

REVIEW

Novel innate and adaptive lymphocytes: The new players in the pathogenesis of inflammatory upper airway diseases

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Summary

Host immunity (innate and adaptive immunity) plays essential roles in the pathogenesis of inflammatory upper airway diseases, including allergic rhinitis and chronic rhinosinusitis. Recently, the discovery of novel innate immune cells, particularly innate lymphoid cells, has renewed our view on the role of innate immunity in inflammatory upper airway diseases. Meanwhile, the identification of new subsets of T helper (Th) cells, including Th22, Th9 and follicular Th cells, and regulatory B cells in the adaptive immunity, has broadened our knowledge on the complex immune networks in inflammatory upper airway diseases. In this review, we focus on these newly identified innate and adaptive lymphocytes with their contributions to the immunological disturbance in allergic rhinitis and chronic rhinosinusitis. We further discuss the perspective for future research and potential clinical utility of regulating these novel lymphocytes for the treatment of allergic rhinitis and chronic rhinosinusitis.

1 | INTRODUCTION

Chronic rhinosinusitis (CRS) and allergic rhinitis (AR) are two common chronic inflammatory upper airway diseases. CRS is a heterogeneous disorder characterized by inflammation of the sinonasal mucosa.^{1,2} Clinically, CRS is divided into two phenotypes depending on the presence or absence of nasal polyps (NPs): CRS with NPs (CRSwNP) and CRS without NPs (CRSsNP). Based on the severity of eosinophilic inflammation, CRS can be further subgrouped into eosinophilic and non-eosinophilic type, particularly in Asian patients with CRSwNP.¹ AR is characterized by an IgE-mediated hypersensitivity reaction in nasal mucosa upon aeroallergen exposure.² CRS and AR are frequently associated with lower airway diseases, including asthma and chronic obstructive pulmonary disease (COPD).³ Current treatment protocols for CRS include non-specific management with antibiotics, corticosteroids and surgery, which leave us with substantial residual morbidity.^{1,4} Also, a considerable number of AR patients

are unsatisfied with pharmaceutical medicines and even immunotherapy.² Hence, there appears to be a compelling need for new approaches to treat CRS and AR. The better understanding of immunological pathogenesis of AR and CRS will facilitate active development and testing of target therapies against specific immune axes.

The development of CRS and AR is orchestrated by various structural cells and immune cells, including epithelial cells, endothelial cells, fibroblasts, T cells, B cells, dendritic cells, eosinophils and mast cells.^{1,2} These cells can produce a diversity of mediators, such as eicosanoids, cytokines, chemokines and immunoglobulins, and thus constitute a complex inflammatory and immune network driving the immune disturbance in AR and CRS.^{1,2} Previous studies have demonstrated a central role for T lymphocytes in this immune network.^{1,2} Dysregulation of T helper (Th) 1, Th2, Th17 cell response and impairment of regulatory T cells (Tregs) have been demonstrated in AR and CRS.^{1,2} Particularly, AR and eosinophilic CRSwNP display a Th2-

dominated response.^{1,2} In recent years, biologic agents targeting Th2 pathway and IgE, including humanized anti-IgE, anti-IL-5, and anti-IL-4 and IL-13 receptor antibody, have shown promising effects for the treatment of AR and CRSwNP.^{4,5} These biologics relieve the symptoms and spare symptomatic drug use in AR patients, and shrink the polyps and reduce the need for surgery in patients with CRSwNP.^{5,6} Despite those encouraging results, it seems that only a subset of patients respond well to the biologics targeting Th2 pathway and IgE, suggesting the importance of other pathways in AR and CRSwNP. Indeed, the recent identification of novel lymphocytes supports the complexity of immune networks beyond Th2 response in the pathogenesis of CRS and AR.

In this review, we summarized the emerging role of innate lymphoid cells (ILCs), Th9 cells, Th22 cells, T follicular helper (Tfh) cells and B regulatory cells (Bregs) in the pathogenesis of CRSwNP and AR (Table 1 and Figure 1). Understanding the phenotype, function and regulation of these new players may offer an opportunity to further endotype CRS and AR and to develop novel approaches for patient management.

1.1 | ILCs

ILCs represent an emerging family of non-T, non-B effector cells that lack rearranged antigen-specific receptors, but have conserved effector cell functions. They represent innate versions of T lymphocytes and are likely generated in situ from systemically distributed committed ILC precursors in response to local environmental signals.⁷ ILCs have an important role in innate immunity to pathogenic and non-pathogenic microorganisms or other environmental insults, and in tissue homeostasis, repair and remodelling.⁸ Unlike adaptive immune cells, ILCs are antigen nonspecific and react promptly to a wide range of innate signals.⁸ Similar to CD4⁺ Th cells, at least three ILC subsets originated from common precursor cells have been identified based on the production of signature cytokines and the expression of transcription factors regulating cell differentiation, that is group 1 ILC including natural killer (NK) cell and non-NK group 1 ILC (ILC1), group 2 ILC (ILC2) and group 3 ILC (ILC3).⁸ The existence of parallel universes of adaptive and innate cells indicates a harmonious collaboration between innate and adaptive immunity in the pathogenesis of human diseases.⁸

NK cells represent the prototypical member of ILC family and constitute group 1 ILC with ILC1s (non-NK cells). NK cells and ILC1s share the expression of transcription factor T-bet and the capacity to secrete IFN- γ . However, they are distinguished by a combination of markers. NK cells lack expression of CD127, whereas ILC1s do not express perforin, granzyme B or killer cell immunoglobulin-like receptors.^{8,9} According to the expression intensity of CD56, NK cells can be subclassified into two types: CD56^{dim} and CD56^{bright} NK cells.¹⁰ CD56^{dim} NK cells are the major type and have a high cytolytic capacity, and secrete low levels of cytokines, whereas CD56^{bright} NK cells have profound capacity to secrete cytokines but acquire cytotoxicity only after prolonged activation.¹⁰ NK cells are important source of IFN- γ , but they can also secrete other cytokines.

On the basis of secreted cytokines, NK cells are subdivided into 4 types including NK1, NK2, NK regulatory, and NK22 cells.¹¹⁻¹⁴ NK cells grown in IL-12 and IL-18 (NK1) produce IFN- γ and inhibit IgE production, whereas NK cells grown in IL-4 (NK2) produce IL-5 and IL-13 and stimulate IgE production.^{11,12} NK regulatory cells produce IL-10 and transforming growth factor (TGF)- β and suppress IgE production.¹³ The IL-22-secreting NK22 cells might have a role in the protection of epithelial cell barriers.¹⁴ Although NK cells were originally named for their ability to mediate cytotoxicity towards target cells and to control infection, they can regulate multitude of inflammatory responses in lots of inflammatory diseases. CD56^{high}CD16⁻ NK cells in allergic contact dermatitis skin released IFN- γ and induced keratinocyte apoptosis.¹⁵ NK cells in synovial fluid of patients with rheumatoid arthritis produced IL-22 and TNF- α and induced proliferation of fibroblast-like synoviocytes.¹⁶ El-Shazly et al¹⁷ demonstrated that NK cells infiltrated into the nasal epithelium of allergic CRS patients, but not non-allergic CRS patients, potentially via CX3CL1 and CCL26/CX3CR1 axis. Kim et al¹⁸ showed that peripheral NK cells from patients with CRS had an impaired effector function reflected by decreased ability to degranulate and to produce IFN- γ and TNF- α . This defect was possibly induced by prostaglandin D2 (PGD2), contributed to reduced eosinophil apoptosis, and associated with disease severity in patients with CRS.^{19,20} Although the percentage of CD56⁺ NK cells in peripheral blood mononuclear cells (PBMCs) was increased in AR patients,²¹ the percentage of CD56^{bright} subpopulation in total NK cells has been reported to be decreased in AR patients, which might contribute to reduced IFN- γ production.²² Interestingly, Mesdaghi et al²¹ found increased percentage of IL-4⁺ NK2 cells and non-significantly decreased percentage of IFN- γ ⁺ NK1 cells in AR patients, underscoring a potential imbalance of NK1/NK2 in AR.

ILC1s (non-NK) are analogous to Th1 cells. ILC1s have been implicated in Crohn's disease and virus infection.^{23,24} The frequency of ILC1s was reported to increase in patients with COPD and correlate with disease severity and susceptibility to exacerbation.²⁵ Nevertheless, the roles of ILC1s in human diseases are far from being clarified. The presence and function of ILC1s in AR and CRS remain unexplored.

ILC3s produce IL-17A and/or IL-22. Transcription factor RAR-related orphan receptor (ROR) γ t and GATA3 are involved in their development as an innate counterpart of Th17 cells.⁹ ILC3s are further subclassified into at least 3 subgroups according to the features of cytokine production.⁹ Lymphoid tissue inducer cells, a subset of ILC3s producing both IL-17 and IL-22, participate in lymphoid organogenesis.²⁶ The subgroup producing IL-22 but not IL-17 is involved in host defence in intestinal homeostasis.²⁷ IL-17-producing ILC3s usually do not produce IL-22.²⁸ They express CCR6 and are preferentially recruited to intestinal mucosa and contribute to gut inflammation.²⁸ ILC3s are implicated in lower airway hyperreactivity in an obesity-associated murine asthma model.²⁹ Similar to ILC1s, the potential involvement of ILC3s in AR and CRS has not been reported yet. Interestingly, accumulating evidence indicates that ILC1s and ILC3s have considerable functional plasticity and can

TABLE 1 The expression and function of NK cells, ILC2s, Th22 cells, Th9 cells, Tfh cells and Bregs in AR and CRS

Cell	AR		CRS				References
	Location	Effect	Regulation	Location	Effect	Regulation	
NK cells	Peripheral blood	Peripheral NK1/NK2 cell dysregulation correlates with IgE levels	Not reported yet	Nasal mucosa and peripheral blood	1. Nasal NK cells are increased in allergic CRS patients 2. Peripheral NK cells have impaired effector function	PGD2 attenuates NK cell effector functions	17-21
ILC2s	Peripheral blood and nasal mucosa	Increased after allergen challenge and correlates the local Th2 mediators	Immunotherapy down-regulates peripheral ILC2	Nasal polyps	Correlates with local Th2 cells and eosinophils Consistent source of IL-13	1. IL-25, IL-33 and TSLP up-regulate ILC2 2. Corticosteroids down-regulate ILC2	45-56
Th22 cells	Peripheral blood	Th22 cell frequency correlates with serum IgE levels, and symptom scores	Not reported yet	Nasal polyps	Inhibits inflammation by enhancing mucin 1 expression	<i>Staphylococcus aureus</i> enterotoxin up-regulates IL-22 production	69-73
Th9 cells	Peripheral blood	Correlates with symptom scores	TGF-β and IL-4 up-regulate Th9 cell	Nasal polyps	IL-17RB ⁺ Th9 cell correlates with eosinophilia	IL-25 potentially up-regulates IL-17RB ⁺ Th9 cell	80,83-86
Tfh cells	Peripheral blood	Positively correlates with the symptom scores, quality-of-life scores, and total serum IgE levels	Immunotherapy down-regulates peripheral Tfh cell	Nasal polyps	IL-4 ⁺ Tfh cell frequency correlates with local IgE levels and B cell phenotypes	Not reported yet	94-100
Bregs	Peripheral blood	IL-10 ⁺ Bregs enhance immunotherapy effect in AR	Coadministration of mite allergens with <i>Clostridium butyricum</i> induces peripheral IL-10 ⁺ Bregs	Not reported yet	Not reported yet	Not reported yet	105,106

NK, natural killer; ILC, innate lymphoid cell; Tfh, follicular T helper; Breg, regulatory B cell; AR, allergic rhinitis; CRS, chronic rhinosinusitis; PGD2, prostaglandin D2; TSLP, thymic stromal lymphopoietin; TGF- β , transforming growth factor- β ; IL-17RB, IL-17 receptor B.

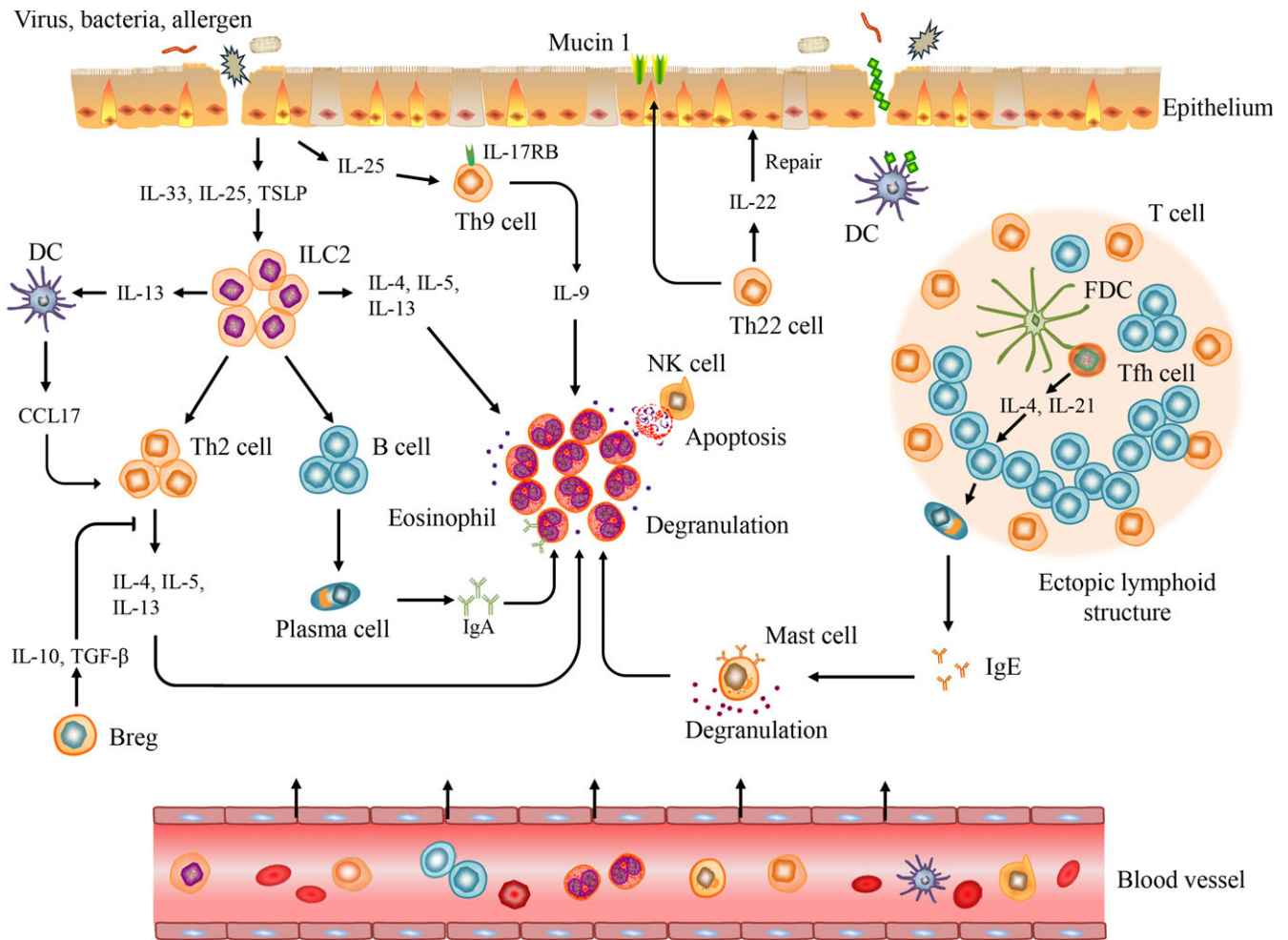


FIGURE 1 An overview of novel innate and adaptive lymphocytes involved in upper airway inflammation. In response to environmental stimuli, epithelial cells secrete interleukin (IL)-33, thymic stromal lymphopoietin (TSLP) and IL-25, which activate group 2 innate lymphoid cells (ILC2s). ILC2s initiate and propagate eosinophilic inflammation through producing type 2 cytokines. ILC2s are able to stimulate dendritic cells (DCs) to produce CCL17 via IL-13, which facilitates Th2 cell chemotaxis to local mucosa. Meanwhile, ILC2s can act as MHCII-dependent antigen-presenting cells to stimulate T cell responses and also able to promote antibody production from B cells. Natural killer (NK) cells demonstrate impaired function to induce apoptosis of eosinophils. Th22 cells play a critical role in protecting barrier surface from damage and promoting the repair of inflamed and injured tissues through IL-22. Additionally, they induce the anti-inflammatory mucin 1 expression. IL-17RB⁺ Th9 cells potentially induce eosinophilia and are regulated by IL-25. IL-4⁺ follicular T helper (Tfh) cells in ectopic lymphoid tissues induce IgE production from B cells. IgE cross-linking leads to degranulation of mast cells. B regulatory cells (Bregs) possess immunosuppressive properties mediated by provision of IL-10 and TGF- β and suppress the function of Th cells

convert into each other in response to specific inflammatory stimuli, such as IL-2, IL-23 and IL-1 β ,³⁰ which complicates the function and phenotype of ILCs.

In contrast to the limited understanding of ILC1s and ILC3s in airway diseases, ILC2s attract a lot of attention given their critical role in early-phase of type 2 immune responses.³¹ ILC2s are initially identified in the gut in the context of helminth infection and have previously been named as natural helper cells and nuocytes.^{32,33} ILC2s require the transcription factor ROR α and GATA3 for their development and differentiation from the common lymphoid progenitors, indicating that they are analogous to Th2 cells.^{34,35} ILC2s produce substantial amount of IL-4, IL-5, IL-9 and IL-13.^{34,35} Epithelial cell-derived IL-33, IL-25 and thymic stromal lymphopoietin (TSLP) are the primary inducers of expansion and activation of ILC2s.³¹

Recently, ILC3-derived IL-2 has been implicated in ILC2 proliferation and survival during type 2 pulmonary inflammation.³⁶ ILC2s are also reversible and up-regulate the expression of ILC1 markers in response to IL-12, IL-1 β and IL-18.^{25,37-39} In spite of their low abundance, ILC2s are the major early source of type 2 cytokines in allergic responses and helminth infection.^{32,33} ILC2s may regulate the function of adaptive immune cells. ILC2s have recently been found to express MHCII and act as MHCII-dependent antigen-presenting cells to stimulate T cell responses in vitro and ILC2-derived interleukin-13 is critical for eliciting production of the Th2 cell-attracting chemokine CCL17 by dendritic cells.^{40,41} ILC2-Th cell interaction through OX40/OX40L enhances Th cell responses.⁴² ILC2s are also able to promote antibody production from B cells.^{43,44} As type 2 responses are the central process in development of AR and

eosinophilic CRSwNP, it is not surprising to find a participation of ILC2s in the pathogenesis of AR and eosinophilic CRSwNP. ILC2s are enriched and activated in polyp tissues.⁴⁵⁻⁴⁸ ST2 (a subunit of IL-33 receptor complex)-positive ILC2s are identified as a consistent source of IL-13 in response to IL-33 stimulation in nasal polyps.⁴⁹ ILC2s correlate with the accumulation of Th2 cells and eosinophils in nasal polyps.^{47,50} In addition, the expression of epithelial cell-derived IL-25, IL-33 and TSLP has been found up-regulated in nasal polyps,⁴ highlighting an ILC2 favouring local milieu in CRSwNP. In asthmatic patients with concomitant CRS, the activation of IL-33/ILC2 axis in nasal mucosa was associated with decline of lung function and recalcitrant status of asthma control, suggesting an important role of nasal ILC2 activation in worsening asthma.⁵¹ Interestingly, corticosteroid treatment is able to induce apoptosis of ILC2s and diminish the accumulation of ILC2s in nasal polyps, which is accompanied by resolution of inflammation.⁴⁶

The studies of AR mainly focus on circulating ILC2s. The frequency of ILC2s in peripheral blood increases significantly upon antigen exposure,^{52,53} representing an egress of ILC2s from bone marrow to peripheral blood.^{52,53} On the contrary, the frequency of peripheral ILCs decreases in AR patients after subcutaneous immunotherapy, which is possibly due to the suppression mediated by IL-10⁺ Tregs.⁵⁴ Tregs suppress the function of ILC2s through IL-10 and TGF- β production and/or inducible T cell costimulator (ICOS)-ICOS ligand interaction,⁵⁵ indicating that Tregs suppress both innate and adaptive immunity by similar mechanisms. Interestingly, it has been discovered that peripheral ILC2s in AR patients highly express a CD2 signalling lymphocyte activation molecule family member, CD84, which mediates adhesion interaction between T lymphocytes and accessory cells.⁵³ As ILC2s may act as antigen-presenting cells, the expression of CD84 on ILC2s possibly sustains the interaction between ILC2s and T cells. Fan et al⁵² discovered that the percentage of peripheral ILC2s was only elevated in AR patients allergic to house dust mite (HDM) but not in those allergic to mugwort, suggesting that different allergens may have distinct immunogenicity for ILC2 induction. Although ILC2s are indicated in regulation of immune responses at mucosal site, only one study has investigated the prevalence of ILC2s in the local mucosa of AR patients so far.⁵⁶ Dhariwal et al⁵⁶ found that following allergen provocation ILC2s were rapidly recruited to nasal mucosa and associated with local type 2 mediator production in AR patients. This is in line with the findings in asthmatic patients where after allergen challenge, D prostanoid receptor 2 (DP2 or CRTH2) positive ILC2s were significantly increased in sputum and correlated with airway eosinophilia.⁵⁷ In addition, Eastman et al⁵⁸ found that ILC2s were recruited to the nasal mucosa in patients with aspirin-exacerbated respiratory disease. Using murine models, two recent researches confirmed the important role of ILC2s in exacerbating type 2 inflammation in AR.^{59,60}

1.2 | Th22 cells

Th22 cells are characterized by the secretion of signature cytokine IL-22, but lack of IL-4, IL-17 and IFN- γ expression. The aryl

hydrocarbon receptor is suggested to be the transcription factor for Th22 cell differentiation.⁶¹ The specific microenvironment for generation of Th22 cells is composed of tumour necrosis factor (TNF)- α and IL-6.^{61,62} Th22 cells play a critical role in protecting barrier surface from damage, and promoting the repair of inflamed and injured tissues to restore their integrity.^{61,63} The percentages of peripheral Th22 cells were increased and positively correlated with insulin resistance in patients with diabetes mellitus,⁶⁴ underscoring an involvement of Th22 cells in chronic inflammation. In atopic eczema, skin-colonized *Staphylococcus aureus* were presented by dendritic cells and recognized by Th22 cells, thus leading to IL-22-mediated tissue remodelling.⁶⁵ In asthma, Th22 cells and IL-22 demonstrate complex functions. Some evidence suggests that IL-22 acts as a pro-inflammatory cytokine, but others pointed to an opposite function.^{66,67} These discrepancies may be due to the difference in the study methods and the time-points to block or exaggerate IL-22 expression in murine models.^{66,67} For example, allergic lung inflammation was drastically inhibited by IL-22 neutralization during immunization, but exaggerated by IL-22 neutralization during challenge in wild-type mice.⁶⁶ Based on the data generated from IL-22-transgenic mice with IL-22 overexpression specifically in lungs, IL-22 was found to function as a negative regulator of allergen-induced airway hypersensitivity reaction.⁶⁷ The inhibitory effect of IL-22 on allergic pulmonary inflammation may partially associate with its function to reduce antigen uptake by DCs and expression of costimulatory molecules on DCs.⁶⁸

In inflammatory upper airway diseases, although the expression and function of IL-22 have been investigated, few studies have specifically addressed the contribution of Th22 cells to AR and CRS at cellular level. No significant change in IL-22 mRNA and protein levels in nasal polyps was found compared to control inferior turbinate mucosa by some studies⁶⁹; however, Noyama et al⁷⁰ discovered an up-regulation of IL-22 mRNA levels in nasal polyps compared to uncinate tissues from non-CRS controls. Moreover, they found that IL-22 was mainly expressed by inflammatory cells in nasal polyps in spite that they did not further define the phenotypes of cells with IL-22 expression.⁷⁰ They demonstrated that the production of IL-22 in nasal polyps was induced by *Staphylococcus aureus* enterotoxin and negatively correlated with local eosinophilia and postoperative computed tomography scores in CRSwNP patients.⁷⁰ IL-22 significantly enhanced mucin 1 mRNA expression in nasal polyps.⁷⁰ Mucin 1 has an anti-inflammatory effect on airway inflammation by inhibiting Toll-like receptor signalling pathway and eosinophil survival.⁷¹ Therefore, the elevated expression of IL-22 may be a negative feedback mechanism to control eosinophilic inflammation and disease development in CRSwNP. As other cell types like Th17 cells and ILC3s can also produce IL-22, it is quite difficult to conclude a definitive role of Th22 cells in CRS without phenotyping the IL-22 producing cells in nasal mucosa.

In children with AR, higher IL-22 mRNA levels in PBMCs were detected compared to healthy individuals, and a positive correlation between IL-22 expression and serum total IgE levels was

demonstrated.⁷² In adult AR patients, Tang et al⁷³ found that the frequency of peripheral Th22 cells was significantly up-regulated in AR patients compared with that in controls, and correlated with nasal congestion and rhinorrhea symptom scores. Moreover, the serum IL-22 levels correlated with the levels of specific IgE against *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*.⁷³ However, whether up-regulated IL-22 has a pathogenic role or functions as a negative feedback mechanism to control inflammation in AR requires future investigations.

1.3 | Th9 cells

Th9 cells are a subset of Th cells producing signature cytokine IL-9.⁷⁴ The induction and activation of Th9 cells require TGF- β and IL-4.⁷⁴ Th9 cells express multiple transcription factors including a high level of E-twenty-six family transcription factor PU.1 and interferon regulatory factor (IRF) 4 and IRF1, and a low level of GATA3.^{74,75} Th9 cells have been implicated in the development of many diseases, including allergic inflammation, tumour and parasitosis.⁷⁵ IL-9 enhances mucus production and mastocytosis in lung.⁷⁶ Adoptive transfer of Th9 cells resulted in allergic airway inflammation and anti-IL-9 blockage ameliorated Th9-mediated asthma in murine models.⁷⁷ IL-9 is highly expressed in the lungs of asthmatic human subjects.⁷⁸ In human allergic contact dermatitis, Th9 cells were increased in the skin lesions and PBMCs from nickel-allergic patients.⁷⁹ With respect to upper airway diseases, Lin et al⁸⁰ found that the expression of IL-9 in submucosal inflammatory cells and IL-9R in epithelial cells was up-regulated in CRSwNP. IL-9 mRNA levels in cultured nasal polyp tissues were up-regulated by IL-4, IL-17A, IL-1 β , whereas suppressed by IFN- γ and IL-27.⁸⁰ It should be noted that in addition to Th9 cells, IL-9 is also produced by other cell types including ILC2s.⁸¹ ILC2 survival is supported by IL-9.⁸¹ As IFN- γ and IL-27 have been reported to inhibit ILC2 function,⁸² it is possible that the downregulation of IL-9 mRNA in nasal polyps by IFN- γ and IL-27 partially works via ILC2s. Interestingly, linuma et al⁸³ have recently found that a subpopulation of polyp Th2 and Th9 cells had up-regulated expression of IL-17 receptor B (IL-17RB) and the increased accumulation of IL-17RB⁺ Th2 and Th9 cells correlated with eosinophilia in nasal polyps. Although no functional study has been conducted in that research, those findings suggest that IL-17RB⁺ Th9 cells potentially contribute to eosinophilia in nasal polyps under the regulation of epithelial cell-derived IL-25 in CRSwNP.⁸³

Wang et al⁸⁴ have found that Th9 cells and transcription factor IRF4 were elevated in the peripheral blood from Chinese AR patients. Levels of TGF- β and IL-4 in PBMCs, the Th9 cell-polarizing cytokines, were much higher in AR patients.⁸⁴ Higher symptom scores were associated with a more severe Th9 response in AR.⁸⁴ Neutralization of IL-9 not only ameliorated symptoms of AR, but also enhanced the effect of allergen-specific immunotherapy in murine models of AR.^{85,86} These results suggest that AR patients have a favourable environment for Th9 differentiation and that Th9 cells potentially participate in the pathology of AR.

1.4 | Tfh cells

Tfh cells are a subset of CD4⁺ Th cells that are specialized in promoting the B cell response and germinal centre (GC) reaction.⁸⁷ These cells exhibit a CXCR5⁺ICOS^{hi} and programmed cell death protein 1 (PD-1)^{hi} surface phenotype, and express high levels of transcriptional factors required for their development and differentiation including B cell lymphoma 6 (Bcl-6), c-musculoaponeurotic fibrosarcoma, IRF4, basic leucine zipper transcription factor, signal transducer and activator of transcription (STAT) 1, STAT3, STAT4, STAT5 and inhibitor of DNA binding proteins, including Id2 and Id3.⁸⁷⁻⁸⁹ Although Tfh cells primarily reside in the GC of secondary lymphoid organs, circulating counterparts of these cells in peripheral blood have been identified.⁸⁹ Differential expression of CXCR3 and CCR6 defines three major subsets within circulating Tfh cells: CXCR3⁺CCR6⁻, CXCR3⁻CCR6⁻ and CXCR3⁻CCR6⁺ cells. CXCR3⁺CCR6⁻ circulating Tfh (Tfh1) cells secrete IFN- γ , but not type 2 or 17 cytokines. IL-4, IL-5 and IL-13 are primarily produced by CXCR3⁻CCR6⁻ circulating Tfh (Tfh2) cells, whereas IL-17A and IL-22 are mainly produced by CXCR3⁺CCR6⁺ circulating Tfh (Tfh17) cells.⁸⁹ Circulating Tfh cells share similar functional properties with bona fide GC Tfh cells and possibly represent a circulating memory compartment of Tfh lineage cells.⁸⁹ In autoimmune diseases, percentages of circulating Tfh cells are increased and correlate with the serum concentrations of autoantibodies.⁹⁰ In addition to inducing B cell response, Tfh cells also possess other functions. Intratumoral Tfh cells have been found to induce CCL17 and CCL22 production from tumour cells, which facilitate recruitment of Tregs and the development of immunosuppressive microenvironment favouring immune surveillance escape and tumour growth.⁹¹ In a mouse asthma model, Tfh cells were found to be plastic in that they were able to develop into IL-4 and IL-13 double-positive Th2 cells in lung.^{92,93}

Local immunoglobulin production may play a critical role in the pathogenesis of CRSwNP.^{1,94} Local IgE antibodies against aeroallergens and *Staphylococcus aureus* enterotoxins are elevated in patients with eosinophilic CRSwNP independent of systemic atopy.⁹⁵ Local IgE can activate nasal mast cells and contribute to eosinophilic inflammation in CRSwNP.⁹⁵ Therefore, understanding the regulation mechanism of local IgE production may have significant therapeutic implications for eosinophilic CRSwNP. Recently, we and others have discovered an accumulation and expansion of CXCR5⁺CD4⁺ Tfh cells in both eosinophilic and non-eosinophilic polyp tissues.^{94,96} We further demonstrated that the frequencies of total Tfh cells and IL-21⁺, IFN- γ ⁺ and IL-17⁺ Tfh cells were increased in both eosinophilic and non-eosinophilic polyps compared to normal inferior turbinate tissues. However, the frequency of IL-4⁺ Tfh cells was only increased in eosinophilic, but not non-eosinophilic, polyp tissues.⁹⁴ In vitro functional analysis showed that IL-4 was involved in polyp Tfh cell-induced IgE production from B cells, and nasal IL-4⁺ Tfh cell frequencies correlated with local IgE levels in vivo.⁹⁴ These results indicate that nasal IL-4⁺ Tfh cells are critical for local IgE production in eosinophilic polyps. The phenotypes of nasal Tfh cells seem to differ from circulating Tfh cells and bona fide Tfh cells in GCs. We found

that nasal Tfh cells expressed ICOS, PD-1 and Bcl-6 at an intermediate level when compared with the highest level in bona fide Tfh cells and the lowest level in circulating Tfh cells, suggesting an immature phenotype of nasal Tfh cells.⁹⁴ We found that polyp Tfh cells also correlated with the change of B cell phenotypes, further supporting a fundamental function of nasal Tfh cells in local humoral immunity in CRSwNP.⁹⁴ We further found that Tfh cells were mainly located in ectopic lymphoid tissues in nasal polyps.⁹⁷ Tfh cells from polyps with ectopic lymphoid tissue formation demonstrated an activated phenotype characterized by up-regulated expression of ICOS and PD-1.⁹⁷ However, the ontogeny and mechanisms underlying the polarization of Tfh cells in inflamed tissues remain to be elucidated.

In AR, Wang et al⁹⁸ found that the frequency of circulating Tfh cells was significantly increased in patients with AR and positively correlated with the symptom scores, rhino-conjunctivitis quality-of-life questionnaires scores and total serum IgE levels. In contrast, Kamekura et al⁹⁹ showed that the percentage of total circulating Tfh cells was comparable between AR patients and controls. However, interestingly, they found that the percentage of CXCR3⁺CCR6⁺ Tfh2 cells was higher, whereas the percentage of CXCR3⁺CCR6⁺ Tfh1 was lower than those of the controls, resembling Th2/Th1 imbalance in AR.⁹⁹ Schulten et al¹⁰⁰ reported that allergen-specific immunotherapy reduced circulating Tfh cells in AR patients, further supporting an involvement of Tfh cells in the immune disturbance in AR. Nevertheless, the presence of Tfh cells in the local mucosa of AR patients has not been reported, despite that local B cell clusters and local Ig class-switch in nasal mucosa have also been demonstrated in AR patients.¹⁰¹

1.5 | Bregs

In addition to producing antibodies, some kinds of B cells also possess immunosuppressive properties mediated by provision of IL-10 and TGF- β , and direct contact with CD4⁺ T cells.¹⁰² This specific population of B cells demonstrating regulatory function is named as Bregs and is involved in maintenance of tolerance of human body.¹⁰² Bregs control the maintenance of Tregs. Several studies showed that mice harbouring a B cell-specific deletion of IL-10 displayed a deficiency of Tregs and an overactivation of pro-inflammatory T cells.¹⁰³ So far, no transcription factor has been identified to define Bregs as a separate lineage, although a Treg-FoxP3-equivalent transcription factor exists.^{102,103} Therefore, multiple Bregs have been described.¹⁰² In mouse, several types of Bregs, including transitional 2-marginal zone precursor cells, CD5⁺CD1d^{hi} B10 cells, CD138⁺ plasma cells, TIM-1⁺ B cells and B-1a cells, have been reported.¹⁰³ In humans, CD27⁺CD24^{high} B10 cells, CD24^{hi}CD38^{hi} Bregs and CD25^{hi}CD71^{hi}CD73^{lo} Bregs have been described.^{102,103} Some murine Bregs are the counterparts of those in human beings. For example, CD5⁺CD1d^{hi} Bregs in mouse spleens parallel with CD27⁺CD24^{hi} Bregs in human blood.¹⁰² They are termed as B10 cells because they are predominant IL-10-producing B cells and suppress effector CD4⁺T cells, monocytes and dendritic cells.¹⁰² It should be emphasized that not all IL-10-producing B cells are regulatory or

suppressive, as they may concomitantly produce pro-inflammatory cytokines such as IL-6.¹⁰⁴

The involvement of Bregs is well established in human inflammatory airway diseases, but little is known about the role of Bregs in airway diseases. Limited studies have shown the involvement of Bregs in AR, but unfortunately, there is no study reporting Bregs in CRS yet. Kim et al¹⁰⁵ found that CD25^{hi} Bregs were lower in individuals with AR than in non-allergic controls. In another report, Kamekura et al⁹⁹ compared peripheral CD3⁺CD19⁺CD24^{hi}CD27⁺ Bregs in patients with AR only and patients with AR and concomitant asthma. They observed a decrease in frequency of Bregs during the disease progression from AR to AR with asthma.⁹⁹ Moreover, in patients with AR and concomitant asthma, the ratio of CXCR3⁺CCR6⁺ Tfh2 cells to Bregs was significantly correlated with levels of fractional exhaled nitric oxide and the number of blood eosinophils, suggesting an imbalance of Tfh2 cell/Bregs in the disease progression from AR to AR with asthma.⁹⁹ In allergen-specific immunotherapy of AR patients allergic to house dust mites, Xu et al¹⁰⁶ found that co-administration of mite allergens with *Clostridium butyricum* induced peripheral IL-10⁺ Bregs and enhanced immunotherapy effect. These findings provide the evidence that Bregs play an important role in immunotherapy-induced immune tolerance in AR patients. Hence, expanding Bregs is a potential promising therapeutic approach to treat AR and other allergy-related diseases.

2 | THERAPEUTIC IMPLICATIONS IN UPPER AIRWAY DISEASES

The therapeutic implications of modulating ILCs, Th22 cells, Th9 cells, Tfh cells and Bregs in AR and CRS are summarized in Table 2.

2.1 | NK cells

NK cell transfer has been investigated for the treatment of several haematologic malignancies and solid tumours.^{107,108} For example, in patients receiving haploidentical hematopoietic stem cell transplantation, infusing with ex vivo expanded NK cells has been proved feasible and effective in preventing leukaemia relapse.¹⁰⁸ These phase I clinical trials raise the possibility of infusing NK cells to improve the NK cell defect in CRS patients. The defect of NK cells in CRS was possibly induced by PGD2, which worked through the D prostanoid receptor 1 (DP1) rather than the DP2 (also termed CRTH2).¹⁹ Therefore, blocking DP1 by ONO-4053, a novel DP1 antagonist, may also have clinical implication to improve NK cell function and alleviate eosinophilic inflammation in CRS.¹⁰⁹

2.2 | ILC2s

As animal and cell experiments have shown a critical role of ILC2s in initiating and amplifying allergic responses, inhibition of ILC2s may have therapeutic potentials for allergen-induced diseases.^{55,56} There

are several ongoing clinical trials investigating the effect of block of ILC2 activating cytokines in allergic diseases. For example, a double-blind, placebo-controlled study showed that treating patients with mild allergy with three monthly doses of AMG157, a humanized anti-TSLP monoclonal immunoglobulin G2 λ , reduced allergen-induced bronchoconstriction and airway inflammation.¹¹⁰ A randomized, double-blind, placebo-controlled, multi-centre clinical trial showed that SB010, a novel DNA enzyme that is able to cleave and inactivate GATA3 mRNA, attenuated both late and early asthmatic responses after allergen provocation in patients with allergic asthma.¹¹¹ It is likely that SB010 not only inhibit Th2 cells, but also ILC2s.¹¹¹ However, it remains unclear whether anti-TSLP or inactivating GATA3 has a therapeutic effect on patients with AR and eosinophilic CRSwNP. ILC2s express CRTH2.⁵⁷ BI 671800, a CRTH2 antagonist, has been demonstrated to inhibit type 2 response-dominated eosinophilic inflammation and relieve AR symptoms induced by allergen exposure in environmental challenge chamber, and had a

favourable safety profile at the dose of 200 mg, twice daily, although its effect in asthmatics remains unclear.^{112,113} It is possible that CRTH2 antagonist partially works through inhibiting ILC2s. Recent studies on monoclonal antibodies against type 2 cytokines demonstrated promising therapeutic effect on both sinus diseases and asthma.¹¹⁴ Dupilumab, IL-4R α antagonist to modulate both IL-4 and IL-13 signalling pathway, has been found to reduce endoscopic polyp burden of CRSwNP patients.¹¹⁴ Mepolizumab binds to IL-5 and prevents it from binding to IL-5R α . Mepolizumab has been shown to reduce the need for surgery in patients with recurrent nasal polyps.⁶ These monoclonal antibodies might significantly influence ILC2 activation by countering the positive feedback between ILC2s and type 2 inflammation. Targeting ILC2s in human allergic diseases is appealing, but many challenges exist. For example, there is a lack of ILC2-specific markers or targets. In addition, targeting upstream cytokines, including IL-33 and IL-25, will likely have effects on multiple cell types or be insufficient to affect ILC2 responses owing to redundancy. Importantly, unintended consequences of targeting repair or homeostatic functions of ILC2s may result in untoward side effects.

TABLE 2 Therapeutic implications of modulating NK cells, ILC2s, Th22 cells, Th9 cells, Tfh cells and Bregs in AR and CRS

Cell	Potential target	References
NK cells	Ex vivo expanded NK cells transfer A novel DP1 antagonist (ONO-4053)	108,109
ILC2s	Anti-TSLP (AMG157), anti-IL-33 and anti-IL-25 antibody Inhibiting transcription factors such as GATA-3 (SB010) CRTH2 antagonist (BI 671800) IL-4R α monoclonal antibody (Dupilumab) IL-5 monoclonal antibody (Mepolizumab)	6,110-114
Th22 cells	Anti-IL-22 monoclonal antibody (ILV-094) IL-12/IL-23p40 antagonist (Ustekinumab) Avoiding diesel exhaust particle	115-117
Th9 cells	Anti-IL-9 monoclonal antibody (MEDI-528) Anti-TNF α antibody (Infliximab) Anti-IL-25 antibody	119-121
Tfh cells	Anti-ICOS ligand antibody (AMG557) Anti-IL-21 antibody (NNC0114-0006) Anti-CXCL13 antibody (VX5) Anti-PD-1 antibody (BMS-936558) Small molecules targeting STAT3 (ISIS-STAT3Rx/AZD9150) CTLA4-IgFc fusion protein (Abatacept)	122-126
Bregs	Ex vivo expanded autologous Bregs transfer	129

NK, natural killer; ILC, innate lymphoid cell; Tfh, follicular T helper; Breg, regulatory B cell; AR, allergic rhinitis; CRS, chronic rhinosinusitis; DP1, D prostanoid receptor 1; TSLP, thymic stromal lymphopoietin; CRTH2, D prostanoid receptor 2; TNF, tumour necrosis factor; ICOS, inducible T cell costimulator; PD-1, programmed cell death protein 1; STAT, signal transducer and activator of transcription; CTLA-4, cytotoxic T lymphocyte-associated protein 4.

2.3 | Th22 cells

IL-22 blocking antibody ILV-094 is currently being investigated in patients with atopic dermatitis.¹¹⁵ Ustekinumab is an IL-12/IL-23p40 antagonist that suppresses Th1, Th17 and Th22 cell activation. In a phase II, double-blind, placebo-controlled study, patients with moderate-to-severe atopic dermatitis received 4 weeks of ustekinumab treatment and IL-22 expression was downregulated after treatment.¹¹⁶ Plé et al¹¹⁷ found that diesel exhaust particle-polycyclic aromatic hydrocarbons and Benzo[a]Pyrene induced production of IL-22 from PBMCs. However, there is controversy about the role of Th22 cells in allergic airway diseases. Therefore, whether the biological agents targeting Th22 and avoiding diesel exhaust have beneficial effect on AR and CRSwNP requires careful investigation.

2.4 | Th9 cells

Several in vitro and in vivo pre-clinical trials explored potential therapeutic effect by targeting the IL-9 pathway in asthma, systemic lupus erythematosus, multiple sclerosis, inflammatory bowel diseases, rheumatoid arthritis, psoriasis and cancers.¹¹⁸ MEDI-528 is a humanized anti-IL-9 monoclonal antibody currently used in phase II trials. Two studies demonstrated an improved quality of life and reduced exacerbation with the use of this agent in asthmatics.¹¹⁹ Infliximab is a chimeric monoclonal antibody that works against tumour necrosis factor alpha (TNF- α), and the trials conducted in patients with Crohn' disease found that infliximab downregulated serum IL-9 levels.¹²⁰ Since in CRSwNP, some Th9 cells have up-regulated expression of IL-17RB⁸³; therefore, it is rational to block IL-25 and alleviate Th9 cell-mediated inflammation in CRSwNP.¹²¹ In addition, transcription factors can be targeted for the regulation of Th9 cells for AR and CRSwNP.

2.5 | Tfh cells

The recent development in Tfh biology highlights the potential for targeting Tfh cells through acting on transcription factor Bcl6, STAT3, chemokine receptor CXCR5, costimulatory and inhibitory molecules inducing PD-1, CD40L, ICOS, CD80 and OX40, and cytokine IL-21, which control migration, development and function of Tfh cells. For example, a phase I trial of anti-ICOS ligand antibody (AMG557) in systemic lupus erythematosus demonstrated the feasibility of administering this antibody to human subjects.¹²² Antibody against IL-21 (NNC0114-0006) was reported as safe and well tolerated in RA patients in a phase 1 trial.¹²³ CXCL13 neutralization antibody (VX5) lead to significant reduction in germinal centres in a T cell-dependent mouse immunization model and inhibited gastric lymphoid follicle formation after *Helicobacter* infection.¹²⁴⁻¹²⁶ In future, these antibodies could be tested in the treatment of AR and CRSwNP patients.

2.6 | Bregs

Induction or transfer of Bregs has not been used in clinic for the treatment of AR and CRSwNP yet. Animal studies have demonstrated that Bregs adoptive transfer suppressed the development of allergic asthma by inhibiting Th2- and Th17-driven inflammation in an IL-10-dependent manner.¹²⁷ Recently, researches on a mouse model of multiple sclerosis have revealed that both CD40 and IL-21 receptor signals drove regulatory B cell expansion by 4-million-fold, and induced IL-10.¹²⁸ More importantly, adoptive transfer of ex vivo expanded regulatory T cells is under phase I clinical trials in patients with recent-onset type 1 diabetes mellitus.¹²⁹ Thus, the ex vivo expanded autologous Bregs may provide a novel and effective treatment for inflammatory upper airway disease.

3 | CONCLUSIVE REMARKS

In this review, we discussed the recent progress on the study of ILCs, Th22 cells, Th9 cells, Tfh cells and Bregs in AR and CRS. The identification of these novel immune cell types significantly advances our understanding of the immunological pathogenesis of CRS and AR. However, in future, several points should be elucidated in the context of AR and CRS. First, in addition to expression of cytokines, the involvement of specific cell types should be more extensively studied at cellular level. Second, the role of ILC1s and ILC3s should be clarified for AR and CRS, particularly for non-eosinophilic CRSwNP. Third, the potential local participation of these novel immune cells in AR should be evaluated given the importance of local immune response in AR development and progression. Fourth, the function of these novel immune cells in AR and CRS needs to be dissected in vivo using animal models and the regulation of these novel immune cells should be further examined in the context of AR and CRS, especially for Th9 cells. Fifth, the ontogeny and mechanisms of polarization of Tfh cells in

inflammatory mucosal sites remain to be elucidated. Although these immune cells are relatively new area of AR and CRS research, they hold promise for the future and the development of novel drugs with new mechanisms of action for AR and CRS treatment.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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