacteriosis or the development of drug-resistant bacteria. Vaccination against gut pathogenic bacteria suppresses lupus progression in (NZW × BXSB) F1 mice without antibiotic-related side effects, but it is not yet available to treat all lupus mouse and patients. Notably, FMT significantly ameliorates disease in lupus mice by restoring the intestinal bacterial balance and intestinal barrier function. Clinical trials of FMT in patients with SLE are promising; however, donor stool screening must be improved to prevent infectious events.

We also propose new insights into the regulation of gut microbiota in SLE, including GCs, autophagy, EV-derived miRNAs, and MSC therapy. First, GCs, which are commonly used in SLE, can ameliorate intestinal dysbacteriosis but have side effects. The regulation of gut microbiota may help enhance the efficacy of GCs in the treatment of SLE and prevent side effects. Second, regulating autophagy and EV-derived miRNAs may treat SLE by regulating the gut microbiota. Finally, promising results have been achieved for the use of MSCs in patients with refractory SLE in current clinical trials. However, disturbed gut microbiota may inhibit the therapeutic effects of MSCs. In contrast, MSCs can ameliorate intestinal dysbacteriosis, restore intestinal barrier function, and inhibit autoimmune progression. In addition, MSC-derived EVs could ameliorate RA in rats by modulating the gut microbiota. Therefore, the gut microbiota may be a target for MSCs in the treatment of SLE. Moreover, the combination of MSC-FMT transplantation has the potential to enhance the effect of MSCs in the treatment of SLE. Therefore, MSC regulation of gut microbiota for the treatment of SLE is a promising direction for future study.

Here, we summarize novel insights into the mechanisms of microbiota dysbiosis in SLE and provide promising therapeutic

strategies, which may help improve our understanding of the pathogenesis of SLE and provide novel therapies for SLE.

AUTHOR CONTRIBUTIONS

QRP and FG wrote the manuscript and designed the figures. YH, AL, SC, JC, and H-FL revised the manuscript. All authors contributed to the article and approved the submitted version.

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Real-Life Cause-Effect Relations Between Urinary IL-6 Levels and Specific and Nonspecific Symptoms in a Patient With Mild SLE Disease Activity

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Background: Little is known about the real-time cause-effect relations between IL-6 concentrations and SLE symptoms.

Methods: A 52-year-old woman with mild SLE activity collected her entire urine for the determination of IL-6/creatinine and protein/creatinine levels (ELISA, HPLC) for a period of 56 days in 12 h intervals (total: 112 measurements). Additionally, she answered questionnaires (VAS) on oral ulceration, facial rash, joint pain, fatigue and tiredness and measured her temperature orally twice a day. Time-series analyses consisted of ARIMA modeling and cross-correlational analyses (one lag = 12 h, significance level = $p < 0.05$).

Results: Statistical analyses showed that increased urinary IL-6 concentrations preceded increased urinary protein levels by 36–48 h (lag3: r=+.225; p=.017) and that, in the opposite direction of effect, increased urinary protein preceded urinary IL-6 decreases by 12–24 h (lag1: r=–.322; p<.001). Moreover, urinary IL-6 increases co-occurred with increased oral ulceration (lag0: r=+.186; p=.049); after 48–60 h, however, IL-6 increases showed a strong tendency to precede oral ulceration decreases (lag4: r=–.170; p=.072). Increases in facial rash preceded decreases in urinary IL-6 after 84–96 h (lag7: r=–.215; p=.023). As to fatigue, increases in urinary IL-6 co-occurred with decreased fatigue (lag0: r=–.193; p=.042); after 84–96 h, however, IL-6 increases preceded fatigue increases (+lag7: r=+.189; p=.046). Finally, joint pain, tiredness and body temperature did not significantly correlate with urinary IL-6 concentrations in either direction of effect.

Conclusions: The results of this evaluation point to real-life feedback mechanisms between immune activity and SLE symptoms. Comparison with a previous evaluation of this patient suggests a counterregulatory mechanism between Th1 activity and IL-6. These findings are preliminary and require replication to draw firm conclusions about the real-time relation between IL-6 and SLE disease activity.

Keywords: lupus, interleukin-6, proteinuria, oral ulcer, facial rash, integrative single-case design, time-series analysis

INTRODUCTION

The functional role of interleukin-6 (IL-6) in systemic lupus erythematosus (SLE) is uncertain and in need of clarification. IL-6 is a pleiotropic cytokine released into circulation in almost all situations of perturbation of the homeostasis of the organism (1). Moreover, IL-6 is a broad-spectrum cytokine that plays a role in various biological activities. It is involved not only in the activation of the immune system but also in regenerative processes, in the regulation of metabolism, in the maintenance of bone homeostasis and in many neural functions (2).

IL-6 is therefore a good candidate when looking for crucial pathogenic players involved in the protean clinical outcomes of human SLE (3). However, results on the connection between IL-6 and clinical manifestations of SLE have been inconsistent. Some investigations have revealed that elevated IL-6 levels reflect disease activity including American College of Rheumatology (ACR)-based symptoms such as fatigue, joint pain, proteinuria, fever, photosensitivity, rash and renal disorder (4), whereas others have failed to find any significant correlations (5).

An important aspect in the immunopathophysiology of SLE is an imbalance in T helper type 1 (Th1) and T helper type 2 (Th2) subsets as well as a dysregulation between T effectors (Th1, Th2, T helper type 17 [Th17]) and T regulatory (Treg) cells (6, 7). However, as with the literature on the association between IL-6 and SLE symptoms, previous reports on Th1/Th2 ratio in SLE have been inconsistent, with some studies describing a predominance of Th1 cytokines (8, 9), while others have reported a predominance of Th2 cytokines (10, 11). Therefore, a mutual contribution of Th1/Th2 ratio to SLE pathology has been hypothesized, with different cytokine patterns at different time points (12).

We propose that the divergent findings concerning IL-6 and SLE as well as Th1/Th2 ratio and SLE may be related to fundamental methodological problems associated with the negligence of the highly dynamic character of IL-6 (13) and of SLE symptoms (14). Conventional methodological approaches are designed to reveal whether variables are concurrently related or not and therefore focus on absolute values rather than on temporal relations between consecutive realizations of variables. Consequently, such research designs cannot properly deal with questions of the temporal delay of cause-effect relations between variables and the temporal pattern of such relations (15). Moreover, IL-6 and SLE symptoms may influence each other in both directions of effect (16), again something that cannot be targeted by conventional methodology (15).

Such cause-effect relations between cytokines and lupus symptoms have already been shown for soluble tumor necrosis factor receptor 55kD (sTNF-R55) – a Th1 cytokine associated with clinical and subclinical SLE disease activity (17) – in a previous study applying the integrative single-case design (18). The integrative single-case design is different from conventional methodology in that it uses time-series analysis and qualitative tools to investigate real-life cause-effect relationships between various biological, psychological and social variables under conditions of "life as it is lived" (15). The study mentioned above was conducted with a 52-year-old woman with infrequently occurring, minor SLE symptoms not requiring steroidal or immunosuppressive drug therapy. In order to preserve the patient's normal routine as much as possible, proteinuria and cytokine levels were determined in 12 h urine samples to serially monitor these parameters non-invasively. Moreover, instead of having to see a physician every 12 hours, the patient used 100 mm visual analogue scales (VAS) to self-determine the presence of several specific (i.e. oral ulcers, facial rash and joint pain) and nonspecific SLE symptoms (i.e. fatigue, tiredness and body temperature). This procedure contributes to the high ecological validity of the study design.

In that study, we found that sTNF-R55 showed bidirectional cause-effect relations when cross-correlated with SLE symptoms (18). In particular, increased urinary sTNF-R55 concentrations preceded decreased urinary protein levels by 36–48 h, and, in the opposite direction of effect, increased urinary protein levels preceded increased urinary sTNF-R55 concentrations by 24–36 h. Furthermore, increases in urinary sTNF-R55 levels preceded increases in oral ulcers by 36–48 h, and increases in oral ulceration preceded decreases in urinary sTNF-R55 levels by 36–48 h. These cross-correlations in both directions of effect indicate feedback loops between sTNF-R55 and SLE symptoms. For example, elevated sTNF-R55 levels may have inhibited clearance of protein from circulation, while decreased protein clearance may have then resulted in decreased sTNF-R55 concentrations either per se or *via* an as yet unknown counterregulatory mechanism (18).

Similar bidirectional mechanisms might characterize the relationship between IL-6 and SLE symptoms. Both TNF-α and IL-6 are able to regulate the Th1/Th2/Th17/Treg balance, which plays a prominent role in autoimmune pathogenesis (6) *via* feedback loops, and therefore contribute to the maintenance of immunological homeostasis (19, 20).

The present article deals with a re-evaluation of the above-mentioned integrative single-case study and takes advantage of

the opportunity to not only cross-correlate IL-6 and SLE symptoms (i.e. proteinuria, oral ulcers, facial rash, joint pain, fatigue, tiredness, body temperature) in the same patient but also to compare them with the findings on the relation between sTNF-R55 and SLE symptoms described above (18). Ultimately, this research strategy allows us to investigate the potentially diverse functional roles of sTNF-R55 and IL-6 in SLE.

PATIENT AND METHODS

Study Design

At study start, the patient was thoroughly examined psychologically as well as physically, the latter to ensure that she was in clinical remission (according to the Systemic Lupus Activity Measure [SLAM]). Then, during the following 56 days, the patient collected her entire urine in 12 h intervals (from approx. 8 a.m. to 8 p.m. and from approx. 8 p.m. to 8 a.m.; total: 112 time intervals) in two canisters per day (containing 0.5 g Na-Metabisulfite and 0.5 g Na-EDTA to prevent urine sedimentation and oxidation) and froze aliquoted urine samples at –20°C. She also filled out questionnaires twice a day at approx. 8 a.m. and 8 p.m. Each week, the patient brought the frozen urine samples to the laboratory where they were stored at –70°C. During each of these weekly visits, an in-depth psychological interview was conducted to identify the previous week's incidents. In addition, a physical examination including a hemogram was performed to check general health and signs of SLE disease activity (SLAM). A more detailed description of the study design is given in (21).

Patient Description and Disease History

The patient is a 52-year-old white post-menopausal woman and a non-smoker. Eight years prior to the study start in 1997, the diagnosis SLE was made by a senior internist (P.K.) and a senior dermatologist (N.S.) according to the following ACR criteria: kidney involvement (histological evaluation of chronic mesangial proliferative glomerulonephritis, WHO classification IIIa) with microscopic hematuria; arthralgia; urticarial vasculitis; oral ulcers; facial rash. Moreover, she showed decreased complement C4 (hypocomplementemia), leukopenia and enhanced antinuclear antibodies (ANA, 1:2560); analyses of antinuclear anti-double-stranded DNA antibodies (ds DNA) were negative.

Pharmacologic treatment lasted three years and consisted primarily of steroids (4–20mg) in combination with other non-steroidal anti-inflammatory medication (paracetamol). The patient did not tolerate antimalarials; moreover, she refused further immune suppressive therapy (e.g. azathioprine, mycophenolate, cyclophosphamide) although her disease fulfilled WHO classification IIIa for SLE. Nevertheless, her laboratory values improved (no proteinuria, no pathological urine sediment) during pharmacologic treatment. The patient attended psychotherapy for three years following diagnosis.

During regular check-ups between first diagnosis in 1989 and study start in 1997, the following minor clinical disease manifestations related to SLE had been identified: oral ulcers, urticarial vasculitis lesions at various body sites (e.g. facial rash), small joint pain, fatigue, tiredness and fever. These symptoms did not require steroidal or immunosuppressive drug therapy and were treated by the patient symptomatically (e.g. mouth rinsing with hexetidin solution). At study start, the patient presented with elevated ANA (1:160, ds DNA negative, SS-Ro-antibody positive) with the above-mentioned mild clinical symptoms, which did not require steroid treatment.

Measurement of Small Joint Pain, Oral Ulceration, Facial Rash, Fatigue, Tiredness, and Body Temperature

In the morning and in the evening (i.e. in 12 h intervals), the patient used VAS/notes to indicate the following: small joint pain; mucosal and cutaneous manifestations such as oral ulcers and facial rash; fatigue; and tiredness. These measurements are part of the DIARI, a paper-and-pencil questionnaire that also includes drug/medication use and potential signs of a cold, flu, etc. (15). In addition, she measured her body temperature orally within 120 sec. using a commercially available mercury thermometer with a scale interval of 0.1°C (model no. 1711, Scheiber GmbH, Kreuzwertheim, Germany). The data were used to construct time series dealing with small joint pain, oral ulceration, facial rash, fatigue, tiredness and body temperature.

Measurement of Urinary IL-6 and Creatinine Levels

Urine samples were stored at –70°C until analysis. We measured the 112 consecutive urinary IL-6 levels in one single run using ELISA as recommended by the manufacturer (Endogen, IBL, Hamburg). Urinary IL-6 concentrations were expressed in microgram per molar (µg/mol) creatinine. Urinary creatinine levels were measured applying High Pressure Liquid Chromatography (HPLC) (Model LC 550; Varian Associates, Palo Alto, CA) as previously described (22). We used a new aliquot for each of the three independent determinations.

Measurement of Urinary Protein

The urinary protein level in each 12 h urine sample was measured in milligram per deciliter (mg %) using the benzethonium chloride method (23) at 505 nm with a Hitachi 911 analyzer (Roche). Values were expressed as miligram per micromolar (mg/µmol) creatinine (HPLC).

Time-Series Analysis

A detailed description of the statistical analyses used in this study is given in (15). In short, cross-correlational analyses between IL-6 levels and the ACR criteria under study were performed at lag0 and at higher lags up to +/–7 using SPSS-Trends™ 26.0 (24). We controlled for spurious cross-correlations due to trends (e.g. circadian rhythm) and serial dependencies (e.g. autoregression) by cross-correlating residuals series after autoregressive integrated moving average (ARIMA) modeling of time series (25). In case the mean of a series needed to be stabilized, a

deterministic trend was either removed from the series, or the series was differenced. In case the variance of a series needed to be stabilized, the series was transformed (e.g. log, square root). Transformation of time series was also used to improve model specification. Moreover, time series which did not need to be modeled and which were found to be not normally distributed were transformed before cross-correlating. Based on experience with our previous studies, binary time series were not modeled. Time series with missing values were linearly interpolated before further analysis. The level of statistical significance was set at $p < 0.05$.

RESULTS

Mean, standard deviation and range of all variables under study are shown in **Table 1**. All time series were complete (i.e. 112 measurements) except for the tiredness time series, which had one missing value (at 12 h unit 52). The patient indicated a mild facial rash on 26 of 112 12 h units (23%) (maximum intensity: 22%; maximum duration: 60 h). Moreover, she had mild oral ulcers (maximum intensity: 19.9%; maximum duration: 24h) on 6 of 112 12 h units (5%). Facial rash and oral ulcers were expressed in binary time series. **Figures 1A, B** show the time series of urinary IL-6 concentrations (µg/mol creatinine) and urinary protein concentrations (mg/µmol creatinine). In the middle of the study period (during 12 h units 45–54), the patient was diagnosed with acute paranasal sinusitis (21). Comparison of grouped time series data using Mann-Whitney U test revealed that only tiredness levels differed significantly during sinusitis compared to before and after sinusitis. The time series of tiredness remained heteroskedastic even after log transformation (data not shown). Neither the weekly clinical check-ups nor the 12 h notes taken by the patient revealed any signs of infection and/or SLE exacerbation during the study period.

Table 2 shows a summary of the results of this evaluation. The urinary IL-6 time series is best described by an ARIMA model with stochastic as well as deterministic seasonal components corresponding to an 8-lag (96 h) rhythm. The cross-correlogram shown in **Figure 2A** reveals that increased urinary IL-6 concentrations significantly preceded increased urinary protein levels by 36–48 h (+lag3: r=+.225; p=.017) and that, in the opposite direction of effect, increased urinary protein levels significantly preceded decreased urinary IL-6 concentrations by 12–24 h (–lag1: r=–.322; p<.001). **Figure 2B** shows that increased urinary IL-6 levels co-occurred with increased oral ulceration (lag0: r=+.186; p=.049); after 48–60 h, however, IL-6 increases showed a strong tendency to precede decreases in oral ulceration (+lag4: r=–.170; p=.072). **Figure 2C** shows that increases in facial rash preceded decreases in urinary IL-6 concentrations by 84–96 h (–lag7: r=–.215; p=.023). As to fatigue, increased urinary IL-6 levels co-occurred with decreased fatigue (lag0: r=–.193; p=.042); after 84–96 h, however, IL-6 increases preceded increased fatigue (+lag7: r=–.189; p=.046) (data not shown). Finally, joint pain, another specific SLE symptom (**Figure 2D**), as well as tiredness and body temperature did not significantly correlate with urinary IL-6 levels, in either direction of effect.

DISCUSSION

While the involvement of IL-6 in B and T cell immunopathology of SLE is undisputed (26, 27), it still remains to be determined whether IL-6 is related to immune-associated ACR symptoms in SLE (4, 5). Accordingly, the special design of this study (e.g. time-series analysis on 112 12 h measurements) allowed us to show in a patient with SLE i) normal or even reduced mean urinary IL-6 concentrations (see **Table 1**) (17), ii) an 8-lag (96 h) circasemiseptan (about-half-weekly) (28) rhythmic pattern in the urinary IL-6 time series (see **Table 2**), and iii) clear interdependencies between the 12 h variations in urinary IL-6 concentrations and the 12 h variations in SLE symptoms. Specifically, cross-correlational analyses revealed that IL-6 either co-occurred with SLE symptoms (oral ulceration, fatigue), preceded (urinary protein) or followed (urinary protein, facial rash, fatigue) SLE symptoms, with temporal delays of up to 96 h. Such complex interrelations between IL-6 and SLE symptoms are new to autoimmune research and need careful interpretation. Specifically, findings from time series

TABLE 1 | Descriptive statistics of urinary IL-6 concentrations and SLE-specific and SLE-nonspecific symptoms (N=112 consecutive measurements).

Parameter	Mean ± SD	Range
Urinary IL-6 (µg/mol creatinine)	1.36 ± 1.04	0.00 – 6.06
Urinary IL-6 (pg/ml)	0.01 ± 0.007	0.00 – 0.04
Urinary IL-6 (pg/h)	0.79 ± 0.67	0.00 – 4.20
Urinary protein (mg/µmol creatinine)	3.20 ± 2.25	0.08 – 14.5
Urinary protein (mg/dl)	3.48 ± 2.58	0.10 – 10.0
Urinary protein (mg/h)	1.76 ± 1.09	0.07 – 6.38
Oral ulceration (%)	0.59 ± 2.79	0.00 – 19.9
Facial rash (%)	2.67 ± 5.60	0.00 – 22.0
Body temperature (°C)	36.7 ± 0.25	36.1 – 37.4
Tiredness (%)	7.77 ± 5.86	0.00 – 38.0
Fatigue (%)	35.2 ± 17.2	6.00 – 77.0
Joint pain (%)	20.2 ± 11.5	5.00 – 54.0

SD, Standard deviation; IL-6, Interleukin-6.

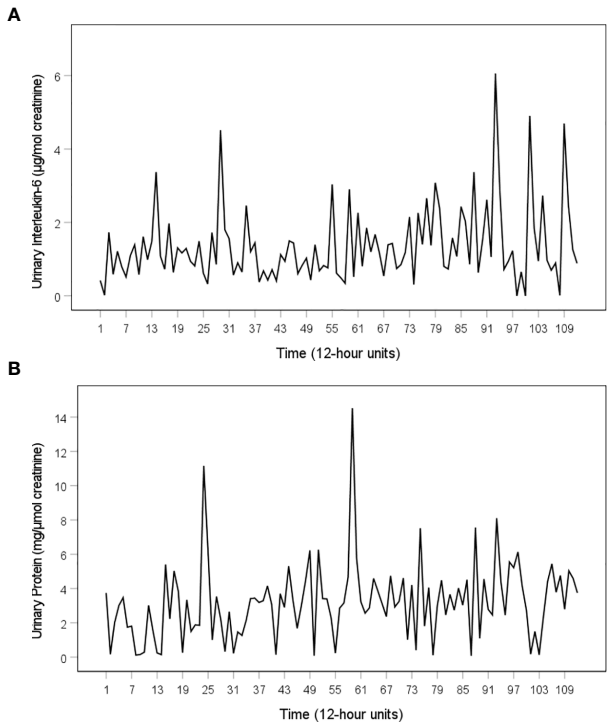

FIGURE 1 | Time series of urinary IL-6 levels and urinary protein levels of the SLE patient under study. **(A)** Time series of urinary IL-6 (µg per mol creatinine), **(B)** Time series of urinary protein (µg per mol creatinine). Both time series cover a period of 56 days. During this time, the patient collected her full urine output in 12 h intervals, resulting in a total of 112 12 h measurements. The 112 12 h units consist of daytime intervals (from 8:00 a.m. to 8:00 p.m., uneven numbers) and nighttime intervals (from 8:00 p.m. to 8:00 a.m., even numbers).

analyses cannot be compared easily with findings from conventional group statistics, which typically do not provide information on temporal delays, temporal patterns and directions of effect (15).

Given that proteinuria is one of the key features of lupus nephritis, our finding of an increase in urinary protein levels following IL-6 increases after 36–48 h confirms, in principle, results from laboratory studies. For example, in an experimental study on IL-6-knockout mice, Cash and colleagues (29) observed delayed lupus nephritis with a marked reduction of proteinuria compared to IL-6-intact control mice. The positive correlation between IL-6 levels and oral ulceration at lag 0 found in this study is also in line with group studies on this topic. Marques and colleagues, for example, showed a stronger positive expression of IL-6 in mucosal biopsies of lupus patients compared to the specimens of normal controls (30).

With regard to fatigue, our study found a negative correlation at lag 0 between urinary IL-6 levels and fatigue intensities as well as a positive correlation at +lag 7 (data not shown). This positive correlation at +lag 7, although describing a long temporal delay of 84–96 h, is principally in line with current experimental literature demonstrating that IL-6 triggers increases in fatigue and other symptoms of so-called sickness behavior (16). The other IL-6 result in regard to fatigue, namely the negative correlation at lag 0, is not in line with conventional research but is consistent with other findings from our working group. In studies on breast cancer patients, for example, we recently showed that increases in urinary IL-6 concentrations preceded fatigue decreases by 48–60 h (31) and that increased levels of urinary neopterin preceded fatigue increases by 24 h (32) and 60–72 h (33). Neopterin is a cellular immune parameter closely linked to the Th1 immunity (22). IL-6 has been shown to have well-defined anti-inflammatory properties and to promote Th2 responses often opposing Th1 activity (34). Thus, the current study's finding of a decrease in fatigue co-occurring with IL-6 increases (in SLE) both replicates our previous findings on the temporal IL-6–fatigue relation (in breast cancer) (31) and is in line with our previous observations on the temporal relation between neopterin and fatigue (in breast cancer) (32, 33).

This study not only showed that urinary IL-6 changes co-occurred with or preceded changes in SLE symptoms but also that, in the opposite direction of effect, SLE symptoms preceded

TABLE 2 | Summary of findings including ARIMA models and cross-correlation results between IL-6 concentrations and SLE-specific and SLE-nonspecific symptoms.

		Urinary IL-6 SAR(2), deterministic season, s=8, sqt	
Urinary protein (0,0,0), cube root	–lag1: r=–.322; *p*<.001		+lag3: r=+.225; *p*=.017
Oral ulceration not modeled		± lag0: r=.186; *p*=.049	+lag4: r=–.170; *n.s.*
Facial rash not modeled	–lag7: r=–.215; *p*=.023		
Joint pain AR(1), deterministic trend			
Fatigue deterministic trend		± lag0: r=–.193; *p*=.042	+lag7: r=.189; *p*=.046
Tiredness SMA(4), ln			
Body temperature deterministic season, s=2, ln			

+Lag means that IL-6 levels precede SLE symptom, –lag means that SLE symptom precedes IL-6 levels. Lag0 in this study can mean concurrency, positive lag (within 12 h) or negative lag (within 12 h). IL-6, interleukin-6; AR, Autoregressive; SMA, Seasonal Moving Average; SAR, Seasonal Autoregressive; s, seasonality; n.s., not significant; sqt, square root; ln, natural logarithm.

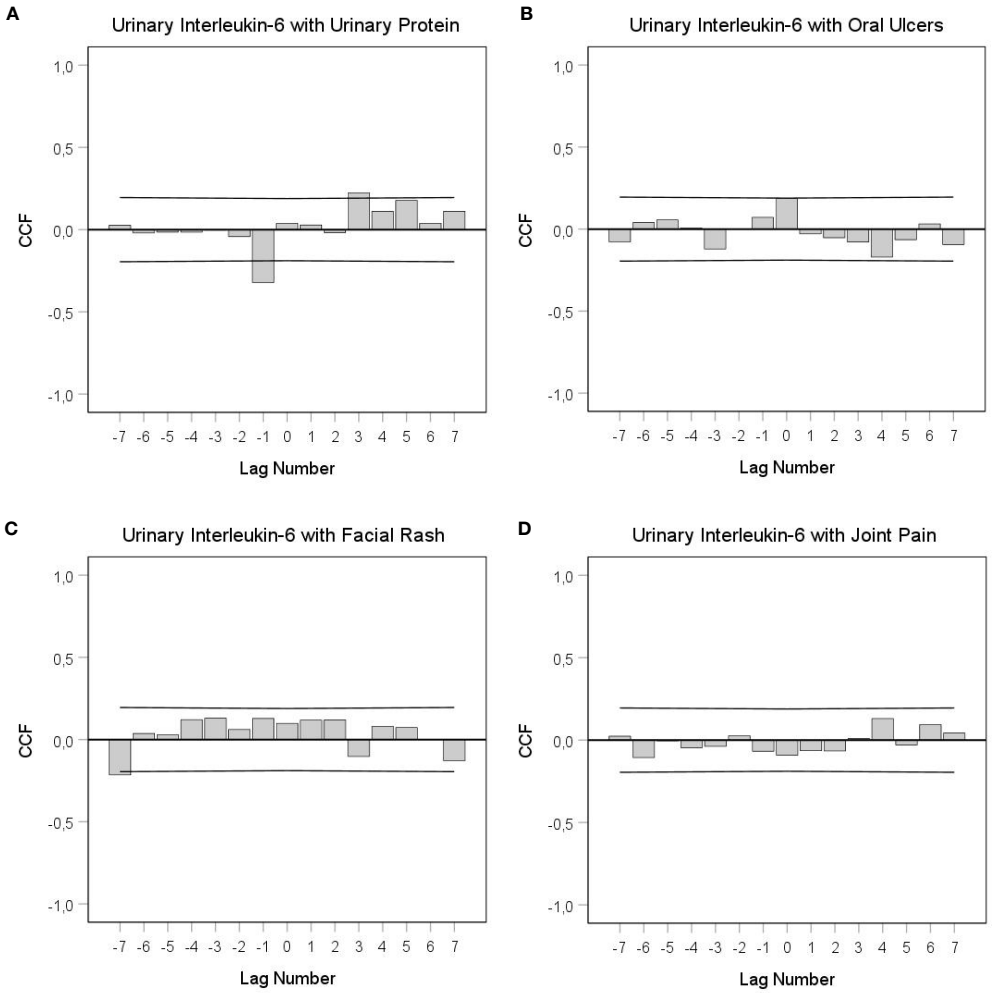

FIGURE 2 | Cross-correlational functions (CCF) between urinary IL-6 levels and SLE-specific symptoms. **(A)** IL-6 with urinary protein, **(B)** IL-6 with oral ulcers, **(C)** IL-6 with facial rash, **(D)** IL-6 with small joint pain. Each lag represents a time interval of 12 h. Cross-correlation coefficients (bars) that reach the upper or lower limits of the 95% confidence intervals (lines) are significant at $p < 0.05$. *A* positive lag significance means that urinary IL-6 levels precede SLE-related symptoms; a negative lag significance means that SLE-related symptoms precede urinary IL-6 levels. Lag0 in this study can mean concurrency, positive lag (within 12 h) or negative lag (within 12 h). Clearly in **(A)**, but perhaps also in **(B)**, there is a change in the sign of the cross-correlation function between positive and negative lags, which indicates negative feedback loops.

IL-6. Such findings are especially difficult to interpret from a conventional research perspective when, as is the case for urinary IL-6 and urinary protein, significances in both directions of effect (negative and positive lag) are found in the CCF (**Figure 2A**). We are convinced that such findings can only be interpreted properly when they are not considered in isolation. Instead, a broader look at the dynamic and possibly functional interdependencies between variables is required (18). In this regard, both long time delays and bidirectional effects between time series variables might indicate feedback mechanisms under real-life conditions (18, 35). Indeed, the cross-correlational constellation seen in **Figure 2A**, in which a negative (positive) value in one process becomes a positive (negative) value after having interacted with the other process, and vice versa, is an indicator of a negative feedback loop in the SLE patient under study (18, 35, 36). This negative feedback loop between urinary IL-6 and urinary protein could be read as follows: Increased protein clearance from circulation might have either per se or *via* an as yet unknown mechanism led to decreased IL-6 concentrations after 12–24 h (see the negative lag and negative correlation in **Figure 2A**), and suppressed IL-6 levels could have then resulted in decreased protein clearance with a temporal delay of 36–48 h (see the positive lag and positive correlation in **Figure 2A**).

The CCF between IL-6 and oral ulceration in **Figure 2B** shows a significantly positive correlation at lag 0 and a strong tendency toward negative significance at +lag 4. Similarly, the CCF between IL-6 and fatigue shows a significantly negative correlation at lag 0 and a positive significance at +lag 7 (data not shown). Both CCFs might indicate negative feedback loops when the following two conditions are met: i) In our study, a lag 0

significance can mean either that two variables are correlated without any directional effect between them or that one variable preceded the other within a time frame of 12 h. For a feedback loop, therefore, we need to assume that oral ulceration and/or fatigue preceded urinary IL-6 concentration changes by up to 12 h; ii) as to the relation between IL-6 and oral ulcers, the non-significant negative correlation at +lag 4 is in fact a meaningful finding when we keep in mind that both significant coefficients and temporal patterns of [non-significant] coefficients are important in the interpretation of integrative single-case studies (15).

Interestingly, the results on IL-6, urinary protein and oral ulcerations show inverted dynamics compared to a previous evaluation of the same integrative single-case study focusing on sTNF-R55 (18). In that evaluation, elevated urinary sTNF-R55 concentrations were preceded by increases in urinary protein and decreases in oral ulcers and were followed by decreases in urinary protein and increases in oral ulcers. In the current evaluation, by contrast, increased levels of urinary IL-6 were preceded by decreases in urinary protein and increases in oral ulcers and were followed by increases in urinary protein and decreases in oral ulcers. These findings indicate a counterregulatory temporal dynamic between sTNF-R55 and IL-6, which might be due to the mutual inhibition of Th1/Th2 subsets or to different pathways that these messenger molecules take (classical signaling/trans-signaling). In our two evaluations, therefore, we found cytokine markers of different Th subsets positively and negatively correlated with SLE symptoms at different points in time, thereby reinforcing the theory that SLE activity is a consequence of disturbed immunological balance (7, 9). Furthermore, these temporal dynamics might explain the inconsistencies in previous reports on the Th1/Th2 ratio in SLE (8–12).

Our current finding of a decrease in urinary IL-6 concentrations 84–96 h after increases in facial rash may be attributable to the emotionally painful experience of having a visible rash on the patient's face (37). In this regard, the patient's facial rash may have been a stressor that triggered a decrease in urinary IL-6 levels after 84–96 h (see **Figure 2C**). This reaction is similar to the evidence of stress-mediated neopterin responses found in this and other integrative single-case studies (15, 21). Specifically, in another evaluation of this patient (21), emotionally painful incidents were followed by ultimate increases in urinary neopterin concentrations after 60–72 h. As noted above, neopterin is a Th1 indicator (22); thus, the stress-mediated effects of neopterin oppose those of IL-6, again underscoring the different functional roles of neopterin and IL-6 with regard to the Th1/Th2 dichotomy.

Unlike findings from conventional group research (16), this integrative single-case study showed no significant effect of IL-6 on facial rash, small joint pain, tiredness and body temperature (see **Table 2**). Some of the null findings of this study could be attributed to the fact that no objective measurement of SLE symptoms was applied. This approach is based on the assumption that interfering too much in a patient's everyday reality endangers the high ecological validity of this kind of investigation. For example, appointments with a specialist every 12 h to objectively measure the symptoms under investigation would have dominated the patient's everyday routine. Furthermore, regular meetings with a specialist could have influenced the patient's symptoms through placebo and/or nocebo effects. Such measures, therefore, would have interfered with the natural ebb and flow of the dynamic relation between immune factors and SLE symptoms. Nonetheless, in future studies, photographs of the skin taken by the patient in 12 h intervals would be a useful addition to objectify mucosal and skin lesions.

A further limitation of this evaluation is that only IL-6 was examined. Investigating additional inflammatory parameters (e.g. IL-1α, IL-1β) (16) could yield further insides, e.g. into the relation between immune factors and SLE symptoms as well as into Th1/Th2 regulation in SLE. The same holds true for transcription factors responsible for T-cell differentiation, which could be assessed in future studies through their measurement in urinary sediment.

For further evaluation of possible feedback processes between immune factors and SLE symptoms, multivariate time-series statistics (e.g. vector autoregressive modelling, impulse response analysis) could help to identify Granger causality and to properly disentangle the temporal sequence of the events within such regulatory circuits (38).

This study has exploratory character, and findings are based on only one patient (n=1). Therefore, we do not yet know whether the relations between immune factors and SLE symptoms found in this patient in disease remission also apply for patients experiencing acute disease activity. Thus, replications are needed before firm conclusions can be drawn.

Nevertheless, a considerable advantage of the integrative single-case study design is that it enables us to account for the dynamic nature of neuroimmunological processes (e.g. temporal delays and patterns, feedback mechanisms) under real-life conditions. These basic insights into the dysfunctional physiology of SLE would not be possible applying conventional laboratory and/or quasi-experimental approaches to this topic. Moreover, our results on the dynamic relation between cytokine levels and SLE symptoms call into question the use of pre-post designs in complex clinical research topics. The following considerations support this assertion: (1) A lack of conventional statistical correlation between a cytokine level and an SLE symptom (analogue to a lag 0 correlation) does not automatically mean that the cytokine is not connected with this symptom; (2) a single significant correlation between a cytokine level and an SLE symptom does not allow inferences on the functional role of a cytokine; (3) assuming that delayed effects between cytokine levels and SLE symptoms may differ across patients, an averaging of results would lead to inconsistencies and a lack of generalizability.

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 study, involving a human participant, was reviewed and approved by the Ethics Committee of the Medical Faculty of the University of Innsbruck. The patient provided her written informed consent to participate in this study. She also gave written informed consent for the publication of any potentially identifiable data included in this article.

AUTHOR CONTRIBUTIONS

CS: development of the study design, statistical analysis, interpretation of findings, writing of the manuscript. LS: interpretation of findings, writing of the manuscript. EC: interpretation of findings, writing of the manuscript. PK: patient recruitment, clinical and diagnostic work. NS: clinical and diagnostic work. FO-P: statistical analysis. MS-K: urinary protein measurement. DF: urinary IL-6 and creatinine measurement. All authors contributed to the article and approved the submitted version.

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REVIEW
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LncRNA Expression Profiles in Systemic Lupus Erythematosus and Rheumatoid Arthritis: Emerging Biomarkers and Therapeutic Targets

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Systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) are two common multisystem autoimmune diseases that share, among others, many clinical manifestations and serological features. The role of long non-coding RNAs (lncRNAs) has been of particular interest in the pathogenesis of autoimmune diseases. Here, we aimed to summarize the roles of lncRNAs as emerging novel biomarkers and therapeutic targets in SLE and RA. We conducted a narrative review summarizing original articles on lncRNAs associated with SLE and RA, published until November 1, 2021. Based on the studies on lncRNA expression profiles in samples (including PBMCs, serum, and exosomes), it was noted that most of the current research is focused on investigating the regulatory mechanisms of these lncRNAs in SLE and/or RA. Several lncRNAs have been hypothesized to play key roles in these diseases. In SLE, lncRNAs such as GAS5, NEAT1, TUG1, linc0949, and linc0597 are dysregulated and may serve as emerging novel biomarkers and therapeutic targets. In RA, many validated lncRNAs, such as HOTAIR, GAS5, and HIX003209, have been identified as promising novel biomarkers for both diagnosis and treatment. The shared lncRNAs, for example, GAS5, may participate in SLE pathogenesis through the mitogen-activated protein kinase pathway and trigger the AMP-activated protein kinase pathway in RA. Here, we summarize the data on key lncRNAs that may drive the pathogenesis of SLE and RA and could potentially serve as emerging novel biomarkers and therapeutic targets in the coming future.

Keywords: lncRNA, profile, systemic lupus erythematosus, rheumatoid arthritis, biomarker, therapeutic target

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1 INTRODUCTION

Systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) are two common multisystem autoimmune diseases that share many clinical manifestations, serological profiles, immunological characteristics, and transcriptomes, for example shared type I interferon (IFN)-stimulated genes of peripheral blood mononuclear cell (PBMC) transcriptomes (1). Furthermore, the co-occurrence of SLE and RA within the same person or within members of a nuclear family indicates that they shared common etiological factors (2–4). In addition to the traditional treatment options with hormones and immunosuppressants (5, 6), a large variety of biological drugs is now available for the treatment of SLE and RA (7–9), however, the clinical response and functional remission rate of these drugs are still not satisfactory. Therefore, treatment strategies for SLE and RA need further improvement by adopting different approaches (9, 10).

In the human genome, 98% of the products are non-coding RNAs (11), and those with a size length greater than 200 nucleotides (NT) are defined as long non-coding RNAs (lncRNAs) (12). LncRNAs have poor protein-coding potential (13–15), except for certain micropeptides or polypeptides that can perform specific biological functions (16). It is well known that the regulation of gene expression *via* lncRNAs occurs mainly through variable interactions with DNA, RNA, and proteins (17, 18), and are thus involved in a variety of important regulatory processes, such as the silencing of the X-chromosome, chromatin modifications, transcriptional activation interference, and post-transcriptional modifications (19). The role of lncRNAs is of particular interest in the pathogenesis of autoimmune diseases (20, 21). They could participate in inflammatory pathways in autoimmune diseases and promote the release of inflammatory factors such as TNF-α, IL-6 (22), IL-8, IL-1β (23), IFN-I (24) to aggravate or alleviate diseases. In addition, lncRNAs are widely found in many bodily fluids and are highly stable in the plasma, potentially serving as biomarkers for multiple diseases (25).

Abbreviations: AKT/mTOR, protein kinase B and mammalian target of rapamycin; AMPK, AMP-activated protein kinase; ceRNA, competitive endogenous RNA; DSCR9, down syndrome critical region; FcγR, receptor of immunoglobulin G; FLS, fibroblast-like synoviocytes; FOXD2-AS1, FOXD2 adjacent opposite strand RNA 1; FRAT1, the frequently rearranged in advanced T cell lymphomas-1; GAS5, growth arrest-specific 5; G-MDSCs, myeloid-derived granulocyte suppressor cells; hnRNP K, heterogeneous nuclear ribonucleoprotein K; hnRNP Q, heterogeneous nuclear ribonucleoprotein Q; IFN-I, type I interferon; ITSN1-2, intersectin1-2; IκBα, inhibitory κBα; LTB_4, leukotriene B4; lncRNA, long non-coding RNA; MALAT-1, metastasis associated lung adenocarcinoma transcript 1; MAPK, mitogen-activated protein kinase; MEG3; maternally expressed gene 3; NEAT1, nuclear-enriched abundant transcript 1; NF-κB, nuclear factor kappa B; NOD2/RIP2, the nucleotide-binding oligomerization domain 2; PBMC, peripheral blood mononuclear cell; PTEN, phosphatase and tensin homolog deleted on chromosome 10; RA, rheumatoid arthritis; RASF, rheumatoid arthritis synovial fibroblasts; ROCK2, Rho associated coiled-coil containing protein kinase 2; SIRT1, silent mating type information regulation 2 homolog 1; SLE, systemic lupus erythematosus; SDC1, Syndecan 1; Tfh cells, T follicular helper cells; YPEL4, Yippee-like-4; YY1, Ying Yang 1.

Here, we aimed to summarize the roles of lncRNAs as emerging novel biomarkers and therapeutic targets in SLE and RA. We conducted a narrative review and summarized original articles on lncRNAs associated with SLE and RA patients, published until November 1, 2021.

2 LncRNAs AND SLE

It is well known that genetic and environmental risk factors are key players involved in the pathogenesis of SLE (26, 27), and the multi-organ involvement, highly heterogenic clinical characters, and differences in the degree of severity lead to major challenges in its diagnosis and treatment (28–30). Recently, increasing evidence shows that many lncRNAs are dysregulated and may have a key role in the development of SLE (31). Transcriptome sequencing results revealed a large number of novel lncRNAs in PBMC, serum and exosomes of SLE patients and animal models. Their potential use as biomarkers and their correlation with clinical features were also studied. Studies focusing on the expression profiles of novel lncRNAs in PBMCs and serum from SLE patients and animal models revealed their potential as biomarkers as well as regulatory mechanisms in SLE.

2.1 The Expression Profiles of LncRNAs in the PBMCs of SLE

Recently, lncRNAs derived from PBMCs of patients with SLE have been a research hotspot because of their large presence and rich variety. Abnormal numbers and functions of PBMCs are significantly related to SLE pathogenesis (32, 33). A study showed that 137 lncRNAs-derived from PBMC were identified as differentially expressed in normal controls (n=15) and SLE patients (n=15) *via* microarray technology, with 83 upregulated and 54 downregulated lncRNAs. Among them, two lncRNAs, ENST00000604411.1 and ENST00000501122.2, were significantly upregulated, while another two, lnc-HSFY2-3:3 and lnc-SERPINB9-1:2, were significantly downregulated in patients with SLE. The study showed that the upregulated ENST00000604411.1 could lead to X chromosome inactivation by protecting the active-X from ectopic silencing, and thus playing a pathogenic role in SLE (34). In addition, the levels of the two upregulated lncRNAs were positively correlated with the clinical activity index (SLEDAI score) of SLE patients (ENST00000604411.1 (r=0.593, P=0.020), ENST00000501122.2 (r=0.539, P=0.038), suggesting that the levels of these two lncRNAs could be used to evaluate the disease activity in SLE patients (34). LncRNA TCONS_00483150 in PBMCs was significantly decreased in patients with SLE compared with health controls, and its expression was significantly correlated with anti-Rib-P autoantibody, which may be anovel biomarker for the diagnosis of SLE (35). It has also been reported that lncRNAs taurine-upregulated gene 1 (TUG1), linc0949, nuclear-enriched abundant transcript 1 (NEAT1), and linc0597 were expressed at lower levels in the PBMCs of SLE patients (31, 36, 37). Among them, TUG1 was further reduced in patients with lupus nephritis, and its expression was negatively correlated with

the SLEDAI score (r=0.904, P< 0.001). NEAT1 is known as an early lipopolysaccharide (LPS) response lncRNA that can modulate the innate immune response *via* the toll-like receptor (TLR) signaling pathway (38, 39). In addition, the levels of NEAT1 expression in PBMCs of SLE patients was significantly increased and was positively correlated with the disease activity. Furthermore, NEAT1 was found to affect the expression of inflammatory chemokines and cytokines by activating the late mitogen-activated protein kinase (MAPK) signaling pathway, which could regulate the immune response of T and B cells, and participate in the development of SLE, thus providing a potential therapeutic target for SLE (37). Another study showed that the up-regulated NEAT1 was negatively correlated with Th1/Th2 balance, which might affect the occurrence and progression of SLE (40). Hence, lncRNAs NEAT1, linc0949, and linc0597 are expected to be promising diagnostic markers for SLE, whereas TUG1 is expected to be a clinical diagnosis and disease activity marker.

Additionally, for the expression of lncRNAs in PBMCs, lnc5150 was lower in patients with SLE (n=76) than in healthy controls (n=71) (41). The expression of lncRNA AC007278.2 was high in SLE patients and could modulate the expression of inflammatory chemokines and cytokines. The study showed that ACC007278.2 could promote B cell maturation by down-regulating its target gene *CCR7* and T follicular helper cells, participating in SLE. Therefore, AC007278.2 may be used as a molecular biomarker for the diagnosis and treatment of SLE (42). Compared to healthy controls, metastasis-associated lung adenocarcinoma transcript 1 (MALAT-1), which is mainly expressed in human monocytes, was significantly increased in SLE patients, and could modulate the silent mating type information regulation 2 homolog 1 (SIRT1) pathway directly (43). Another study reported that MALAT1 also could participate in type I interferon-mediated SLE by up-regulating OAS2, OAS3 and OAS-like (OASL) in $CD4^+$ T cells (24).

LncRNA growth arrest-specific 5 (GAS5) regulates growth arrest, apoptosis, cell cycle, and replication in T cell lines and non-transformed lymphocytes (44). GAS5 was reported to be related with an increased risk of development of SLE in a murine model (45). Also, GAS5 has been found to be involved in disease progression in SLE patients (46) and may be involved in the development of SLE *via* the MAPK signaling pathway (25). These results indicate that PBMC-derived lncRNAs may play a vital role in the pathogenesis of SLE, but the specific mechanisms remain unclear.

Recently, the genetic significance of lncRNAs in many autoimmune diseases has been investigated, and most of the susceptibility loci for SLE were found to be located in noncoding regions of the genome (47, 48). A novel SLE susceptibility locus in a lncRNA gene (SLEAR) was identified at the single-nucleotide polymorphism rs13259960, which can result in decreased SLEAR production in PBMCs from patients with SLE. Moreover, it could interact with RNA binding proteins and thus affect the downstream target genes. In addition, the level of SLEAR expression was correlated with the percentage of PBMC death in patients with SLE (47). The rs145204276 ID/DD genotypes in the promoter region of the LncRNA-GAS5 gene may have a protective effect against SLE by up-regulating LncRNA-GAS5 expression and its targets miR-21 and phosphatase and tensin homolog deleted on chromosome 10 (PTEN) (48). Two functional promoter variants in linc00513, significantly overexpressed in SLE, were reported to be possible candidates in promoting genetic susceptibility to SLE (49). Till now, these studies are very few, and several still need large-scale data verification to provide novel insights into the genetics of SLE.

Abnormal proliferation and activation of B cells can produce large quantities of autoantibodies, which are deposited in the kidney and other tissues, further inducing inflammation and tissue damage. This is considered the core of the pathogenesis of SLE (50, 51). Among all SLE treatments targeting B cells, belimumab is the only biological agent approved by the FDA (52). Recently, Dimitrioset et al. reported that CD19-targeted chimeric antigen receptor (CAR) T-cell therapy was successful in refractory SLE, and the rapid disappearance of dsDNA autoantibodies during CD19 CAR-T cell therapy suggested CD19-targeted plasmablasts as the major source of these antibodies (53).

The activation of type I interferon (IFN-I) in B cells is also closely related to the pathogenesis of SLE (54, 55). Recently, based on this theory, SLE treatment has mainly focused on blocking IFN-1 or its receptor (56), or targeting improved B cell survival to reduce the level of immunoglobulin G (IgG) autoantibodies (57–59). It has been reported that myeloid-derived granulocyte suppressor cells (G-MDSCs) promote B cell IFN-1 signal activation in lupus MRL/LPR mice (60). TLRs or interferon-α (IFN-α) can induce the expression of B cell activating factor (BAFF) (61, 62). LncRNA NEAT1 was highly expressed in G-MDSCs of lupus MRL/LPR mice, and G-MDSCs enhanced TLRs or IFN-α to produce BAFF (60). Furthermore, BAFF enhanced the activation of B cell IFN-1 signaling by inhibiting the expression of cytokine signal transduction inhibitor 3, which is involved in the occurrence and development of SLE. NEAT1 deficiency alleviated the symptoms of lupus and inhibited the activation of IFN-1 signaling in B cells of pristane-induced lupus mice, indicating that lncRNA NEAT1 plays a key role in the activation of B cell IFN-1 signaling pathway (60). LincRNA00892 also has been reported possibly activated $CD4^+$ T and B cells by targeting heterogeneous nuclear ribonucleoprotein K (hnRNP K) and subsequently up-regulating the expression of CD40L, thereby playing a pathogenic role in SLE (63). These data suggest that lncRNA is involved in modulating B cell activation and the production of autoantibodies, thus providing a new theory and intervention strategy for SLE.

Accumulating evidences have demonstrated that T cells are central in the pathogenesis of SLE (64, 65). LncRNAs uc001ykl.1 and ENST00000448942 in T cells from SLE patients (n=24) were downregulated compared to normal controls (n=21), and their expression was correlated with the erythrocyte sedimentation rate (ESR) (66). LncRNA GAS5 has been reported to possibly upregulate the adenovirus E4

promoter-binding protein (E4BP4) by inhibiting miR-92a-3p, attenuating the self-reactivity of $CD4^+$ T cells in SLE, playing a protective role in SLE (67). Therefore, targeting lncRNAs expressed in T cells and their signaling pathways may be a potential therapy for SLE.

2.2 The Expression Profiles of LncRNAs in the Serum and Plasma of SLE

LncRNAs are stable in serum and plasma and may serve as novel non-invasive biomarkers for SLE (68). The expression of linc-DC and GAS5 has been found to be decreased in the plasma of SLE patients (n=163) compared with health controls (n=80), while linc0597 is increased (68). Another study identified 1873 lncRNAs derived from the plasma of SLE patients through gene ontology analysis, with 221 upregulated and 1652 downregulated lncRNAs ($lg|FC| \geq 2.0$ and $P \leq 0.05$), of which Yippee-like-4 (YPEL4) was related to the receptor immunoglobulin G (FcγR) pathway (69). The FcγR mediates the interaction between immune complexes and immune cells and participates in the activation and regulation of a variety of immune responses, which play important roles in humoral immunity and cellular immunity. The combination of FcγR and the IgG Fc segment could stimulate immune cells to release inflammatory mediators, activate $CD4^+$ and $CD8^+$ T cells, and amplify humoral and cellular immunity, thereby promoting the pathogenesis of SLE (70). However, the molecular mechanism of action has not yet been identified. In another study, compared with the normal control group, 1315 significantly differentially expressed lncRNAs ($lg|FC| \geq 2.0$ and $P \leq 0.05$) were found in the plasma of SLE patients (n=24) (68), with significantly increased levels of linc0597, lnc0640, and lnc5150 and significantly decreased levels of GAS5 and lnc7074. However, the molecular mechanism of action has not yet been identified. These lncRNAs may be involved in the regulation of the MAPK signaling pathway, promoting the inflammatory response in SLE, and could be used as novel potential diagnostic biomarkers (68). This panel of five lncRNAs (linc0597, lnc0640, lnc5150, GAS5, lnc7074) had a high accuracy for the diagnosis of SLE (AUC=0.966), and could also be used to distinguish SLE from RA patients (AUC=0.683 and 0.910, respectively) (25). Subsequently, in the external validation phase, the expression levels of these five lncRNAs were investigated in thirty RA patients and thirty-one SLE patients. The results showed that the levels of GAS5 and linc0597 were significantly lower in SLE patients in the testing group than in RA patients, while no significant differences were found in the levels of lnc7074, lnc-DC, lnc0640, and lnc5150 between the two groups, which may be different from other autoimmune diseases (Sjogren's syndrome) (25). Finally, the co-expression analysis found that GAS5, lnc0640 and lnc5150 may be involved in the pathogenesis of SLE *via* the MAPK signaling pathway. The competitive endogenous RNA (ceRNA) network showed that the forementioned five lncRNAs bind competitively with miRNAs and regulate the expression of their target genes, hence their aberrant expression may have a vital role in SLE pathogenesis. Therefore, it is hypothesized that analyzing the ceRNA network in SLE may help expand the understanding of transcriptomes (especially non-coding transcriptomes) and improve the understanding of the pathogenesis, diagnosis, and treatment of SLE (71, 72).

2.3 The Expression Profiles of LncRNAs in the Exosomes of SLE

Exosomes are endocytic membrane-derived vesicles, measuring 30–120 nm in length, and participate in the communication among cells and in the delivery of contents (e.g., proteins, lipids, nucleic acids) to target cells (73–75). Evidence indicates that exosomal non-coding RNAs play a vital role in the pathogenesis of autoimmune diseases, such as SLE and RA (76, 77).

With recent research findings, the role of lncRNAs in SLE has gradually become clear. The abnormal expression of lncRNAs in patients with SLE can be used as a potential biomarker to assist in SLE diagnosis and treatment. However, the specific mechanisms need to be confirmed. In addition, more evidence is needed to investigate the other roles of lncRNA in SLE, such as whether it is related to clinical features, diagnosis, and prognosis, and whether it can be used to evaluate the clinical treatment effect on SLE. These findings will provide novel ideas and directions for lncRNA research.

3 LncRNA AND RA

RA is a typical chronic systemic autoimmune disease dominated by inflammatory synovitis. Genetics, smoking, air pollution, and gender are all considered risk factors for RA (78, 79). Its pathogenesis is complex, and pro-inflammatory factors such as interleukin (IL)-1, IL-17, IL-22, tumor necrosis factor alpha (TNF-α), IL-6, and matrix metalloproteinase (MMP) have been confirmed to be related to the development of RA (80–84). Recently, emerging studies have found that lncRNAs play a critical role in the pathogenesis of RA (85–88). In addition, many lncRNA disorders are related to RA disease activity, indicating that the role of lncRNA is conducive to the clinical diagnosis of RA and may serve as a new target for its treatment.

3.1 The Expression Profiles of LncRNAs in the PBMCs of RA

Studies have shown that lncRNA HOTAIR derived from both serum and PBMCs is significant highly expressed in RA and could be used as a novel biomarker for its diagnosis (89, 90). In addition, it may also play a vital role in RA pathogenesis. The expression of HOTAIR in chondrocytes stimulated by LPS was significantly reduced. Overexpression of HOTAIR reduced the rate of LPS-induced cell proliferation and inhibited inflammatory cytokine (IL-17, IL-23) production. The overexpression of HOTAIR also inhibited the activation of nuclear factor kappa B (NF-κB) in chondrocytes stimulated by LPS by blocking p65 nuclear transport, resulting in the reduction of IL-1β and TNF production (91). This suggests that regulating the expression of HOTAIR may be a potential treatment strategy for RA.

The lncRNA GAS5 is related to several autoimmune diseases. The expression of GAS5 in PBMCs and fibroblast-like synoviocytes (FLS) is lower in the serum of patients with RA (n=35) than that in normal controls (n=35) (92, 93). Moreover, GAS5 can be used as a ceRNA to directly target miR-222-3p, upregulate the expression level of Sirt1, and inhibit the proliferation and inflammation of RA-FLS. It is also reported that the overexpression of lncRNA GAS5 in the PBMCs of patients with RA can activate the AMP-activated protein kinase (AMPK) pathway, negatively regulate the expression of IL-6 and IL-17, and alleviate RA disease activity (94). These findings suggest that GAS5 activation is a potential target for RA treatment. Compared with healthy controls (n=20), the expression of lncRNAs MIR22HG and ENST00000619282 is significantly increased, while the expression of lncRNAs down syndrome critical region (DSCR9), LINC01189 and MAPKAPK5-AS1 is significantly decreased in PBMCs from patients with RA (n=20). According to gene ontology analysis, these significantly altered lncRNAs are mainly involved in the regulation of autophagy and apoptosis (95).

Some lncRNAs can act as ceRNAs to regulate miRNA function and are involved in RA progression (96). Compared with normal controls (n=40), the expression level of lncRNA HIX003209 in the PBMCs of patients with RA (n=43) was higher and positively correlated with the expression levels of TLR2 and TLR4 in macrophages (97). Further studies have found that HIX003209 can reversibly promote the proliferation and activation of macrophages by modulating the inhibitory effect of the κBα (IκBα)/NF-κB signaling pathway. In contrast, HIX003209 can act as a ceRNA to participate in TLR4-mediated inflammatory responses by binding to miR-6089 in macrophages (98). This suggests that the HIX003209-miR-6089-TLR4 signaling pathway may be a novel target for the treatment of RA.

3.2 The Expression Profiles of LncRNAs in the FLS of RA

The FLS is a key effector cell type responsible for the inflammation of the synovium and destruction of bone and cartilage. It can mediate the production of inflammatory mediators and matrix degrading enzymes and play a critical role in the occurrence and development of RA (99–101).

In the synovial tissue of patients with RA (n=30), a total of 349 lncRNAs were significantly upregulated, and 806 were significantly downregulated ($lg|FC| \geq 2.0$ and $P \leq 0.05$) compared with those in the normal control group (n=30). Among these lncRNAs, the levels of lnc-AL928768.3 and lnc-AC091493.1 expression were positively correlated with the RA-DAS28 score and the level of CRP, which is considered to be a novel diagnostic marker and activity index of RA. These lncRNAs can regulate their target mRNAs [e.g., Syndecan 1 (SDC1), leukotriene B4 (LTB_4)], and are thus implicated in the abnormal immune response of RA and in promoting the proliferation of FLS *via* multiple pathways (102). In terms of promoting RA inflammation, the level of lncRNA Fer-1-like family member 4 (FER1L4) in FLS and synovial tissues (STs) of patients with RA was low, whereas NLR family CARD domain containing 5 (NLRC5) was highly expressed (103). NLRC5 promotes RA progression by modulating the NF-κB signaling pathway (104). In contrast, overexpression of FER1L4 reduced the expression of NLRC5 and inflammatory factors. This suggests that FER1L4 may be a potential therapeutic target for RA (105). LncRNA linc00152 was reported to be up-regulated in RA-FLS, which could promote TAK1 expression by targeting miR-103a and thus activate the NF-κB pathway. Also, transcription factor Ying Yang 1 (YY1) could also directly promote linc00152 expression, thus forming a linc00152/NF-κB feedback loop that could promote RA-FLS inflammation (106). LncRNA FOXD2 adjacent opposite strand RNA 1 (FOXD2-AS1) was found to promote the proliferation and invasion of RA-FLS by regulating the miR-331-3p/PIAS3 pathway (107). LncRNA LERFS (lowly expressed in rheumatoid fibroblast-like synoviocytes) could promote synovial aggression and joint destruction by interacting with heterogeneous nuclear ribonucleoprotein Q (hnRNP Q) (108). LncRNA ZNF667-AS1 was reported to be down-regulated in RA-FLS, and its overexpression could play a protective role in RA by sponging miR-523-3p, thus inactivating the JAK/STAT signaling pathway (109). The down-regulated expression of the lncRNA X-inactive specific transcript (XIST) was found to inhibit the proliferation of synovial fibroblasts (SFs) by promoting the miR-126-3p/NF-κB pathway, thereby playing a protective role in RA (110). Therefore, targeting these lncRNAs in the FLS of RA may be used as a new strategy for RA therapy.

Comparing the expression profile of FLS-derived lncRNAs from patients with RA and healthy controls, p38 inhibited cutaneous squamous cell carcinoma associated lincRNA (lncRNA PICSAR) was found to be highly expressed in the FLS and synovial fluid of patients with RA. When PICSAR small-interfering RNA was used to reduce the expression of PICSAR, the levels of IL-6, IL-8, and MMP-3 were significantly reduced. Thus, PICSAR may be act as the ceRNA of miR-4701-5p and then promote the proliferation, invasion, and migration of RA FLS (111).

In vitro, overexpression of lncRNA maternally expressed gene 3 (MEG3) reversed both the high expression of miR-141 in LPS-stimulated chondrocytes and the production of IL-23. In animal experiments, overexpression of lncRNA MEG3 inhibited the protein kinase B (PKB; also known as AKT) and mammalian target of rapamycin (mTOR) (AKT/mTOR) signaling pathway. This suggests that lncRNA MEG3 can also be used as a ceRNA to inhibit inflammation by downregulating miR-141 and AKT/mTOR signaling pathways (112). In addition, in a CFA-induced rat RA model, MEG3 was low in synovial tissue and FLS, while the level of NLRC5 was increased, suggesting that MEG3 may potentially regulate the progression of RA by targeting NLRC5 (113).

LncRNA-H19 is highly expressed in the FLS of patients with RA (114). In a collagen-induced arthritis (CIA) mouse model, the expression of lncRNA-H19 was closely associated with the proliferation of synovial cells, and knocking down lncRNA-H19 could inhibit the proliferation of MH7A human synovial cells.

LncRNA-H19 can act as a ceRNA of miR-124a to inhibit the expression of CDK-2 and MCP-1 (115, 116). As already known, miR-124A may participate in the pathogenesis of RA through several molecular mechanisms. miR-124A can suppress the proliferation and inflammation of RA-FLS by targeting the phosphatidylinositol 3-kinase (PI3K)/NF-κB pathway (117). The methylation of miR-124a helps attenuate IL-1β-mediated RA-FLS proliferation and the expression of TNF-α (118). Also, miR-124a was found to inhibit the proliferation and invasion of RASFs by decreasing the expression of MMP3/13 and IL-1 (119). It has also been reported that the expression of lncRNA-H19 was inhibited by liver X receptor (LXR) agonists, suggesting that LXR may have an anti-arthritis function (120). Therefore, targeting the lncRNA-H19 and its downstream signaling pathway or using LXR agonists may be new strategies for RA treatment.

In addition, many other lncRNAs have been reported to be involved in the pathogenesis of RA. Overexpression of lncRNA zinc finger antisense 1 (ZFAS1) was found to upregulate miR-27a, and thereby promote the migration and invasion ability of

TABLE 1 | LncRNAs implicated in SLE and RA.

LncRNAs	Site	Expression	Signaling	References
SLE				
GAS5*	PBMC/Serum	DOWN	MAPK signaling pathway	(25)
NEAT1*	PBMC	UP	MAPK signaling pathway	(37)
TUG1*	PBMC	DOWN	Unknown	(36)
ENST00000604411.1	PBMC	UP	X chromosome inactivation	(34)
ENST00000501122.2	PBMC	UP	Unknown	(34)
TCONS_00483150	PBMC	DOWN	Unknown	(35)
lnc5150	PBMC/Serum	DOWN	MAPK signaling pathway	(25)
AC007278.2	PBMC	UP	Unknown	(42)
MALAT-1	PBMC	UP	SIRT1 signaling pathway	(43)
uc001ykl.1	B cell	DOWN	Unknown	(66)
ENST00000448942	B cell	DOWN	Unknown	(66)
YPEL4	Serum	UP	FcγR pathway	(69)
linc0949	PBMC	DOWN	Unknown	(31)
linc0597	PBMC/Serum	DOWN	Unknown	(31)
lnc0640	Serum	UP	MAPK signaling pathway	(68)
lnc7074	Serum	DOWN	MAPK signaling pathway	(68)
linc-DC	Serum	UP	Unknown	(68)
RA				
HOTAIR	Chondrocytes	DOWN	NF-κB signaling	(91)
HOTAIR	PBMC/Serum exosomes	UP	Unknown	(89, 90)
GAS5*	PBMC/Serum/S-erum exosomes	UP	AMPK pathway	(89)
GAS5*	FLS	DOWN	SIRT1 signaling pathway	(94)
MIR22HG	PBMC	UP	Unknown	(95)
ENST00000619282	PBMC	UP	Unknown	(95)
DSCR9	PBMC	DOWN	Unknown	(95)
LINC01189	PBMC	DOWN	Unknown	(95)
MAPKAPK5-AS1	PBMC	DOWN	Unknown	(95)
HIX003209	PBMC	UP	IκBα/NF-κB/HIX003209-miR-6089-TLR4	(98)
lnc-AL928768.3	STs	UP	Unknown	(102)
lnc-AC091493.1	STs	UP	Unknown	(102)
FER1L4	FLS/STs	DOWN	NF-κB signaling	(103, 104)
linc00152	FLS	UP	NF-κB signaling	(106)
FOXD2-AS1	Serum/STs	UP	miR-331-3p/PIAS3 pathway	(107)
LERFS	FLS	DOWN	Unknown	(108)
ZNF667-AS1	FLS	DOWN	JAK/STAT signaling	(109)
XIST	FLS	DOWN	miR-126-3p/NF-κB signaling	(110)
PICSAR	FLS	UP	Unknown	(111)
MEG3	Chondrocytes	UP	AKT/mTOR	(112)
lncRNA-H19	FLS	UP	PIK3/NF-κB pathway	(114, 117)
ZFAS1	FLS	UP	Unknown	(121)
ITSN1-2	FLS	DOWN	NOD2/RIP2	(122)
RP11-83J16.1	FLS	UP	Unknown	(124)
NEAT1*	PBMC exosomes	UP	Unknown	(129)
LUST	Serum exosomes	UP	Unknown	(89)
anti-NOS2A	Serum exosomes	UP	Unknown	(89)
SNHG4	Serum exosomes	UP	Unknown	(89)
HAR1B	Serum exosomes	UP	Unknown	(89)
TUG1*	Serum exosomes	UP	Unknown	(89)

*DOWN is downregulated, UP is upregulated. The lncRNAs marked with * are shared both SLE and RA.*

RA-FLS, suggesting a pathogenic role of ZFAS1 in RA (121). Low expression of lncRNA intersectin1-2 (ITSN1-2) inhibits the nucleotide-binding oligomerization domain 2 and receptor-interacting protein 2 (NOD2/RIP2) signaling pathway and reduces the proliferation and inflammation of RA-FLS (122). Overexpression of the lncRNA downregulated in liver cancer stem cells (DILC) can induce FLS apoptosis and downregulate the expression of IL-6, thereby reducing RA inflammation (123). Increased expression of lncRNA RP11-83J16.1 in FLSs from RA patients has been identified, which could regulate the levels of the frequently rearranged in advanced T cell lymphomas-1 (FRAT1) and β-catenin expression and thus promote cell proliferation, migration, invasion, and decreased apoptosis in RA-FLS (124). Compared with healthy controls (n=40), the expression of lncRNA PlncRNA-1 was downregulated in the serum and fibroblasts of active RA patients (persistent symptoms) (n=34), but not in inactive RA patients (long term of no or few symptoms after active RA) (n=36). In addition, PlncRNA-1 plays a central role in RA possibly by regulating on TGF-β1 expression (125). In summary, these lncRNAs may act as therapeutic targets for RA.

3.3 The Expression Profiles of LncRNAs in the Exosomes of RA

Recently, lncRNAs have been found to be enriched in exosomes (126), which can be released by almost all cells, and are present in bodily fluids, thus making them attractive targets for biomarker research (127). LncRNA NEAT1 was reported to be highly expressed in RA and PBMC-derived exosomes in patients with RA (n=5), that could contribute to the pathogenesis of RA through the delivery of lncRNA NEAT1. Furthermore, the study also highlighted that lncRNA NEAT1 shuttled by PBMC-derived exosomes plays a critical role in the development of RA by regulating the miR-23a/MDM2/SIRT6 axis (128). Subsequent studies have also shown that, compared with the exosomes from normal controls (n=20), there was a significant increase in the expression of NEAT1 in the exosomes of patients with RA (n=68).

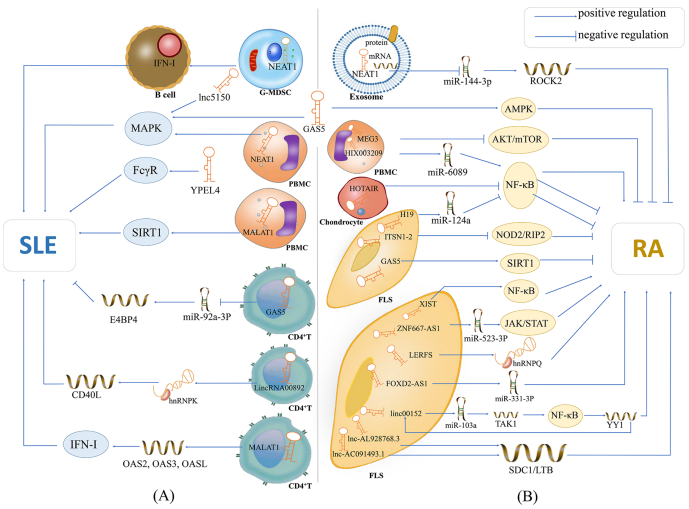

FIGURE 1 | The potential mechanisms of lncRNAs in SLE and RA. **(A)** lncRNA NEAT1 is overexpressed in G-MDSCs and induces the promotion of G-MDSCs on IFN-I signaling activation of B cells, contributing to the pathogenesis of SLE; lnc5150 and GAS5 in serum participate in the regulation of the MAPK signaling pathway, and promote the inflammatory response of SLE; lncRNA YPEL4 in serum promotes the onset of SLE through FcγR-mediated phagocytosis; lncRNA NEAT1 in PBMCs affects the expression of inflammatory mediators through activating the MAPK signaling pathway; lncRNA MALAT1 is overexpressed in PBMCs and can modulate the SIRT1 pathway directly, then promote the inflammatory response of SLE; lncRNA GAS5 in $CD4^+$ T cells can upregulate E4BP4 by inhibiting miR-92a-3p and attenuating the self-reactivity of $CD4^+$ T cells, playing a protective role in SLE; LincRNA00892 can activate $CD4^+$ T by targeting hnRNP K and subsequently up-regulating the expression of CD40L, thereby playing a pathogenic role in SLE; lncRNA MALAT1 in $CD4^+$ T cells can participate in type I interferon-mediated SLE by up-regulating OAS2, OAS3 and OASL. **(B)** lncRNA NEAT1 shuttled by PBMC-derived exosomes plays critical role in the development of RA by acting as a ceRNA for miR144-3p to restrict its function, and thus increase the expression of the miR144-3p-targeted gene ROCK2; lncRNA GAS5 in the serum of patients with RA activates the AMPK pathway; lncRNA MEG3 acts as ceRNA to inhibit inflammation by down-regulating AKT/mTOR signaling pathways; lncRNA HIX003209 in LPS-treated chondrocytes promotes the proliferation and activation of macrophages by modulating the inhibitory effect of the IκBα/NF-κB signaling pathway; lncRNA HOTAIR inhibits the activation of NF-κB in chondrocytes and reduce inflammation of RA; lncRNA-H19 acts as the ceRNA of miR-124a to inhibit the proliferation and invasion of RASF; lncRNA ITSN1-2 inhibits the NOD2/RIP2 signaling pathway and reduces the proliferation and inflammation of RA-FLS; GAS5 in FLS acts as a ceRNA to directly target miR-222-3p, upregulates the expression of Sirt1 and inhibits the proliferation and inflammation of RA; lncRNA XIST can inhibit the proliferation of SFs by promotion of of miR-126-3p/NF-κB pathway, thereby playing a protective role in RA; lncRNA ZNF667-AS1 is overpressed in RA-FLS, which plays a protective role in RA by sponging miR-523-3p and thus inactivation of JAK/STAT signaling pathway; LncRNA LERFS is lowly expressed in RA-FLS and can promote synovial aggression and joint destruction by interacting with hnRNP Q; lncRNA FOXD2-AS1 can promote the proliferation and invasion of RA-FLS through regulating the miR-331-3p/PIAS3 pathway; lncRNA linc00152 is up-regulate in RA-FLS, which can promote TAK1 expression by targeting miR-103a and thus activate the NF-κB pathway; lncRNA AL928768.3 and lnc-AC091493.1 can regulate their target mRNAs (e.g., SDC1, LTB), and thus implicate in the abnormal immune response of RA or promote the proliferation of FLS *via* multiple pathways in patients with RA. Also, transcription factor YY1 can promote linc00152 expression directly, and thus forming a linc00152/NF-κB feedback loop, which can promote RA-FLS inflammation.

Also, NEAT1 might act as a ceRNA for miR144-3p to restrict its function, and thus increase the expression of the miR144-3p-targeted gene (Rho associated coiled-coil containing protein kinase 2, ROCK2) in $CD4^+$ T cells, promoting the progression of RA (129). Another study showed that the levels of a set of lncRNAs, HOTAIR, Luca-15 Specific Transcript (LUST), anti-NOS2A, MEG, TUG1, NEAT1, Small Nucleolar RNA Host Gene 4 (SNHG4), Highly Accelerated Region 1B (HAR1B), and GAS5, have higher expression levels in seral exosomes of patients with RA (n=28) than in the seral exosomes of normal controls (n=10) (89). Hence, these molecules are likely to serve as biomarkers for RA. However, nowadays, little is known about the exact downstream signaling pathways of exosomal lncRNAs in modulating inflammatory response and autoimmunity. Further studies are warranted to fill this research gap.

4 THE SIMILARITIES AND DIFFERENCES IN LncRNAs BETWEEN SLE AND RA

Studies showed that some lncRNAs can regulate both SLE and RA, but the mechanisms involved are different. For example, GAS5 may participate in the pathogenesis of SLE through the MAPK pathway, but it regulates the progression of RA by activate the AMPK pathway (25, 94). Overexpressed NEAT1 in the G-MDSCs from the lupus murine model could lead to BAFF secretion and thus promote the activation of B cells so as to accelerate the progression of SLE, while the delivery of NEAT1 by PBMCs-derived exosomes could promote the development and progression of RA *via* the microRNA-23a/MDM2/SIRT6 axis (60, 128). However, apart from these similarities and differences in lncRNAs between SLE and RA, their function and molecular mechanisms are still not well understood. Although both diseases are closely related to autoimmune inflammation, different organs are involved in the pathogenesis of SLE and RA; in SLE kidneys, blood cells, skin, brain, heart, lungs, and joints are mainly affected (21), while RA commonly affects the joints in the hands, wrists, knees, etc. (130). Therefore, further studies are needed to reveal the similarities and differences between lncRNAs in SLE, RA, and also other autoimmune diseases. The lncRNAs implicated in SLE and RA are shown in **Table 1**.

5 FUTURE PERSPECTIVES

Recently, the studies focusing on investigating the role of lncRNAs in autoimmune diseases have significantly increased. However, the current studies are mainly focused on the possible role of lncRNAs as biomarkers, by screening their expression profiles in diagnostic data or by monitoring the activity of autoimmune diseases. Conversely, information on the role of their biological function and molecular mechanisms is still relatively scarce.

In addition to being potential biomarkers in SLE and RA, lncRNAs were found to participate in the modulation of the inflammatory and autoimmune responses, which are shown in **Figure 1**. However, the upstream regulatory mechanism of the abnormal expression of these lncRNAs in SLE and RA is still unclear, and there is a lack of studies addressing such question. Moreover, the downstream regulatory mechanism of these lncRNAs in SLE and RA still needs further investigation. These studies may greatly improve our understanding of the pathogenesis of human autoimmunity and provide novel therapies for autoimmune diseases.

AUTHOR CONTRIBUTIONS

HW, SXC, AFL, KYS, STW and SJW wrote the manuscript and designed the figure. PW, WL, and QP revised the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Humanized Mouse Models of Systemic Lupus Erythematosus: Opportunities and Challenges

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Animal models have played a crucial role in the understanding of the mechanisms and treatments of human diseases; however, owing to the large differences in genetic background and disease-specific characteristics, animal models cannot fully simulate the occurrence and progression of human diseases. Recently, humanized immune system mice, based on immunodeficient mice, have been developed that allow for the partial reconstruction of the human immune system and mimic the human *in vivo* microenvironment. Systemic lupus erythematosus (SLE) is a complex disease characterized by the loss of tolerance to autoantigens, overproduction of autoantibodies, and inflammation in multiple organ systems. The detailed immunological events that trigger the onset of clinical manifestations in patients with SLE are still not well known. Two methods have been adopted for the development of humanized SLE mice. They include transferring peripheral blood mononuclear cells from patients with SLE to immunodeficient mice or transferring human hematopoietic stem cells to immunodeficient mice followed by intraperitoneal injection with pristane to induce lupus. However, there are still several challenges to be overcome, such as how to improve the efficiency of reconstruction of the human B cell immune response, how to extend the lifespan and improve the survival rate of mice to extend the observation period, and how to improve the development of standardized commercialized models and use them. In summary, there are opportunities and challenges for the development of humanized mouse models of SLE, which will provide novel strategies for understanding the mechanisms and treatments of SLE.

Keywords: systemic lupus erythematosus, immunodeficient mouse, humanized SLE mouse, autoantibodies, pro-inflammatory cytokines, lupus nephritis

INTRODUCTION

Systemic lupus erythematosus (SLE) is a typical autoimmune disease characterized by excessive activation of T and B cells, producing a large number of autoantibodies and pro-inflammatory cytokines that result in tissue and organ damage (1). At present, there are few clinically approved traditional therapeutic drugs and biologic therapies for SLE (2, 3). Animal models have made great

contributions to the study of SLE pathogenesis and the development of new drugs. Based on the study of spontaneous (4–6) or induced (7–9) lupus-prone mouse model, considerable progress has been made in understanding the pathogenesis of SLE. In these models, disease phenotypes similar to patients with SLE can be observed, including the imbalanced immune responses of T and B cells, the production of a variety of autoantibodies and a large number of pro-inflammatory cytokines, and damage to multiple organs (such as lupus nephritis, etc.) (10). However, the genetic background differences between humans and mice cause the lupus-prone mouse model to have many differences from human SLE, especially when studying the *in vivo* functions of molecules with poor homology between humans and mice (such as non-coding RNA, etc.) (11–13) and Kv1.3 phenotype, etc. (14). The emergence of humanized mice allows for better studies *in vivo*, further clarifies the pathogenesis, and improves the success rate of translational medicine research (such as novel drug discovery, etc.) (15–17). At present, there are two main methods of constructing humanized mouse models of SLE, including transferring human peripheral blood mononuclear cells (PBMCs) or peripheral blood lymphocytes (PBLs) from patients with SLE to immunodeficient mice (18, 19), or transferring human hematopoietic stem cells (HSCs) to immunodeficient mice and then injecting intraperitoneally (*i. p.)* with pristane to induce lupus (20) (**Figure 1**). For these two humanized SLE mouse models, the PBLs/PBMCs humanized mouse model is widely used, but individual differences in SLE patients often lead to inconsistent model parameters and poor uniformity; the HSCs-pristane humanized mouse model can better reproduce the clinical features of human SLE, but there are very few such studies. The differences between two kinds of humanized SLE mice as shown in **Table 1**. The above two humanized SLE mouse models provide opportunities to study the pathogenesis and prevention of SLE *in vivo*, but there are also many challenges.

PBLS/PBMCS HUMANIZED SLE MOUSE MODEL

Development of PBLs/PBMCs Humanized SLE Mouse Model

The main characteristic of humanized mice is the reconstruction of the human immune system in immunodeficient mice. For DKO (BALB-Rag2$^{-/-}$ IL2Rgc$^{-/-}$) mice (4–5 weeks old) engrafted with PBMCs (0.3–0.5×10^{7}) from patients with SLE, the ratio of human CD45^{+} cells to total PBMCs increased from 5–10% (6–7 weeks old) to 20–80% (8–10 weeks old) (21).

It is known that T and B cells interact to promote the progression of lupus (35). T cells mainly promote the development of SLE through the production of pro-inflammatory cytokines and tissue infiltration (36). Humanized mouse models of SLE constructed by engrafting PBLs/PBMCs from patients with SLE have mainly revealed the presence of human T cells (21). A skewed ratio of CD4 to CD8 (lower frequency of CD4^{+} and higher CD8^{+} cells) in the PBMCs of patients with SLE is commonly observed (37, 38). In a humanized mouse model of SLE within 7–8 weeks by

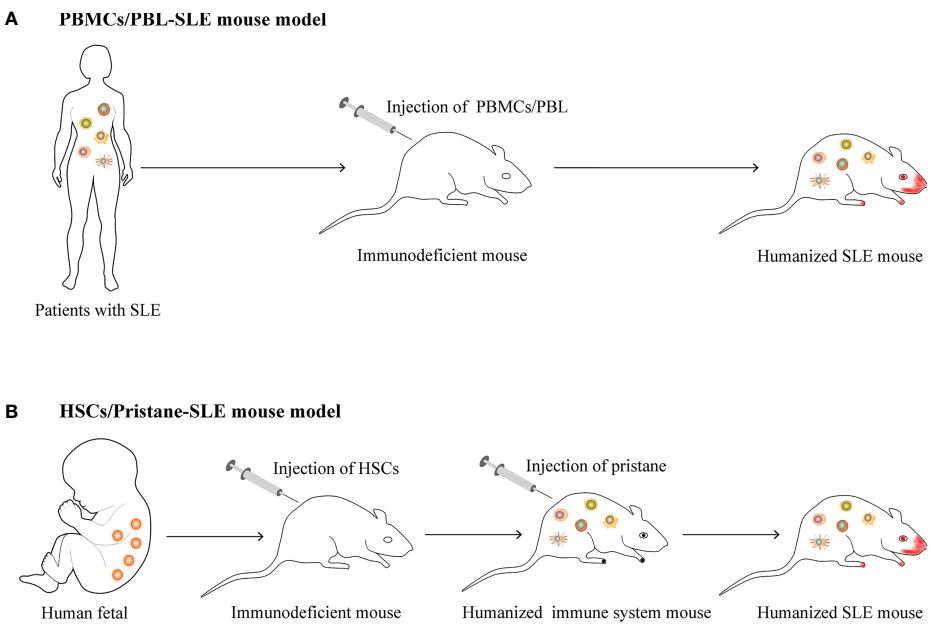

FIGURE 1 | The construction of humanized SLE mouse model. **(A)** Transferring human peripheral blood mononuclear cells (PBMCs) or peripheral blood lymphocytes (PBLs) from patients with SLE to immunodeficient mice. **(B)** Transferring human hematopoietic stem cells (HSCs) to immunodeficient mice and then injecting intraperitoneally (*i. p.*) with pristane to induce lupus.

TABLE 1 | The differences between two kinds of humanized SLE mice.

	PBLs/PBMCs humanized mouse model	HSCs-pristane humanized mouse model
Methods	PBLs/PBMCs from patients with SLE were injected intravenously or intraperitoneally into immunodeficient mice	Human HSCs were injected intravenously into immunodeficient mice followed by pristane intraperitoneally
Immune cells	Human $CD45^+$ cells accounted for 20–80% of peripheral blood (21) Human $CD4^+$ T cells ↓, $CD8^+$ T cells ↑ in peripheral blood (21, 22) Human IL-17^+ Tfh cells in spleen ↑ (23)	Human T cells, B cells, and NK cells in peripheral blood of mice ↓ (20) Human $CD19^+$ $CD20^-$ $CD27^{hi}$ $CD38^{hi}$ plasmablasts/plasma in peripheral blood and spleen of mice ↑ (20) Human $CD27^+$ memory B cells and $CD2^{7-}$ IgD^- B cells in peripheral blood and spleen of mice ↑ (20) Human $CD27^-$ IgD^+ naïve/transitional B cells in peripheral blood and spleen of mice ↓ (20)
Auto-antibodies	Human IgG ↑ (24, 25) Human IgG I ↑ (22) Human IgG II ↑ (26) Human IgG, IgA, IgM ↑ (27) Human anti-dsDNA↑ and mostly IgG I, IgG II (28) Human anti-dsDNA ↑ (16, 17, 23, 29–33) Human anti-Ro, anti-La, anti-RNP ↑ (34) Human anti-ssDNA, anti-RNA, anti-histone, anti-nucleosome ↑ (15)	Human anti-nuclear autoantibodies (anti-dsDNA, anti-histone, anti-RNP70, anti-SM, anti-SSA IgGs) ↑ (20)
Pro-inflammatory cytokines	Human IL-10 ↑ (27) Human IFN-γ, IL-4 ↑ (15) Human IFN-γ, IL-10 ↑ (16, 31) Human IFN-γ, IL-10 ↑, TGF-β ↓ (29)	Human IFN-γ, IL-6, IL-8, IL-18, MCP-1 ↑ (20)
Renal function	Proteinuria ↑ (14) Proteinuria ↑ and human IgG deposition in glomeruli (15, 21, 23, 32) Human IgG, IgA deposition in glomeruli (22) Human IgG, IgA, IgM deposition in glomeruli (17) Human IgG, IL-17A deposition in glomeruli (30) Human IgG, C3 deposition in glomeruli (29)	Proteinuria ↑ (20) Human $CD45^+$ cells, IgG, and IgM deposition in glomeruli (20)
Survival rates	Survival rates ↓ (14, 21, 25, 26)	Median survival at 13 weeks (20)

"↑/↓" in PBLs/PBMCs humanized mouse models represent an increase or decrease compared to healthy PBLs/PBMCs controls.
"↑/↓" in HSCs-pristane humanized mouse models represent an increase or decrease compared to no pristane controls.

engrafting PBMCs ($0.3–0.5\times10^7$) from patients with SLE, $CD3^+$ cells were found in the $CD45^+$cells of PBMCs from both SLE-DKO and ND-DKO mice. While in SLE-DKO mice, a significantly lower frequency of $CD3^+CD4^+$cells (5.5% ± 2.1%) and a higher frequency of $CD3^+CD8^+$ cells (79.4 ± 3.6%) was reported; this contrasted with a more typical distribution of $CD3^+CD4^+$ (66.2 ± 2.5%) and $CD3^+CD8^+$ (16.5 ± 2.1%) cells in the ND-DKO mice (21). In addition, a similar study showed that among T cells, the ratio of $CD4^+CD8^-$ cells to $CD4^-CD8^+$cells were 3:1, and 1:2 at one- and two-months post engraftment, respectively (22). This skewed distribution could also be detected in humanized SLE mice, which supports the hypothesis that this model mimics the characteristics of human SLE.

Previous studies have demonstrated that the effective B cell helper activity of Th17 cells was an important function of pro-inflammatory T cells (39, 40). The increased percentage of human IL-17^+ Tfh cells was detected in the spleens of NSG mice (8 weeks old) engrafted with PBMCs (1×10^7cells/mouse) from patients with active lupus, while this process could be halted by the knockdown of RORγ in human $CD4^+$ T cells (23). In this instance, Th17 could also be detected in humanized SLE mice, and RORγ therapy targeting $CD4^+$ T cells is expected to become a novel strategy.

In SLE, B cells mainly play a role in antibody production, antigen presentation, and cytokine expression (41). NK cells are producers of various cytokines and chemokines (e.g., IFN-g, TNF-a, CCL5, CCL3, and CCL4), which amplify and recruit an inflammatory response through various mechanisms, further contributing to the progression of SLE (42). Myeloid cells (e.g., neutrophils, dendritic cells) have also been reported to be key factors in SLE (43, 44). However, the reconstruction efficiency of B, NK, and myeloid cells in the PBLs/PBMCs humanized SLE mouse model is not satisfactory and remains a huge challenge today. In this mice model, it was found that human $CD3^+$ populations were detected in the $CD45^+$cells in PBMCs of SLE-DKO mice at 3–4 weeks post engraftment, while other human immune cells such as B cells, NK cells, and myeloid cells were rare or undetectable (21). Therefore, improving the reconstruction efficiency of B cells, NK cells, and myeloid cells is a significant challenge to develop this model better.

The success of reconstruction in humanized SLE mice with engraftment of human PBLs/PBMCs can also be assessed based on the expression of human IgG, with successful engraftment indicated by equal to or higher than 200 μg/mL of human IgG in the sera two weeks following PBLs/PBMC administration (28). As previously reported, the average serum level of human IgG was approximately 3000 μg/mL post-PBLs (3×10^7 cells/mouse) from patients with SLE were injected *i.p.* into SCID mice (8–10 weeks old) (28). A similar study showed that approximately 500 μg/mL of human IgG in the serum of SCID mice (9–13 weeks old) engrafted with PBMCs (3×10^7 cells/mouse) from patients with SLE could be detected (29).

The ratio of human CD4/CD8 also can affect the production of IgG in humanized SLE mice. When this ratio increased from a

lower ratio (less than 0.5) to a higher ratio (greater than 1.5), serum levels of human IgG could be detected (24). In addition, *in vitro* activation of human PBMCs also led to ten times higher IgG production *in vivo* compared with PBMCs without activation (24). Therefore, for the variation in human IgG production in humanized mice, the key effector may be the phenotype and activation status of human PBLs/PBMCs.

For the autoantibodies produced by this PBLs/PBMCs humanized SLE mouse model, several main autoantigens (e.g., dsDNA, Ro, RNP, anti-La, etc.), and subtypes of IgGs (e.g., IgG I, IgG II) can be detected. Human IgG could be detected in the serum of established humanized SLE mice at two weeks and reached maximum levels at two months after the reconstruction of the model with *i.p.* injection of PBLs (1.5×10^7 cells/mouse) from patients with SLE into SCID mice (22).

Anti-dsDNA autoantibodies are representative autoantibodies for the diagnosis and modeling of the disease activity of SLE (45). A previous study showed that higher levels of human IgG I and IgG II anti-dsDNA autoantibodies were detected in the serum of humanized SLE mice (10–12 weeks old), after the establishment of this model with *i.p.* injection of PBMCs (3×10^7 cells/mouse) from patients with SLE (SLEDAI score 5.88 ± 4.18) into SCID mice (8–10 weeks) (28).

In addition, humanized SLE mice produce autoantibodies against other autoantigens. After the establishment of humanized SLE mice with *i.p.* injection of $2–5\times10^7$ PBMCs from patients with SLE into SCID mice (5–7 weeks old), antibodies against human anti-Ro, anti-RNP, and anti-La in serum could be detected at 4–6 weeks after transplantation (34). DKO mice (4–5 weeks old) were used for engraftment of ($0.3–0.5\times10^7$ cells/mouse) PBMCs from patients with SLE, and the antibodies of humans (e.g., anti-dsDNA antibody, ANA antibodies, ACL IgG) in serum could be detected at 4–8 weeks post engraftment. Importantly, SLE patients with a high level of antiphospholipid antibodies (>80 GCL) showed high ACL IgG levels in all DKO mice engrafted with their PBMCs. Additionally, all mice had detectable ACL IgG at two to three different times within two to four weeks post engraftment (21). Thus, the engrafted mice showed most of the antibodies in patients with SLE and reflected an accurate phenocopy of certain autoantibodies.

Treatment of PBLs/PBMCs Humanized SLE Mouse Model

Autoantibodies and Pro-Inflammatory Cytokines

In terms of intervention studies, the production of autoantibodies and pro-inflammatory cytokines in humanized SLE mice can also be attenuated or eliminated by drugs or other factors.

The protein annexin A1 (ANX A1) is a modulator of the immune response involving several cell types, and its expression in activated B and T cells is abnormal in autoimmune disease (46–48). In one study, the levels of autoantibodies s (e.g., anti-ssDNA, anti-RNA, anti-histone, and anti-nucleosome IgG), inflammatory cytokines (e.g., IFN-γ and IL-4), and disease symptoms were significantly reduced in anti-ANX A1 antibody-treated humanized SLE mice (12 weeks old NSG mice engrafted with 1×10^7 PBMCs/mouse from patients with SLE), compared to the humanized SLE mice treated with the isotype control antibody (15).

Myeloid-derived suppressor cells (MDSCs) with immunosuppressive functions are a group of highly heterogeneous populations derived from myeloid progenitors (49). It has been reported that MDSCs have a pathogenic role in promoting the development of autoimmune diseases (50–52). For example, mouse MDSCs can promote the differentiation of Th17 cells (53). However, the role of MDSCs in Th17 differentiation and the pathogenesis of autoimmune diseases in humans is relatively unknown. In a previous study, a humanized SLE mouse model was established by intravenous injection of PBMCs from patients with active SLE into immunodeficient non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice. To investigate the function of MDSCs and Arg-1 in disease progression, the NOD/SCID mice were injected with unaltered PBMCs, MDSC-depleted PBMCs, or unaltered PBMCs plus nor-NOHA (the Arg-1 inhibitor). The study showed that all mice (4–5 weeks old NOD/SCID mice engrafted with $0.5–1\times10^7$ PBMCs/mouse from patients with SLE) injected with unaltered PBMCs had detectable human autoantibodies within four to five weeks (30). However, mice receiving MDSC-depleted PBMCs showed significantly less severe symptoms, indicating that MDSCs are necessary for disease progression *in vivo*. In addition, the deleterious role of MDSCs was possibly dependent on Arg-1, because its inhibitor significantly delayed disease progression in NOD/SCID mice (30). The above research indicates that targeting MDSCs or Arg-1 is expected to alleviate SLE disease progression.

Based on the suppressive activity of complement receptor type 1 on human lymphocytes, the co-crosslinking of this receptor on B cells with the B-cell receptor (BCR) can inhibit the activation and proliferation of B cells, and this receptor may be a novel therapeutic target for negative signal delivery (54, 55). Humanized SLE mice (8 weeks old SCID mice engrafted with 1×10^7 PBMCs/mouse from patients with SLE) were treated with anti-human DNA-like chimeras, which contained a monoclonal antibody against human inhibitory complement receptor type 1. The results showed that anti-dsDNA antibodies were directly eliminated. The specific clearance of autoreactive B cells not only limited the production of anti-dsDNA IgG, but also limited the activation and proliferation of autoreactive T cells. Additionally, the levels of pro-inflammatory cytokines IL-10 and IFN-γ were also reduced (16, 31). The same study showed that anti-human DNA-like chimeras could prevent the production of anti-dsDNA IgG antibodies (32). Anti-human DNA-like chimeras had an ideal therapeutic effect in humanized SLE mice, and they are expected to enter clinical research as a drug.

Two synthesized peptides (based on the sequence of CDR1 and CDR3 of the pathogenic murine anti-DNA 16/6Id) were reported to be immunodominant T cell epitopes in normal (e.g., BALB/c, SJL) and lupus-prone (NZB×NZW) F_1 mice (56–58). Treatment with these peptides improved clinical symptoms and decreased autoantibody production in spontaneous and induced SLE (59–61). Treatment with hCDR1 significantly decreased the serum levels of human anti-dsDNA antibodies and decreased the

serum levels of IFN-γ and IL-10, while increasing TGF-β production in humanized SLE mice (8–10 weeks old SCID mice engrafted with 3×10^7 PBLs/mouse from patients with SLE) (29). However, this treatment did not affect anti-tetanus toxoid antibodies. Therefore, the effect of hCDR1 treatment may be restricted to SLE-associated responses, and the hCDR1 peptide is a potential novel candidate for SLE treatment.

One potential therapeutic strategy for SLE is antisense/ribozyme, which specifically inhibits the expression of the target mRNA without severe side effects (62, 63). In a study, humanized SLE mice (SCID mice engrafted with 0.5×10^7 PBLs/mouse from patients with SLE) treatment with the chemically modified ribozyme (RZ-I) not only decreased anti-DNA antibody production in these humanized SLE mice but also inhibited IgG deposition in the kidneys of these mice (17). Therefore, a novel therapeutic strategy for SLE may be based on the usefulness of chemically modified ribozymes.

Whether the delivery of IL-2 and TGF-β, which are deficient in SLE, mediated by nanoparticles (NPs) to mouse $CD2^+$ and $CD4^+$ cells, could induce a tolerogenic immune response and then protect mice from a lupus-like disorder was investigated (64, 65). Humanized SLE mice (8–12 weeks old NSG mice engrafted with 1×10^7 PBMCs/mouse from patients with SLE) treated with T cell-targeted NPs loaded with IL-2/TGF-β showed significantly reduced serum levels of human IgG and improved skin morphology (25). Therefore, NPs may provide a novel therapeutic strategy *in vivo* for the suppression of proinflammatory responses in SLE and other autoimmune diseases.

In another study, the binding of XmAb5871 (the Fc domain of one anti-human CD19 antibody) with FcγRIIb promoted the engagement of FcγRIIb with the BCR complex (66). This antibody stimulated phosphorylation of the ITIM of FcγRIIb and suppressed BCR-induced calcium mobilization. It also allowed for the proliferation of human B cells, costimulatory molecule expression on B cells from healthy persons and patients with SLE, as well as the proliferation of B cells induced by LPS, IL-4, or B cell-activating factor (BAFF) (67). Another study involved anti-XmAb5871 treatment performed on humanized SLE mice (6–12 weeks old SCID mice engrafted with $1–3\times10^7$ PBMCs/mouse from patients with SLE). It was found that anti-XmAb5871 inhibited the activation of B cells and the total human IgG2 level (26). In addition, anti-XmAb5871 substantially inhibited anti-tetanus titer *in vivo* (26). Thus, anti-XmAb5871 should be considered a novel B cell-targeted immunosuppressive therapeutic strategy for SLE.

AS101 as an immunomodulator can significantly decrease serum levels of human IgG, IgA, IgM (e.g., anti-dsDNA IgG, anti-Sm IgG), and IL-10 in humanized SLE mice (SCID mice engrafted with 1.5×10^7 PBMCs/mouse from patients with SLE) (27).

In addition, treatment of humanized SLE mice (6–10 weeks old SCID mice engrafted with 1.5×10^7 PBMCs/mouse from patients with SLE) with an anti-IL-6 monoclonal antibody inconsistently decreased the serum concentration of anti-dsDNA IgG produced by PBMCs from patients with SLE. In contrast, administration of an anti-IL-10 monoclonal antibody consistently decreased autoantibodies produced by SLE PBMCs (33).

Lupus Nephritis

The kidney is one of the most involved organs in SLE (lupus nephritis) (68). Approximately 50% of patients with SLE have clinical renal involvement with lupus nephritis (69), and humanized SLE mice also show similar renal disease. SLE-DKO mice have mild proteinuria at 4–6 weeks after implantation of PBMCs ($0.3–0.5\times10^7$) from patients with SLE and human IgG deposits in the glomeruli, and the glomeruli were enlarged, showing severe capillary thrombosis and endothelial cell necrosis. Multifocal acute tubular necrosis with hyaline casts was also observed (21). The overall appearance of the kidney was similar to that of a human lupus class IV-G proliferative nephritis. It has also been reported that 1.5×10^7 PBLs of patients with SLE were injected *i.p.* into SCID mice. The kidney tissue showed that human IgA and IgG were granular and circularly deposited along the mesangium and capillaries, and proteinuria occurred (14, 22). It can be seen that the humanized mice modeled by the PBMCs of patients with SLE also displayed kidney lesions, which were similar to spontaneous and induced mouse models and are closer to clinical patients.

The intervention of humanized lupus mice can reduce pathological changes in their kidneys. Anti-ANX A1 treatment of humanized SLE mice (12 weeks old NSG mice engrafted with 1×10^7 PBMCs/mouse from patients with SLE) reduced the proteinuria of the mice, significantly reduced cell infiltration in the kidney, and no immune complex deposition was observed (15). NOD/SCID mice receiving MDSC-depleted PBMCs showed a substantial decrease in proteinuria levels, IL-17A, and human IgG deposition in glomeruli and mesangial cell proliferation (30). The proteinuria level of humanized SLE mice (8–10 weeks old SCID mice engrafted with 3×10^7 PBLs/mouse from patients with SLE) was significantly reduced after hCDR1 treatment; however, IgG and C3 deposits in the kidney sections were detected in only one (6%) in 17 mice treated with hCDR1 (29). Treatment with RZ-I reduced the level of proteinuria, inhibited the production of anti-DNA, and there was no glomerular IgG, IgM, or IgA deposition in humanized SLE mice (SCID mice engrafted with 0.5×10^7 PBLs/mouse from patients with SLE) (17). Targeting immunogenic self-DNA-specific Tfh cells through human RORγ knockdown in $CD4^+$ T cells and IL-17 neutralization effectively eliminated the levels of kidney inflammation, IgG deposition, and proteinuria in humanized SLE mice (8 weeks old NSG mice engrafted with 1×10^7 PBMCs/mouse from patients with SLE) (23). In anti-human DNA-like chimera treatment, this has also been proven to considerably reduce immune complex deposition and improve kidney disease (16, 31, 32). The application of humanized lupus mice has allowed for the increase in attempts to treat lupus nephritis and has guided researchers in the clinical development of new drugs and treatment measures.

Lifespan and Survival Rates

Immunodeficient mice transplanted with PBMCs from patients with SLE generally die spontaneously after four weeks. In contrast, the survival rate of mice modeled with normal human PBMCs was significantly higher than that of lupus patients (21).

It has been reported that specific treatment of humanized SLE mice can improve their survival rate. PBMCs from patients with lupus nephritis were pretreated with Kv1.3-NPs and then transferred 0.8×10^7 PBMCs into 6–10 weeks old NSG mice. It was found that this pretreatment increased the survival rate of PBMC-humanized mice with lupus nephritis by 66% compared with those in the non-treated PBMCs group (14). Pretreated T cells with NPs loaded with IL-2/TGF-β further improved the survival rate of humanized SLE mice (8–12 weeks old NSG mice engrafted with 1×10^7 PBMCs/mouse from patients with SLE) compared to those of the non-treated T cell group (25). Treatment with an anti-XmAb5871 antibody inhibited the activation of B cells in humanized SLE mice (6–12 weeks old SCID mice engrafted with $1–3\times10^7$ PBMCs/mouse from patients with SLE), and significantly improved the survival rate compared with non-treated mice (26). Therefore, further studies are required to extend the lifespan and improve the survival rate of humanized SLE mice.

In summary, specific intervention for humanized SLE mice can significantly reduce the levels of autoantibodies and pro-inflammatory cytokines, improve renal function, and prolong the life span (**Table 2**).

HSCS-PRISTANE HUMANIZED SLE MOUSE MODEL

In the HSCs-pristane humanized SLE mouse model, NSG mice (within three days after birth) were sublethally irradiated with 1 Gy γ-rays first and then transplanted with human $CD34^+$ HSCs (1×10^5 cells/mouse) by intra-hepatic injections. The results showed that these humanized mice consistently achieved a good reconstitution of the human immune system, with reconstitution levels in the blood (42.1%), and higher levels in the tissues at 12 weeks, including the spleen (82.8%), mesenteric lymph nodes (97.4%), and liver (89.0%). Subsequently, pristane was injected

TABLE 2 | The therapeutic effect on humanized SLE mice.

Treatment	Immunodeficient mice	Age of the mice	Number of cells	Inclusion criteria	Results
Anti-annexin A_1 antibody (15)	NSG mice	12 weeks old	1×10^7 PBMCs	Positive anti-nuclear autoantibodies (ANA), positive IgG autoantibodies against dsDNA and proteinuria	Human anti-ssDNA, anti-RNA, anti-histone, anti-nucleosome ↓ Human IFN-γ, IL-4 ↓ Proteinuria ↓ Human IgG deposition in glomeruli ↓
MDSC-depleted PBMCs (30)	NOD/SCID mice	4–5 weeks old	$0.5–1\times10^7$ PBMCs	SLE patients with active disease (SLEDAI, 9; dsDNA, 1:10) and lupus nephritis	Human anti-dsDNA ↓ Proteinuria ↓ Human IgG, IL-17A deposition in glomeruli ↓ Renal mesangial cell proliferation ↓
DNA-like chimera (16)	SCID mice	8 weeks old	1×10^7 PBMCs	At least four ARA (American Rheumatism Association) criteria for SLE, combined with high titers of anti-nuclear and anti-dsDNA IgG antibodies	Human anti-dsDNA ↓ Human IFN-γ, IL-10 ↓ Human IgG deposition in glomeruli ↓ Human T cell activation ↓
hCDR1 (29)	SCID mice	8–10 weeks old	3×10^7 PBLs	the disease activity index (SLEDAI) was between 2 and 14 (mean 5.7 ± 5.12)	Human anti-dsDNA ↓ Human IFN-γ, IL-10↓, TGF-β ↑ Proteinuria ↓ Human IgG, C3 deposition in glomeruli ↓
RZ-I (17)	SCID mice	\	0.5×10^7 PBLs	Patients diagnosed with active lupus nephritis or those with inactive SLE	Human anti-dsDNA ↓ Proteinuria ↓ Human IgG deposition in glomeruli ↓
(anti-CD3 AB-) T-cell targeted NPs encapsulating IL-2/TGF-β (25)	NSG mice	8–12 weeks old	1×10^7 PBMCs	\	Human IgG ↓ Improve skin shape Survival rates ↑
XmAb5871 (26)	SCID mice	6–12 weeks old	$1–3\times10^7$ PBMCs	the Safety of Estrogens in Lupus Erythematosus National Assessment SLE disease activity index	Human IgG II ↓ Human anti-tetanus titer ↓ Survival rates ↑
AS101 (27)	SCID mice	\	1.5×10^7 PBMCs	the American Rheumatology Association criteria for SLE	Human IgG, IgA, IgM (e.g., anti-dsDNA IgG, anti-Sm IgG) ↓ Human IL-10 ↓
IL-10 mAb, IL-6 mAb (33)	SCID mice	6–10 weeks old	1.5×10^7 PBMCs	the American Rheumatology Association criteria for SLE	Human anti-dsDNA ↓ (IL-10 mAb was more effective than IL-6 mAb
ROR knockdown in $CD4^+$ T cells or IL-17 neutralization (23)	NSG mice	8 weeks old	1×10^7 PBMCs	Patients with new onset and untreated SLE (mean ± SD age 29.1 ± 12.6 years) who did not have other autoimmune diseases or infectious	Human anti-dsDNA ↓ Proteinuria↓ Human IgG deposition in glomeruli ↓
Kv1.3-NPs (14)	NSG mice	8–12 weeks old	0.8×10^7 PBMCs	Positive diagnosis for lupus nephritis	Survival improved by 66%

"↑/↓" in humanized mouse models of SLE represent an increase or decrease compared to healthy PBLs/PBMCs controls.

i.p. into 12–13 weeks old humanized NSG mice and normal NSG mice. Pristane injection induced the hyperactivation of B cells, as shown by the increased expression of CD86, a B cell activation marker. In addition, the percentage and absolute number of $CD19^+CD20^-CD27^{hi}CD38^{hi}$ plasmablasts/plasma cells in the peripheral blood and spleen of pristane-injected humanized NSG mice. Moreover, a relative expansion in the percentage of $CD27^+$ memory B cells and $CD27^-IgD^-$ B cell populations and a reduction in the $CD27^-IgD^+$ naïve/transitional B cell compartment were found in these pristane-injected humanized mice. Finally, pristane-injected humanized mice showed the activation of both $CD4^+$ and $CD8^+$ T cells, a marked reduction in both $CD4^+$ and $CD8^+$ T cells with a naïve phenotype, and an increased percentage of T cells with an effector memory phenotype in the peripheral blood, spleen, mesenteric lymph nodes, and peritoneal lavage, indicating a systemic proinflammatory condition (20). For the production of autoantibodies and pro-inflammatory cytokines by these pristane-injected humanized mice, the total levels of human IgG and IgM and human anti-nuclear autoantibodies (e.g., anti-dsDNA antibody, anti-histone antibody, anti-RNP70 antibody) were detected. In particular, human anti-dsDNA IgG can be detected as early as four weeks after the injection of pristane and gradually increased to eight weeks. The serum levels of human pro-inflammatory cytokines (e.g., IFN-γ, IL-8, IL-6) also increased significantly in the plasma and peritoneal lavage fluid (20). Lupus nephritis is the most severe manifestation of organ involvement in patients with SLE (70). It is characterized by the deposition of immune complexes in the glomerulus and infiltration of leukocytes, leading to proteinuria (71). In the upper pristane-injected humanized mice, focal to diffuse global glomerular enlargement by mesangial/endocapillary proliferation and increased glomerular cellularity and human $CD45^+$ cells in the glomeruli were reported. All of these were not observed in NSG mice injected with pristine alone (20). For lung injury, the upper pristane-injected humanized mice showed increased multifocal serosal and subpleural inflammation with fibrosis, as well as perivascular interstitial and intra-alveolar mononuclear cell infiltrate (20). For the survival rate, the upper pristane-injected humanized mice showed significantly earlier mortality (median survival at 13 weeks) after pristane injection. NSG mice injected with pristine alone appeared healthy, and there was no mortality during the observation period (20 weeks after pristane injection) (20).

The above HSCs-pristane humanized SLE mouse model provided another strategy for the development of a humanized SLE mouse model. This model is more consistent with the clinical characteristics of SLE patients and reflects the interaction of various immune cells, which is an ideal mouse lupus model. At present, there are few intervention studies based on this model, and more follow-up studies are needed to confirm its stability and clinical value.

THE IMPROVEMENT OF HUMANIZED SLE MOUSE MODEL

In the development of humanized SLE mice, PBLs/PBMCs humanized SLE mouse models are widely used, but individual differences in patients with SLE often lead to inconsistent parameters and poor uniformity. This model can better study human T cells, but the effect of human B cell reconstruction is poor, the level of human NK cells is low, and the differentiation of human myeloid cells is lacking.

Regarding the poor reconstruction of human B cells, the reason may be that human T cells proliferate too fast, and the proportion of human B cells decreases as time increases (72). In addition, some reports have shown that certain proteins related to B cell survival showed weak cross-reaction between mice and humans, and there was a lack of signal supporting human B cell survival in mice (73). It has been reported that the lentiviral vector carrying the human IL-7 gene was overexpressed in $Rag2^{-/-}\gamma C^{-/-}$ mice, and the serum level of human IL-7 in mice was maintained at a high level during the observation period of six months. Overexpression of human IL-7 significantly increased the proportion of T and B cells in peripheral blood (74). It has also been reported that the proportion of human B cells can be increased by injecting recombinant human BLyS protein into humanized mice (73).

The low level of human NK cells in humanized mice may be due to the lack of relevant cytokines that support the survival of human NK cells in mice, resulting in a short survival time (72). To solve this problem, a study was conducted involving human IL-15 and Flt3l vectors that were injected into humanized mice, and it was found that the NK cell reconstruction level was significantly improved (75). In addition, the induced human NK cells normally express both activation and inhibition, causing NK cell-dependent liver damage and having the ability to kill target cells *in vitro*. The above results indicate that the reconstructed human NK cells were functional (75).

Regarding the problem of poor myeloid differentiation, it has been reported that human neutrophils, monocytes, and dendritic cells (DCs) were significantly increased after the injection of human G-CSF into NOG mice (76). Similarly, NOD/SCID mice were injected with human SCF, IL-3, GM-CSF, and TPO for two weeks, and the development of lymphocytes and myeloid cells was significantly improved (77). The injection of human FLT3L in NOD/SCID mice significantly increased the number and function of DCs (78). In addition, Nsg-sgm3 mice were constructed using a transgenic technique to express human SCF, GM-CSF, and IL-3. The results showed that the reconstruction level of myeloid cells was significantly improved, especially in DCs (79).

Another major challenge is that although PBLs/PBMCs humanized SLE mouse model can better simulate the clinical characteristics of patients with SLE, their lifespan and survival rate are significantly lower than those of spontaneous or induced lupus-prone mouse models, which may lead to a narrow period for observation or treatment. One study found immunodeficient mice transplanted with high lupus activity PBMC had a low survival rate and transplanted with low lupus activity PBMC had a high survival rate (21). Therefore, in future research, determining a consistent standard and unifying it is an important direction to better construct a humanized SLE mouse model.

Another HSCs-pristane humanized SLE mouse model irradiates mice before modeling. This can provide more "space" for humanized construction through irradiation or pretreatment

TABLE 3 | The improvement of humanized SLE mice.

Treatment	Results
Irradiation	2~3 Gy pre-irradiation → Mouse immune system ↓ (80)
Chemical reagent	CD122 antibody or IL-2R antibody → Mouse NK cells ↓ (80) Cl2MDP → Mouse macrophages ↓ (81)
Cytokines	Human G-CSF → Human neutrophils, monocytes, and dendritic cells ↑ (76) Human SCF, IL-3, GMCSF, TPO → Human lymphocytes and myeloid cells ↑ (77) Human FLT3L → Human dendritic cells ↑ (78)
Proteins	Recombinant human BLyS protein → Human B cells ↑ (73)
Viral vector	Lentiviral vectors overexpress human IL-7 → Human T cells and B cells ↑ (74)
Gene expression plasmid	Human IL-15 and Flt3l gene expression plasmid → Human NK cells ↑ (75)
Genetic engineering	Human SCF, GM-CSF and IL-3 gene knockin → Myeloid cells (especially dendritic cells) ↑ (79)

"↑/↓" in humanized mouse models represent an increase or decrease compared to non-intervention control.

with chemical reagents. A previous study compared the efficiency of transplantation with irradiation and found that human immune cells could survive better by pre-radiation 2–3 Gy to NOD/SCID mice before injection of human HSCs (80). It has also been found that mouse NK cells could be knocked out using CD122 or IL-2R antibodies (80). Cl2MDP can knock out mouse macrophages and obtain a better reconstruction of the human immune system (81). This modeling method will theoretically better reproduce the clinical features of human SLE, but there are still few research reports.

Based on the above, the treatment of humanized mice can significantly increase the number of human B, NK, and myeloid cells, and better reconstruct the human immune system (**Table 3**). However, these interventions have rarely been used in humanized SLE mice. It can be seen that the humanized SLE mouse model still has a long way to go.

FUTURE PERSPECTIVES

Currently, the pathogenesis of SLE is still not well known, and the clinically approved traditional therapeutic drugs, as well as biologic therapies for SLE are still very few. The successful development of a humanized SLE mouse model has provided a new path for the study of SLE. However, there are still many challenges to overcome, such as how to better reconstruct the B-cell immune response and how to extend the lifespan and survival rate of mice to extend the period of medical treatment. In summary, to further improve humanized SLE mouse models and develop standardized or even commercialized models, these models can better clarify the pathogenesis of SLE and provide new strategies for the prevention and treatment of SLE, especially the development of new drugs.

AUTHOR CONTRIBUTIONS

JC, SL, and HZ wrote the manuscript and designed the figures. LY, FG, SC, AL, QRP, CY, H-fL, and QJP revised the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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The Interactions Between Autoinflammation and Type 2 Immunity: From Mechanistic Studies to Epidemiologic Associations

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Autoinflammatory diseases are a group of clinical syndromes characterized by constitutive overactivation of innate immune pathways. This results in increased production of or responses to monocyte- and neutrophil-derived cytokines such as interleukin-1β (IL-1β), Tumor Necrosis Factor-α (TNF-α), and Type 1 interferon (IFN). By contrast, clinical allergy is caused by dysregulated type 2 immunity, which is characterized by expansion of T helper 2 (Th2) cells and eosinophils, as well as overproduction of the associated cytokines IL-4, IL-5, IL-9, and IL-13. Traditionally, type 2 immune cells and autoinflammatory effectors were thought to counter-regulate each other. However, an expanding body of evidence suggests that, in some contexts, autoinflammatory pathways and cytokines may potentiate type 2 immune responses. Conversely, type 2 immune cells and cytokines can regulate autoinflammatory responses in complex and context-dependent manners. Here, we introduce the concepts of autoinflammation and type 2 immunity. We proceed to review the mechanisms by which autoinflammatory and type 2 immune responses can modulate each other. Finally, we discuss the epidemiology of type 2 immunity and clinical allergy in several monogenic and complex autoinflammatory diseases. In the future, these interactions between type 2 immunity and autoinflammation may help to expand the spectrum of autoinflammation and to guide the management of patients with various autoinflammatory and allergic diseases.

Keywords: autoinflammation, autoinflammatory diseases (AID), allergy, type 2 immune response, type 2 immunity

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INTRODUCTION

Diseases of immune dysregulation affect up to 40% of the global population and can have devastating consequences including organ failure and death (1, 2). Conceptually, disorders of immune activation are divided into three major categories. Autoimmune diseases are caused by inappropriate antigen-specific immune responses to self-antigens, and inflammation is largely promoted by lymphocytes (3). Allergic diseases are also mediated by inappropriate activation of lymphocytes, but the immune responses are against foreign antigens, or allergens (4, 5). By contrast, autoinflammatory diseases are caused by activated myeloid cells that mediate antigen-independent innate immune pathology (3). Although this is a useful conceptual framework, many autoimmune

diseases are driven by a combination of innate and adaptive immune dysregulation (6, 7). The role of autoinflammatory pathways in autoimmune diseases has become a major area of investigation, uncovering novel interactions between innate and adaptive immunity (6, 7).

While the boundaries between autoimmunity and autoinflammation have become less clear over time, less work has been done on the intersection of allergy and autoinflammation. In general, autoimmune and autoinflammatory responses have been thought to primarily repress allergic inflammation, and vice versa (8). This is largely due to the Th1-Th2 (T helper 1 – T helper 2) paradigm, where Th1 and Th2 cells have counterregulatory roles. Th1 cells are associated with Type 1 immune responses, which are also characterized by activated myeloid lineage cells, and which are associated with autoimmunity and autoinflammation (9). However, over the past several decades it has become clear that autoinflammatory-associated cytokines and pathways can promote allergy-associated type 2 immune responses (5, 10). In this review, we explore the interactions between autoinflammation and type 2, or allergy-associated, inflammation. We begin by providing a brief overview of autoinflammation and type 2 inflammation, including the human diseases associated with both immune responses. We then review the role of autoinflammation-associated cytokines and pathways in type 2 responses, and the role of type 2 immune factors in autoinflammation. Finally, we summarize results from studies exploring the prevalence of type 2 clinical and immunologic phenotypes in patients with monogenic and complex autoinflammatory diseases.

PART 1: AN OVERVIEW OF AUTOINFLAMMATION AND TYPE 2 INFLAMMATION

Autoinflammation Results From Inappropriate Innate Immune Activation

The concept of autoinflammatory disease was coined in 1999 to describe a group of immune dysregulatory diseases characterized by recurrent episodes of fever and systemic inflammation. In contrast to autoimmune diseases, autoinflammatory disorders are typified by constitutive activation of myeloid cells rather than antigen-specific T cell or B cell responses (3). Given the central role of myeloid cells in the innate arm of immune responses, the concept of "autoinflammation" was subsequently broadened to characterize primary disorders of the innate immune system. This approach was further advanced by the discovery of monogenic autoinflammatory diseases caused by mutations in genes critical for innate immune function (11–16).

One useful framework for characterizing monogenic autoinflammatory diseases is by the innate immunologic pathways that are dysregulated by disease-causing mutations. Many autoinflammation-associated genes are critical to the inflammasome and IL-1β production pathway (**Figure 1**). This includes the *MEFV* gene, which causes the prototypical autoinflammatory disease Familial Mediterranean Fever (FMF). Other examples of inflammasome-regulating genes and associated autoinflammatory diseases include *MVK* (hyper-IgD syndrome; HIDS), *NLRP3* (Cryopyrin-associated periodic fever syndrome; CAPS), *PSTPIP1* (Pyogenic arthritis with pyoderma gangrenosum and acne; PAPA), *WDR1* (periodic fever, immunodeficiency, and thrombocytopenia; PFIT), *IL1RA* (Deficiency of IL-1RA; DIRA), and *NLRC4* (Macrophage activation syndrome; MAS). Inflammasomes are innate immune sensors; upon activation, they form multimeric complexes that cleave the protease caspase-1, which in turn cleaves and activates IL-1β and IL-18. Consequently, inflammasomopathies are characterized by overproduction of IL-1β, and affected patients respond clinically to inhibitors of IL-1β and its receptor (3).

Another group of diseases is caused by mutations in the tumor necrosis factor (TNF)/NF-κB signaling pathway, which modulates innate and adaptive immune responses (**Figure 2**) (17). The prototypical example of TNF-receptor associated periodic fever syndrome (TRAPS) is caused by mutations in *TNFRSF1A*, although the pathogenesis of TRAPS is complex and includes TNF-independent mechanisms (18). Downstream of the TNF receptor, ubiquitin-editing enzymes like OTULIN and A20 negatively regulate NF-κB signaling; inactivating mutations cause the autoinflammatory diseases Otulipenia and HA20, respectively (19, 20). Gain-of-function mutations in the *NOD2* and *CARD14* genes also cause autoinflammation due to constitutive activation of NF-κB signaling (21, 22). Although TNF inhibitors can be effective for this group of diseases, NF-κB can also be activated by TNF-independent agonists including IL-1β. Accordingly, some patients with NF-κB associated autoinflammatory diseases require treatment with other immunomodulators, including IL-1 pathway inhibitors (3, 18, 23).

The Type I interferon (IFN) pathway is important for antiviral immunity and for innate immune functions such as natural killer cell activation and antigen presentation (**Figure 3**) (24). Inborn errors of immunity that cause activation of Type I IFN signaling are termed interferonopathies. Proteasome-associated autoinflammatory syndromes (PRAAS) result from mutations in genes encoding proteasome subunits. Proteasome dysfunction induces the unfolded protein response (UPR), resulting in Type I IFN activation and autoinflammation (25). Several monogenic interferonopathies are caused by mutations in genes that modulate intracellular responses to nucleic acids. For example, mutations in the DNA sensor gene *TMEM173* lead to STING-associated vasculopathy with onset in infancy (SAVI) (26). Mutations in interferon-response genes like *STAT2* can also cause autoinflammation due to overactive signaling downstream of Type I IFN (27, 28).

In addition to these canonical dysregulated pathways, autoinflammation can also be caused by mutations in genes important for other innate immune functions. Deficiency of ADA2 (DADA2) is caused by mutations in *CERC1*, which regulates monocyte differentiation (29). Mutations in complement pathway genes like *CFH, C3*, and *CD46* can cause atypical hemolytic uremic syndrome (3, 30–32). Genes that regulate actin polymerization like *WDR1* and *CDC42* are also important for inflammasome assembly; mutations can

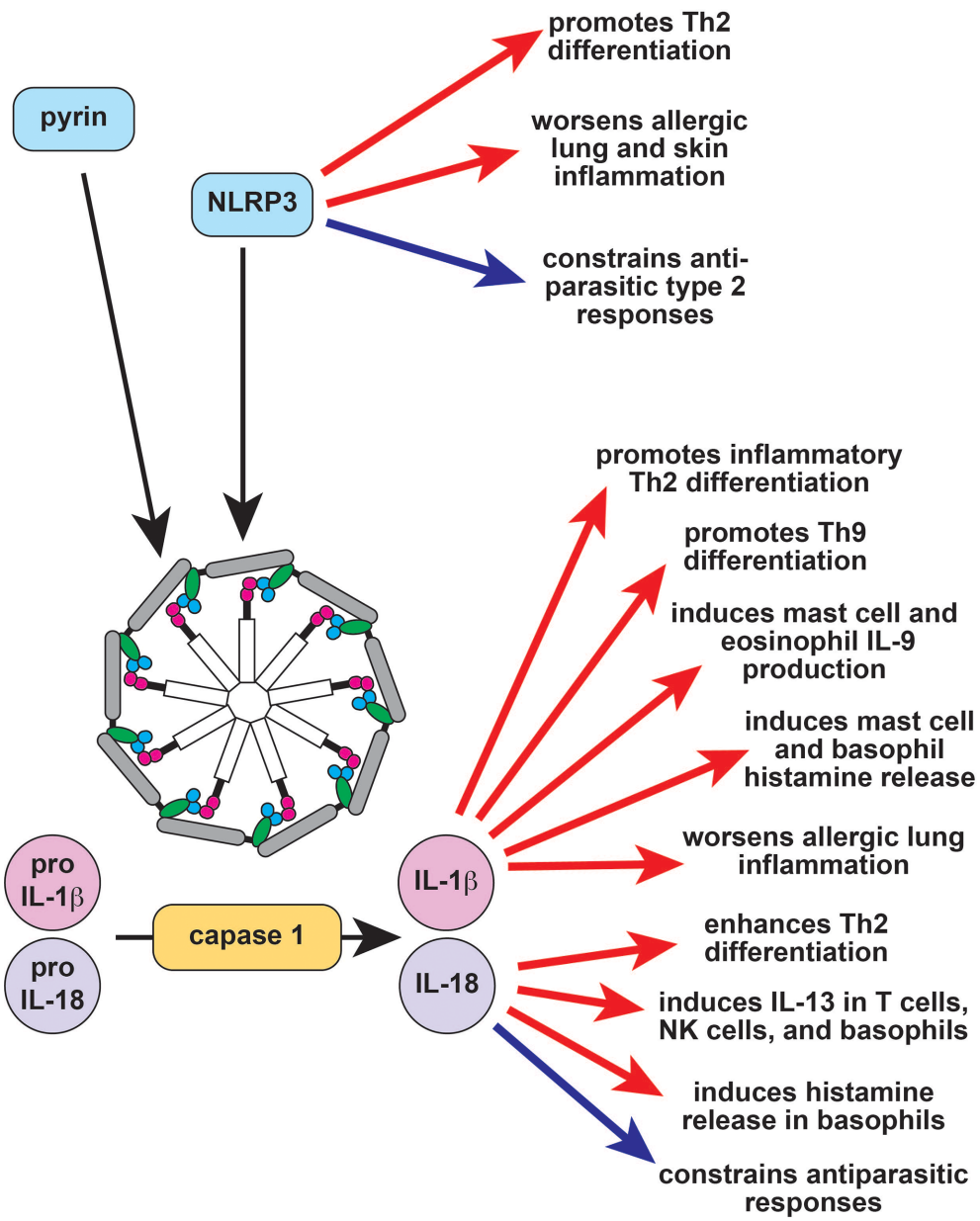

FIGURE 1 | The role of the inflammasome in type 2 immune responses. Inflammasomes are large multimeric signaling molecules that process inactive pro-IL-1β and pro-IL-18 into their active forms. Constitutive activation of the pyrin inflammasome results in Familial Mediterranean Fever (FMF), while activation of NLRP3 causes the autoinflammatory disease cryopyrin-associated periodic fever syndrome (CAPS). NLRP3 induces Th2 differentiation through inflammasome-dependent and independent mechanisms (red arrows) but also acts as a brake on type 2 responses to parasites (blue arrow). IL-1b enhances allergic responses through a variety of effector cells (red arrows), while the effect of IL-18 is context-dependent (red and blue arrows). Th2, T helper 2; IL-9, interleukin 9; IL-13, interleukin 13; NK, natural killer.

therefore cause IL-1β and IL-18-dependent autoinflammation (3, 33, 34). The ripoptosome is a multimeric complex containing RIPK1, FADD, and caspase-8 that is important for regulating the balance between necroptotic and apoptotic cell death; inactivating mutations can therefore cause autoinflammation secondary to increased necroptosis (35–37). Somatic mutations in the ubiquitin-editing gene *UBA1* lead to VEXAS, a treatment-refractory complex autoinflammatory syndrome characterized by activation of multiple immune pathways (38). Finally, a number of complex autoinflammatory diseases including systemic juvenile idiopathic arthritis (sJIA), Behcet's disease, and periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) syndrome are linked to a combination of genetic polymorphisms and environmental

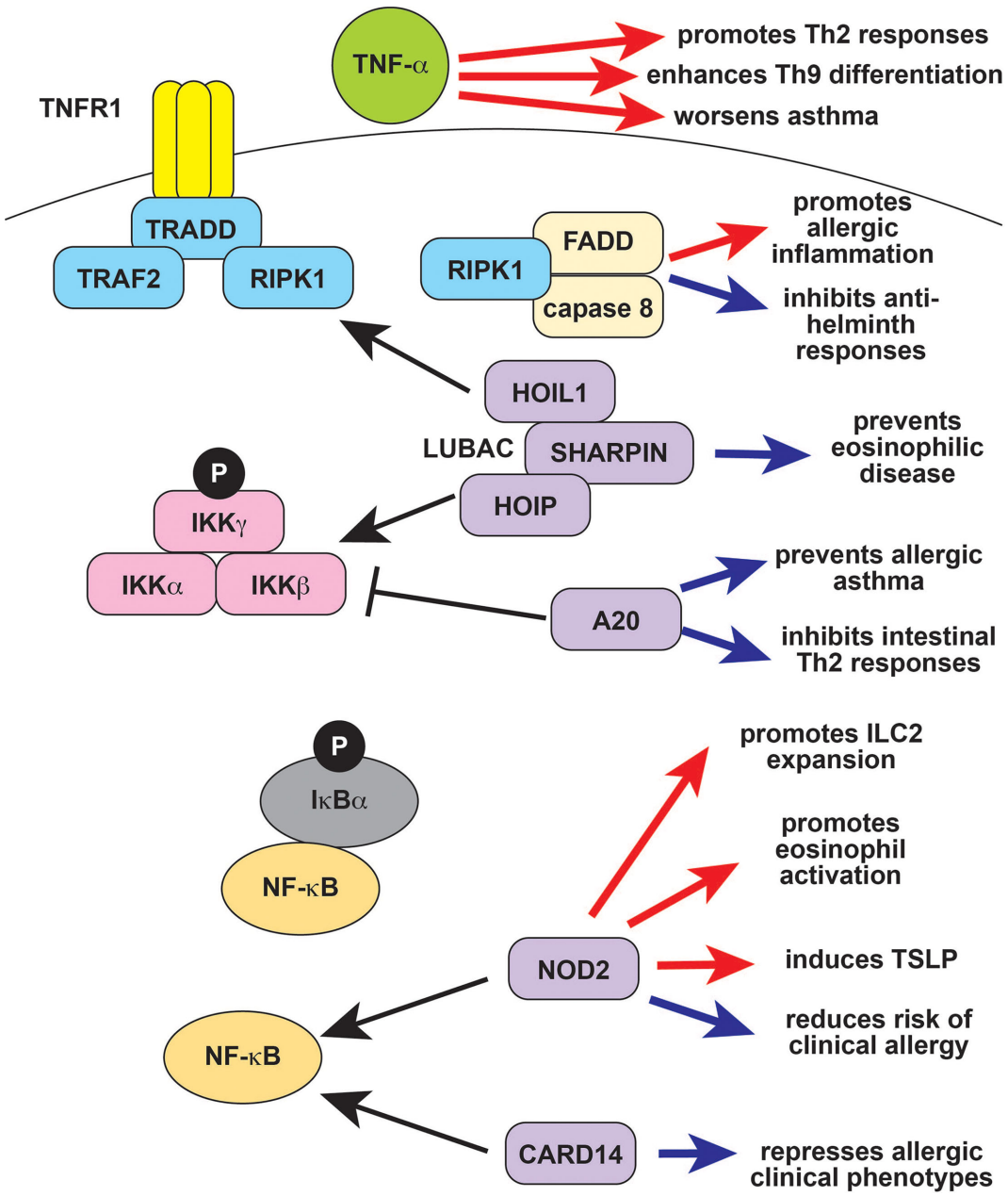

FIGURE 2 | TNF-α and NF-κB signaling in type 2 immune responses. TNF-α exacerbates type 2 diseases like asthma in part by promoting Th9 and Th2 differentiation and function (red arrows). The protease caspase-8 forms the ripoptosome complex together with the TNF signaling molecule RIPK1 and FADD. The ripoptosome is regulates cell death, with caspase-8 and RIPK1 promoting apoptosis over necroptosis, so that defects in RIPK1 result in increased necroptosis and autoinflammation. The ripoptosome promotes type 2 responses in response to environmental allergens (red arrow) but can suppress type 2 responses to parasites (blue arrows). Ubiquitin editing proteins like A20 (*TNFAIP3*) and LUBAC (composed of HOIL-1, HOIP, and SHARPIN) modulate NF-κB signaling by targeting upstream molecules for activation and/or degradation. A20 negatively regulates NF-κB and also prevents allergic asthma as well as other type 2 responses (blue arrows). SHARPIN activates NF-κB, and deficiency results in eosinophilic tissue infiltration (blue arrow). The NF-κB signaling molecules CARD14 and CARD15/NOD2 also modulate type 2 responses. CARD14 prevents allergic disease, and deficiency results in clinical atopy (blue arrow). CARD15/NOD2 is reported to have both positive (red arrows) and negative (blue arrows) effects on type 2 immunity, and its role may be context-dependent. TNF-α, tumor necrosis factor alpha; RIPK1, Receptor Interacting Serine Threonine Kinase 1; FADD, Fas Associated *via* Death Domain; LUBAC, linear ubiquitin chain assembly complex; HOIL-1, Haem-Oxidized IRP2 Ubiquitin Ligase 1; HOIP, HOIL-1L Interacting Protein; SHARPIN, SHANK-associated RH-interacting protein; TNFAIP3, TNF-α induced protein 3; CARD14, caspase recruitment domain-containing protein 14; CARD15, caspase recruitment domain-containing protein 15; NOD2, nucleotide binding oligomerization domain-containing protein 2; Th2, T helper 2; IL-9, interleukin 9; IL-13, interleukin 13; IL-4, interleukin 4; NK, natural killer.

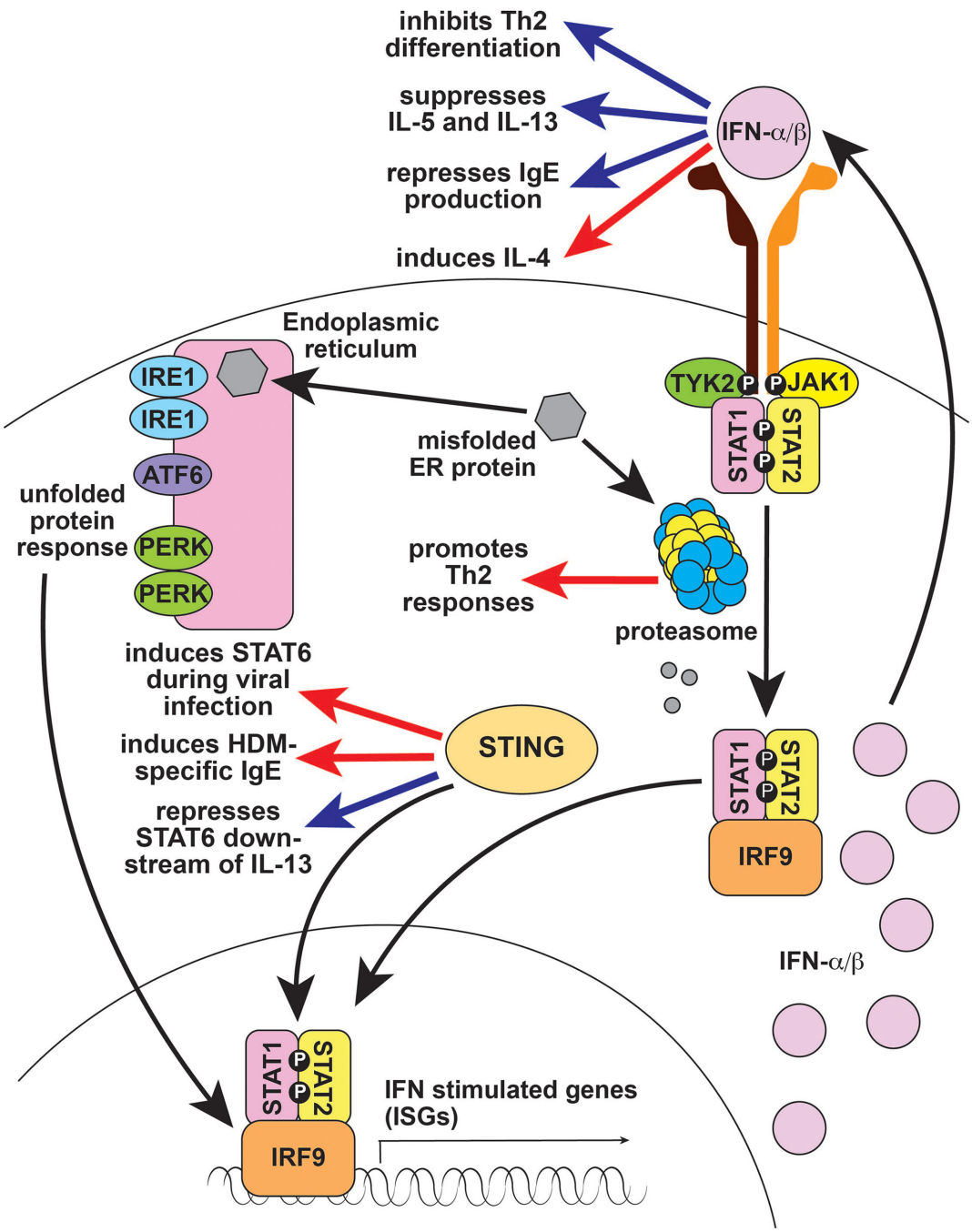

FIGURE 3 | Type 1 interferon signaling in type 2 immune responses. Type 1 IFNs like IFN-α and IFN-β largely suppress type 2 responses (blue arrows), although they are reported to induce IL-4 (red arrow), which can enhance type 2 immunity. The proteasome is important for processing and degrading misfolded endoplasmic reticulum proteins; defects cause unfolded proteins to accumulate, resulting in Type 1 IFN production. The proteasome also regulates antigen processing and presentation which is critical for T cell immunity, including Th2 responses (red arrow). STING is a DNA sensor that activates Type 1 IFN. STING activates STAT6 in response to viral infection and promotes IgE production in response to HDM (red arrow), but also represses IL-13-induced STAT6 activation in subjects with rhinosinusitis (blue arrow). IFN, interferon; ER, endoplasmic reticulum; IRE1, Inositol Requiring Enzyme 1; ATF6, Activating Transcription Factor 6; PERK, PKR-like Endoplasmic Reticulum Kinase; STING, Stimulator of Interferon Genes; TYK2, Tyrosine Kinase 2; JAK1, Janus Kinase 1; STAT1, Signal Transducer and Activator of Transcription 1; IRF9, interferon regulatory factor 9; Th2, T helper 2; IL-4, interleukin 4; IL-5, interleukin 5; IL-13, interleukin 13; IgE, immunoglobulin E, NK, natural killer; HDM, house dust mite.

factors (39, 40). As increased access to next-generation sequencing accelerates gene discovery, the spectrum of autoinflammatory diseases will likely broaden to comprise new mechanisms of innate immune dysregulation.

Type 2 Immunity Is Characterized by Allergy-Associated Effector Cytokines and Cells

Type 2 immunity was originally described as a counter-regulator of Th1-driven immune responses but was subsequently recognized as a distinct immune response with important roles in antihelminth defense, allergy, and wound repair (4, 5). Type 2 immunity is most commonly associated with Th2 cells and their hallmark effector cytokines IL-4, IL-5, and IL-13. However, type 2 inflammation is mediated by many other cell types including alternatively activated macrophages, type 2 innate lymphoid cells (ILC2), eosinophils, basophils, mast cells, and immunoglobulin E (IgE) secreting plasma cells (8). In addition to Th2-effector cytokines, type 2 immune cells secrete and respond to IL-9, IL-33, IL-25, and thymic stromal lymphopoietin (TSLP) (**Figure 4**) (4).

Immune responses have evolved to protect against discrete pathogens; in this context, type 2 immunity is critical to host defense against helminth infections. Accordingly, type 2 immune cells are found at barrier surfaces where they promote goblet cell hyperplasia, mucus secretion, and muscle contraction – all of which induce intestinal worm expulsion (4, 8). Many of these protective mechanisms can also promote tissue remodeling,

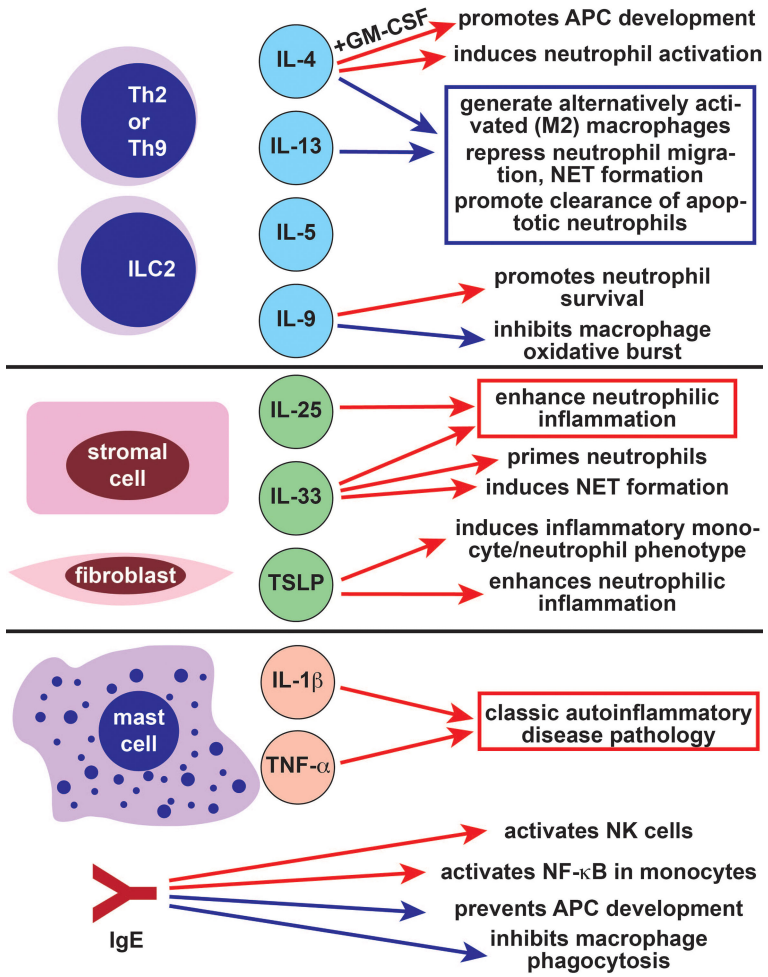

FIGURE 4 | The role of type 2 immune cells and cytokines in autoinflammatory cells and pathways. The Th2- and ILC2-derived cytokines IL-4 and IL-13 largely suppress autoinflammatory pathology by inducing the differentiation of anti-inflammatory M2 alternatively activated macrophages and repressing neutrophil migration (blue arrows). IL-4 also has some positive effects on autoinflammatory cells (red arrows), particularly in combination with GM-CSF. Like IL-4, the Th2/Th9/ILC2-derived cytokine IL-9 has both positive (red arrow) and negative (blue arrow) effects on autoinflammatory cells. The alarmins IL-25, IL-33, and TSLP promote autoinflammatory pathology through their effects on neutrophils and monocytes (red arrows). Mast cells also promote classic autoinflammatory pathology by producing IL-1β and TNF-α. The allergy-associated immunoglobulin IgE can both promote (red arrows) and repress (blue arrows) autoinflammatory disease. Th2, T helper 2; ILC2, type 2 innate lymphoid cell, IL-, interleukin-; GM-CSF, granulocyte macrophage colony stimulating factor; NET, neutrophil extracellular trap; APC, antigen presenting cell; TSLP, thymic stromal lymphopoietin; TNF-α, tumor necrosis factor alpha; IgE, immunoglobulin E; NK, natural killer.

making them important for wound repair after injury (4, 8). Type 2 dependent repair can ultimately result in tissue fibrosis, particularly when these pathways are chronically activated (4). Fibrosis is a highly pathological inflammatory endpoint that can result in significant morbidity and mortality secondary to organ failure. Thus, protective type 2 responses can easily become pathogenic when dysregulated or overactivated.

Consistent with the reciprocal inhibition seen for Th1 and Th2 cells, type 2 immune responses can also protect from autoimmune inflammation. This has largely been described in the context of murine inflammatory models, where Th2 cells and type 2 cytokines ameliorate autoimmune arthritis and encephalitis (8, 41, 42). However, the role of type 2 immunity in human autoimmune disease is complex: the type 2 effector cytokines IL-13 and IL-9, for example, are both thought to promote inflammation in patients with ulcerative colitis and psoriasis (43–46). Th2 cells and IgE can both promote kidney inflammation in patients with systemic lupus erythematosus (47, 48).

Allergic disorders make up the largest group of human diseases characterized by type 2 dysregulation and include asthma, atopic dermatitis, food allergy, and allergic rhinitis (2). Immunologically, allergy is caused by an exaggerated type 2 response to foreign antigens. However, many allergy-associated clinical syndromes have forms in which allergic sensitization cannot be demonstrated (49, 50). In some cases, this might be due to primary dysregulation of type 2 inflammatory cells and mediators. For example, some patients with late-onset eosinophilic asthma are thought to have primary dysregulation of ILC2, which produce type 2 cytokines independent of antigenic stimulation (51). Patients with NARES (nonallergic rhinitis with eosinophilia syndrome) are thought to have a primary eosinophilic disorder in at least some cases (50). In other cases, non-type 2 mediators can promote symptoms that are clinically indistinguishable from allergen-specific type 2 responses. For example, hormonal rhinitis can mimic allergic rhinitis but is caused by hormone-induced nasal vascular engorgement (50).

PART 2: THE ROLE OF AUTOINFLAMMATION IN TYPE 2 IMMUNE RESPONSES

Autoinflammation, Type 2 Immunity, and Clinical Allergy: A Complex Relationship

Type 1 cytokines have long been thought to primarily repress type 2 immunity based on the Th1-Th2 paradigm. Indeed, the type 1 cytokines IFN-γ and IL-12 inhibit Th2 differentiation and type 2 responses to helminth infection (8, 52, 53). However, other autoinflammatory and autoimmune cytokines can amplify type 2 inflammation, worsening type 2-driven pathology (54–56) Additionally, autoinflammatory and autoimmune cytokines can directly promote tissue inflammation, resulting in clinical phenotypes identical to type 2-driven allergic disease (57–59). The heterogeneity of inflammatory mechanisms driving common clinical phenotypes can present substantial barriers to understanding the crosstalk between type 2 inflammation and autoinflammation in human disease. To help address this complexity, one can approach the role of autoinflammation in type 2-mediated disease using the innate immunologic pathways that are used to categorize monogenic autoinflammatory diseases: inflammasomes, TNF-α, Type I IFN, and newer pathways including necroptosis.

Inflammasomes and Associated Cytokines in Type 2 Immunity

The pyrin inflammasome does not appear to have a major role in type 2 immune responses, and a recombinant pyrin domain was found to attenuate allergic inflammation in mice by suppressing NF-κB activation (60). By contrast, NLRP3 directly promotes Th2 differentiation independent of its inflammasome function by transcriptionally inducing *Il4* in conjunction with IRF4 (**Figure 1**) (61). The NLRP3 inflammasome can also trigger a Th2-biased response in the context of both infection and allergic inflammation (**Figure 1**) (62–64). NLRP3 activation in bronchial epithelial cells promotes allergic lung inflammation, whereas activation in keratinocytes promotes eczema (63, 65). By contrast, Helicobacter pylori gastric infection protects from allergic asthma by activating NLRP3 in proximal dendritic cells (66). Similarly, helminths induce NLRP3, which then acts as a brake on type 2 responses *via* both inflammasome-independent and inflammasome-dependent mechanisms (**Figure 1**) (67–69). Taken together, these studies suggest that NLRP3 activation may primarily suppress type 2 responses to pathogens but promote dysregulated type 2 responses to environmental allergens.

The end products of inflammasome activation, IL-1α, IL-1β and IL-18, can also regulate type 2 immunity. Single nucleotide polymorphisms (SNPs) in *IL1A, IL1B*, and *IL1R1* are all linked to asthma; accordingly, IL-1α and IL-1β both exacerbate murine allergic airway inflammation (**Figure 1**) (70–75). Type 2 immune cells like eosinophils and mast cells can release IL-1β, airway epithelial cells stimulated with the house dust mite (HDM) allergen can release IL-1α, and IL-1β can be found in allergic tissues, further suggesting that IL-1 has a role in type 2 responses (76–79). This hypothesis is supported by the observation that IL -1β enhances inflammatory Th2 differentiation and helps induce the differentiation of Th9 cells (**Figure 1**) (55, 80–82). IL-1β is also capable of regulating various type 2 innate effector cells to promote tissue inflammation. For example, IL-1β activates human ILC2s in the presence of IL-2, inducing proliferation and effector cytokine production (83, 84). IL-1β also induces histamine release from basophils and mast cells, and histamine enhances IL-1β release, which can induce a positive feedback loop (**Figure 1**) (85, 86). Eosinophils and mast cells stimulated with IL-1β produce IL-9, further supporting the hypothesis that IL-1β can enhance type 2 immune responses to promote allergic pathology (**Figure 1**) (87, 88). A pathogenic role for IL-1 signaling in allergy is further supported by a number of clinical studies demonstrating the efficacy of IL-1 pathway inhibitors in asthma and atopic dermatitis (89–91). Several larger randomized controlled clinical trials have been planned to follow up these encouraging observations but were halted early due to patient

recruitment – particularly in light of the ongoing COVID-19 pandemic (NCT01122914, NCT04035109, NCT03513458).

Like IL-1β, IL-18 is reported to enhance Th2 differentiation and T-cell-derived IL-13 production (**Figure 1**) (54, 69). This effect is IL-4-dependent and may be because IL-18-induces IL-4 production or because it increases T cell sensitivity to IL-4 (54). IL-18 also induces IL-13 in natural killer (NK) cells and in basophils, suggesting that it may contribute to the innate arm of type 2 immune responses (**Figure 1**) (92, 93). In addition to IL-13, IL-18 also induces histamine from basophils and can promote eosinophil development and maturation in combination with IL-5 (94, 95). However, IL-18 can also repress type 2 responses *in vivo*. IL-18-deficient mice develop enhanced allergen-induced eosinophilia, and IL-18-deficient mice are protected from helminth infections (**Figure 1**) (96, 97). This suggests that the role of IL-18 in type 2 immunity may be context-dependent. Indeed, IL-18 can promote either Th1 or Th2 differentiation depending on genetic background and cytokine milieu (98). Similarly, IL-18 represses allergic pathology and IgE production in combination with IL-12 but induces both of these in the absence of IL-12 (95, 99, 100).

TNF-α and NF-κB Signaling in Type 2 Immunity

The inflammatory cytokine TNF-α has a role in both innate and adaptive immunity, underlying the efficacy of TNF inhibitors in patients with autoimmune conditions like rheumatoid arthritis (RA) and autoinflammatory conditions like Deficiency of ADA2 (DADA2) (101, 102). TNF-α and other TNF superfamily cytokines promote the differentiation of Th9 cells, suggesting that they may enhance type 2 immune responses (**Figure 2**) (103, 104). Many TNF superfamily cytokines are costimulatory molecules that more generally modulate division, survival, and activation in T cells. Several of these positively regulate of Th2 differentiation and function due to their role in costimulation (105). TNF-α also enhances the effect of IL-4 on eosinophils and enhances Th2-mediated responses at mucosal sites (**Figure 2**) (56, 106). This may be in part due to effects on non-immune cells that promote type 2 responses. For example, TNF-α and IL-1β synergize to promote airway hyperresponsiveness, which might partly underlie the role of TNF-α in asthma (**Figure 2**) (107, 108). The TNF-α inhibitor etanercept initially showed promise for severe refractory asthma, but a subsequent trial failed to show efficacy (107, 109). Clinical development was ultimately halted due to an increased rate of serious adverse effects, most notably respiratory infections, in a phase 2 trial of golimumab (110). Etanercept and infliximab are reported efficacious for the treatment of atopic dermatitis and have been used as an off-label treatment for severe disease (111, 112).

NF-κB signaling has long been known to play a role in type 2 immune responses, Th2 differentiation, IgE production, and the function of innate type 2 effectors like eosinophils, ILC2s, and mast cells (10, 113–115). Inactivating mutations in NF-κB pathway genes like *CARD11* and *CARD14* cause monogenic immune dysregulatory syndromes that include allergic phenotypes, indicating that physiologic NF-κB signaling can suppress type 2 pathology (**Figure 2**) (116, 117). The clinical phenotype of *CARD14* loss-of-function (LOF) is particularly interesting in the context of autoinflammation, because activating *CARD14* mutations cause a monogenic autoinflammatory disease (22). Similarly, *NOD2 (CARD15)* LOF polymorphisms are associated with an increased risk of clinical allergy and inflammatory bowel disease (IBD), whereas activating mutations cause the autoinflammatory disease Blau syndrome (**Figure 2**) (21, 118, 119). However, NOD2 also induces the type 2 cytokine TSLP, promotes ILC2 expansion, and induces eosinophil activation (**Figure 2**). These studies suggest that, in some cases, NF-kB signaling is primarily an inducer of type 2 immune responses.

The autoinflammation-associated NF-κB signaling repressor A20 (*TNFAIP3*) inhibits airway epithelial cytokine production in response to endotoxin, suppressing type 2 responses to HDM and preventing allergic asthma (**Figure 2**) (120). A20 also has a cell-intrinsic anti-inflammatory role in mast cells, inhibits intestinal Th2 responses, and prevents Th17 differentiation in response to HDM (**Figure 2**) (121–123). These observations may explain the negative associations of *TNFAIP3* expression with allergic asthma, chronic rhinosinusitis, atopic dermatitis, and food allergy (124–127). The SHARPIN protein (Shank-interacting protein like 1) is a part of the LUBAC (linear ubiquitin chain assembly complex), which promotes NF-κB activation and is linked to autoinflammation and complex immune dysregulation. SHARPIN promotes regulatory T cell function, so deficiency promotes systemic inflammation (128). Additionally, SHARPIN deletion causes lymphocyte-independent eosinophilic esophagitis, and keratinocyte-specific deletion causes eosinophilic dermatitis (**Figure 2**) (129–131).

Type I IFN Signaling in Type 2 Immunity

Broadly, type I IFNs inhibit type 2 immune responses: they suppress IL-5 and IL-13 production, inhibit GATA3-dependent Th2 differentiation, and block B cell isotype switching to IgE (**Figure 3**) (24, 132–135). Accordingly, recombinant IFN-α is used to treat Idiopathic Hypereosinophilic Syndromes and Eosinophilic Granulomatosis with Polyangiitis (136, 137). Type I IFNs are also thought to play a role in asthma, where deficiency leads to increased viral infection and enhanced Th2 differentiation, worsening disease (138). However, type I IFNs are also reported to induce IL-4 production and to promote murine eosinophilic rhinosinusitis, possibly by increasing eosinophil recruitment (**Figure 3**) (134, 139). It remains to be determined whether these functions have any role in promoting type 2 immunity-related human diseases.

In addition to modulating type I IFNs through the unfolded protein response, the proteasome is important for antigen processing and presentation (140). Consequently, defects in the proteasome result in a general loss of T-cell-dependent immunity. Taken together with the antagonistic role of type I IFN on type 2 responses, it is not surprising that defects in the proteasome are associated with reduced Th2 responses (**Figure 3**) (141). By contrast, the DNA sensor STING activates the type 2 associated signaling molecule STAT6 in response to viral infection, although the result is enhanced antiviral immunity rather than a type 2 immune response (**Figure 3**) (142). STING also promotes HDM-induced IgE production by enhancing the function of T follicular

helper cells (**Figure 3**) (143). However, STING represses IL-13-induced STAT6 phosphorylation in subjects with rhinosinusitis by increasing expression of the STAT6 inhibitor SOCS1 (suppressor of cytokine signaling 1) (**Figure 3**) (144). SOS1 induction may also underlie the observation that STING signaling in ILC2s promotes a phenotypic shift to Type 1 ILC (ILC1) during lung inflammation (145). Taken together, these studies suggest that the role of STING in type 2 immunity is complex and context dependent.

Other Autoinflammation-Associated Pathways in Type 2 Immunity

Cytoskeletal regulators that play a role in inflammasome activation, like CDC42 and WDR1, also play a role in adaptive immunity and nonhematopoietic cells. CDC42 is activated by the atypical guanine nucleotide exchange factor DOCK8, which is linked to autosomal recessive hyper-IgE syndrome (33). CDC42 signaling is also important for mast cell and eosinophil function, and CDC42-deficient invariant natural killer T cells have a defect in IL-4 secretion because CDC42 degradation induces IL-4 secretion in response to lipid antigens (146). Complement activation promotes Th1 differentiation and function, which can indirectly repress type 2 responses, but is not thought to directly regulate Th2-driven responses (147). The complement system can activate innate type 2 effectors like eosinophils and mast cells, however, and may therefore promote some type 2 associated pathology (148, 149). Environmental allergens can activate the ripoptosome to trigger type 2 inflammation through RIPK1 and caspase 8, which shunt cells away from necroptosis and towards apoptosis (**Figure 2**) (36, 150, 151). Caspase-8 can also promote allergic pathology by directly activating IL-1 cytokines (152). However, caspase 8 prevents type 2 immune responses to *Trypanosoma cruzi* infection, leading to increased parasitemia and chronic infection (150). Caspase-8 also promotes epithelial keratinocyte cohesion, so that epidermal-specific deficiency causes a spontaneous eczematoid dermatitis (153). Thus, the effect of the ripoptosome on type 2 immune responses may be context-dependent as for other autoinflammatory mediators.

PART 3: THE ROLE OF TYPE 2 IMMUNITY IN AUTOINFLAMMATORY CELLS AND PATHWAYS

Th2 and ILC2-Derived Cytokines in Autoinflammatory Cells and Pathways

The type 2 cytokines IL-4 and IL-13 have long been studied as modulators of innate immune function due to their role in the generation of alternatively activated macrophages (M2) (**Figure 4**) (154). In contrast to classical activation, which is induced by IFN-γ and characterized by type 1 cytokine production and microbial killing, alternative activation causes macrophages to develop an immunoregulatory function. M2 macrophages are not efficient killers of invading pathogens but produce growth factors and extracellular matrix components, making them important for wound healing (154). They also can generate or maintain type 2 immune responses. In the context of alternative activation, IL-4 promotes tissue resident macrophage activation and accumulation (155). Exposure to IL-4 in combination with GM-CSF (granulocyte-monocyte colony stimulating factor) causes peripheral monocytes to function as antigen presenting cells (**Figure 4**) (156). These monocyte-derived cells phenotypically resemble inflammatory dendritic cells rather than inflammatory macrophages (157). Indeed, the inflammatory macrophage phenotype is promoted by classical activation and inhibited by alternative activation (158). This may be because IL-4 inhibits NF-κB and inflammasome activation in macrophages, reducing responsiveness to lipopolysaccharide (158).

IL-4 also has a role in neutrophil biology and can even be produced by neutrophils (159). While IL-4 can induce neutrophil activation and phagocytosis, it also inhibits neutrophil migration (**Figure 4**) (160, 161). IL-4 also represses the formation of neutrophil extracellular traps (NETs), an important mechanism used for pathogen killing (162). Like IL-4, IL-13 inhibits neutrophil migration to inflamed tissues, although IL-13 also enhances production of several neutrophil effector proteins including IL-8 (163, 164). Finally, the IL-4 and IL-13 activated signaling molecule STAT6 is importance for clearance of apoptotic neutrophils, which promotes resolution of inflammatory responses (165). Taken together, these data suggest that type 2 cytokines primarily repress pathways associated with autoinflammation in macrophages and neutrophils.

Like IL-4 and IL-13, the type 2 cytokines IL-5 and IL-9 are derived primarily from T helper cells and ILC2s. While neither IL-5 nor IL-9 is implicated in alternative activation of macrophages, both cytokines can modulate the function of monocytes and neutrophils. IL-5 receptor is expressed on neutrophils, including airway-resident neutrophils from asthma patients, although its function in neutrophils is not well characterized (166, 167). IL-5 indirectly regulates dendritic cells by inducing eosinophils, which repress plasmacytoid dendritic cell derived type I IFN production (168). IL-9 represses autoinflammation-associated responses by inhibiting oxidative burst and TNFα release in LPS-stimulated human monocytes and alveolar macrophages (**Figure 4**) (169, 170). However, IL-9 can also promote neutrophil survival and neutrophil-derived IL-8 release, enhancing type 1 inflammatory responses (171, 172). This suggests that the role of IL-9 in autoinflammation is complex and context-dependent.

Alarmins in Autoinflammatory Cells and Pathways

Type 2 innate cytokines, or alarmins, are produced by epithelial cells, endothelial cells, stromal cells, and fibroblasts in response to injury. These alarmins include IL-25, IL-33 and TSLP; they activate ILC2, Th2, eosinophils, mast cells, and other type 2 effectors (5, 10, 173, 174). Because activated ILC2 and Th2 cells produce large amounts of IL-4 and IL-13, alarmins can indirectly promote ILC2-dependent immunosuppressive functions in neutrophils (175, 176). In some cases, alarmins can also directly regulate neutrophils and monocytes. For example, IL-33 primes neutrophils so that they are rapidly recruited to sites of infection and inflammation, whereas IL-25 promotes neutrophilic airway infiltration (**Figure 4**) (177–180). IL-33 overexpression causes spontaneous neutrophilic

arthritis and sterile inflammation possibly due to increased NET formation (181). The alarmin TSLP enhances neutrophilic inflammation and induces a proinflammatory phenotype in circulating monocytes and neutrophils (**Figure 4**) (182, 183). Further supporting its role in neutrophil-mediated host defense, TSLP enhances neutrophilic microbicidal activity against methicillin-resistant *Staphylococcus Aureus* (184). Together, these data suggest that type 2 alarmins can promote autoinflammatory pathology in some contexts.

Mast Cells and High Affinity IgE Receptor in Autoinflammatory Cells and Pathways

Mast cells produce IL-1β; which is cleaved and activated by caspase 1, caspase 8, and serine proteases (**Figure 4**) (185). Mast cell IL-1β production is NLRP3-dependent, suggesting that mast cells may have a role in NLRP3-associated autoinflammatory processes. Accordingly, patients with *NLRP3* mutations develop cold-induced histamine-independent urticariform lesions, and mast cells are a major source of IL-1β in affected skin (77, 79). Mast cells also produce IL-1β in patients with the adult-onset autoinflammatory disease Schnitzler's syndrome, in subjects with chronic recurrent multifocal osteomyelitis, and have been found in inflamed joints from patients with FMF (186–188). In mice, mast cells promote sterile joint and central nervous system inflammation (185, 189). Mast cell derived TNF-α induces urticariform rashes in patients with *NLRP3* mutations, although the role of mast cell derived TNF-α in other autoinflammatory diseases is not known (**Figure 4**) (190).

IgE is a critical inducer of many type 2 effector cells, including mast cells, through its high affinity receptor Fc epsilon RI. Fc epsilon RI is also expressed and functional in several type 1 innate effector cells. IgE crosslinking suppresses monocyte function by blocking phagocytosis and preventing differentiation into dendritic cells (**Figure 4**) (191, 192). Simultaneously, engagement of Fc epsilon RI activates NF-κB in monocytes and dendritic cells, which promotes secretion of IL-6, IL-10, and TNF-α (192, 193). Macrophage Fc epsilon RI engagement also reprograms alternatively activated tumor-resident macrophages to be more proinflammatory, enhancing their antitumoral functions (194). The functions of IgE and its receptor are not as well characterized in other type 1 innate cells. However, Fc epsilon RI is expressed in both dendritic cells and neutrophils, where it delays neutrophil apoptosis (195–197). IgE can also activate NK cells through the lower affinity Fc gamma RIII receptor (198). Future studies will be needed to further characterize the roles of IgE and its receptors in autoinflammation-associated innate immune cells.

PART 4: THE EPIDEMIOLOGY OF ALLERGY IN AUTOINFLAMMATORY DISEASES

The Epidemiology of Allergy in Monogenic Autoinflammatory Diseases

One way to investigate the interaction between autoinflammatory pathways and type 2 immunity is to investigate the prevalence of allergic clinical and immunological phenotypes in subjects with monogenic autoinflammatory diseases (**Table 1**). Because single gene mutations promote activation of discrete innate pathways, this approach can assess the *in vivo* roles of dysregulated autoinflammatory pathways in regulating human type 2 immune responses (199). This question has been most extensively studied in FMF, perhaps because it was the first autoinflammatory disease to be linked to a causative gene (16) (**Table 1**). Several studies have suggested that FMF protected against asthma and atopy, potentially due to protective linkage of *MEFV* with asthma associated genes like *IL4RA* (199–201). Although one study suggested that Turkish FMF patients may have elevated total serum IgE relative to healthy volunteers, this result was not seen in other cohorts, where there was a trend towards reduced serum IgE (199, 201, 202). Taken together, these results suggest that activation of the pyrin inflammasome attenuates human type 2 immune responses.

By contrast, the autoinflammatory disease CAPS, caused by *NLRP3* mutations, is associated with peripheral eosinophilia and eosinophilic skin infiltration (199, 203) (**Table 1**). Eosinophilia correlates with CAPS disease activity, suggesting that *NLRP3* activation promotes eosinophilia (199). This is consistent with the role of NLRP3 and IL-1β in promoting the differentiation and function of type 2 effectors like Th2 cells, mast cells, and eosinophils. CAPS is also characterized by an increased prevalence of eczema, asthma, and allergic rhinitis relative to both the general population and FMF (199). This is consistent with the observation that NLRP3 activation exacerbates murine models of asthma and eczematous dermatitis (63, 65). Finally, the urticariform lesions of CAPS are characterized by IL-1β and TNF-α producing mast cell infiltration, once again linking the NLRP3 inflammasome to type 2 effector activation in humans (77, 79, 190). Overall, these results suggest that in humans, constitutive activation of the NLRP3 inflammasome promotes type 2 immune responses. Because helminth infections are extremely uncommon in countries with highly developed CAPS cohorts, it remains to be determined whether NLRP3 activation suppresses type 2 responses to pathogens in humans, as it does in murine models (67–69).

CDC42 is a plasma membrane associated GTPase involved in diverse processes including cell division, phagocytosis, and epithelial cell morphology (204). Mutations are linked to NOCARH (neonatal onset of pancytopenia, autoinflammation, rash, and episodes of HLH) an IL-1-responsive autoinflammatory disease with features of macrophage activation syndrome (MAS) (33, 205). CDC42 alternates between an inactive cytosolic form and an active plasma membrane bound form; mutations affecting trafficking alter the subcellular localization independent of the protein's activation state (33). This, in turn, alters the partners that bind to CDC42, ultimately leading to NF-κB overactivation and autoinflammation (204). In addition to autoinflammation, one patient with NOCARH also developed mild hypereosinophilia and hyper-IgE, although no clinical allergic diagnoses were reported (204) (**Table 1**). As additional patients are identified, careful phenotyping will be needed to determine whether type 2 immune activation is a common feature of NOCARH.

TABLE 1 | Associations of monogenic autoinflammatory diseases with type 2 clinical and immunological phenotypes.

Disease	Gene(s)	Type 2 Phenotype
FMF	*MEFV*	Reduced prevalence of asthma (199–201) Increased prevalence of rhinosinusitis (199) Elevated total serum IgE relative to healthy volunteers (202) Reduced total serum IgE relative to healthy volunteers (201) Reduced mean absolute eosinophil count relative to healthy volunteers (199)
CAPS	*NLRP3*	Increased prevalence of hypereosinophilia, asthma, eczema, and rhinosinusitis relative to healthy volunteers (199, 203) Increased mean absolute eosinophil count relative to healthy volunteers (199) Th2 cell expansion (199)
NOCARH	CDC42	Mild hypereosinophilia and hyper-IgE (204)
TRAPS	*TNFRSF1A*	Increased prevalence of allergic rhinitis, eosinophilic GI disease relative to healthy volunteers (199) Th2 cell expansion (199)
CANDLE	*POMP* *PSMA3* *PSMB10* *PSMB4* *PSMB8* *PSMB9* *PSMG2*	Increased prevalence of eczema, eosinophilic GI disease relative to healthy volunteers (199) Reduced prevalence of asthma relative to healthy volunteers (199) Reduced mean absolute eosinophil count relative to healthy volunteers (199)
DADA2	*CERC1*	Increased prevalence of eczema, allergic rhinitis relative to healthy volunteers (199) Reduced mean absolute eosinophil count, total serum IgE relative to healthy volunteers (199)
HA20	*TNFAIP3*	Increased prevalence of eczema, allergic rhinitis, eosinophilic GI disease relative to healthy volunteers (199) Th9 cell expansion (19, 199)
HIDS	*MVK*	Increased prevalence of allergic rhinitis, eosinophilic GI disease relative to healthy volunteers (199) Reduced total serum IgE, mean absolute eosinophil count relative to healthy volunteers (199)
PAPA	*PSTPIP1*	Reduced mean absolute eosinophil count relative to healthy volunteers (199)

Far less is known about the prevalence of type 2 immune activation in other autoinflammatory diseases. In one systematic population study, clinical diagnoses of allergic rhinitis were highly prevalent in almost all autoinflammatory diseases, including FMF (199). This included diseases with reduced clinical laboratory markers of type 2 inflammation relative to the general population, like HIDS and DADA2. This might be because autoinflammation-associated cytokines like IL-1β and TNF-α can promote sinus mucosal thickening independent of type 2 immune activation (206). Thus, in some cases, autoinflammatory pathology may mimic type 2 associated disease, and this may be a potential confounder in epidemiologic studies.

The Epidemiology of Allergy in Complex Autoinflammatory Diseases

Unlike their monogenic counterparts, complex autoinflammatory diseases are linked to multiple genetic and environmental factors that contribute to their pathogenesis. Behcet's disease is a heterogeneous and complex autoinflammatory disease that manifests with orogenital ulcers, pustular skin disease, arthritis, eye disease, gastrointestinal inflammation, and vascular complications (207). Genetic studies have identified a number of risk alleles that overlap with both recurrent aphthous stomatitis and PFAPA syndrome, allowing the three syndromes to be grouped together as Behcet's spectrum disorders (39). Amongst the susceptibility loci shared by Behcet's spectrum disorders are multiple genes associated with Th1-driven immunity, such as *STAT4* and *IL12A* (39). Th1 cells are thought to repress Th2 cells, and perhaps for this reason patients with Behcet's disease were found in several studies to have lower rates of allergic sensitization and lower IgE levels than the general population (201, 208) (**Table 2**). However, a separate group of studies reported increased rates of atopy and elevated levels of type 2 cytokines in subjects with Behcet's spectrum disorders, particularly children with PFAPA (209–212). Moreover, the IgE-blocking monoclonal antibody has been reported to alleviate symptoms and reduce autoinflammation in one subject with concurrent Behcet's disease and asthma (213) (**Table 2**). These disparate findings may be partly due to the genetic and phenotypic heterogeneity of patients with Behcet's spectrum disorders, which can vary substantially between cohorts with different ancestries (207).

Systemic juvenile idiopathic arthritis (sJIA) is another complex autoinflammatory disease with genetic and phenotypic links to both autoinflammation and autoimmune inflammation (40). Children with allergic disease were found to be at a higher risk of developing JIA in a Taiwanese cohort, although sJIA was not differentiated from other forms (214). Atopy may also be a risk factor for increased disease severity in sJIA, although this has only been investigated in one small prospective study (215). Adult-onset Still's disease (AOSD) is an adult-onset clinical syndrome that phenotypically resembles sJIA (216). Cases of AOSD have been reported in association with elevated serum IgE, IL-4, and clinical atopy, but the prevalence of these features has not yet been systematically investigated (216, 217).

CONCLUSIONS

Although autoinflammation and type 2 immunity have traditionally thought to counter-regulate each other, a growing body of literature demonstrates that the relationship between type 1 and type 2 immune responses is more nuanced than this

TABLE 2 | Associations of complex autoinflammatory diseases with type 2 clinical and immunological phenotypes.

Disease	Type 2 Phenotype
Behcet's spectrum disorders (Behcet's disease, PFAPA, aphthous stomatitis)	Lower rates of allergic sensitization and lower IgE levels than the general population (201, 208) increased rates of atopy and elevated levels of type 2 cytokines PFAPA (209–212). Clinically responds to omalizumab (anti-IgE) (213)
sJIA/AOSD	Atopy is a risk factor for severe disease in sJIA (215) Positive association of AOSD with elevated serum IgE, IL-4, and clinical atopy (case reports) (216, 217)

canonical view would suggest. Some autoinflammatory cytokines, like IL-1β and TNF-α; enhance the differentiation and function of type 2 effector cells and exacerbate allergic pathology. Others, like type 1 IFN, largely repress type 2 inflammation but can promote type 2 cytokine production in certain contexts. And some autoinflammatory signaling molecules like NLRP3 may constrain type 2 responses in the context of parasitic infection, while inducing type 2 immunity in the setting of allergic inflammation. These observations suggest that the role of autoinflammation in type 2 immunity may rely on a broad array of genetic and environmental factors involved in driving the immune response.

Similarly, the role of type 2 immunity in the pathogenesis of autoinflammation is complex and context-dependent. While Th2- and ILC2-derived type 2 cytokines like IL-4 and IL-13 generally repress type 1 inflammation, they can promote neutrophil activation in certain context. Moreover, alarmins like IL-33 and TSLP clearly induce autoinflammatory effectors like neutrophils and monocytes, causing local and systemic inflammation. Mast cells, which are generally considered type 2 effectors, have a clear role in *NLRP3*-associated autoinflammatory diseases and may play a role in diseases linked to other genes like *MEFV*. Future investigation will be required to determine the roles played by type 2 cytokines and effectors in modulating pathology in subjects with monogenic and complex autoinflammatory diseases.

Clinical epidemiology studies in patients with autoinflammatory diseases paint a similarly nuanced picture. Given the role of *NLRP3* in promoting allergic pathology, for example, it is not surprising that the phenotypic spectrum of CAPS comprises eosinophilia and clinical allergy in addition to systemic autoinflammation. In other syndromes, autoinflammation appears to have a negative effect on type 2 immunity – most notably for FMF – although the mechanisms are not well-defined. Finally, in some cases, it appears that autoinflammatory pathology can mimic allergic disease, causing a phenotype that is indistinguishable from clinical allergy but that is not mediated by type 2 effectors. These observations have clinical implications for subjects with autoinflammatory diseases, where type 2 directed therapies have been reported effective in some cases. They may also have repercussions for subjects with clinical allergy-associated diagnoses like asthma, where non-allergic endotypes are unlikely to respond to type 2 directed therapies. The ability of autoinflammatory cytokines and mediators to both potentiate and clinically phenocopy type 2 pathology suggests that some of these patients might benefit from autoinflammation-directed treatments. In the future, dissecting the interactions between these two not-so-separate arms of the immune response should help to refine our understanding of – and improve treatments for – monogenic and complex immune dysregulatory disorders.

AUTHOR CONTRIBUTIONS

MS, AS, and DS organized and composed the manuscript. MS created tables. DS created figures and provided supervision. All authors contributed to the article and approved the submitted version.

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AMG853, A Bispecific Prostaglandin D_2 Receptor 1 and 2 Antagonist, Dampens Basophil Activation and Related Lupus-Like Nephritis Activity in Lyn-Deficient Mice

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Systemic lupus erythematosus is a complex autoimmune disease during which patients develop autoantibodies raised against nuclear antigens. During the course of the disease, by accumulating in secondary lymphoid organs (SLOs), basophils support autoreactive plasma cells to amplify autoantibody production. We have recently shown that murine lupus-like disease could be controlled by 10 days of oral treatment with a combination of prostaglandin D_2 (PGD_2) receptor (PTGDR) antagonists through the inhibition of basophil activation and recruitment to SLOs. Importantly, inhibiting solely PTGDR-1 or PTGDR-2 was ineffective, and the development of lupus-like disease could only be dampened by using antagonists for both PTGDR-1 and PTGDR-2. Here, we aimed at establishing a proof of concept that a clinically relevant bispecific antagonist of PTGDR-1 and PTGDR-2 could be efficient to treat murine lupus-like nephritis. Diseased Lyn-deficient female mice received treatment with AMG853 (vidupiprant, a bispecific PTGDR-1/PTGDR-2 antagonist) for 10 days. This led to the dampening of basophil activation and recruitment in SLOs and was associated with a decrease in plasmablast expansion and immunoglobulin E (IgE) production. Ten days of treatment with AMG853 was consequently sufficient in reducing the dsDNA-specific IgG titers, circulating immune complex glomerular deposition, and renal inflammation, which are hallmarks of lupus-like disease. Thus, bispecific PTGDR-1 and PTGDR-2 antagonists, such as AMG853, are a promising class of drugs for the treatment or prevention of organ damage in systemic lupus erythematosus.

Keywords: SLE, AMG853, PGD_2, basophils, PTGDR

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INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic remitting–relapsing autoimmune disease affecting mainly women of child-bearing age (1). These relapses are associated with the increased detection of autoreactive immunoglobulin titers [of immunoglobulin G (IgG), IgA, IgM, and IgE isotypes] mainly raised against nuclear antigens such as double-stranded DNA (dsDNA) or ribonucleoproteins (RNPs) (2). Autoreactive antibodies can form circulating immune complexes (CICs) together with autoantigens and complement factors, the deposition of which in target organs can lead to chronic inflammation and organ failure (3). About 25%–50% of SLE patients develop lupus nephritis, which is evidenced by the glomerular deposition of CIC and which can evolve toward fibrosis, glomerular dysfunction, and kidney failure (4). No efficient specific treatment is currently available for SLE patients, and flares of the disease are usually contained with high doses of corticosteroids and immunosuppressive drugs that are not devoid of serious side effects. Maintenance therapy is recommended after lupus nephritis flares to prevent further relapses and end-stage renal disease. These immunosuppressive therapies can be deleterious and are associated with high morbidity (4, 5). There is an urgent need to develop safe alternatives to maintain the remission state and/or prevent the occurrence of kidney involvement in SLE patients.

Autoantibodies and CICs are considered the main pathogenic factors in the pathophysiology of SLE. Beyond their direct effects on the targeted organs, CICs can activate some innate immune cells through Fc receptors and/or nucleic acid receptors. For instance, CICs induce the production of type I interferon (IFN) by plasmacytoid dendritic cells, the production of B-cell-activating factor of the tumor necrosis factor (TNF) superfamily (BAFF) by monocytes and macrophages, and the release of autoantigens by neutrophils through NETosis (neutrophil extracellular traps). Thus, these immune cells participate in the amplification of autoantibody production by providing key cytokines or antigens to autoreactive B and T cells (1, 3).

We previously showed that basophils contributed to SLE disease amplification by promoting the production of autoantibodies after their accumulation in secondary lymphoid organs (SLOs), both in several lupus-like mouse models and in SLE patient cohorts (6–10). In addition, we demonstrated that IgE, autoreactive IgE, and type 2 immunity contributed to the pathophysiology of lupus disease both in lupus-like mouse models and in SLE patients (8, 10–13). Basophils can be activated by numerous inflammatory mediators, including prostaglandin D_2 (PGD_2), the titers of which were increased in active SLE patients and lupus-prone mice (10). Indeed, combined treatment with antagonists targeting each PGD2 receptors (PTGDR), e.g., PTGDR-1 (laropiprant) and PTGDR-2 (CAY10471), was sufficient in reducing basophil recruitment to SLOs, plasmablast accumulation, autoreactive antibody production, CIC glomerular deposition, and kidney inflammation in less than 10 days, in both genetic spontaneous and inducible lupus-like nephritis mouse models (10). However, these effects could have been due to particular features of the antagonists used. Indeed, laropiprant showed inverse agonist and pharmacochaperone properties by inhibiting PTGDR-1 constitutive cAMP production and by stabilizing its expression in the plasma membrane (14), while CAY10471 showed an extremely low dissociation rate from PTGDR-2 ("insurmountable" antagonist) (15). AMG853 (vidupiprant) is a bispecific antagonist of both PTGDR-1 and PTGDR-2 without such particular properties that showed good safety and tolerability profiles in clinical trials (16). AMG853 could represent a promising alternative to preventing or limiting basophil accumulation in SLOs and breaking the basophil-dependent amplification loop of autoantibody production.

Here, we evaluated the efficacy of AMG853 in dampening the lupus-like disease in aged $Lyn^{-/-}$ female mice. AMG853 treatment reduced basophil and plasmablast accumulation in SLOs, autoreactive antibody titers, CIC glomerular deposition, and the kidney inflammation in this model. Overall, we established a proof of concept that AMG853, a clinically relevant bispecific PTGDR-1 and PTGDR-2 antagonist, can control lupus-like inflammation in a manner similar to the combination of PTGDR-1- and PTGDR-2-specific antagonists.

MATERIALS AND METHODS

Mice and Treatments

C57BL/6J wild-type (WT) mice were purchased from Charles River Laboratories. $Lyn^{-/-}$ mice (17) on a pure C57BL/6J genetic background were bred in our local animal facility in specific and opportunistic pathogen-free conditions and maintained in specific pathogen-free conditions during the experiments. For *ex vivo* experiments, spleen from 10- to 12-week-old WT mice were used. Only 40- to 50-week-old female mice were used in the *in vivo* experiments. Mice received treatment by oral gavage with 14 mg kg^{-1} day^{-1} of AMG853 (Tocris, Bio-Techne, Noyal Châtillon sur Seiche, France) or vehicle (10% ethanol in tap water) daily for 10 days. Blood was harvested under isoflurane anesthesia in the retro-orbital sinus on day −1 of the treatment procedure or by intracardiac puncture immediately after sacrifice on day 10. Mice were euthanized in a controlled-released CO_2 chamber. The study was conducted in accordance with the French and European guidelines and was approved by the local ethics committee comité d'éthique Paris Nord no. 121 and the Ministère de l'enseignement supérieur, de la recherche et de l'innovation under the authorization number APAFIS#14115.

Human Sample Handling

Blood samples were collected from adult healthy volunteers. The study was approved by the Comité Régional de Protection des Personnes (CRPP, Paris, France) under the reference ID-RCB 2014-A00809-38. Written informed consent was obtained from all individuals. All samples were collected in heparin blood collection tubes (Becton Dickinson, Franklin Lakes, NJ, USA) and processed within 2 h as previously described (10). Blood was centrifuged at 600 × *g* for 10 min at room temperature and plasma was removed. ACK (ammonium–chloride–potassium)

lysing buffer (150 mM NH_4Cl, 12 mM $NaHCO_3$, 1 mM EDTA, pH 7.4) was added to the blood and incubated for 5 min at room temperature and an additional 5 min on ice. Twenty-five milliliters of phosphate-buffered saline (PBS) was added and the cells then centrifuged (500 × *g*, 5 min). This step was repeated three times. After lysis of the red blood cells, basophils were purified to >95% by magnetic negative selection following the manufacturer's instructions using the Human Basophils Enrichment kit (STEMCELL Technologies, Vancouver, Canada).

Ex Vivo Primary Cell Stimulation

Human blood basophils and mouse splenocytes were cultured in a culture medium (RPMI 1640 with Glutamax and 20 mM HEPES, 1 mM Na-pyruvate, and 1× non-essential amino acids; all from Life Technologies, Carlsbad, CA, USA), 100 µg/ml streptomycin and 100 U/ml penicillin (GE Healthcare, Chicago, IL, USA), and 37.5 µM β-mercaptoethanol (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 20% heat-inactivated fetal calf serum (Life Technologies) at 37°C and 5% CO_2. For human basophils, 10,000 cells in 200 µl medium per point were used. For mouse splenocytes, 2 million cells in 200 µl medium per point were used. The cells were pretreated or not with 1 µM AMG853 (Tocris, Bio-Techne) over 15 min at 37°C and 5% CO_2. Then, 1 µM PGD_2 (Cayman Chemicals, Ann Arbor, MI, USA) was added or not to the cells for 20 h. At the end of the incubation, the cells were harvested and stained as described in the following section.

Flow Cytometry

Single-cell suspensions from the spleen or peripheral lymph nodes (LNs; pooling inguinal, axillary, and cervical LNs) were prepared as previously described (10). For murine cells, unspecific antibody-binding sites were saturated with a blocking buffer containing 10 µg/ml of anti-CD16/CD32 antibody clone 2.4G2 (BioX Cell, Lebanon, NH, USA) and 100 µg/ml of polyclonal rat IgG, polyclonal mouse IgG, and polyclonal Armenian hamster IgG (Innovative Research Inc., Novi, MI, USA) in fluorescence-activated cell sorting (FACS) buffer (PBS, 1% bovine serum albumin, 1 mM EDTA, and 0.05% sodium azide). Mouse cells were stained in optimized concentrations of fluorophore-conjugated monoclonal antibodies, the list of which is available in **Supplementary Table S1**. Basophils were defined as $CD45^{lo}CD3^{-}CD19^{-}CD117^{-}CD200R3^{+}CD49b^{+}Fc\varepsilon RI\alpha^{+}CD123^{+}$ cells among $CD45^{+}$ viable singlets. Plasmablasts were defined as $CD45^{+}CD3^{-}SSC^{lo}CD138^{+}CD19^{+}$I-A/I-$E^{+}$. The ratio of the geometric mean fluorescence intensity (gMFI) of the marker of interest to the isotype control gMFI was normalized to the mean of the values from WT animals treated with vehicle in each experiment and is expressed in arbitrary units (a.u.). Human basophils were stained with the antibodies listed in **Supplementary Table S1**. Human basophils were defined as $Fc\varepsilon RI\alpha^{+}CD123^{+}CCR3^{+}$ cells. For all flow cytometry experiments, dead cells were stained in PBS with Ghost 510 viability dye (TONBO Bio., San Diego, CA, USA) and were excluded from the analysis. Before staining, unspecific antibody-binding sites were saturated with a blocking buffer containing 100 µg/ml of polyclonal rat IgG, polyclonal mouse IgG, polyclonal goat IgG, and polyclonal human IgG (Innovative Research Inc.) in FACS buffer. Flow cytometry acquisition was realized using a Becton Dickinson 5-laser LSR II Fortessa X-20 and data analysis using FlowJo vX (Treestar, BD Biosciences, Franklin Lakes, NJ, USA).

Tissue Analyses and Miscellaneous Assays

Both kidneys were collected. The left kidney was embedded in OCT (CellPath, Powys, UK) and snap-frozen in liquid nitrogen before immunofluorescence analyses. Thereafter, 4-µm acetone-fixed cryosections were blocked in 10% fetal calf serum and stained with fluorescein isothiocyanate (FITC)-conjugated anti-mouse C3 (Cedarlane, Ontario, Canada) or Alexa Fluor® 488-conjugated anti-mouse IgG F(ab)′2 (Jackson ImmunoResearch, West Grove, PA, USA), or their respective isotype controls, before being mounted in Immu-Mount (Thermo Fischer Scientific, Waltham, MA, USA) and analyzed by fluorescent microscopy (Leica DMR; Leica Microsystems, Wetzlar, Germany). The ratio of specific glomerular fluorescence to the tubulointerstitial background was then measured using ImageJ software (NIH, Bethesda, MD, USA), averaging 30 glomeruli per mouse for each sample. Half of the right kidney was homogenized in PBS containing protease inhibitors (Thermo Fischer Scientific) and centrifuged for 10 min at 10,000 × *g* at 4°C. Supernatants were harvested and stored at −80°C until kidney cytokine analyses by ELISA. Mouse interleukin 1β (IL-1β) and IL-4 ELISAs (Duoset; R&D Systems, Minneapolis, MN, USA) were performed as per the manufacturer's instructions after diluting the kidney extracts to 5 mg/ml of proteins (Pierce BCA Protein Assay, Thermo Fischer Scientific). The other half of the right kidney was fixed in 10% formalin (Sigma-Aldrich), embedded in paraffin, and 5-µm sections were stained by hematoxylin and eosin or Masson's trichrome and then imaged with a conventional optical microscope (Leica DMD108, Leica Microsystems).

Serum IgE was quantified using a Mouse IgE ELISA Kit (Bethyl Laboratories, Montgomery, TX, USA) with the samples diluted 1:10 or 1:20 and the anti-dsDNA IgG using a homemade method as previously described with the samples diluted 1:50 (10). Absorbance at 450 nm and its correction at 570 nm were assessed by an Infinite 2000 PRO plate reader (Tecan, Männedorf, Switzerland).

Statistics

We applied Student's unpaired *t*-tests to compare the differences of one variable between two groups when the distributions were Gaussian and the Mann–Whitney *U* test for non-parametric distributions. When more than two groups were compared (**Figure 1**), one-way ANOVA coupled with Tukey's multiple comparisons test was used. Paired two-way ANOVA coupled with Holm–Sidak's posttest was used to analyze the effects of two variables, such as treatment and time, on the same individuals. Individual mice were always represented as a dot, with the mean ± SEM or bars indicating variability from day 0 (D0) to D10 of treatment. Statistical calculations were done using Prism v9 software (GraphPad Software, San Diego, CA, USA).

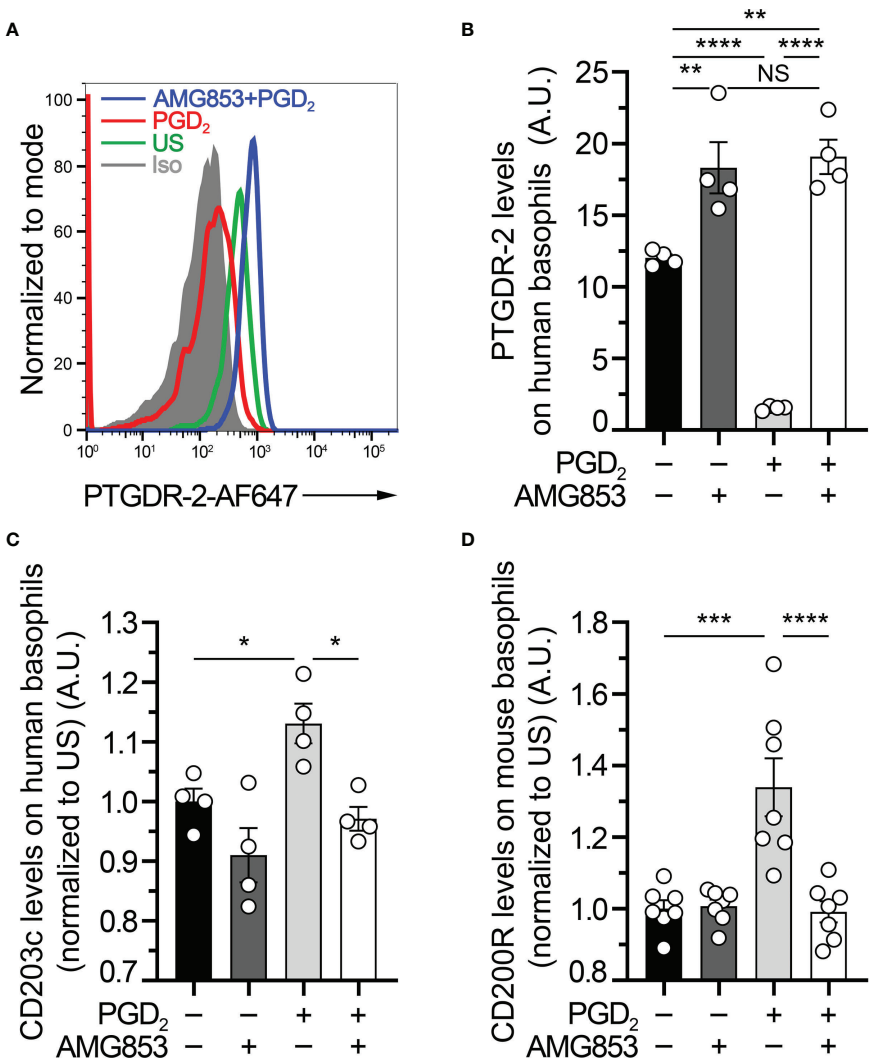

FIGURE 1 | AMG853 blocks *ex vivo* prostaglandin D_2 (PGD_2)-induced basophil activation. **(A)** Representative FACS analysis of the expression levels of PTGDR-2 (also CD294 or CRTH2) on purified human blood basophils (as described in *Materials and Methods*) after 20 h of incubation without (–) or with (+) 1 μM PGD_2 and with or without 1 μM of the bispecific PTGDR-1 and PTGDR-2 antagonist AMG853. *Gray-filled histogram*: isotype control signal; *green line*: unstimulated (US) control; *red line*: PGD_2-stimulated basophils; *blue line*: AMG853-treated and PGD_2-stimulated basophils. Human basophils were defined as $Fc\varepsilon RI\alpha^+CD123^+CCR3^+$ cells. **(B)** PTGDR-2 levels on purified human basophils treated as indicated in **(A)**. **(C)** CD203c levels on purified human basophils treated as indicated in **(A)** and normalized to the mean of the US conditions. **(D)** CD200R1 (CD200R) levels on mouse basophils from splenocytes from wild-type (WT) mice incubated for 20 h as indicated in **(A)** (as described in *Materials and Methods*) and normalized to the mean of the US conditions. **(B–D)** Results were from at least two independent experiments. Individual values are indicated *inside bars* representing the mean ± SEM. Statistical analysis used one-way ANOVA coupled with Tukey's multiple comparisons posttest between the indicated groups. $^{NS}p > 0.05$; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$.

RESULTS

AMG853 Blocks *Ex Vivo* PGD_2-Induced Basophil Activation

PTGDR-2, also known as CD294 or chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2), is internalized following engagement by PGD_2 (10, 18). Human basophil stimulation by PGD_2 induced an upregulation of the basophil activation marker CD203c (10, 19). To validate the efficacy of AMG853 in blocking the PGD_2-induced basophil activation, purified human basophils were pretreated or not with 1 μM of AMG853 and then stimulated with 1 μM PGD_2 over 20 h. AMG853 pretreatment prevented PGD_2-induced PTGDR-2 internalization and led to its accumulation on the surface of basophils (**Figures 1A, B**), suggesting that the levels of PTGDR-2 detected in unstimulated conditions were lowered by an autocrine effect of culture-induced PGD_2 production by basophils, as previously shown (10). PGD_2 induced an increase in the basophil expression of CD203c, an effect that was completely blocked by AMG853 pretreatment (**Figure 1C**).

CD200R (or CD200R1) is a recognized mouse basophil activation marker (20, 21). A similar blocking effect of AMG853 was observed on the PGD_2-induced CD200R overexpression by mouse spleen basophils (**Figure 1D**).

Together, these results showed that AMG853 could indeed prevent the PGD_2-induced activation of both human and mouse basophils *ex vivo*.

AMG853 Treatment Dampens Basophil Accumulation and Activation in SLOs During Lupus-Like Disease

Lyn$^{-/-}$ mice have a peripheral basophilia associated with an IgE-, IL-4- and basophil-dependent T helper type 2 (TH2) bias (10, 11). With aging, the basophils of *Lyn*$^{-/-}$ mice accumulate in SLOs, which support autoreactive humoral immunity, IgE class switching, and the development of a spontaneous lupus-like disease (8, 10, 22, 23). To evaluate the efficacy of AMG853 in reducing the severity of lupus-like disease in sick *Lyn*$^{-/-}$ mice, 40- to 50-week-old WT and *Lyn*$^{-/-}$ female mice received treatment by oral gavage with 14 mg kg^{-1} day^{-1} of AMG853 for 10 days. Such treatment led to a dramatic decrease in the detection of basophils in both the spleen and LNs of aged and diseased *Lyn*$^{-/-}$ mice (**Figures 2A–C** and **Supplementary Figure S1**), confirming the PTGDR-dependent accumulation of basophils in SLOs during the course of the disease in *Lyn*$^{-/-}$ mice.

The expression of the mouse basophil activation marker CD200R was increased on the surface of basophils in the pristane-induced lupus-like mouse model (9). Similarly, the expression of CD200R was increased on the surface of basophils in the spleen and LNs of aged *Lyn*$^{-/-}$ mice compared to their WT counterparts, and 10 days of AMG853 treatment decreased its levels on both *Lyn*$^{-/-}$ spleen and LN basophils (**Figures 2D, E**).

Altogether, these results demonstrated that AMG853 treatment was efficient in dampening basophil activation and accumulation in SLOs in aged and diseased *Lyn*$^{-/-}$ mice.

AMG853 Treatment Dampens Plasmablast Accumulation, Autoantibody Titers, and TH2 Environment During Lupus-Like Disease

Basophils are known to promote humoral responses through antibody-secreting cell support (8–10, 24, 25). We previously showed that basophils promoted the number and maturation of autoreactive plasmablast in lupus-like mouse models (8–10, 12). Plasmablasts produce autoantibodies of various isotypes, including autoreactive IgG and IgE, which are described as

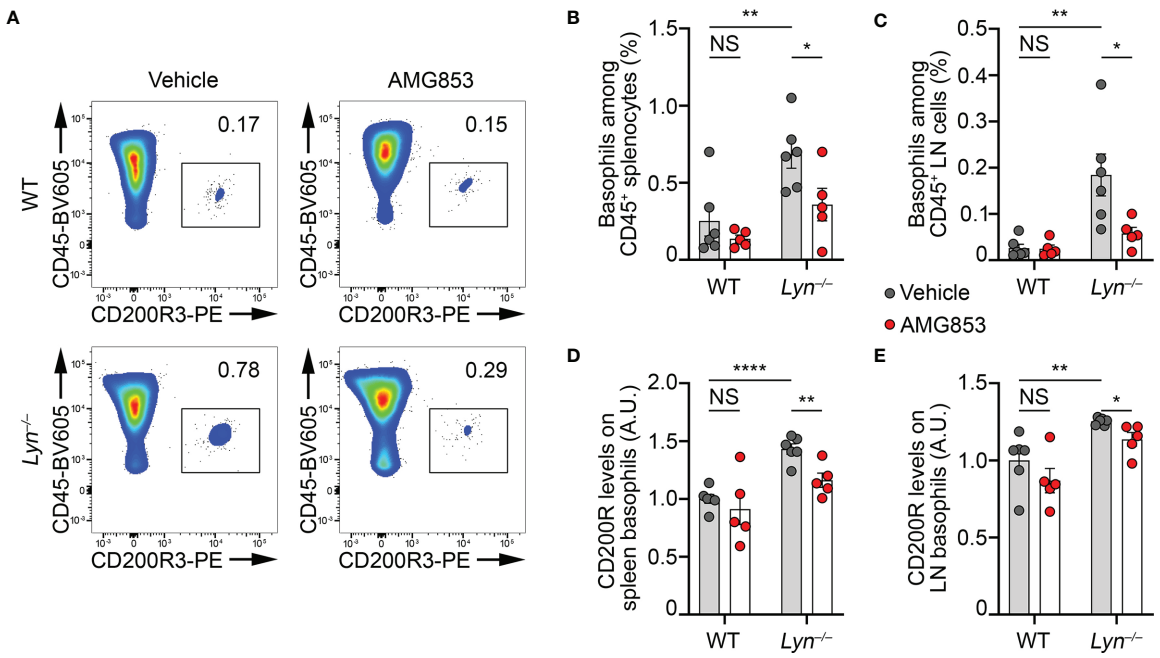

FIGURE 2 | AMG853 dampens the accumulation and activation of basophils in secondary lymphoid organs from *Lyn*$^{-/-}$ mice. Aged (40–50 weeks) wild-type (WT) and *Lyn*$^{-/-}$ female mice were treated for 10 days by oral gavage with AMG853 (n = 5 per genotype, *red filled circles*) or vehicle (10% EtOH in tap water; n = 6 per genotype, *gray filled circles*). Basophil recruitment in the spleen **(A, B)** and peripheral lymph nodes (LNs) **(C)** was assessed by flow cytometry, as exemplified in **(A)** and described in *Materials and methods*. Basophil activation was assessed by measuring the expression levels of CD200R by flow cytometry. The ratio of the geometric mean fluorescence intensity (gMFI) of CD200R signal to the isotype control gMFI was normalized to the mean of the values from WT animals treated with vehicle in each experiment and expressed in arbitrary units (a.u.). Basophil activation was assessed in the spleen **(D)** and LNs **(E)**. Results were from three independent experiments. Individual values are indicated *inside bars* representing the mean ± SEM. Statistical analysis used unpaired Student's *t-tests* between the indicated groups. $^{NS}p > 0.05$; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{****}p < 0.0001$.

contributing pathogenic factors during SLE (2). As AMG853 dampened the activation and recruitment of basophils to SLOs (**Figures 1**, **2**), we next sought to verify whether AMG853 would also decrease the accumulation of plasmablasts in SLOs and the titers of circulating anti-dsDNA autoantibodies. As anticipated, AMG853 treatment led to a dramatic decrease in the proportions of plasmablast (defined as $CD45^+CD138^{hi}CD19^+I\text{-}A/I\text{-}E^+$) in the LNs of diseased *Lyn*$^{-/-}$ mice (**Figures 3A, B**).

As a consequence, while vehicle-treated *Lyn*$^{-/-}$ mice tended to increase their anti-dsDNA IgG autoantibody levels during the 10 days of experiment, AMG853-treated *Lyn*$^{-/-}$ mice showed significantly reduced titers of anti-dsDNA IgG autoantibodies over the same period (**Figure 3C**).

Basophils control a constitutive TH2 skewing in *Lyn*$^{-/-}$ mice in an IgE- and IL-4-dependent manner, which contributes to the development of lupus-like nephritis (8, 11). The serum IgE titers

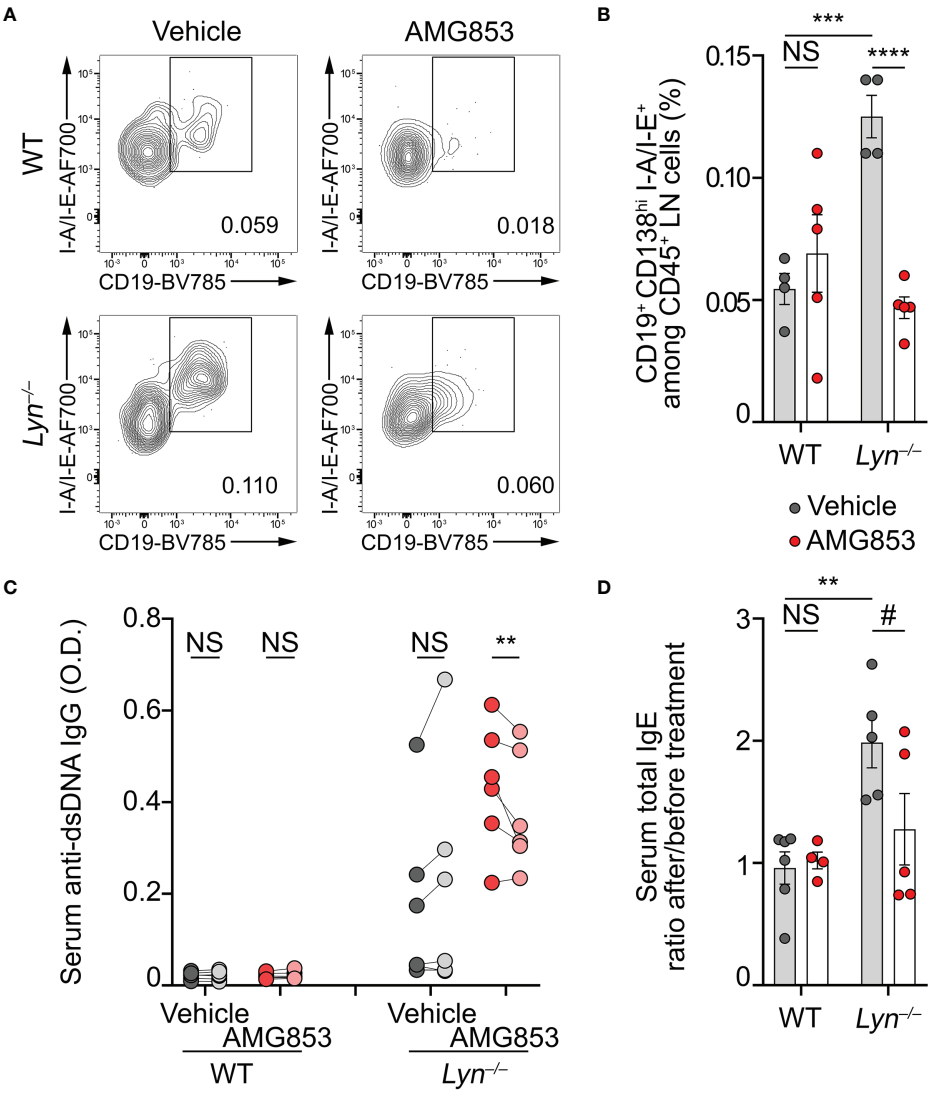

FIGURE 3 | AMG853 dampens plasmablast accumulation, autoantibody titers, and the TH2 environment in *Lyn*$^{-/-}$ mice. Aged (40–50 weeks) wild-type (WT) and *Lyn*$^{-/-}$ female mice were treated daily for 10 days by oral gavage with AMG853 (n = 5 per genotype, *red filled circles*) or vehicle (10% EtOH in tap water; n = 4 per genotype, *gray filled circles*). **(A, B)** Plasmablasts quantified in peripheral lymph nodes (LNs) from the indicated animals by flow cytometry, as exemplified in **(A)** (pre-gated on living $CD45^+CD138^{hi}$ singlets) and summarized in **(B)**. Plasmablasts were defined as $CD45^+CD138^{hi}CD19^+I\text{-}A/I\text{-}E^+$ cells among $CD45^+$ viable singlets from LN cells. **(C)** Anti-dsDNA IgG autoantibody titers from serum samples harvested before (*dark gray-* and *dark red-filled circles*) and after (*light gray-* and *light red-filled circles*) treatment of each mouse with either vehicle (*gray filled circles*) or AMG853 (*red filled circles*). Individual values for each mouse before and after treatment are represented and linked. *O.D.*, optical density. **(D)** Total IgE titers were determined by ELISA in the serum from the indicated individuals. The ratio between individual values after treatment and values before treatment are represented. Results are from two **(B)** or three **(C, D)** independent experiments. **(B, D)** Individual values are shown *inside bars* representing the mean ± SEM. **(B, D)** Statistical analyses by unpaired Student's *t*-tests between the indicated groups. **(C)** Statistical analyses done using two-way analysis of variance (ANOVA) followed by Holm–Šídák's multiple comparisons test. $^{NS}p > 0.05$; $^{\#}p = 0.08$; $^{**}p < 0.001$; $^{***}p < 0.001$; $^{****}p < 0.0001$.

reflected this basophil- and IL-4-dependent TH2 skewing (8). We next measured the total IgE levels as a surrogate marker of the TH2 environment in sera from WT and *Lyn*$^{-/-}$ mice treated with vehicle or AMG853. During the course of the experiments, vehicle-treated *Lyn*$^{-/-}$ mice showed a rise in their IgE titers, whereas most of the AMG853-treated *Lyn*$^{-/-}$ mice had decreased total IgE serum levels, evidencing the induced reduction of the TH2 component of the disease. Importantly, WT mice were not affected by the treatment (**Supplementary Figure S2** and **Figure 3D**).

Altogether, these results gave evidence of the efficacy of targeting PTGDR with the bispecific antagonist AMG853 on reducing both autoantibody-producing cells and autoantibody titers.

AMG853 Treatment Dampens Immune Complex Deposition, TH2, and Pro-Inflammatory Cytokine Environment in Lupus-Like Nephritis

Beyond the efficacy of AMG853 on the accumulation of basophils and autoantibody-producing cells in SLOs, we next sought to verify whether these effects were associated with a reduction in lupus-like nephritis activity in *Lyn*$^{-/-}$ mice. As CIC glomerular deposition facilitates inflammation in lupus-like nephritis, we quantified the IgG and complement component C3 deposits in the glomeruli of WT and *Lyn*$^{-/-}$ mice treated or not with AMG853. AMG853 treatment led to a marked decrease in CIC detection in the glomeruli of treated *Lyn*$^{-/-}$ mice compared to their vehicle-treated counterparts (**Figures 4A–C**).

We previously showed that basophil depletion in aged *Lyn*$^{-/-}$ mice led to a dramatic decrease in glomerular CIC deposition and the kidney content of pro-inflammatory cytokines (8, 10). In line with the effects on the accumulation of basophils and the production of autoantibodies, AMG853 treatment significantly reduced the contents of TH2 and pro-inflammatory cytokines (IL-4 and IL-1β) in the kidneys of *Lyn*$^{-/-}$ mice (**Figures 4D, E**). Of note is that treatment with AMG853 over 10 days was not long enough to ameliorate the glomerular histological lesions observed in aged *Lyn*$^{-/-}$ mice with established disease (**Supplementary Figure S3**).

These results validated the efficacy of AMG853 treatment in reducing both kidney CIC glomerular deposits and renal inflammation in diseased *Lyn*$^{-/-}$ mice.

DISCUSSION

We previously demonstrated the contribution of basophils and the TH2 environment, including IgE and autoreactive IgE, in

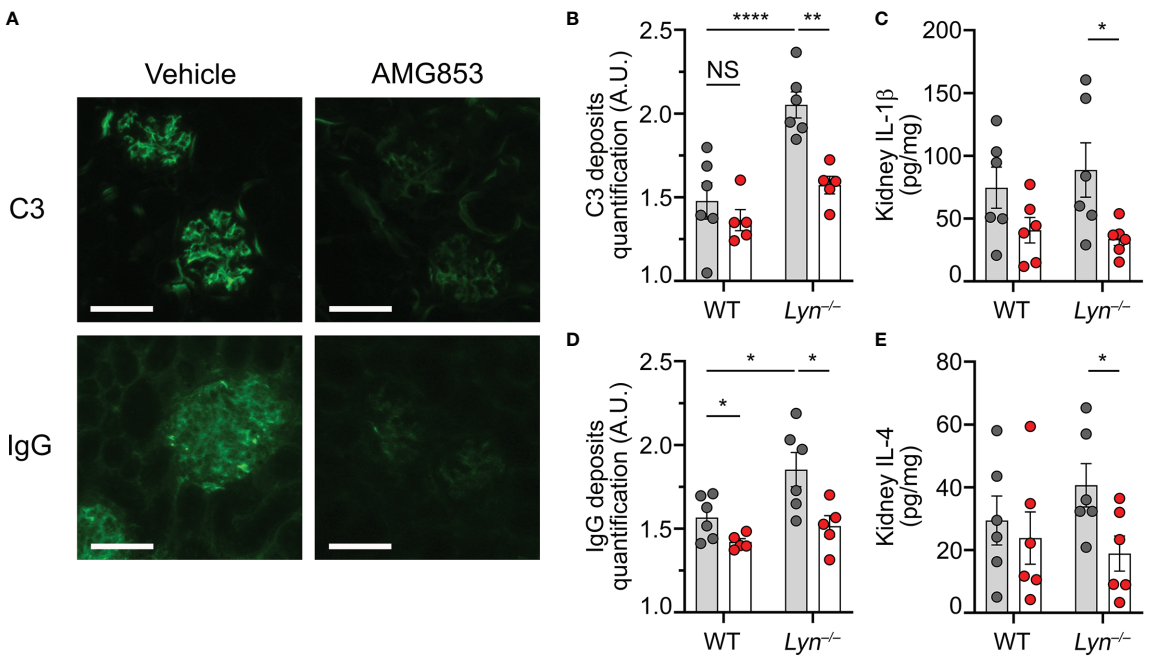

FIGURE 4 | AMG853 dampens lupus-like nephritis in *Lyn*$^{-/-}$ mice. Aged (40–50 weeks) wild-type (WT) and *Lyn*$^{-/-}$ mice were treated daily for 10 days by oral gavage with AMG853 (*n* = 5–6 per genotype, *red filled circles*) or vehicle (10% EtOH in tap water; *n* = 6 per genotype, *gray filled circles*). **(A–C)** Cryosections of kidneys (4 µm) analyzed by immunofluorescence for C3 and IgG staining as exemplified for *Lyn*$^{-/-}$ mice in **(A)** (*scale bar* = 60 µm) and quantified in **(B, C)** as arbitrary units (a.u.) corresponding to the ratio of glomerular measured fluorescence intensity to the interstitial background fluorescence intensity. **(D, E)** Protein extracts from kidneys assessed for total protein and cytokine contents by bicinchoninic acid (BCA) assay and ELISA, respectively, as described in *Materials and methods*. **(D, E)** Content of IL-1β **(D)** or IL-4 **(E)** in kidney extracts expressed in picograms per milligram of renal proteins. **(B–E)** Results from three independent experiments presented as individual values in *bars* representing the mean ± SEM. Statistical analyses used unpaired Student's *t*-tests between the indicated groups. $^{NS}p > 0.05$; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{****}p < 0.0001$.

immune dysregulation leading to the amplification of SLE and lupus nephritis activity. These findings constitute a promising area of new therapeutic strategies for SLE in preclinical and clinical studies (6, 8–10, 12, 26–29). We have recently identified PTGDRs as promising therapeutic targets by assessing, successfully, the efficacy of the combination of two single antagonists, one targeting PTGDR-1 (laropiprant) and the other targeting PTGDR-2 (CAY10471), in ameliorating the autoimmune and renal parameters in lupus-like disease and the importance of the PGD_2 axis in the pathogenesis of lupus (10). However, AMG853, as a single molecule with a bispecific antagonist activity, would be more easily validated in clinical trials than combined treatments. To develop the translation into clinics of our previous results, we explored the proof of concept that AMG853 was efficient in dampening lupus-like disease in mice.

Here, we provided evidence that AMG853, a bispecific antagonist targeting both PTGDRs, was effective in blocking PGD_2-induced human and mouse basophil activation *ex vivo* and in controlling basophil recruitment to SLOs, humoral autoimmunity, IgE production, CIC glomerular deposition, and kidney inflammation in aged $Lyn^{-/-}$ mice with established lupus-like nephritis. Then, we provided the proof of concept that this antagonist bispecific for PTGDR1 and PTGDR2 is efficient in dampening the symptoms of lupus-like nephritis in $Lyn^{-/-}$ mice.

Altogether, the increased total IgE titers in the sera of SLE patients, autoreactive IgE, basophil activation and accumulation in SLOs, and the dysregulation of humoral immunity in SLE patients underlined the key role of type 2 immunity in disease activity amplification and increased risks of relapse. As initially developed for atopic diseases, especially with lung involvement, PTGDR antagonists obviously represent a logical approach to control this TH2 side of the disease. If AMG853 failed to show any benefits as an add-on to corticosteroid therapy for patients with moderate to severe asthma, it was well tolerated without any reported serious adverse events over 12 weeks of treatment (16). However, corticosteroid therapy is known to affect the basophil compartment (30), which suggests that any effects of AMG853 on the activation of basophil in the context of asthma may have been missed in this trial. Thus, AMG853 appeared safe as maintenance therapy for lupus nephritis patients at risk of relapse since these patients are in dire need to reduce the morbidity of their long-term immunosuppressive and corticoid treatments. Breaking the basophil-, IgE-, and PGD_2-dependent amplification loop of SLE might indeed lead to preventing the occurrence of disease flares and also prevent or limit the development of lupus nephritis.

In conclusion, the present study identified AMG853 as a promising candidate to further develop the targeting of PTGDRs in SLE patients. This approach was successfully implemented in $Lyn^{-/-}$ mice in this study, but it needs to be validated in other lupus-prone mouse models such as MRL-Fas^{lpr} or NZBxNZW F1 mice, which constitute lupus-like mouse models with different pathophysiological origins (31). These validations in other preclinical models might strengthen the concept and allow clinical development of the proposed approach. Another question needs to be addressed. Indeed, here and in previous studies, we analyzed the effects of PTGDR blockade on mice with established lupus-like disease (10). It will be of primary interest to determine in longer-term studies whether PTGDR blockade during the early stage of lupus-like nephritis prevents its development and could then be developed as a preventive therapy for SLE patients as well. These additional preclinical studies might allow developing this approach in clinical studies in the near future.

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 the Comité Régional de Protection des Personnes (CRPP, Paris, France) under the reference ID-RCB 2014-A00809-38. The patients/participants provided written informed consent to participate in this study. The animal study was reviewed and approved by Comité d'éthique Paris Nord no. 121—Ministère de l'enseignement supérieur, de la recherche et de l'innovation under authorization number APAFIS#14115.

AUTHOR CONTRIBUTIONS

CP and NC conceived the project, designed and conducted the experiments, and wrote the manuscript. NC directed the project. JT, YL, and CS conducted the experiments. CP and NC had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors approved the final version of the article.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.824686/full#supplementary-material

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Conflict of Interest: CP and NC are co-inventors of the patent WO2016128565A1 related to the use of PTGDR-1 and PTGDR-2 antagonists for the prevention or treatment of systemic lupus erythematosus. NC holds another patent related to compositions and methods for treating or preventing lupus (W020120710042).

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.

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MINI REVIEW
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T Helper 2-Associated Immunity in the Pathogenesis of Systemic Lupus Erythematosus

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Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease that mainly affects women in their reproductive years. A complex interaction of environmental and genetic factors leads to the disruption of immune tolerance towards self, causing overt immune activation and production of autoantibodies that attack multiple organs. Kidney damage, termed lupus nephritis, is the leading cause of SLE-related morbidity and mortality. Autoantibodies are central to propagating lupus nephritis through forming immune complexes and triggering complements. Immunoglobulin G (IgG) potently activates complement; therefore, autoantibodies were mainly considered to be of the IgG isotype. However, studies revealed that over 50% of patients produce autoantibodies of the IgE isotype. IgE autoantibodies actively participate in disease pathogenesis as omalizumab treatment, a humanized anti-IgE monoclonal antibody, improved disease severity in an SLE clinical trial. IgE is a hallmark of T helper 2-associated immunity. Thus, T helper 2-associated immunity seems to play a pathogenic role in a subset of SLE patients. This review summarizes human and animal studies that illustrate type 2 immune responses involved during the pathology of SLE.

Keywords: autoimmunity, SLE, lupus nephritis, Th2, IL-4, IgE, autoantibody

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INTRODUCTION

Systemic Lupus Erythematosus (SLE) is an autoimmune disease that affects multiple organs such as the skin, joints, kidney, heart, and brain (1). SLE mainly affects women in their reproductive years and shows higher prevalence in African, Hispanic, or Asian ethnicities. SLE prognosis has seen significant improvement in the last half-century, with survival probability reaching 92% during a 10-year period (2). However, patients with renal disease, termed lupus nephritis, have a poorer prognosis than those without renal complications (3). Thus, a large proportion of research is focused on understanding the mechanisms surrounding lupus nephritis. Autoantibodies are the primary cause for renal damage *via* induction of inflammation in the kidneys through immune complex deposition and complement activation (4). Indeed, autoantibodies specific for double-stranded DNA (anti-dsDNA) show a positive correlation with disease severity (5). Especially, autoantibodies of the IgG1 and IgG3 isotypes are considered pathogenic due to their superior ability to activate complement and

engage Fc receptors (6). More than 50% of SLE patients, however, also produce autoantibodies of the IgE subclass, and these antibodies showed a strong association with disease severity (7). Moreover, treatment of SLE patients with monoclonal antibodies specific for IgE (omalizumab) improved disease activity in a randomized clinical trial (8). Thus, it seems IgE is also involved in the pathogenesis of lupus nephritis.

IgE is a type 2-associated immunoglobulin typically associated with allergic disease. Therefore, most IgE and type 2 immunity-associated responses have been described in the context of allergic disease. Briefly, exposure to allergens causes epithelial cells to produce alarmins (9). Alarmins activate type 2 innate lymphoid cells (ILC2) and dendritic cells (DCs) to produce type 2 cytokines and to induce T cell differentiation, respectively. Activated DCs induce the differentiation of T helper 2 (Th2) cells and T follicular helper (Tfh) cells. Th2 cells recruit innate immune cells such as eosinophils and mast cells to allergic sites, whereas Tfh cells activate B cells to produce IgE. Subsequently, IgE binds to allergens located in allergic sites, which are then recognized by innate immune cells *via* their IgE receptor, FcεRI. This interaction activates innate cells to produce effector molecules that propagate inflammation (10).

The type 2-associated immune response found in SLE shows similarities and differences with the response seen in allergic disease. This review discusses the type 2-associated immune response found in SLE patients and describes how this immune response is shaped during the induction phase and damages end organs during the effector phase (**Figure 1**).

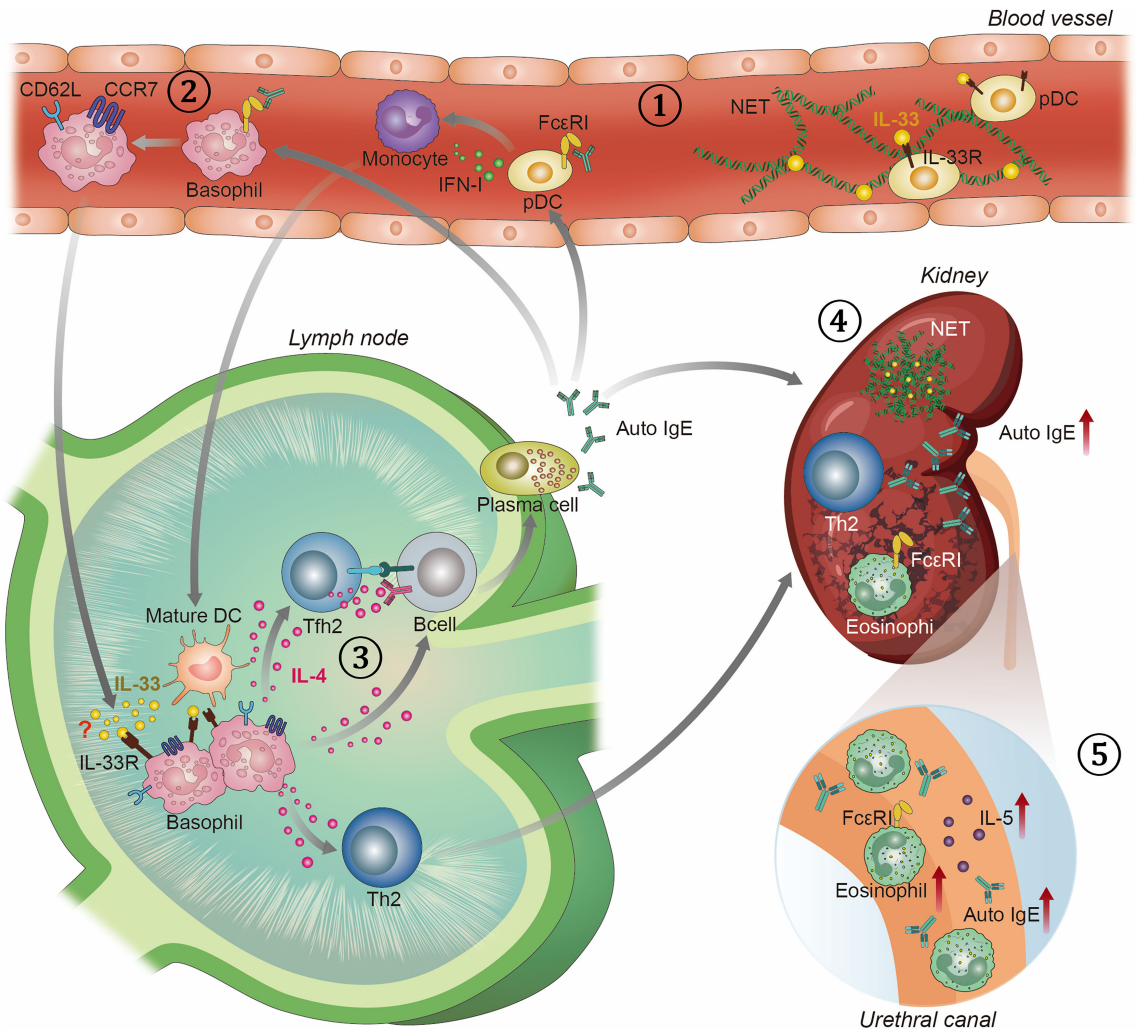

FIGURE 1 | Overview of T helper 2 associated immune response in SLE. 1) SLE patients have elevated levels of NETs decorated with bioactive IL-33 and auto-IgE in blood. They are potent in activating pDCs to produce IFN-I, which are known to play key role in the pathogenesis of SLE. Activated pDCs stimulate monocytes to differentiate into mature DCs. 2) Auto-IgE also stimulates basophils to drain into SLOs by upregulating CD62L and CCR7. 3) In SLO, basophils polarize T cells into Th2 and Tfh2 cells, and activate B cells in IL-4 dependent manner. Tfh2 cells promote the differentiation of B cells into IgE autoantibody-producing plasma cells. IgE autoantibodies, in turn, activate pDCs, basophils, and eosinophils. 4) Once activated, Th2 cells and eosinophils infiltrate into the kidney and augment lupus nephritis. In the kidney, NETs decorated with IL-33 and auto-IgE are also found. 5) Eosinophils, auto-IgE, and IL-5 cytokines are also detected in the urine of SLE patients.

INDUCTION PHASE

Alarmins

Alarmins act as the first line of defense in our body's outer and inner extremities. They are constitutively expressed by structural and immune cells and are rapidly released upon sensing environmental triggers, such as viral infections, tobacco smoke, pollutants, and physical damage (11). Thus, alarmins play crucial roles in shaping the initial response of our immune system. However, excessive release of alarmins is detrimental to our health and can contribute to various pathologies such as tumorigenesis, allergy, and autoimmunity (11). For example, exposure to allergens or viral infections in the lung causes a rapid release of alarmins by epithelial cells, leading to the development of asthma. Asthma is a type 2-mediated inflammatory disease, and the alarmins interleukin-25 (IL-25) and interleukin-33 (IL-33) play essential roles in shaping the Th2 response signature found in asthma.

Interleukin-25

IL-25 is produced by both epithelial cells and immune cells and is involved in the recruitment and activation of Th2 cells, ILC2, B cells, eosinophils, and mast cells. The IL-25 receptor is expressed on multiple immune cells and structural cells, such as endothelial cells, epithelial cells, and fibroblasts. Thus, once released, IL-25 acts in an autocrine feed-forward mechanism (12). Currently, there are two reports on the role of IL-25 in association with SLE (**Tables 1** and **2**). Both reports claim IL-25 expression is increased in the serum of SLE patients, especially in patients with active disease severity and lupus nephritis (13, 14). Moreover, serum IL-25 levels showed a positive correlation with disease severity and anti-dsDNA autoantibodies, suggesting IL-25 might play a pathogenic role in SLE patients (13, 14). However, the second report claims IL-25 plays a protective role in SLE as injection of recombinant IL-25 ameliorated symptoms in MRL/Lpr mice, while the addition of recombinant IL-25 to SLE PBMC cultures suppressed secretion of inflammatory cytokines (13). Moreover, serum levels of IL-25 had a negative correlation with serum levels of IL-5 and IgE in SLE patients (14). Thus, the current evidence suggests IL-25 is highly induced during SLE pathogenesis; however, IL-25 seems to play an immunosuppressive role and is not involved in inducing type 2 responses in SLE patients.

Interleukin-33

IL-33 is a nuclear factor expressed in the nuclei of structural cells and innate cells, which is released upon cell damage and necrosis (39). Once released, IL-33 propagates Th2 immune responses by activating ILC2s, Th2 cells, DCs, eosinophils, mast cells, and basophils. IL-33 also signals through structural cells, such as endothelial cells, epithelial cells, and fibroblasts (40). IL-33 signals through the heterodimeric complex consisting of interleukin-1 receptor-like 1 (IL1RL1 or ST2) and IL-1R accessory protein (IL-1RAcP). Due to its strong induction of type 2 responses, IL-33 is a central player in driving allergic disease (39). However, IL-33 is also involved in maintaining tissue homeostasis and tissue repair *via* activating $ST2^+$ regulatory T (Treg) cells and ILC2s to produce amphiregulin (Areg) (41). In a different light, IL-33, in the presence of IL-12, can also indirectly promote type 1 responses by activating Th1, CD8 T cells, NK cells, and NKT cells to produce interferon-γ (IFN-γ) (39). Thus, IL-33 plays multiple roles in physiology and its function comes in various flavors in a context-dependent manner.

In SLE patients, serum levels of IL-33 proteins were elevated in SLE patients compared to healthy controls (42). However, serum IL-33 levels did not correlate with most clinical and

TABLE 1 | Type 2-associated immune response in SLE patients.

Factor	Role in pathogenesis of SLE (Human)	Reference(s)
IL-25 (Alarmins)	- Increased serum IL-25 protein levels in SLE patients - Serum IL-25 had positive correlation with disease severity and anti-dsDNA autoantibodies in SLE patients - Recombinant IL-25 suppressed inflammtory cytokine secretion from SLE PBMC cultures - Serum IL-25 cytokine had negative correlation with serum IL-5 and IgE levels in SLE patients	(13, 14)
IL-33 (Alarmins)	- Increase of neutrophil extracellular traps (NETs) decorated with IL-33 in blood of SLE patients - NETs with IL-33 showed positive correlation with SLEDAI scores - NETs with IL-33 were detected in inflamed skin and inflamed kidney of SLE patients	(15)
Basophil	- Basophil counts were reduced in circulation and accumulated in lymphnodes and spleen of SLE patients - Basophils are potential primary source of IL-4 in secondary lymphoid organ - Basophils isolated form SLE patients induced anti-dsDNA IgG and anti-dsDNA IgE *in vitro*	(16, 18)
Th2 cells	- Frequency of Th2 cells were increased in blood of SLE patients - Th2 cells were detected in kidney biopsies of Lupus nephritis patients - IL-4 levels positively corrlated with hypercellullarity in nephritis patients	(16, 19, 20)
Tfh cells	- The number of Tfh and Th17 cells positively correlated with disease activity in SLE patients - Low dose of rhIL-2 administration ameliorated disease activity in patients by increasing the number of Tregs and decreasing the number of Tfh and Th17 cells	(21–24)
Tfh2 cells	- The frequencies of Tfh2 cells were significantly higher in active lupus patients than healthy control - The number of Tfh2 cells positively correlated with SLEDAI scores in SLE patients - Tfh2 cells were highly activated in SLE patients and were better at inducing plasma cell differentiaiton in vitro than control group	(25, 26)
Anti-dsDNA IgE	- 50-60% of cohort were positive for autoreactive IgE, and antibody titers correlated with SLEDAI scores and active nephritis - 35% of lupus nephritis patients showed IgE deposition in kidney and had poorer prognosis. - Patients with IgE deposition showed upto 71% increase in occurence rate of lupus nephritis	(7, 27)
Eosinophil	- Increase in urinary eosinophils and IL-5 in lupus nephritis patients. - Eosinophil level showed correlation with renal function and SLE disease activity. - Patients with eosinophiluria and tissue eosinophil infiltration were more likely to progress to end-stage kidney disease. - Blood eosinophil levels between SLE patients and healthy controls were comparable.	(28, 29)

TABLE 2 | Type 2-associated immune response in SLE mouse model.

Factor	Role in pathogenesis of SLE (Mouse)	Reference(s)
IL-25 (Alarmins)	- Injection of recombinant IL-25 ameliorated lupus symptoms in MRL/Lpr mice	(13)
IL-33 (Alarmins)	- Treatment with IL-33 inhibitory antibodies alleviated lupus symptoms in MRL/Lpr mice - Early tratment of recombinant IL-33 alleviated SLE symptoms in NZB/W F1 mice	(30, 31)
Basophil	- Activated basophils and IgE autoantibodies play role in autoantibody production and lupus nephirtis development in *Lyn-/-* mice - IgE autoantibodies aggravated disease in *FcγRIIB-/-, FcγRIIB-/- (x) Yaa* and MRL/Lpr mouse models of SLE	(16, 17, 32–34)
Th2 cells	- Frequency of Th2 cells were increased in *Ets1ΔCD4* mouse model of SLE - Th2 cells were detected in the kidneys of NZM2410 mouse model of SLE - IL-4 neutralizing antibodies administration or STAT6 deletion in NZM2410 mice resulted in the abrogation of nephritis symptoms - IL-4 transgenic B6C3F1 mice developed glomerulosclerosis associated with collagen deposition	(20, 26, 35–37)
Tfh2 cells	- IL-4 neutralization significantly decreased the frequencies of Tfh2 cells in *Ets1ΔCD4* mice while alleviating splenomegaly and reducing IgE autoanibody in serum	26
Extrafollicular helper T cells	- Involved in propagating autoantibody production in extrafollicular zone in MRL/Lpr mice	(38)
Anti-dsDNA IgE	- IgE autoantibodies were shown to be involved in aggravating disease in *Lyn-/-*, *FcγRIIB-/-*, *and FcγRIIB-/- (x) Yaa* mice	(7, 16)

laboratory characteristics of patients, including SLE disease activity index (SLEDAI) scores and anti-dsDNA autoantibody titers. In another study, Mok et al. found comparable levels of IL-33 protein in the serum of SLE patients and healthy controls (43). Thus, it seems the systemic release of IL-33 does not play a role during the pathogenesis of SLE. On the other hand, Georgakis et al. discovered an increase of neutrophil extracellular traps (NETs) decorated with bioactive IL-33 in the blood of SLE patients, which showed a positive correlation with SLEDAI scores (15). NETs decorated with IL-33 were potent in activating plasmacytoid dendritic cells (pDCs) to produce type 1 interferons (IFN-I), which play key roles during SLE pathogenesis (15, 44). Interestingly, this group also detected IL-33-decorated NETs in inflamed skin and inflamed kidney of SLE patients. However, the role played by IL-33-decorated NETs at these sites requires further investigation. Mouse studies mirror discoveries made in humans as treatment of IL-33 inhibitory antibodies alleviated lupus symptoms in MRL/Lpr mice (30). It will be interesting to evaluate whether the therapeutic effect of IL-33 neutralization is due to suppression of IL-33-decorated NETs in MRL/Lpr mice. There are, however, some discrepancies in animal models as early treatment of recombinant IL-33 to NZB/W F_1 mice alleviated SLE symptoms (31). As explained earlier, the effect of IL-33 is highly context-dependent and it seems IL-33 is pathogenic in SLE only when in complex with NETs. Although the role of IL-33 decorated NETs for activating pDCs and inducing IFN-I has been identified, their roles in inducing type 2 immune responses in SLE requires further investigation.

Basophil

Basophils are one of the rarest cell types in the body, making up less than 1% of the circulating white blood cell population. Due to their rarity, basophils were considered less important for the pathogenies of SLE. However, the role of basophils has been re-examined in the past few decades. The first evidence was provided by a study on Lyn-deficient mice ($Lyn^{-/-}$) mice. Lyn is a Src family protein tyrosine kinase and it is reported that $Lyn^{-/-}$ mice develop spontaneous autoimmunity and lupus nephritis in old age (16). This study further demonstrated that activated basophils and IgE autoantibodies play essential roles in amplifying autoantibody production and the development of lupus nephritis. Mechanistically, basophils were activated by autoreactive IgE and recruited to secondary lymphoid organs (SLO) where they promoted Th2 cell differentiation and the production of autoantibodies (16) (**Figure 1**). The role of basophil in aggravating SLE was further demonstrated in $Fc\gamma RIIB^{-/-}$, $Fc\gamma RIIB^{-/-}$ *(x) Yaa* and MRL/Lpr mouse models of SLE as well as in the pristine-induced model of SLE (17, 32–34). This phenomenon was also discovered in SLE patients where basophil counts were reduced in circulation while they accumulated in the lymph nodes and spleen of SLE patients (16, 17). The recruitment of basophils to SLO requires activation by factors such as autoreactive IgE and Prostaglandin D2 (PDG2) (16, 34). Upon activation, basophils upregulate CD62L, CCR7, and CXCR4 which leads to their recruitment to SLOs (17).

Once recruited into SLOs, basophils are involved in activating T cells, B cells, and pDCs. IL-4 is indispensable for the induction of Th2 cells, yet the initial source of IL-4 has remained elusive. Currently, basophils are considered the potential primary source of IL-4 *in vivo* (18). Thus, basophils are highly effective in polarizing Th2 cell differentiation while inhibiting differentiation into Th1 cells (45). Similarly, T follicular helper type 2 (Tfh2) cells also require IL-4 for its differentiation (26). Therefore, basophils might also play a central role during the induction of Tfh2 cells; however, the main cell types involved during the differentiation of Tfh2 cells are yet to be determined. Tfh2 cells are the main cell type that induces IgE class switching of B cells, therefore, the induction of Tfh2 cells is a key step for the induction of autoreactive IgE responses. This is discussed in more detail later. In addition to the production of Th2 related cytokines, basophils can also express IL-6 upon activation. IL-6 is required for driving Th17 differentiation, and indeed Pan et al. demonstrated basophils isolated from SLE patients but not from controls could induce the differentiation of Th17 cells (17).

In addition to activating T cells, basophils are also involved in activating B cells. Upon activation, basophils upregulate expression of molecules such as B-cell activating factor (BAFF), and A proliferation-inducing ligand (APRIL), thereby interacting with B cells in a contact-dependent manner (46). Moreover, IL-4 is a survival factor and differentiation factor for B cells. Thus, basophil derived IL-4 is important for the survival of B cells and the differentiation of B cells to plasma cells, and isotype switching of B cells to IgE isotype (47). Indeed, culture of basophils isolated from SLE patients with B cells induced the secretion of anti-nuclear IgG and anti-nuclear IgE, in the absence of T cells (17). Thus, basophils are involved in the differentiation and activation of both T cells and B cells in SLOs.

T Helper 2 Cells

Th2 cells mainly mediate type 2 immune responses. Type 2 immune responses are critical in eradicating extracellular parasites but are also involved in diseases such as asthma, food allergy, and atopic dermatitis. IL-4, IL-5, and IL-13 are the main effector cytokines secreted by Th2 cells. IL-5 signals through the IL-5R, which is highly expressed in eosinophils and partly in basophils and mast cells. However, the function of IL-5 is quite specific for the biology of eosinophils and is involved in the activation, survival, and differentiation of eosinophils (48). IL-4 and IL-13 drive most of the hallmarks of Th2 responses such as IgE production, M2 macrophage differentiation, smooth muscle contractility, mucus production, and recruitment of innate cells to the site of inflammation (49). IL-4 and IL-13 play redundant roles as they are usually co-expressed by lymphocytes, and they share the IL-4 receptor alpha (IL-4Rα). In terms of IgE production, both IL-4 and IL-13 play important roles. Indeed, *Il4* $^{-/-}$ and *Il13* $^{-/-}$ mice show partial IgE reduction while *Il4*$^{-/-}$/*Il13*$^{-/-}$ double knockout mice and *Il4r*$^{-/-}$ mice show the highest reduction in IgE levels (35).

Th2 cells produce a high amount of IL-4 and IL-13, therefore, they were considered important for the shaping of humoral immune responses (50). However, the concept of Th2 mediated-IgE production and B cell activation must be revisited since recent studies prove that Tfh2 cells are the main contributors to IgE induction (26, 51, 52). Nevertheless, Th2 cells and Tfh2 cells share many characteristics, such as the expression of GATA-3 and the requirement of IL-4 cytokine for its differentiation. Therefore, Th2 cells might aid the induction of Tfh2 cells in a paracrine manner by providing a steady amount of IL-4 in the T cell zone. Moreover, the frequency of Th2 cells is increased in SLE mouse models and SLE patients, implying that they may have a role in the pathogenesis of SLE, be it independent of B cell activation (16, 26). Indeed, the role of Th2 cells in end-organ damage is further discussed below.

T Follicular Helper 2 Cells

One of the critical features of SLE is the generation of autoantibodies against nuclear components. They form immune complexes (ICs) and cause chronic systemic inflammatory autoimmune disease. Antigen-specific autoreactive antibody-producing B cells develop in germinal centers (GCs) in which B cells undergo somatic hypermutation, selection, and differentiation into antibody-producing plasma cells and long-lived memory B cells (53). T follicular helper (Tfh) cells play critical roles in mediating GC reactions, from GC formation to the induction of high-affinity antibody-producing plasma cells. Since patients with SLE have somatically mutated high-affinity autoantibodies in serum, Tfh cells are likely to be involved with the pathogenesis of SLE (54, 55). The number of Tfh and Th17 cells positively correlated with disease activity in SLE patients, suggesting these cells mediate pathogenic responses during SLE (21–23). A clinical trial partially confirmed this hypothesis as administration of low-dose recombinant human IL-2 (rhIL-2) ameliorated disease activity in patients by increasing the number of regulatory T cells (Tregs) and decreasing the number of Tfh and Th17 cells (24).

Blood Tfh cells can be further subtyped into Th1, Th2, and Th17 subsets according to the expression of CXCR3 and CCR6 (51). Among these subsets, Tfh2 cells are involved in the pathogenesis and etiology of SLE. The frequencies of Tfh2 cells are significantly higher in active lupus patients, and the number of Tfh2 cells positively correlates with SLEDAI scores in SLE patients (25, 26). Tfh2 cells express GATA-3 and secrete high amounts of IL-4 (26). IL-4 acts as both an inducing factor and an effector molecule of Tfh2 cells as IL-4 neutralization significantly decreased the frequencies of Tfh2 cells in SLE mice while alleviating splenomegaly and reducing IgE autoantibody titers in SLE mice (26). In addition to IL-4, interferon-α (IFN-α) is also described to enhance the generation of circulatory Tfh2 cells in a model of adenovirus infection (56). The primary function of Tfh2 cells is the induction of IgE. Among the three Tfh subsets, only Tfh2 cells could promote IgE induction *in vitro* (51). Additionally, Tfh2 cells have superior capabilities to induce IgG production from B cells compared to Tfh1 cells. In line with this finding, Le Coz et al. and our group have observed Tfh2 cell frequencies have a positive correlation with serum anti-dsDNA IgG and anti-dsDNA IgE titers (25, 26). Additionally, Tfh2 cells were highly activated in SLE patients compared to the control group, as they had higher IL-4, IL-5, and IL-13 expressions and were better at inducing plasma cell differentiation of B cells *in vitro* (26). The exact mechanism underlying activation of Tfh2 cells in SLE patients is unknown, and requires further investigation.

Extrafollicular Helper T Cells

IgE$^+$ B cells follow a unique path of maturation compared to IgG$^+$ B cells. IgE$^+$ B cells experience a short period in germinal centers (GC) and quickly exit GCs to complete their maturation process in the extrafollicular zone (57). Therefore, IgE$^+$ plasma cells (PCs) come in two waves. An initial wave of IgE$^+$ PCs directly differentiate in the extrafollicular zone and have B cell receptors that have not undergone somatic hypermutations (SHM). The second wave of IgE$^+$ PCs arises from IgE$^+$ germinal center B cells (GC B) and have B cell receptors that have undergone SHM (57). As anti-dsDNA IgE and autoreactive IgE require SHM, we can confer that autoreactive IgE arises from GC-derived PCs. The cell types involved in the extrafollicular B cell response are not well described; however, the role of

extrafollicular helper T cells has been recently described in the context of lupus.

Extrafollicular helper T cells have been detected in the spleens of MRL/Lpr mice (38). These cell types were important for driving the formation of extrafollicular plasmablasts in MRL/Lpr mice *via* CD40L, Icos, and IL-21. Similar to their Tfh cell counterparts, extrafollicular helper T cells required Bcl6 for their differentiation. However, they express distinct markers from Tfh cells: $CD4^{+}PSGL\text{-}1^{low}CXCR5^{low}CXCR4^{+}$. Extrafollicular helper T cells are also found in human tonsils, and they are $CD4^{+}PSGL1^{hi}PD\text{-}1^{hi}CXCR5^{hi}$ cells (58). They exhibit a transcriptionally distinct phenotype from Tfh cells and promote memory B cells to produce immunoglobulins *via* CD40L, IL-10, and IL-21. It is interesting to note that extrafollicular T helper cells express CXCR4 and that basophils activated by PGD2 turn on the same chemokine receptor. Thus, basophils have a high possibility of localizing at the extrafollicular region. In addition, considering the importance of IL-4 for the induction of IgE in B cells, the expression of IL-4 in extrafollicular helper T cells must be addressed in the future.

EFFECTOR PHASE

T Helper 2 Cells

Once Th2 cell differentiation is completed within secondary lymphoid organs, they are recruited to the site of inflammation to elicit effector functions. Th2 cells are detected in kidney biopsies of lupus nephritis patients as detected by immunohistochemistry and in the kidneys of the NZM2410 mouse model of SLE (19, 36). IL-4 levels correlated with hypercellularity in nephritis patients suggesting Th2 cells play an active role in disease progression. Similarly, administration of IL-4 neutralizing antibodies or deletion of STAT6 in NZM2410 mice resulted in the abrogation of nephritis symptoms (20). Of note, IL-4 seems to be involved in glomerulosclerosis *via* direct interaction with kidney cells (36, 59). In a different model of IL-4 transgenic B6C3F1 mice, mice developed glomerulosclerosis associated with collagen deposition (37).

Alternatively, Th2 cells might implement its effector functions by recruiting and maintaining eosinophils in the kidney *via* releasing IL-5 cytokine in the kidney microenvironment. IL-5 is indispensable for the recruitment and survival of eosinophils. Interestingly, urinary IL-5 and eosinophiluria were increased in patients with lupus nephritis, suggesting IL-5 mediated recruitment of eosinophils occurs in nephritis patients (28). Thus, Th2 cells might mitigate effector functions *via* two tracks by interacting with kidney cells to induce glomerulosclerosis or secreting IL-5 to recruit eosinophils to the kidney.

Anti-dsDNA IgE

The role of autoreactive IgE in SLE patients has been discussed since the 1970s in line with their abilities to promote basophils (60). IgE is the least abundant immunoglobulin isotype in a healthy individual and is commonly known to trigger type I hypersensitivity. Elevated levels of IgE reflect type 2 immune response since type 2 cytokines, such as IL-4, induce IgE production (61). Among 117 US and 79 French patients with SLE, about 50~60% of the cohort were positive for autoreactive IgE. These antibody titers correlated with disease activity and active nephritis, suggesting IgE is involved during the pathogenesis of SLE (7). Indeed, anti-dsDNA IgE levels show comparable disease predictive ability as anti-dsDNA IgG, while the combination of both parameters enhances prediction (7). Moreover, anti-dsDNA IgG and anti-dsDNA IgE are each risk factors for SLE in an independent fashion, suggesting they do not share identical pathways (47).

In line with human studies, there is ample evidence from mice studies highlighting the pathogenic role of IgE autoantibodies in SLE. IgE autoantibodies were involved in aggravating disease in *Lyn*$^{-/-}$, *Fc*γ*RIIB*$^{-/-}$, and *Fc*γ*RIIB*$^{-/-}$*/Yaa* mice (7, 16). Deficiency of IgE in SLE mouse models resulted in the decrease of autoantibodies and amelioration of organ pathology. Mechanistically, IgE autoantibodies were involved in the activation of basophils (which is discussed above) and the activation of pDCs (62). pDCs were found to express FcϵRI, which is the high-affinity IgE receptor. Thus, pDCs could uptake dsDNA-IgE complexes in a FcϵRI dependent fashion, and sensed DNA in a toll-like receptor 9 (TLR9) dependent manner. Upon DNA sensing pDCs secreted a high amount of IFN-I. This pathway was physiologically relevant as the IFN-α inducing capacity of SLE serum was reduced upon IgE neutralization, *in vitro* (62).

While the role of IgE autoantibodies in amplifying inflammation is well studied, the role of IgE autoantibodies in mediating end organ damage is still elusive. A study conducted with lupus patients revealed that 35% of lupus nephritis patients showed IgE deposition in the kidney, and these patients had a poorer prognosis. Moreover, among patients with IgE deposition, the occurrence rate of lupus nephritis increased by up to 71% (27). This strongly suggests that IgE has a pathogenic role in the kidney. IgE is different from other immunoglobulins, as they cannot activate complement pathways. Instead, they act through binding and crosslinking high-affinity IgE (FcϵRI) receptors expressed on the surface of mast cells, basophils, and to a lesser extent on eosinophils (63, 64). As IgE are deposited in inflamed kidneys of lupus mice and patients, they are suspected to mediate local immune reactions and inflammation. However, the exact role played by IgE in the kidney remains to be addressed.

Eosinophil

Eosinophils are innate immune leukocytes stimulated by IL-5, granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-33. They are the effector arm in Th2 related immune responses such as parasite infection and in Th2 associated diseases such as asthma and allergic responses (65, 66). Eosinophils are distributed in the bone marrow, blood, spleen, thymus, gastrointestinal tract, and uterus during steady state (67). However, under pathogenic conditions, they infiltrate into inflamed tissues and contribute to organ destruction by mediating local cytotoxic actions through the secretion of granule proteins (68). A recent study with lupus nephritis

patients discovered an increase in urinary eosinophils along with the detection of eosinophil cationic protein and IL-5. Eosinophils also correlated with renal function and SLE disease activity, and authors suggest using urine eosinophil levels as a biomarker for active lupus nephritis (28). Furthermore, patients with eosinophiluria and tissue eosinophil infiltration were significantly more likely to progress to end-stage kidney disease. However, the precise relation between eosinophiluria and lupus nephritis is poorly understood. Blood eosinophil levels are also comparable between SLE patients and healthy controls. Nevertheless, these are exciting observations that call for further research into understanding the role of eosinophils in mitigating glomerular damage (28, 29, 69).

CONCLUSION

Over 50% of SLE patients display a type 2-associated immune response, as evidenced by the detection of IgE autoantibodies. These IgE autoantibodies were clinically meaningful as IgE neutralization with omalizumab treatment alleviated disease severity. This review illustrates the players involved during the induction and effector phase of type 2 immunity in the context of SLE pathology. The induction phase likely consists of the release of IL-33-decorated NETs from neutrophils. NETs decorated with IL-33 can activate multiple innate cells containing IL-33R, such as pDCs and basophils. Upon activation, basophils express CD62L and CCR7 and are recruited to SLOs. In SLOs, basophils aid in polarizing T cells towards Th2 cells and Tfh2 cells while also activating B cells. Tfh2 cells are indispensable for activating and producing IgE-producing plasma cells. The exact mechanism leading to the induction of autoreactive IgE plasma cells is unknown. However, it will most likely be a result of loss of quality control in germinal centers or in the extrafollicular zone. Once IgE autoantibodies are produced, they further activate pDCs and basophils, forming a positive feedback loop. Alternatively, circulating IgE autoantibodies are deposited in the kidney. At this site, IgE can interact with eosinophils to signal the release of cytotoxic molecules such as cationic proteins. Th2 cells also infiltrate into the interstitial region of the kidney, where they directly interact with kidney cells to induce fibrosis and glomerulosclerosis. The success of IgE targeting therapy in patients has sparked interest in understanding the role of Th2 related responses in SLE. There are many missing links in this field; however, additional research will draw a clearer map of the immune response in type-2 subtyped SLE patients and will pave the way for developing novel therapies for Th2 associated SLE.

AUTHOR CONTRIBUTIONS

HK, CK, and S-HI conceptualized the article, reviewed the literature, and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Th2 to Th1 Transition Is Required for Induction of Skin Lesions in an Inducible and Recurrent Murine Model of Cutaneous Lupus–Like Inflammation

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Cutaneous lupus erythematosus (CLE) is an autoimmune skin disease characterized by a strong IFN signature, normally associated with type I IFNs. However, increasing evidence points to an additional role for IFNγ, or at least a pathogenic T effector subset dependent on IFNγ, for disease progression. Nevertheless, Th2 effector subsets have also been implicated in CLE. We have now assessed the role of specific T cell subsets in the initiation and persistence of skin disease using a T cell-inducible murine model of CLE, dependent on KJ1-26 T cell recognition of an ovalbumin fusion protein. We found that only Th2-skewed cells, and not Th1-skewed cells, induced the development of skin lesions. However, we provide strong evidence that the Th2 disease-initiating cells convert to a more Th1-like functional phenotype *in vivo* by the time the skin lesions are apparent. This phenotype is maintained and potentiates over time, as T cells isolated from the skin, following a second induction of self-antigen, expressed more IFN-γ than T cells isolated at the time of the initial response. Transcriptional analysis identified additional changes in the KJ1-26 T cells at four weeks post injection, with higher expression levels of interferon stimulated genes (ISGs) including *CXCL9, IRF5, IFIH1*, and *MX1*. Further, injection of IFN-γ-/- T cells faied to induce skin disease in mice. We concluded that Th2 cells trigger skin lesion formation in CLE, and these cells switch to a Th1-like phenotype in the context of a TLR7-driven immune environment that is stable within the T cell memory compartment.

Keywords: cutaneous lupus erythematosus, CD4+ helper T cell, Lupus flare, CXCR6, Th1 & Th2

INTRODUCTION

Cutaneous lupus erythematosus (CLE) is an autoimmune disease with a broad range of skin and mucosal tissue manifestations (1) that may or may not overlap with systemic lupus erythematosus (SLE). A high frequency of patients with SLE may develop skin lesions; however, not all CLE patients exhibit systemic disease or even progress to SLE (2). Additionally, CLE can be refractory to SLE treatments (3). Hence, pathogenic mechanisms need to be further explored to identify the unique and shared features of CLE and SLE, and to provide essential insights for treating both conditions.

A clear understanding of the immune parameters that contribute to CLE have been hampered by the lack of an appropriate experimental model, such that CLE has received less attention than SLE in terms of etiology and treatment. Well-characterized SLE animal models, including MRL/lpr, NZB/W, and BSXB mouse strains, have been used to study the development of skin lesions. However, in these strains, the onset of the cutaneous disease is variable, colony dependent, usually takes a long time (~6 months) to develop and lacks a number of the critical features of human CLE (4). Recently, we have developed an inducible murine model of CLE that recapitulates human CLE by a number of criteria, including interface dermatitis, mucin deposition, lupus band reaction, erythema, scaling and hair loss (5). This model depends on the doxycycline induction of an OVA-peptide-containing pseudo-autoantigen (TGO), in combination with the adoptive transfer of activated OVA-specific DO11 T cells, and thereby allows us to explore the pathogenic activity of defined T cell subsets.

Early studies considered SLE an autoantibody/immune complex driven disease and focused on Th2 effector cells. However, the current literature points to a key role for skin-localized Th1 cells in lupus pathogenesis in both humans and mice (6–12). The serum levels of IFN-γ, TNF-α, and IL-12 are significantly higher in SLE patients than in healthy controls (11). The Th1-biased inflammation is most likely enhanced by type-I IFN secretion mainly from plasmacytoid dendritic cells (pDC) (13). By contrast, atopic dermatitis is normally associated with Th2 cells and psoriasis is driven by Th17 cells (14).

The TGO model was originally envisaged as a model of SLE since expression of the OVA pseudo-autoantigen is under the control of a reverse transactivator (rtTA) driven by an invariant chain promoter, and therefore likely to be expressed by all MHCII+ cells, not just MHCII+ cells in the skin. Based on the critical role of autoantibody/autoantigen immune complexes in lupus pathogenesis, all our initial studies used activated Th2-skewed T cells to initiate the disease process. The rapid onset of cutaneous lesions was unexpected, as was the finding that DO11 T cells isolated 4 weeks post transfer expressed a Th1 phenotype and produced IFNγ (5). The current study was therefore undertaken to better understand the role of Th2 vs Th1 cells in the development and recurrence of CLE. Unexpectedly, we found that an initial injection of Th1 cells failed to trigger cutaneous lesions, even though the injected DO11 T effector cells were present in the spleen, LN and to some extent the skin. Together, our data point to the distinct ability of Th2 cells to migrate to the skin and the unexpected plasticity of these Th2 cells to acquire a Th1-like phenotype when exposed to an immune environment perturbed by a TLR7-driven type I IFN response.

RESULTS

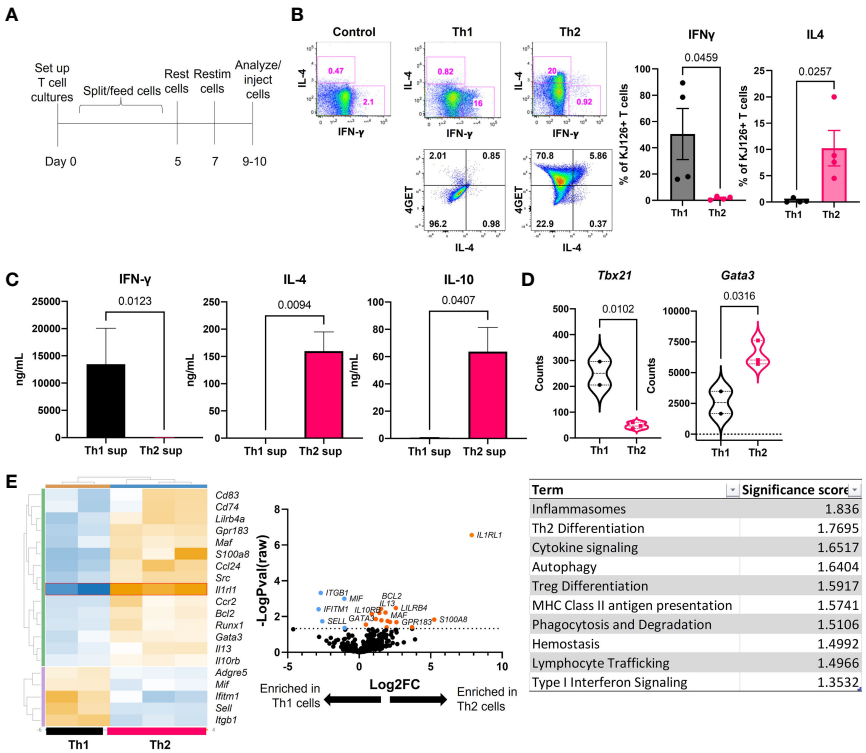
Analysis of *In Vitro* Skewed DO11 T Cells Confirms Cytokine and Gene Expression Associated with Th1 and Th2 Differentiation

Ii-TGO mice were generated by intercrossing mice that express an invariant chain promoter-driven reverse transactivator (Ii-rtTA) transgene with mice that express a Tet-regulated ovalbumin fusion protein (TRE-TGO). Upon Dox administration, these mice express an OVA fusion protein that incorporates the transferrin receptor transmembrane domain to facilitate efficient trafficking to endocytic compartments. To compare the pathogenic potential of distinct T cell subsets, OVA-specific DO11 T cells were activated *in vitro* with OVA-peptide and APCs under Th1 or Th2 skewing conditions, expanded in the presence of IL-2, and then restimulated 2-3 days prior to i.v. injection (**Figure 1A**). In some studies, we used T cells derived from DO11 mice that had been intercrossed with the IL-4 reporter line, 4get (15). We confirmed the functional phenotype of the Th1 and Th2 cells at the time of injection by flow cytometry. Only the Th1 cells expressed IFNγ, and only the Th2 cells expressed IL-4. In addition, the majority of the OTII 4get Th2 cells expressed GFP while few if any of the OTII 4get Th1 cells were GFP+, confirming their commitment to the Th1 lineage (**Figure 1B**). Cytokine concentrations in culture fluids collected after the *in vitro* restimulation were determined by ELISA, and the results confirmed the expected phenotype; Th1 supernatants contained high levels of IFNγ and the Th2 supernatants contained IL-4 and IL-10 (**Figure 1C**).

In addition, RNA extracted from the restimulated T cells was analyzed by NanoString™ Mouse Immunology code set. *Tbx21* (Tbet) was enriched in Th1 cells and *Gata3* was enriched in Th2 cells (**Figure 1D**). Additional genes were differentially upregulated in the two populations, including increased *S100a8, Il13, Il10rb*, and *Runx1* in Th2 cells and increased *Mif, Itgb1*, *Ifitm1* and *Sell* in Th1 cells (heatmap) (**Figure 1E**). The highest upregulated differentially expressed gene (DEG) in Th2 cells was *Il1rl1* (volcano plot). Gene set analysis (GSA) revealed Th2 differentiation, lymphocyte trafficking and type I interferon signaling terms were different between Th1 and Th2 cells using a cutoff significance score of 1.3.

Injection of Th2 Cells, and Not Th1 Cells, Induces Skin Disease in Lupus-Prone Mice

We reported previously that sublethally irradiated (400R) IiTGO mice, provided with Dox chow and injected with activated Th2 cells, developed lupus-like skin lesions (5). To determine whether Th1 cells could induce CLE as efficiently as Th2 cells, TLR9$^{-/-}$ Ii-TGO recipients were sublethally irradiated (400R) and provided with Dox chow 6-18 hrs prior to i.v. injection of Th1 or Th2 DO11



Term	Significance score
Inflammasomes	1.836
Th2 Differentiation	1.7695
Cytokine signaling	1.6517
Autophagy	1.6404
Treg Differentiation	1.5917
MHC Class II antigen presentation	1.5741
Phagocytosis and Degradation	1.5106
Hemostasis	1.4992
Lymphocyte Trafficking	1.4966
Type I Interferon Signaling	1.3532

FIGURE 1 | Generation and functional phenotype of activated DO11 T cells. **(A)** Timeline for the generation of activated Th1 and Th2 subsets. **(B)** Cytokine production by the injected Th1 and Th2 cells shown by flow cytometric analysis of IL-4 vs IFNγ (top) and GFP vs IL-4 (bottom). **(C)** Culture supernatants from the restimulated Th1 and Th2 cells determined by ELISA (n=5-6 Th1 and 9-11 Th2). **(D)** NanoString analysis of master regulator transcription factors expressed by *in vitro* activated T cells. **(E)** NanoString heatmap, volcano plot and Gene Set Analysis (GSA) of restimulated Th1 and Th2 cells analyzed by Rosalind software (n=2 Th1 and n=3 Th2 RNA samples pooled from 2 separate experiments; two-tailed student's t tests significant as indicated).

T cells (**Figure 2A**). Skin lesions developed in the Th2 injected mice 3-4 weeks post transfer, but not in the mice injected with Th1 cells, as shown in images of representative mice (**Figure 2B**) and compiled skin score data from 4 experiments (**Figure 2C**). The absence of skin lesions in the Th1-injected mice was not due to the failure of KJ1-26 cells to survive or engraft the recipients, as indicated by splenomegaly (**Figure 2D**) and the initial weight loss in the Th1-injected mice (**Figure 2E**). To further compare the extent of engraftment of the injected DO11 Th1 and Th2 T cells, single cell suspensions obtained from the skin, LN and spleen of the injected mice were analyzed by flow cytometry using the KJ1-26 anti-clonotypic antibody. We found more KJ1-26+ cells in Th2 injected mice in all 3 tissues (**Figure 2F**).

Th1 and Th2 cells are known to express distinct sets of chemokine receptors and Th1 and Th2 cells use different ligands and chemokine receptors to enter the skin (14). In addition to their role in cell migration, chemokine receptors impart functional capacity on T cells. Therefore, we examined DEGs between pre- and post-injection T cells, as well as Th1 and Th2 skewed cells, to better understand factors that might be contributing to T cell function in our model. CXCR6 was significantly higher in Th2 vs. Th1 cells by both NanoString array and flow cytometry (**Figure 2G**). However, there were no significant differences in the expression of other chemokine receptors, based on both Nanostring and flow panels, that have been reported to distinguish Th2 cells from Th1 cells (e.g., CXCR3, CCR4, CCR5, or CCR8) (**Figure 3G**), even though the cells were clearly skewed to the Th2 subset, based on cytokine production and expression of the 4get reporter (**Figure 1**).

To confirm the presence of the ligands for CXCR6 and CLA in CLE mouse skin, we queried our NanoString dataset which compared RNA isolated from the skin of Th2 injected TLR9$^{-/-}$ Ii-TGO mice (skin score 3-4) to the skin of TLR9+/+ IiTGO mice (no disease) (5). We found significant increases in *CXCL16* (ligand for CXCR6) and *SELL* (ligand for CLA) but not *SELE* (another ligand for CLA) in TLR9-/- versus WT skin (**Figure 2H**). Taken together, these data suggest that Th2 skewing promotes skin infiltration by patterning expression of skin-homing molecules, thereby allowing the Th2 cells to follow CXCL16-CXCR6 chemokine and CLA-L-Selectin integrin signals to impart functional capacities in skin.

Antigen-Specific Th2 Cells Switch to Th1-Like Cells *In Vivo*

Next, we compared the injected KJ1-26 Th2 cells to KJ1-26 T cells isolated from lesional skin 4-5 weeks post injection to identify changes in gene expression that developed during the post-injection time frame. Antigen-specific T cells were enriched

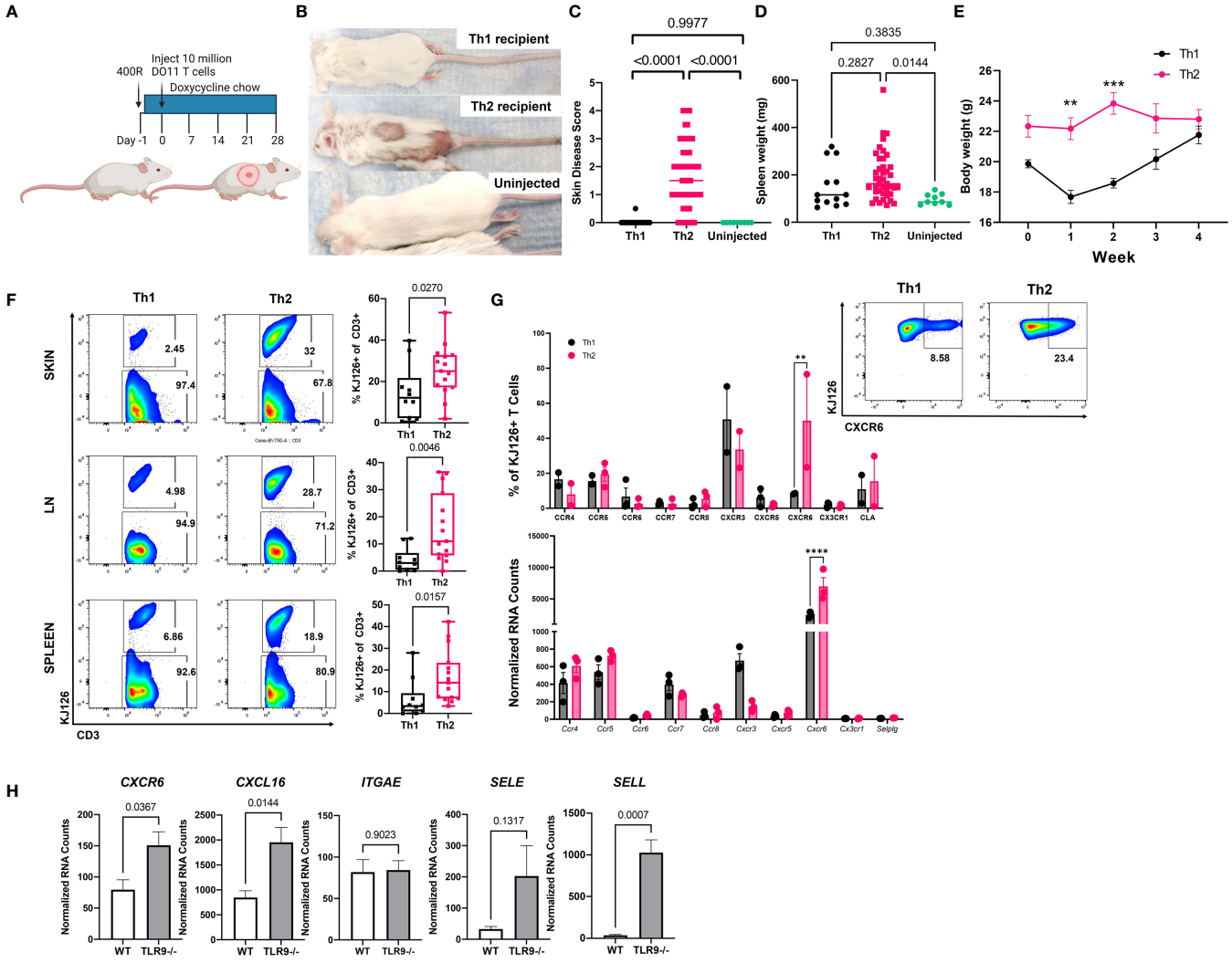


FIGURE 2 | Injection of Th2, but not Th1, induces lupus-like skin lesions in mice. **(A)** Timeline for the initial induction of skin lesions created with BioRender.com. **(B)** Representative images of mice injected with Th1 or Th2 cells, compared to uninjected control. **(C)** Skin disease scores, **(D)** spleen weights and **(E)** body weights of mice (n=15 Th1, 45 Th2 and 9 uninjected mice pooled from 4 separate experiments, one and two way ANOVAs significant as indicated.) **(F)** T cell engraftment assessed by flow cytometry analysis of cell suspensions recovered from skin, lymph node (LN) and spleen tissue (n=10 Th1 and n=15 Th2 mice pooled from 2 separate experiments; two-tailed students t tests significant as indicated). **(G)**. Assessment of chemokine receptor expression by RNA (top) and flow cytometry (bottom) of *in vitro* activated Th1 and Th2 cells. **(H)** Reanalysis of gene expression in total skin of TLR9-/- versus WT control mice from Mande et al., (5) to assess ligand expression. $^{**}p<0.01$, $^{***}p<0.001$ and $^{****}p<0.0001$.

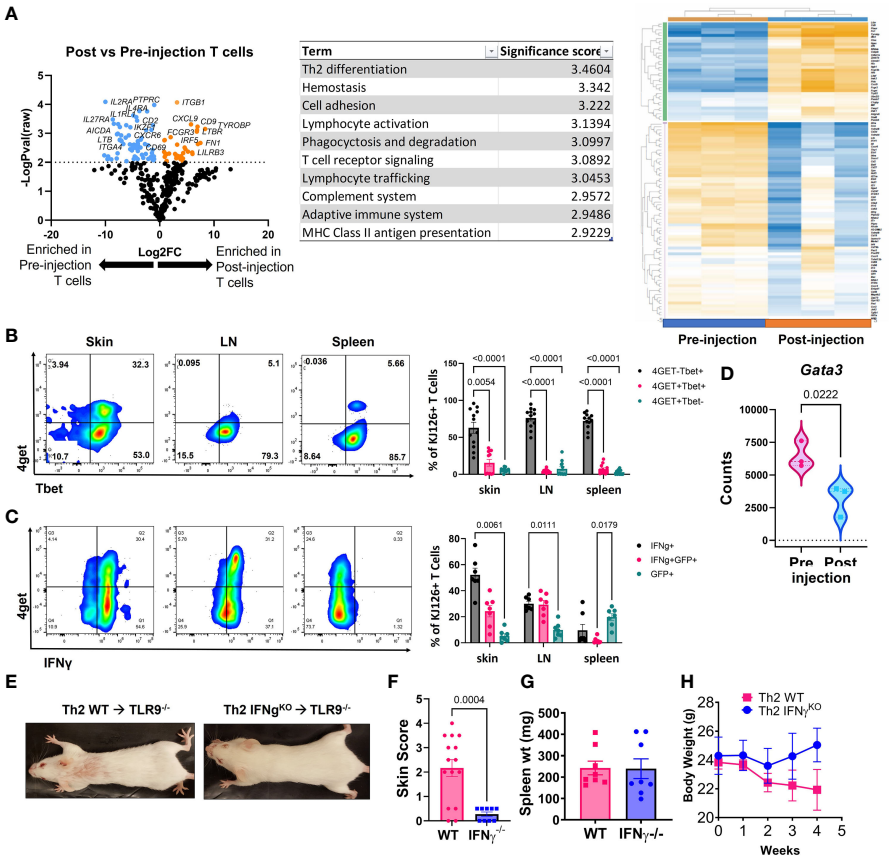


FIGURE 3 | Analysis of post-injection T cells reveals a Th1-shift, and IFNγ is required for development of skin lesions. **(A)** NanoString analysis of post vs pre-injection T cells including volcano plot, GSA and heatmap generated with Rosalind software (n=3 pre-injection and n=3 post-injection enriched T cells pooled from 2 separate experiments). **(B)** Flow cytometry analysis of 4get reporter and Tbet expression in skin, lymph node (LN) and spleen of mice exhibits a Th1 switch post-injection (n=7 mice pooled from 2 separate experiments; two-way ANOVA with Tukey's post-tests significant as indicated). **(C)** Flow cytometry analysis of 4get reporter and IFNγ expression in skin, lymph node (LN) and spleen of mice exhibits a Th1 switch post-injection (n=12 mice pooled from 2 separate experiments; two-way ANOVA with Tukey's post-tests significant as indicated). **(D)** Assessment of *Gata3* RNA expression in pre- vs post-injection Th2 cells (n=3 pre-injection and n=3 post-injection T cells pooled from 2 separate experiments; one-tailed student's t test significant as indicated). **(E)** Representative images of mice injected with Th2 skewed WT or IFNγ-/- DO11 cells. **(F)** Skin disease scores, **(G)** spleen weights and **(H)** body weights of mice (n=15 WT and 9 IFNγ-/- DO11 recipient mice pooled from 2 separate experiments, student's t tests significant as indicated).

from the skin using KJ1-26 magnetic beads for positive selection. NanoString analysis of RNA isolated from these cells revealed 150 differentially expressed genes (DEGs) between injected and 4 wk post-injection KJ1-26+ T cells. T cells isolated from lesional skin showed the upregulation of interferon-stimulated genes (ISGs), including *IRF5*, *IFIH1*, and *MX1* (**Figure 3A**). The data also showed decreased expression of Th2-related genes including *IL4RA*. These data, combined with the observation that the Th2 upregulated other ISGs led us to further examine the cytokine profile of post-injection DO11 4get T cells.

We tracked 4get expression in both *in vitro* and *ex vivo* from T cells, in addition to staining for IFNγ. By 4 weeks post Th2 cell injection a high proportion of KJ1-26+ T cells in the skin and skin-draining LN (sdLN) exhibited a Th1-like phenotype, as defined by Tbet and IFN-γ expression (**Figures 3B, C**). Intriguingly, ~75% of the T cells in the skin were Tbet+/IFNg+ and of these, 25% also were also GFP$^+$. Lower GFP detection in combination with Tbet staining may be due to the transcription factor fixation/nuclear permeabilization protocol, which is more harsh than the reagents used for intracellular cytokine staining. *Gata3*, a master regulator of Th2-associated gene expression, was also reduced in the post-injection T cells expression (**Figure 3D**). Together, these data indicate that the injected KJ1-26+ T cells switch from a Th2 to a Th1-like subset and acquire the capacity to produce IFNγ.

DO11 IFN-γ Production Is Required for Skin Disease

To understand the significance of IFN-γ to development of skin disease, we skewed WT or IFN-$\gamma^{-/-}$ DO11 T cells towards a Th2 phenotype and injected them into host mice. IFN-$\gamma^{-/-}$ DO11 failed to induce skin lesions in mice (**Figures 3E, F**), despite inducing splenomegaly and an initial drop in body weight (**Figures 3G, H**). These data indicate that the switch towards a

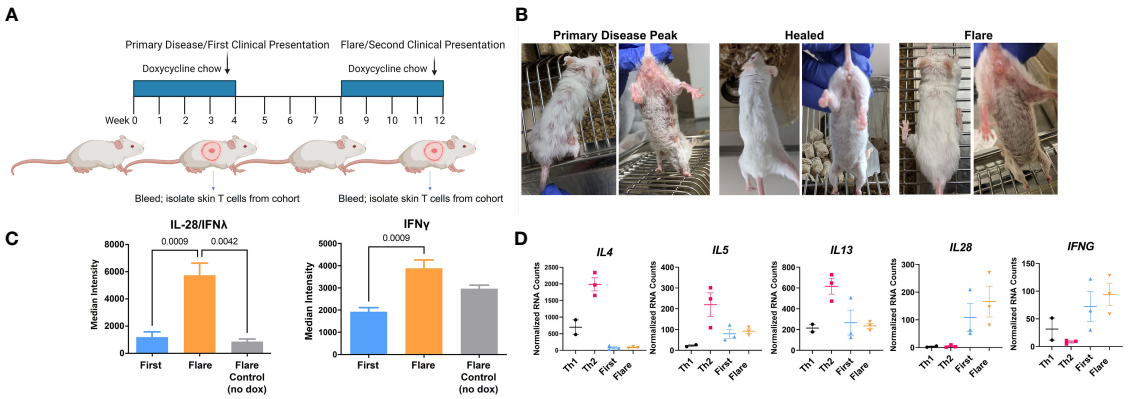


FIGURE 4 | *In vivo* acquired Th1 phenotype is maintained by memory T cells. **(A)** CLE flare model diagram created with BioRender.com. **(B)** Sample clinical photographs of initial induction and reinduction of skin lesions. **(C)** RayBiotech array analysis of serum from mice at the time of the initial occurrence of skin lesions and the reinduction of skin lesions. **(D)** Comparison of *in vitro* activated T cells and KJ-126+ T cells recovered from the initial and reinduced skin lesion by NanoString gene expression analysis (n=2-3 cultured cell batches and 3 mice per group pooled from 2 independent experiments).

Th1 phenotype is required for the development of skin disease in this model.

DO11 Memory T Cells Maintain Their Th1 Phenotype Following Reinduction of Skin Disease

CLE is cyclical, with relapsing and remitting flares of cutaneous lesions. To model disease flares, mice that had developed skin disease were taken off Dox chow to allow the skin lesions to heal. Dox chow was then readministered to these mice 4 weeks later to reinduce the model autoantigen (**Figure 4A**). Within two weeks, skin disease recurred (**Figure 4B**) without the injection of additional DO11 T cells. Sera collected during the initial clinical presentation and at the time of flare were assayed by protein array to determine whether cytokine titers increased in the mice with flares. We found increases in IL28 (IFNλ) and IFN-γ in flares versus initial clinical presentation (**Figure 4C**). Cytokine titers in the sera of flare control mice, which did not receive the second course of Dox chow, were comparable to those detected during the initial response.

To determine whether these cytokines could be T cell derived, RNA isolated from the cultured Th1 and Th2 cells was compared to T cells isolated from the skin during the initial and flare responses. Gene transcription was assessed with the NanoString probe set as in **Figure 3**. We found that T cells isolated from the skin showed minimal expression of *IL4, IL5* and *IL13*, but did express *IL28* and *IFNγ*, both of which trended higher at time of flare (**Figure 4D**) Taken together, these data indicated that the Th1-like phenotype, established during the primary response, persisted in the T cell memory compartment.

DISCUSSION

In the current study we have explored the role of specific T cell subsets in an SLE-like model of inflammatory skin disease. We found that the injection of *in vitro* skewed Th2 cells induced skin lesions under conditions where *in vitro* skewed Th1 cells did not. Nevertheless, antigen-specific T cells isolated from lesional skin 4 weeks post the initial induction of disease displayed a Th1-like and not Th2-like phenotype, and the acquired Th1 phenotype persisted in memory T cells even when the autoantigen levels decreased upon withdrawal of doxycycline chow.

The injected Th2 cells preferentially expressed CXCR6, consistent with reports that CXCR6 mediates skin homing. The ligand for CXCR6, CXCL16, was recently reported to be elevated in juvenile SLE patients, and was strongly associated with alopecia, malar rash, and nephritis (16). CXCL16 is produced by keratinocytes (17, 18), and induced by TLR7 ligation (19). TLR7 plays a key role in the current CLE mouse model (5) as well as in human CLE [reviewed in (20)]. Further, CXCL16 is constitutively expressed by keratinocytes and is upregulated by UV light, a known trigger of CLE (18). Recent studies have also identified CXCR6 on skin effector T cells in the context of melanoma (21) and on skin resident memory T cells (Trm) in melanoma-associated vitiligo (22).

Th2 cells were long believed to be stably committed effector cells that were resistant to Th1 skewing conditions. However, there is precedent for Th2 cells to retain a degree of plasticity that enables them to adapt to changes in their microenvironment [reviewed in (23)] Examples include the role of increased type I IFN signaling following lymphocytic choriomeningitis (LCMV) viral challenge (24, 25). The Th2 cells used to induce disease in the current study produced IL-4 and failed to produce IFNγ, but they were only cultured with skewing antibodies and cytokines during the initial culture period and may not have been fully committed to the Th2 lineage (26). We hypothesize that in the immune microenvironment of CLE mice, TLR7-driven production of type I IFNs by pDCs, keratinocytes or other innate immune effector populations may skew the adoptively transferred Th2 cells towards a Th1 phenotype. Despite their inability to produce either IL4 protein or IL4 transcripts, a

significant number of 4-GET KJ1-26+ cells in the CLE mice still expressed GFP, perhaps reflecting an active IL-4 promoter. Nevertheless, expression of *Gata3*, the master regulator of Th2 differentiation was significantly reduced in cells isolated from the lesional skin at weeks when compared to cultured Th2s. Hence, future studies will explore the histone methylation status of the Th1 or Th2 associated promoters during different stages of CLE pathogenesis.

Nevertheless, we cannot rule out the possibility that non-Th2 cells present in the initial innoculum further developed *in vivo* into disease inducing Th1 effector cells. We believe this is unlikely, since we have never found that the injection of Th1 cells led to the development of skin disease. It is also possible that activated Th2 cells have a selective survival advantage *in vivo* since they do not express the high levels of FasL found on Th1 cells, but in preliminary studies, DO11 Fas-deficient (lpr/lpr) Th1 cells still failed to induce skin disease.

Our findings showed higher levels of other serum cytokines including IL-12p70, IL-28 (also known as IFN-λ2/3), and TNF-α during CLE flare compared to the initial response. IFNλs have type I IFN-like activity and act primarily on epithelial cells. There are reports of high IFNλ and the IFNλ receptor in keratinocytes of CLE lesional skin (27). Elevated blood levels of IFNλ3, as well as increased *IFNL2* and *IFNL3* mRNA have been detected in blood CD4+ T cells of lupus-prone mice and patients (28, 29). TNF family members are elevated in lupus patients (30) and are shed at higher rates preceding flares (31). Further, elevated serum levels of IL-4, IL-5, IL-6, and IFN-γ precede autoantibody positivity in systemic lupus patients (32). Taken together, these studies of serum cytokines mirror what we observed in our flare model and support the idea that a plastic T cell pool may promote clinical manifestations in human patients.

In conclusion, our findings highlight the role of Th2 in the initiation of skin lupus in mice. We hypothesize that differences in chemokine receptors and ligands expressed on Th2 vs. Th1 cells enable Th2 cells to enter the skin and establish disease. *In vivo*, Th2 cells acquire an IFN-γ^+ phenotype associated with the establishment and maintenance of skin disease. We also found that the IFN-γ producing function of Th cells is potentiated during the flare. One interesting implication of the current study is that Th2 cells, responding to foreign allergens in the skin, may on occasion recognize other self or foreign epitopes, and in the context of an ongoing inflammatory response, trigger the onset of SLE. The ability to readily induce flares in this model also point to persistent autoreactive T resident memory cells as potential therapeutic targets.

MATERIALS AND METHODS

Mice

All mice were housed in pathogen-free facilities at UMMS, and procedures were approved under protocol #2096 by the UMMS Institutional Animal Care and Use Committee and in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. Mice used for these studies were on the Balb/c background. Age and sex-matched mice were used, and both male and female mice of all strains were tested to avoid gender bias. Replicate experiments were performed two to five times.

Recipient TLR9KO Ii-TGO and WT Ii-TGO controls were generated as previously described (5). BALB/c DO11 mice (C.Cg-Tg [DO11.10]10 Dlo/J; Jackson Laboratory stock no. 003303) or Rag-/- DO11 mice (C.Cg-Rag1tm1Mom Tg (DO11.10)10Dlo/J; stock no. 030666) bred to IL-4/GFP-enhanced transcript (4get) mice (C.129-Il4tm1Lky/J; stock no. 004190) were used as T cell donors.

T Cell Skewing

Magnetic bead-purified DO11 CD4+ T cells (BD IMag magnetic particles) were activated using OVA peptide–pulsed (323-339, *In vivo*gen) irradiated spleen cells (as source of APCs) as described previously (33). Th1 cells were cultured with recombinant mouse IFNγ (10ng/mL) and anti-IL4 antibody (10ug/mL); Th2 cells were cultured with recombinant mouse IL-4 (10ng/mL) and anti-IFNγ (XMG2.1 10ug/mL) and anti-IL12p40 (10ug/mL). All cells (including unskewed Th0) received recombinant mouse IL-2 from J2 supernatant to promote survival and expansion. Cells were split on day 2, fed IL-2 on day 3, and split again on day 4. By day 7, the cells had rested and were re-activated, but not re-skewed, with another batch of OVA-pulsed splenocytes. Cells were harvested on day 10 at the peak of activation post-restim.

RayBiotech Array

Supernatants from restimulated T cells and/or serum from CLE mice were assayed in the RayBiotech Th1/Th2/Th17 mouse Quantibody array per the manufacturer's protocol. Slides were shipped for scanning array service and data were analyzed by taking the median fluorescence intensity minus the background fluorescence from blank control wells. Data are deposited on GEO Database under accession # GSE186095.

NanoString Analysis

RNA was extracted from polarized cultured T cells, and from post-injection CD3 column-enriched (Miltenyi biotech) T cells from CLE mouse skin using Qiagen RNEasy mini kits. RNA was hybridized for ~18h (BioRad CFX thermocycler) and assayed in the NanoString mouse Immunology panel per the manufacturer's instructions. Data were analyzed with Rosalind software using NanoString partner analysis. Data are deposited on GEO Database under accession # GSE185355.

CLE Induction

10^7 activated and skewed T cells were injected i.v. into sublethally irradiated (4 Gy) age- and sex-matched TLR9WT or TLR9KO Ii-TGO recipient mice. To induce expression of the TGO transgene in the MHCII cells, mice were fed with 200 mg/kg of Dox chow (Bio-Serv). For CLE flares, mice were kept on chow for 4-5 weeks, allowed to heal for 4 weeks, then Dox chow was reintroduced.

Flow Cytometry

Single-cell suspensions obtained from spleen, sdLNs, and skin were analyzed by flow cytometry using fluorochrome-conjugated mAbs listed in **Table S1**. Zombie Aqua or Zombie NIR (Biolegend) was used to distinguish live and dead cells. Intracellular staining was carried out on cells incubated with Brefeldin A (Biolegend) in all tissue digestion and FACS staining buffers, approximately for 4 hours. Cells were permeabilized and fixed with transcription factor staining buffer (Invitrogen) or Cytofix/Cytoperm (BD Biosciences) and subsequently incubated with fluorochrome-conjugated mAb to mouse IFN-γ (clone XMG1.2, eBioscience), IL4 (clone 11B11, Biolegend), Tbet (clone 4B10, Biolegend), or GATA3 (clone TWAJ, eBioscience). Flow cytometric analysis was carried out using a Cytek Aurora, and analysis was conducted with FlowJo software 9.7.6 (TreeStar).

Cell Isolation From Skin

Cells were isolated from the skin as described previously (5). Briefly, shaved dorsal skin was harvested, minced, and digested for 45 minutes at 37°C with 2.0 mg/ml collagenase XI from Clostridium histolyticum (Sigma-Aldrich), 0.5 mg/ml hyaluronidase from bovine testes (Sigma-Aldrich), and 0.1 mg/ml DNAse (Sigma-Aldrich). Single cells were washed with 10% cRPMI, filtered through a 100 μm filter, and stained for flow cytometry staining as described above. For samples to be used for ICS, Brefeldin A was added to the digestion buffer and surface stain cocktail. For enrichment of antigen-specific T cells from skin, we used PE-conjugated KJ1-26 antibody and a PE positive selection kit (Miltenyi biotech).

Statistics

Statistical analyses were performed using Prism software version 7.0 (GraphPad). Experiments are reported as mean ± SEM. Data were analyzed using a 2-tailed Student's t test for comparison between 2 data sets. Multiple comparisons were analyzed by 1-way ANOVA and 2-way ANOVA, followed by Tukey's multiple-comparison *post hoc* test. Differences were considered significant at a P value of less than 0.05.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/geo/, SuperSeries #GSE186096, containing accession # GSE186095 and GSE185355.

ETHICS STATEMENT

The animal study was reviewed and approved by University of Massachusetts Chan Medical School Institutional Animal Care and Use Committee.

AUTHOR CONTRIBUTIONS

Conceptualization: AM-R and JR; Methodology: AM-R and JR; Software Programming: N/A; Validation/Verification: GR, N-SH, and KA; Formal analysis: N-SH, PM, GR, and JR; Investigation: N-SH, PM, TB, KP, SS, SM, KH, KA, AM-R, and JR; Resources: JR and AM-R; Data Curation: N-SH, PM, and JR; Writing - Original Draft: N-SH and JR; Writing - Review and Editing: All authors; Visualization: N-SH, PM, KH, TB, JR, and AM-R; Supervision: JR and AM-R; Project administration: JR and AM-R; Funding acquisition: JR and AM-R. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.883375/full#supplementary-material

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Conflict of Interest: Author PM is employed by Q32 Bio Inc. JR is an inventor on patent application #15/851,651, "Anti-human CXCR3 antibodies for the Treatment of Vitiligo" which covers targeting CXCR3 for the treatment of vitiligo; and on patent #62489191, "Diagnosis and Treatment of Vitiligo" which covers targeting IL-15 and Trm for the treatment of vitiligo.

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.

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