



Group 2 innate lymphoid cells and eosinophilic chronic rhinosinusitis

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Purpose of review

Chronic rhinosinusitis (CRS) is a heterogeneous disease and is recently classified into two phenotypes, eosinophilic CRS (ECRS) and non-ECRS. ECRS is characterized by Th2-biased eosinophilic inflammation, and non-ECRS is characterized by Th1-biased neutrophilic inflammation. Group 2 innate lymphoid cells (ILC2s) rapidly produce large amounts of Th2 cytokines and exert critical roles in Th2-type immune responses. We summarize our current knowledge about the pathogenic roles of ILC2s in ECRS.

Recent findings

The prevalence of ILC2s is increased in nasal polyps, and it is positively correlated with the number of infiltrating eosinophils. Epithelium-derived cytokines (IL-33, IL-25, and thymic stromal lymphopoietin), cysteinyl leukotrienes, and prostaglandin D₂ stimulate the production of Th2 cytokines from ILC2s, which drives eosinophilic inflammation in nasal mucosa. Regulation of ILC2s would be a novel therapeutic approach for the refractory and/or recurrent cases of ECRS.

Summary

Increased ILC2s play a pivotal role in the pathophysiology of ECRS by producing large amounts of Th2 cytokines, which lead to Th2-type eosinophilic inflammation in nasal polyps.

Keywords

chronic rhinosinusitis, eosinophils, group 2 innate lymphoid cells, IL-5, nasal polyp

INTRODUCTION

Chronic rhinosinusitis (CRS) is one of the most common nasal diseases in adults, characterized by persistent symptomatic inflammation of nasal and paranasal mucosa that lasts longer than 12 weeks [1]. Unremitting inflammation can last for years, and CRS affects approximately 12% of the population in Europe and the United States [1,2^{••}]. CRS was classified into two major subtypes: CRS without nasal polyps (CRSsNP) and CRS with nasal polyps (CRSwNP) [1]. However, there are different phenotypes of inflammation in CRS between European and East Asian countries. In Europe, CRSwNP is characterized by Th2-biased eosinophilic inflammation, and CRSsNP is characterized by Th1-biased neutrophilic inflammation. On the other hand, in East Asian countries, more than half of CRSwNP cases exhibit neutrophilic inflammation, although the prevalence of eosinophilic inflammation has recently increased [3–5]. A recent study suggested that genetic factors or genetic/environment interactions play a role in the development of noneosinophilic nasal polyps in East Asian countries [6]. Therefore, a new classification of CRS based on its

phenotypes (eosinophilic CRS (ECRS) and non-ECRS) has been proposed by the Japanese Epidemiology Survey of Refractory Eosinophilic Chronic Rhinosinusitis Study (JESREC study) [7].

The recent discovery of innate lymphoid cells (ILCs) suggests important roles for these cells in immunity, tissue development, and remodeling [8^{••}]. In contrast to T cells and B cells, ILCs lack antigen receptors such as T-cell receptor and B-cell receptor and are activated by several cytokines and/or lipid mediators. ILCs are classified into three groups analogous to the subsets of T-helper lymphocytes. Group 1 ILCs (ILC1s) produce Th1 cytokines (i.e., IFN- γ) and express transcription factor T-bet [9], whereas group 2 ILCs (ILC2s) are capable of

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Curr Opin Allergy Clin Immunol 2019, 19:18–25

DOI:10.1097/ACI.0000000000000496

KEY POINTS

- The prevalence of ILC2s is increased in nasal polyps of ECRS.
- Increased IL-33, IL-25, TSLP, cysLTs, and PGD₂ in nasal polyps may be involved in the development of the eosinophilic inflammation by stimulating ILC2s to release a large amount of Th2 cytokines.
- Cross-talk between ILC2s and eosinophils in nasal polyps may be critical for amplification and maintenance of Th2-type inflammation.
- Blocking ILC2-derived Th2 cytokines may provide a potential therapeutic target for the treatment of refractory and/or recurrent cases of ECRS.

producing Th2 cytokines and display transcription factor GATA binding protein 3. Group 3 ILCs (ILC3s) are associated with IL-17 and/or IL-22 production and are dependent on transcription factor retinoic acid-related orphan receptor gamma t. Human ILCs are highly heterogeneous cell types between individuals and tissues [10]. All ILC subsets were present in nasal polyps, and ILC2s were dominant and increased when compared with peripheral blood and tonsil from CRSwNP patients, or with the sinus tissues from CRSsNP patients or control patients [11²²,12]. In this article, the role of ILC2s in the pathophysiology of CRS, particularly ECRS, will be reviewed.

CHARACTERISTICS OF EOSINOPHILIC CHRONIC RHINOSINUSITIS

CRS shows remarkable heterogeneity at the clinical and molecular levels, and the current classification of CRS into CRSsNP and CRSwNP might not adequately reflect the pathophysiologic diversity of CRS. Tomassen *et al.* [13] classified 173 CRS patients into 10 clusters on the basis of immune markers such as IL-5, IFN- γ , IL-17A, TNF- α , IL-22, IL-1 β , IL-6, IL-8, eosinophilic cationic protein (ECP), and IgE and found that high IL-5 and ECP clusters showed nasal polyp phenotypes with strongly increased asthma prevalence. These IL-5 and eosinophil-predominant clusters may represent ECRS subtype of CRS classified by the JESREC study.

ECRS is characterized by serum and tissue eosinophilia, bilateral multiple polyposis, sticky and glue-like rhinorrhea, ethmoid sinus-dominant disease, and comorbidity with asthma. ECRS is intractable even after endoscopic sinus surgery (ESS). To diagnose ECRS, the JESREC scoring system was established by assessing unilateral or bilateral disease, the

presence of nasal polyps, blood eosinophilia, and dominant computed tomography (CT) shadow of ethmoid. Histologically, number of eosinophils in submucosa of ethmoid cavity or nasal polyps greater than or equal to 70/high power field (HPF $\times$ 400) was defined as ECRS. Significantly higher recurrence rates of nasal polyps after ESS were found in ECRS patients compared with non-ECRS patients. ECRS was classified into mild, moderate, and severe cases, according to the factors of recurrence: eosinophil rates of peripheral blood greater than 5%, ethmoid-dominant CT shadow, and comorbidity of bronchial asthma or aspirin intolerance. More severe cases had higher recurrence rates after ESS [7].

FUNCTION AND REGULATION OF GROUP 2 INNATE LYMPHOID CELLS

ILC2s are a recently discovered lymphocyte population that promotes features of allergic airway diseases including asthma and CRSwNP through the production of Th2 cytokines. ILC2s were originally isolated from mice and characterized by several investigators, and they were independently designated as natural helper cells, nuocytes, and innate helper 2 cells [14–16]. In 2013, this cell population was defined as ILC2s [17]. ILC2s do not express conventional cell surface markers for T cells, B cells, natural killer (NK) cells, myeloid cells, and dendritic cells; thus, they were designated as lineage-negative (Lin[−]) [18]. Human ILC2s were first discovered within nasal polyps in 2011 [19]. To date, ILC2s have been described in mice and humans at varying body sites and represent a critical innate cellular source of Th2 cytokines (including very high amounts of IL-5 and IL-13, but also IL-4, IL-9, granulocyte-macrophage colony-stimulating factor (GM-CSF), and amphiregulin). The early induction of these cytokines promotes tissue eosinophilia, mucus production from goblet cells, activation of T cells, B cells, and macrophages, mastocytosis, muscle contractility, tissue repair, metabolic homeostasis, obesity, and dietary stress [20–24]. Owing to their innate characteristics and immediate action, ILC2s are thought to be particularly important at barrier surfaces such as nasal mucosa [25].

Initial studies have shown that the epithelium-derived cytokines such as IL-33, IL-25, and thymic stromal lymphopoietin (TSLP) induce Th2 cytokine production from ILC2s. Increasing evidence has revealed that activating factors for human ILC2s are IL-1 β [11²²,26], TNF superfamily (TNF-like ligand 1A and glucocorticoid-induced TNF receptor-related protein ligand) [27–29], cysteinyl leukotrienes [cysLTs; leukotriene C₄, leukotriene D₄, and leukotriene E₄ (LTE₄)] [30,31²³], prostaglandin D₂ (PGD₂) [32],

and retinoic acid [33]. IL-2 and IL-7 induce the proliferation and development of ILC2s [20], and IL-4 and IL-9 act as regulators of ILC2 activation [34,35]. On the other hand, negative regulators for human ILC2s are lipoxin A₄ [36], IL-10 [37], TGF- β [37], prostaglandin E₂ [38], prostaglandin I₂ [39], and vitamin D [33]. All of these have anti-inflammatory activities in allergic inflammation. In addition, IL-27 [40[■]], type I and type II interferons (IFN- β and IFN- γ) [40[■],41], and β 2-adrenergic receptor pathway [42] inhibit ILC2 activity.

GROUP 2 INNATE LYMPHOID CELLS IN NASAL POLYPS

Mjösberg *et al.* [19] first found human ILC2s in nasal polyps. They defined human ILC2s as CD45⁺Lin⁻CD127⁺CRTH2⁺CD161⁺ cells. Their lineage (Lin) included CD1a, CD3, CD4, CD11c, CD14, CD19, CD34, CD56, CD94, CD123, T-cell receptor alpha/beta, T-cell receptor gamma/delta, blood dendritic cell antigen 2, and high affinity immunoglobulin epsilon receptor subunit alpha (FcER1 α). The prevalence of ILC2s (described as % of CD45⁺ cells) was significantly increased in nasal polyps ($n=4$) when compared with nasal tissues in healthy controls ($n=4$) [19]. These nasal polyp-derived ILC2s produced IL-5, IL-13, GM-CSF, IL-4, and IL-9 in response to IL-33 and IL-2 [43]. Shaw *et al.* [44] reported the increased prevalence of ILC2s (Lin⁻CD127⁺CRTH2⁺ cells, described as percent of Lin⁻ cells) in inflamed ethmoid sinus mucosa from CRSwNP ($n=4$) when compared with those from CRSsNP ($n=3$), without using gating for CD45⁺, a marker of all lymphoid cells. Miljkovic *et al.* [45] also reported the increased prevalence of ILC2s (CD45⁺Lin⁻CD127⁺CRTH2⁺CD161⁺ cells, described as percent of CD45⁺ cells) in nasal polyps of CRSwNP ($n=16$) when compared with sinus mucosa of control patients ($n=7$), and it was positively correlated with allergic patients and was negatively correlated with previous surgeries. Walford *et al.* [46] reported the increased prevalence of ILC2s (CD45⁺Lin⁻CRTH2⁺ cells, described as % of CD45⁺ cells) in eosinophilic nasal polyps ($n=8$) when compared with noneosinophilic nasal polyps ($n=10$) or sinus mucosa of CRSsNP ($n=7$). Treatment with systemic corticosteroid reduced the ILC2s in eosinophilic nasal polyps by 50% in CRSwNP patients. Dexamethasone reduced Th2 cytokine production and proliferation of ILC2s in lungs of fungal allergen-challenged mice and induced apoptosis of ILC2s *in vivo* and *in vitro* [46]. Ho *et al.* [47] defined ECRS as greater than 10 eosinophils in nasal polyp/HPF, and the prevalence of ILC2s (CD45⁺Lin⁻CD127⁺CD4⁻CD8⁻CRTH2⁺CD161⁺ cells, described as % of CD45⁺ cells) in nasal polyps was significantly

elevated in ECRS patients ($n=13$) when compared with non-ECRS patients ($n=13$). In addition, the prevalence of ILC2s in nasal polyps was positively correlated with tissue and blood eosinophilia, allergic status, asthma status, and nasal symptom scores [47]. We examined the prevalence of ILC2s (CD45⁺Lin⁻CD127⁺CRTH2⁺ cells, described as % of CD45⁺Lin⁻ cells) in nasal polyps from ECRS and non-ECRS patients according to the JESREC study. The prevalence of ILC2s was significantly increased in ECRS patients ($n=16$) compared with non-ECRS patients ($n=18$), and it was positively correlated with the number of infiltrating eosinophils [48]. Poposki *et al.* [12] revealed that ILC2s (CD45⁺Lin⁻CD127⁺CRTH2⁺CD161⁺ cells) in nasal polyps, but not ILC2s in blood, spontaneously released IL-5 and IL-13 without additional stimulation, indicating that ILC2s in nasal mucosa are already activated in CRSwNP. Additionally, the prevalence of ILC2s is positively correlated with tissue concentration of IL-5 and IL-13 in nasal polyps [12]. Taken together, ILC2s may play an important role in the development of Th2-type eosinophilic inflammation in nasal polyps. However, the gating strategy of ILC2s is slightly different in each experiment, and some markers such as CD123 and FcER1 α were not included, but were used in the original article by Mjösberg. It is possible that a small amount of Th2 effector cell types such as basophils, mast cells, and eosinophils are mixed into the ILC2 populations in some experiments.

ILC2s might be directly recruited into the nasal mucosa through peripheral blood circulation [43]. However, it has been reported that ILCs are tissue-sessile cells, with limited migration of hematogenic ILCs into tissue [49], and that tissue-resident ILC2s would proliferate *in situ* during inflammatory responses [40[■]]. Bal *et al.* [11[■]] reported that ILC2s have the potential to transdifferentiate into ILC1s when exposed to a Th1 inflammatory environment. Nasal polyp-derived ILC2s in the presence of IL-12 produced IFN- γ rather than IL-5 in response to IL-33 and TSLP. Such conversion of tissue-resident ILC2s into ILC1s was reversed by IL-4. Eosinophils were an important source of IL-4, which was enriched in nasal polyps. They also showed that ILC2s colocalized with the accumulated eosinophils in nasal polyps by histological analysis [11[■]]. These results indicate that the composition of the ILC subset might change under the influence of an altered microenvironment, cross-talk between ILC2s and eosinophils in nasal polyps might be critical for the amplification of Th2-type inflammation, and ILC2 phenotype in nasal polyps might be maintained by IL-4.

Aspirin-exacerbated respiratory disease (AERD) is a Th2-immune-mediated respiratory disease associated with nasal polyps and asthma and is defined

by hypersensitivity reactions from cyclooxygenase-1 (COX-1) inhibition [50]. The prevalence of ILC2s was significantly increased in nasal mucosa and was decreased in peripheral blood at the time of COX-1 inhibitor reactions in patients with AERD, concurrently with significant increases in urinary PGD₂ metabolite and LTE₄ levels. The prevalence of ILC2s in nasal mucosa was positively correlated with reaction severity [51[■]]. These results suggest that ILC2s may be recruited from peripheral blood to the nasal mucosa during COX-1 inhibitor-induced reactions of AERD with enhanced production of PGD₂ and cysLTs.

ROLE OF GROUP 2 INNATE LYMPHOID CELLS IN NASAL POLYPS

There is increasing evidence that epithelium-derived cytokines, IL-33, IL-25, and TSLP, are key regulators of Th2-type inflammation and activate ILC2s through their receptors, ST2, IL-17 receptor B, and TSLPR (TSLP receptor) [43,52,53]. We have previously reported that mRNA of IL-25 and TSLP, and the protein concentration of IL-33 and IL-25, were significantly increased in nasal polyps of ECRS patients when compared with those of non-ECRS patients or uncinate tissues of control patients. Immunohistological analysis revealed that tissue expression of IL-33, IL-25, and TSLP were all increased in nasal epithelial cells of ECRS patients. The mRNA expression of TSLP and IL-25 was positively correlated with disease severity evaluated by CT scores in ECRS patients, and the protein concentration of IL-33 was positively correlated with the number of infiltrating eosinophils in nasal polyps [54]. These positive correlations between IL-33/IL-25/TSLP and eosinophilic inflammation in nasal polyps agreed with the results of previous experiments [55–58]. These epithelium-derived cytokines are released from cultured nasal epithelial cells in response to stimulation with house dust mite, *Alternaria*, protease from *Staphylococcus aureus*, trypsin, and papain [44,48,54,59]. We confirmed a significant increase in the secretion of IL-33, IL-25, and TSLP from cultured nasal epithelial cells of ECRS patients when compared with those of non-ECRS patients or control patients [54]. These results indicate that IL-33, IL-25, and TSLP are upregulated in nasal polyps of ECRS patients, and are involved in the development of the eosinophilic inflammation by stimulating ILC2s to release large amounts of Th2 cytokines (Fig. 1).

CysLTs and PGD₂ are well known lipid mediators of allergic diseases, and are potent stimulators of ILC2s [31[■],32]. They are mainly released from mast cells [60,61], and their functional receptors, CysLT1

and CRTH2, are both expressed by human ILC2s [31[■],32]. Tissue concentrations of cysLTs and PGD₂ were elevated in eosinophilic nasal polyps when compared with nasal tissues from CRSsNP patients or healthy controls [62–64]. Expression of PGD₂ synthase was also increased in nasal polyps and was positively correlated with eosinophil infiltration [65]. These results indicate that increased cysLTs and PGD₂ in nasal polyps of ECRS patients stimulate ILC2s to produce Th2 cytokines and are also important for the development of eosinophilic inflammation (Fig. 1).

ILC2s express the receptor for IL-1β [19], and IL-1β directly induces the production of Th2 cytokines from human ILC2s [11[■],26]. IL-1β belongs to the IL-1 family and is critical for host defense responses to infection and injury [66,67]. IL-1β can be produced by monocytes, macrophages, dendritic cells, B cells, and NK cells [66], and its concentration was elevated in eosinophilic nasal polyps [68–70]. However, the role of IL-1β in the pathophysiology of ECRS is poorly understood, because IL-1β induced the transdifferentiation of ILC2s into the ILC1-like phenotype, and would be a negative regulator of ILC2s [26]. IL-12, TGF-β, and IFN-γ are other potential negative regulators of ILC2s, although the concentrations of these cytokines are not elevated in nasal polyps [4,12].

ILC2s contribute to the Th2-biased inflammation in eosinophilic nasal polyps. The prevalence of Th2 cells was also elevated in nasal polyps from eosinophilic CRSwNP patients when compared with non-eosinophilic CRSwNP patients [71]. High amounts of Th2 cytokines such as IL-4, IL-5, and IL-13 were found in eosinophilic nasal polyps, at both the mRNA and protein levels [13,72–76]. IL-4 and IL-13 are key cytokines that control mucus production in epithelial cells, IgE responses in B cells and plasma cells, activation of macrophages, and tissue remodeling [77]. IL-5 is important in the chemotaxis, activation, and survival of eosinophils [78,79]. Recent studies showed that eosinophils in full activation status induce inflammation, tissue damage, and microbial killing, whereas eosinophils in partial activation status contribute to immune regulation and tissue repair [80]. Eosinophil–epithelial cell interactions play a crucial role in the initiation and development of eosinophilic inflammation. We previously demonstrated that coculture of human airway epithelial [NCI-H292 (human pulmonary mucoepidermoid carcinoma cell line)] cells and eosinophilic human eosinophil cell line-1 cells or peripheral eosinophils stimulated secretion of the mucin 5AC mucin, IL-8, and profibrotic cytokines such as platelet-derived growth factor and vascular endothelial growth factor, from epithelial cells, leading to goblet cell metaplasia

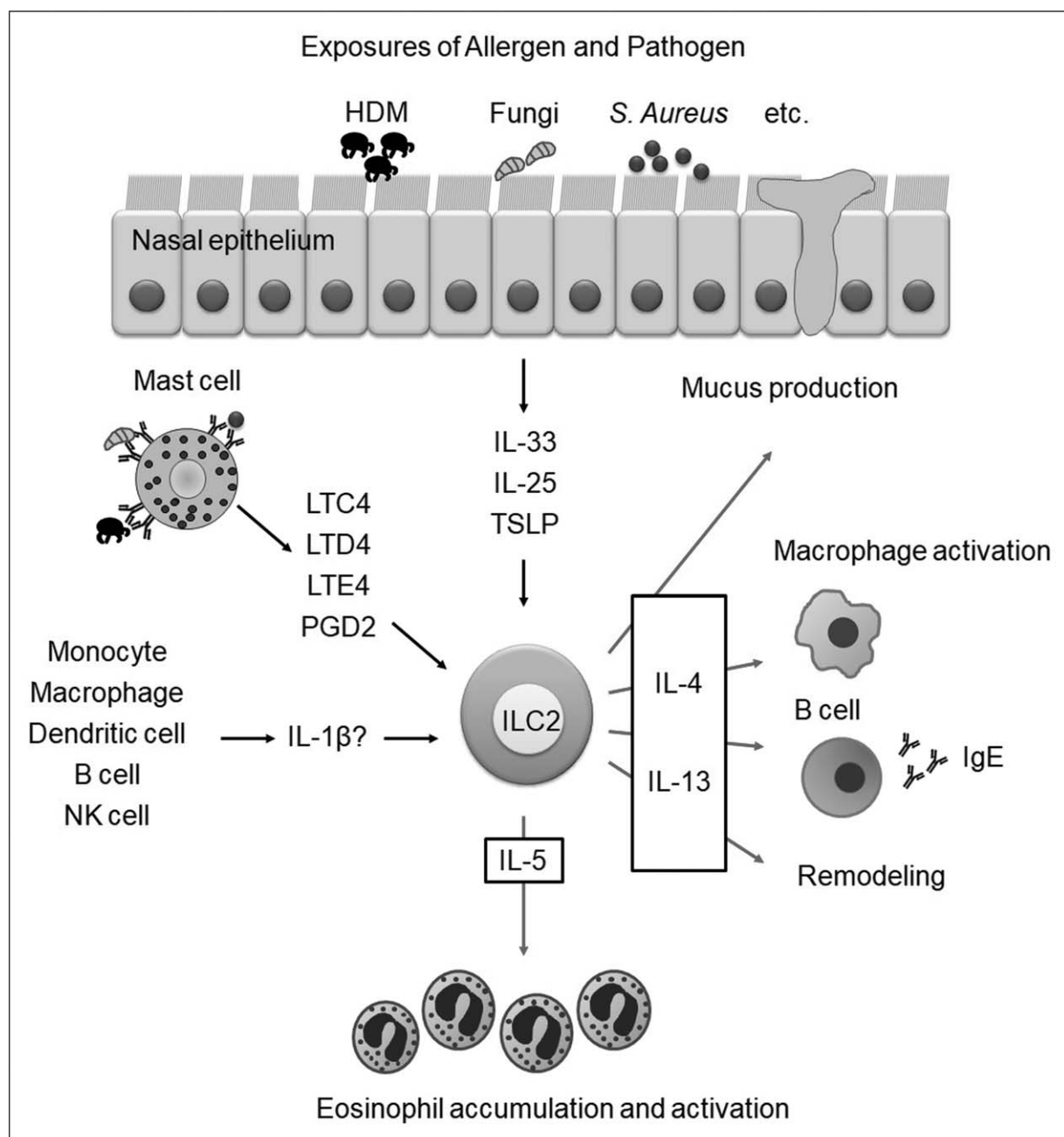


FIGURE 1. Role of ILC2s in Th2-type eosinophilic inflammation in nasal mucosa of ECRS patients. Epithelium-derived cytokines (IL-33, IL-25, and TSLP), and mast cell-derived cysLTs (LTC₄, LTD₄, and LTE₄) and PGD₂ stimulate the production of Th2 cytokines (IL-4, IL-5, IL-13, etc.) from ILC2s, leading to eosinophilic inflammation, IgE production, mucus production, and tissue remodeling. cysLTs, Cysteinyl leukotrienes; ECRS, eosinophilic chronic rhinosinusitis; HDM, house dust mite; ILC2s, group 2 innate lymphoid cells; LTC₄, leukotriene C₄; LTD₄, leukotriene D₄; LTE₄, leukotriene E₄; PGD₂, prostaglandin D₂; *S. aureus*, *Staphylococcus aureus*; TSLP, thymic stromal lymphopoietin.

and tissue remodeling [81]. Clinical effects of antihuman IL-4 receptor α subunit monoclonal antibody (dupilumab) and antihuman IL-5 monoclonal antibodies (mepolizumab and reslizumab) have been examined for severe cases of CRSwNP. Dupilumab improved the clinical symptoms of CRSwNP patients evaluated by nasal symptom scores and polyp scores through the inhibition of IL-4 and IL-13 signaling

[82]. Mepolizumab and reslizumab also improved clinical symptoms by reducing polyp size in 50–60% of CRSwNP patients [83,84]. These results suggest that Th2 cytokines released by ILC2s play pivotal roles in the pathophysiology of ECRS (Fig. 1) and that blocking these pathways provides a potential therapeutic target for the treatment of refractory and/or recurrent cases of ECRS.

PERIPHERAL BLOOD GROUP 2 INNATE LYMPHOID CELLS AND EOSINOPHILIC CHRONIC RHINOSINUSITIS

ILC2s can be recruited to the nasal mucosa through peripheral blood circulation. We previously examined the prevalence of blood ILC2s in CRS patients with different conditions, including asthma and allergic rhinitis. The prevalence of blood ILC2s was not increased in CRS patients when compared with control patients, and it was not different between patients with ECRS and non-ECRS. The percentage of blood eosinophils was significantly higher in ECRS patients when compared with non-ECRS patients; however, the prevalence of blood ILC2s was significantly lower in patients with higher blood eosinophilia and in those with asthma. On the other hand, it was significantly higher in patients with allergic rhinitis and with higher serum IgE levels [48].

The numbers of blood ILC2s in allergic asthma patients were reported to be greater than the numbers in allergic rhinitis patients and healthy controls [85]. Smith *et al.* [86] reported that significantly greater numbers of Th2 cytokine-producing ILC2s were detected in the blood of patients with severe asthma when compared with mild asthmatics, despite high-dose oral corticosteroid therapy. Jia *et al.* [87] reported that the prevalence of IL-13⁺ILC2s was significantly higher in uncontrolled asthma patients when compared with partly controlled patients and that effective treatment of uncontrolled patients resulted in a marked decrease in blood IL-13⁺ILC2 when their symptoms were well controlled. The prevalence of blood ILC2s was also increased during the pollen season in patients with grass pollen allergic rhinitis, and this increase was inhibited by subcutaneous grass pollen immunotherapy [88]. Increased prevalence of blood ILC2s was demonstrated in allergic rhinitis patients after nasal cat allergen exposure [89]. These results suggest that the increased prevalence of blood ILC2s may reflect type 1 allergic immune responses and severity of asthma status, although it is not increased in ECRS patients.

CONCLUSION

Growing evidence suggests a critical role for ILC2s in the pathophysiology of ECRS by producing large amounts of Th2 cytokines. Although the regulation of ILC2s appears to be very complex, numerous studies have suggested that epithelium-derived cytokines such as IL-33, IL-25, and TSLP and eicosanoids such as cysLTs and PGD₂ are important for the activation of ILC2s. Regulation of ILC2s is a novel therapeutic approach for refractory and/or recurrent cases of ECRS.

Acknowledgements

None.

Financial support and sponsorship

The work was supported by the Japan Society for the Promotion of Science (JSPS; grant number JP26861371).

Conflicts of interest

There are no conflicts of interest.

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