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Group 2 Innate Lymphoid Cells in Airway Diseases

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Group 2 innate lymphoid cells (ILC2s) are increasingly recognized as a key controller of type 2 inflammation, and are well known to be highly elevated in human airway type 2 inflammatory diseases including allergic rhinitis, chronic rhinosinusitis with nasal polyps, and asthma. ILC2-mediated production of type 2 cytokines initiates and amplifies airway inflammation via activation of eosinophils, B cells, mast cells, macrophages, fibroblasts, and epithelial cells in these diseases. ILC2s require at least three major signals to fully activate and robustly produce type 2 cytokines. IL-1 family cytokines (IL-1β, IL-18, IL-33), IL-25, and TNF superfamilies (TNF, TL1A, GITR-L, RANK-L) activate the NF-κB and AP-1 pathways that initiate production of IL-5 and IL-13. Lipid mediators (LTC_4, LTD_4, PGD_2) and neuropeptide NMU promote production of IL-4 through the NFAT pathway. IL-2 and IL-7 family cytokines (IL-2, IL-7, IL-9, TSLP) activate the STAT5 pathway that induces survival of ILC2s and enhances cytokine production. The activation of STAT5 is necessary to potently induce cytokine- and lipid mediator-mediated production of type 2 cytokines. Inhibitory pathways for ILC2s have also become clearer. Type I and II interferons and IL-27 inhibit ILC2 functions through the activation of STAT1. Suppression mediated via β_2-adrenergic receptor agonists, PGE_2, and PGI_2 occurs through cAMP and PKA. Glucocorticoid, testosterone, IL-10, and TGF-β are also able to inhibit ILC2-mediated production of type 2 cytokines. Blockage of ILC2 activators, activation of inhibitory pathways of ILC2s, and suppression of ILC2-mediated pathways including type 2 cytokines (IL-5, IL-13, IL-4Ra) may become therapeutic strategies for airway type 2 inflammatory diseases.

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Innate Lymphoid Cells

Innate lymphoid cells (ILCs) are increasingly recognized as a key component of both innate immunity, to protect from pathogens, and pathological processes for many diseases. ILCs are classified into three major subsets: group 1 ILCs (ILC1s), ILC2s and ILC3s, and each ILC group has several

ABBREVIATIONS: ADRB2 = β_2-adrenergic receptor; AR = androgen receptor; AREG = amphiregulin; CGRP = calcitonin gene-related peptide; CRS = chronic rhinosinusitis; CRSsNP = CRS without nasal polyps; CRSwNP = CRS with nasal polyps; CysLT1R = cysteinyl leukotriene receptor 1; γ_c = IL-2R common γ chain; GITR = glucocorticoid-induced TNFR-related protein; ICOS = inducible T-cell costimulator; IFN = interferon; ILC = innate lymphoid cell; ILC2 = group 2 ILC; LT = leukotriene; MAPK = mitogen-activated protein kinase; NMU = neuromedin U; NP = nasal polyp; PG = prostaglandin; RANK = receptor activator of NF-κB; RANK-L = RANK ligand; SCC = solitary chemosensory cell; TL1A = TNF-like cytokine 1A; TNFRSF = TNF receptor superfamily; TNFSF = TNF superfamily; TSLP = thymic stromal lymphopoietin; VIP = vasoactive intestinal peptide

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subsets.[1-3] All ILCs develop from common innate lymphoid progenitors, which are differentiated from the common lymphoid progenitors.[2] Although ILCs lack antigen receptors (T-cell receptor [TCR], B-cell receptor [BCR]), ILCs produce high levels of helper T-cell cytokines via antigen-independent stimuli including cytokines and lipid mediators. ILC1s are characterized by the expression of T-bet and production of the helper T type 1 (Th1) cytokine interferon (IFN)-γ. ILC2s express GATA3 and produce Th2 cytokines including IL-5 and IL-13. ILC3s are characterized by the expression of RORγt (retinoic acid receptor-related orphan nuclear receptor γ, thymus-specific isoform) and the production of type 3 (Th17) cytokines including IL-17A and IL-22. Therefore, ILCs are recognized as the innate counterpart of T lymphocytes in that ILC1s, ILC2s, and ILC3s mirror $CD4^+$ Th1 cells, Th2 cells, and Th17 cells, respectively.

ILC2s

The presence of ILC2s was initially implicated in mice by showing that IL-25 and IL-33 induce type 2 cytokines in RAG-deficient mice.[4,5] These studies suggested that non-B/non-T cells are responsible for IL-25- and IL-33-mediated production of type 2 cytokines. In 2010, four independent groups characterized type 2 cytokine-producing non-B/non-T cells in mice, and these cells are now categorized as a single population termed ILC2s by nomenclature.[6] Human ILC2s are currently recognized as $CD45^+$ lymphocytes that are Lineage (CD1a, CD3, CD4, CD16, CD19, CD34, CD94, CD303, FcεRI) negative $CD127^+CD161^+CRTH2^+$.[1,7]

ILC2-mediated inflammation is mainly via the production of type 2 cytokines including IL-4, IL-5, IL-9, and IL-13 (Fig 1).[1,8-10] IL-5 primarily induces eosinophilia. IL-4 and IL-13 are key factors that control IgE responses in B cells, mucus production and remodeling in epithelial cells, induction of eosinophil chemoattractants including eotaxin-3 (CCL26) in epithelial cells and fibroblasts, and the induction of M2 macrophages. IL-9 promotes mast cell growth and goblet cell metaplasia. ILC2s not only produce type 2 cytokines but also release many other factors including amphiregulin (AREG), which controls fibrosis and tissue repair and remodeling.[1,8] Therefore, accumulation and activation of ILC2s is now considered a key event for many type 2 inflammatory diseases (Fig 1).

Activation of ILC2s by Cytokines

ILC2s express a wide variety of cytokine receptors on the cell surface. IL-25 and IL-33 were initially identified as major type 2 cytokine inducers in ILC2s, and receptors for IL-25 (IL-17RA and IL-17RB [IL-25R]) and IL-33 (IL-1RL1 [IL-33R] and IL-1RAP) are highly expressed on ILC2s. IL-25 is an IL-17 family cytokine that is released from epithelium, especially from a unique population of epithelial cells called tuft cells in the gut and solitary chemosensory cells (SCCs) and brush cells in the airway.[11-13] IL-33 is released from epithelial cells and other cells including fibroblasts and endothelial cells.[8] IL-25 and IL-33 activate AP-1 and nuclear factor (NF)-κB pathways via mitogen-activated protein kinases (MAPKs) and TRAF6, respectively.[14-16] In addition to IL-33, receptors for other IL-1 family cytokines, IL-1R1 and IL-18R, are expressed on ILC2s and IL-1β and IL-18 induce IL-5 and IL-13 in ILC2s.[17-20]

Members of the TNF superfamily (TNFSF) also provide strong signals in ILC2s. TNF-like cytokine 1A (TL1A [TNFSF15]) induces production of IL-5 and IL-13 in ILC2s via death receptor 3 (DR3 [TNF receptor superfamily 25; TNFRSF25]).[21,22] Nagashima et al[23] found the expression of glucocorticoid-induced TNFR-related protein (GITR [TNFRSF18]) on ILC2s and an agonistic antibody against GITR enhanced IL-33-mediated induction of IL-5 and IL-13 in human ILC2s. Since many TNFRSFs, including DR3 and GITR, share the NF-κB and MAPK signal pathways, our group[24] recently screened TNFRSFs on ILC2s and ligands (TNFSFs) that activate human ILC2s. We discovered that TNF receptor II (TNFRII [TNFRSF1B]) and receptor activator of NF-κB (RANK [TNFRSF11A]) are expressed on human ILC2s; and that their ligands, TNF and RANK ligand (RANK-L [TNFSF11]), induce production of type 2 cytokines in human ILC2s. These results suggest that IL-25, IL-1 family cytokines, and TNFSFs are major activators and inducers of type 2 cytokines in ILC2s, mainly via NF-κB and AP-1 (MAPK) pathways (Fig 2).

Although administration of IL-25 or IL-33 induces potent type 2 inflammation in mice in vivo, IL-25 or IL-33 alone is a weak inducer of IL-5 and IL-13 in ILC2s in vitro.[4,5,25-27] This suggests that these cytokines are important initiators of ILC2-mediated type 2 inflammation; however, similar to T lymphocytes, ILC2s may require costimulatory signals that fully activate ILC2s. ILC2s also express receptors for IL-2 and IL-7 family cytokines including IL-2 (IL-2Rα, IL-2Rβ, and IL-2R common γ [γ_c]), IL-7 (IL-7Rα and γ_c), IL-9

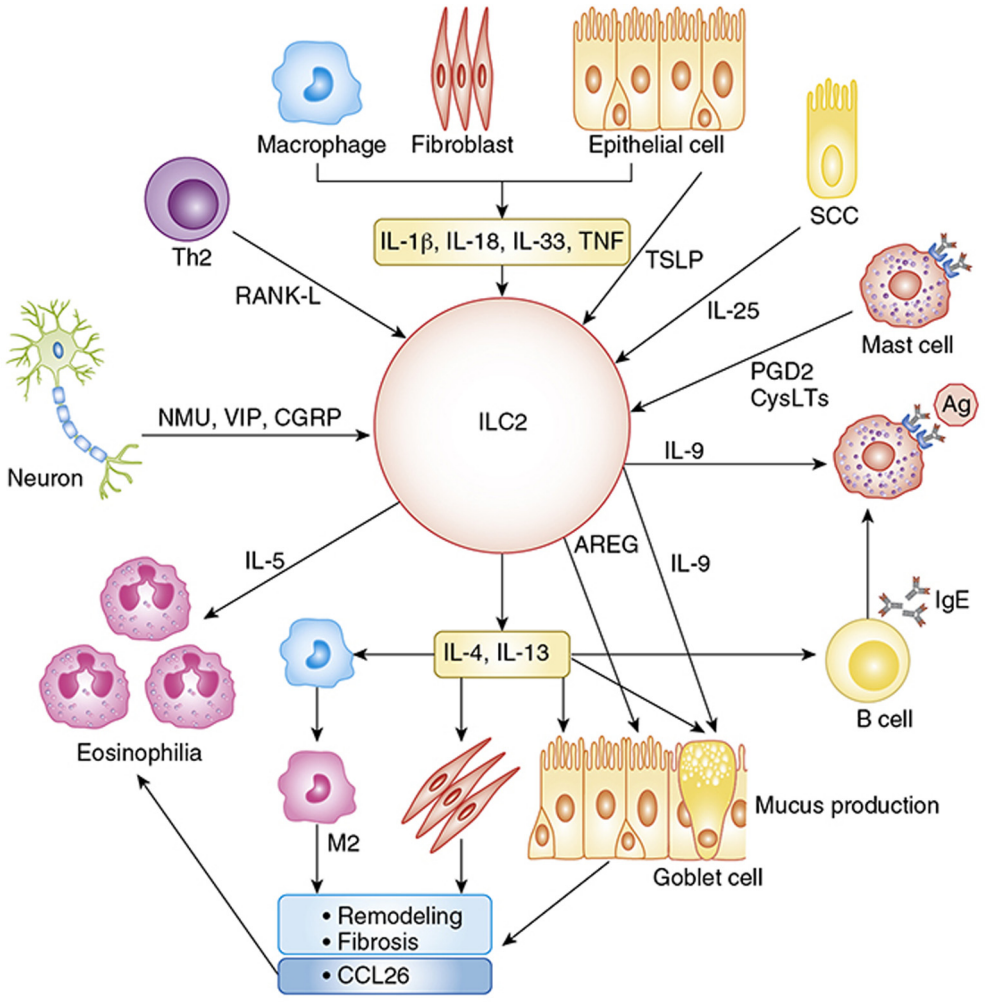

Figure 1 – *Group 2 innate lymphoid cell (ILC2s) contribute to airway inflammation via the production of type 2 cytokines and amphiregulin. ILC2s produce type 2 cytokines in response to stimulation with several cytokines such as TNF and IL-1 family cytokines from epithelial cells, fibroblasts, and macrophages; TSLP from epithelial cells; IL-25 from SCCs and RANK-L from Th2 cells; lipid mediators from mast cells; and neuropeptides from neurons. IL-5 promotes eosinophilia and IL-9 induces goblet cell metaplasia and mast cell growth. AREG controls fibrosis and remodeling in epithelial cells. IL-4 and IL-13 activate macrophages, B cells, fibroblasts, epithelial cells, and goblet cells to induce eosinophil recruitment, mucus production, remodeling, fibrosis, and IgE-mediated reactions. Ag = antigen; AREG = amphiregulin; CGRP = calcitonin gene-related peptide; CysLTs = cysteinyl leukotrienes; M2 = M2 macrophage; NMU = neuromedin U; PGD_2 = prostaglandin D_2; RANK-L = receptor activator of NF-κB ligand; SCC = solitary chemosensory cell; Th2 = helper T type 2; TNF = tumor necrosis factor; TSLP = thymic stromal lymphopoietin; VIP = vasoactive intestinal peptide.*

(IL-9R and γ_c), and thymic stromal lymphopoietin (TSLP) (IL-7Rα and CRLF2 [TSLPR]).[8] IL-2, IL-7, IL-9, and TSLP activate ILC2s through similar signaling pathways including STAT1, STAT3, and STAT5, and the activation of STAT5 is considered a key pathway that promotes ILC2 functions.[8] Although these cytokines alone do not induce full activation, they are able to enhance IL-1β-, IL-18-, IL-25-, IL-33-, TNF-, and RANK-L-mediated production of type 2 cytokines in ILC2s.[17-19,24,26] This suggests that IL-2, IL-7, IL-9, and TSLP act as costimulators and enhancers for ILC2-mediated type 2 inflammation via activation of STAT5 (Fig 2).

Activation of ILC2s by Lipid Mediators

Lipid mediators including leukotrienes (LTs) and prostaglandins (PGs) play important roles in homeostasis and inflammation. Importantly, CRTH2, a receptor for PGD_2, and cysteinyl leukotriene receptor 1 (CysLT1R), a receptor for LTC_4 and LTD_4, are highly expressed on ILC2s and their ligands (PGD_2, LTC_4, and LTD_4) induce ILC2 migration and production of type 2 cytokines including IL-4, IL-5, and IL-13 in human ILC2s.[28-33] Activation of CRTH2 and CysLT1R increases cytosolic Ca^{2+} levels and activate the NFAT pathway. Interestingly, unlike cytokines such as IL-33, PGD_2 and

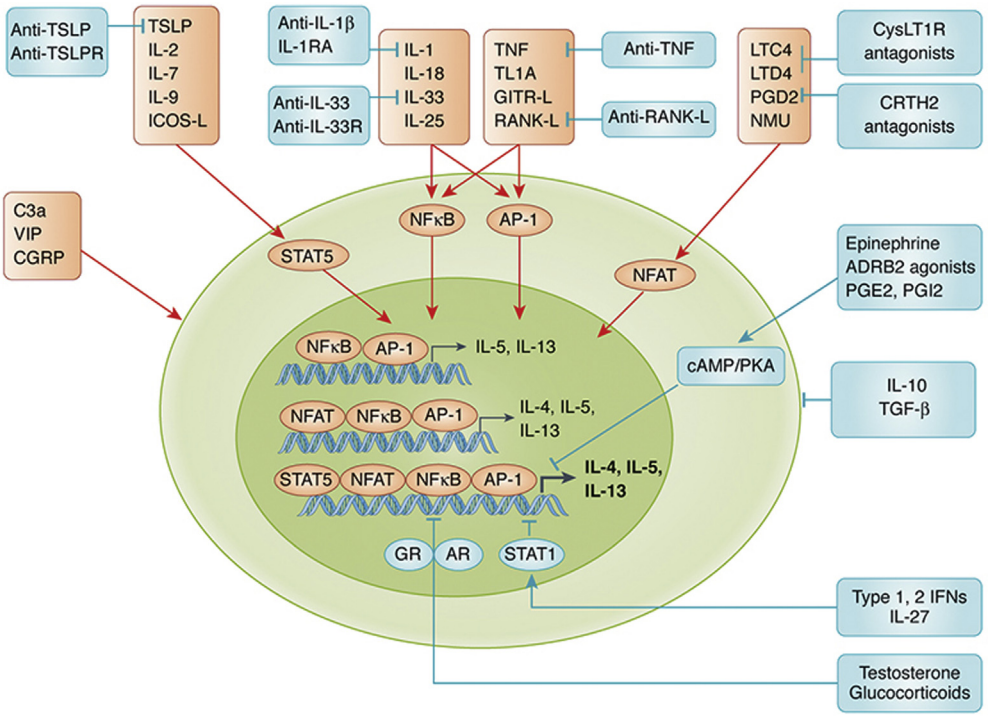

Figure 2 – *Schematic of activation and inhibition pathways for ILC2s. IL-1 family cytokines, IL-25, and TNFSFs weakly induce IL-5 and IL-13 via activation of NF-κB and AP-1. Activation of NFAT by lipid mediators and NMU is required for induction of IL-4. IL-2 and IL-7 family cytokines and ICOS-L act as costimulators and potently enhance cytokine- and lipid mediator-mediated induction of IL-4, IL-5, and IL-13 via the activation of STAT5 in ILC2s. Although C3a, VIP, and CGRP activate ILC2s, at least in mice, their signaling pathways in ILC2s have not been elucidated. Testosterone and glucocorticoids suppress NF-κB-mediated induction of type 2 cytokines via activation of nuclear receptors, AR and GR, respectively. IFN- and IL-27-mediated inhibition of ILC2s occurs via the STAT1 pathway. The activation of cAMP and PKA is involved in ADRB2 agonist-, PGE_2-, and PGI_2-mediated inhibition. IL-10 and TGF-β also suppress functions of ILC2s. Furthermore, monoclonal antibodies and receptor antagonists against activation pathways of ILC2s are commercially available or in clinical trials. ADRB2 = β_2-adrenergic receptor; AR = androgen receptor; cAMP = cyclic AMP; CRTH2 = chemoattractant receptor-homologous molecule expressed on Th2 cells; CysLT1R = cysteinyl leukotriene receptor 1; GITR-L = glucocorticoid-induced TNFR-related protein ligand; GR = glucocorticoid receptor; ICOS-L = inducible T-cell costimulatory ligand; IFN = interferon; IL-1RA = IL-1 receptor antagonist; IL-33R = IL-33 receptor; LTC_4, LTD_2 = leukotrienes C_4 and D_2; NF-κB = nuclear factor-κB; NFAT = nuclear factor of activated T cells; PGD_2, PGE_2, PGI_2 = prostaglandins D_2, E_2, and I_2; PKA = protein kinase A; STAT1, STAT5 = signal transducer and activator of transcription 1 and 5; TL1A = TNF-like cytokine 1A; TNFSF = TNF superfamily; TSLPR = thymic stromal lymphopoietin receptor. See Figure 1 legend for expansion of other abbreviations.*

CysLTs potently induce production of IL-4 in ILC2s.[28,29] This indicates that activation of NFAT may be necessary to induce ILC2-mediated production of IL-4. In Th2 cells, engagement of TCR activates three major transcription factors (NFAT, NF-κB, and AP-1) to drive profound production of IL-4, IL-5, and IL-13.[32] Based on these findings, von Moltke et al[32] proposed that ILC2s also require these three transcription factors and that a combination of cytokines (that activate NF-κB and AP-1) and lipid mediators (that activate NFAT) mimics TCR signaling by inducing all type 2 cytokines including IL-4 in ILC2s (Fig 2).

Activation of ILC2s by Other Factors

In addition to cytokines and lipid mediators, inducible T-cell costimulator (ICOS) and receptors for complement C3a and neuropeptides including neuromedin U (NMU), vasoactive intestinal peptide (VIP), and calcitonin gene-related peptide (CGRP) were found on ILC2s.[34-39] C3a, NMU, and VIP directly induce production of type 2 cytokines in ILC2s, and at least NMU-mediated responses are via MAPK and NFAT pathways in mice.[36-38] Although CGRP alone does not, it significantly enhances the production of IL-5 in the presence of IL-25 or IL-33 in mouse ILC2s.[39] ICOS ligand induces the cell survival of ILC2s via the activation of STAT5.[34] These results suggest that C3a, NMU, and VIP are direct activators and that ICOS ligand and CGRP may play a costimulatory role in ILC2-mediated type 2 inflammation. However, the role of complement and neuropeptides has not been investigated in human ILC2s and will require further studies.

Plasticity and Heterogeneity of ILC2s

ILCs are known to display plasticity and heterogeneity.[1,3] Although IL-1β and IL-18 induce production of type 2 cytokines in ILC2s, IL-1β plus

IL-12 or IL-18 plus IL-12 have the capacity to transdifferentiate human ILC2s into IFN-γ-producing ILC1s.[17,18,40] Furthermore, notch, CysLTs, and a combination of IL-1β, IL-23, and transforming growth factor (TGF)-β seem to convert ILC2s into an IL-17-producing ILC3-like phenotype.[3,41,42]

Recently, Ricardo-Gonzalez et al[19] performed single-cell RNA sequencing on ILC2s sorted from bone marrow, lung, fat, gut, and skin of mice and found that expression of IL-18R1, IL-25R, and IL-33R in ILC2s was enriched in particular tissues although other common ILC2 markers such as GATA3, IL-7Rα, and TSLPR were expressed in the majority of ILC2s across tissues. They found that IL-33R is abundant on bone marrow, lung, and fat ILC2s; IL-25R is on gut ILC2s; and IL-18R1 is highly expressed on skin ILC2s.[19] However, whether ILC2s express distinct activating receptors in human tissue is not known and future study is required.

Presence of ILC2s in Airway Inflammatory Diseases

Animal studies have revealed the importance of ILC2s in airway type 2 inflammatory diseases.[3,9,10] Many groups also have identified accumulation and activation of ILC2s in human airway diseases. We summarize here the presence of ILC2s and their activators in three human airway type 2 inflammatory diseases.

ILC2s in Allergic Rhinitis

Allergic rhinitis is characterized by IgE-mediated type I hypersensitivity with elevation of Th2 cells and type 2 cytokines in nasal mucosa, and is one of the most common allergic diseases in the world.[43] ILC2s in peripheral blood and nasal curettage samples are increased after nasal allergen challenge in patients with allergic rhinitis, and ILC2 numbers in these nasal curettage samples positively correlate with eosinophil numbers and IL-5 concentrations.[44,45] Peripheral blood ILC2s are increased during pollen season in patients with seasonal allergic rhinitis.[46,47] However, patients with seasonal allergic rhinitis who received allergen-specific immunotherapy did not show seasonal elevation of blood ILC2s.[46] These results indicate that ILC2s are elevated locally and systemically in patients with allergic rhinitis and that the accumulation of ILC2s is controlled at least in part by allergic reactions.

During IgE-mediated reactions, mast cells quickly release lipid mediators including LTs and PGD_2 that are elevated in the nasal mucosa of patients with allergic rhinitis.[43,45,48] These lipid mediators may play an initial role in recruitment and activation of ILC2s in allergic rhinitis. In the case of epithelial-derived cytokines, IL-33 is detected in the nasal mucosa but it is still not clear whether IL-33 is elevated in allergic rhinitis.[48] Limited studies have found that IL-25 is elevated in allergic rhinitis.[49] Many groups have found that TSLP is significantly elevated in allergic rhinitis.[48-50] These results suggest that lipid mediators, IL-25, and TSLP may play critical roles in ILC2-mediated type 2 inflammation in allergic rhinitis.

ILC2s in Chronic Rhinosinusitis With Nasal Polyps

Chronic rhinosinusitis (CRS) is a common chronic inflammatory disease of the human upper airway and sinuses, and is frequently divided into two main phenotypes: CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP). In Western countries, CRSwNP is well known to be characterized by type 2 inflammation with eosinophilia and the presence of high levels of type 2 cytokines such as IL-5 and IL-13.[51,52] Nasal polyps (NPs) are one of the first tissues in which human ILC2s were discovered, and many groups have reported the elevation of ILC2s in NPs.[7,35,52-55] Our group has characterized the presence of all major ILC subsets in CRS and found that ILC2s are the predominant ILCs in NPs and are significantly elevated in NPs compared with sinus mucosa of control subjects and patients with CRSsNP.[35] In Asia, about 50% of patients with CRSwNP presented noneosinophilic inflammation in NPs.[52] Tojima et al[55] found that ILC2s are elevated only in eosinophilic NPs in Japan. In contrast to allergic rhinitis and asthma, blood ILC2s do not seem to be elevated in patients with CRSwNP.[35,55] These results suggest that ILC2s may play a role in type 2 inflammation in eosinophilic CRSwNP, but ILC2 activation may be restricted to the nasal mucosa of these patients.

Although our group also found that ILC2s are not only elevated but also activated and releasing type 2 cytokines in NPs in vivo, factors that activate ILC2s in NPs were not clear.[35] Our group[56] recently examined the presence of IL-25, IL-33, and TSLP in CRSwNP in Chicago and reviewed these cytokines in worldwide published CRS studies. We found that IL-25 is almost undetectable, IL-33 is present but shows mixed results, and that only TSLP consistently shows significant elevation in NPs. However, TSLP alone does not induce type 2 cytokines in ILC2s. This suggests that IL-25- and IL-33-independent ILC2 activators may play a role in CRSwNP. Our group[24] recently screened other potential

ILC2 activators and found that RANK-L is significantly elevated in NPs. We also found that RANK-L is expressed on Th2 cells in NPs; and that NP Th2 cells enhance ILC2-mediated production of type 2 cytokines via the RANK-L-mediated pathway. In addition to RANK-L and TSLP, several groups found elevation of lipid mediators including LTC4 and PDG2 in NPs, especially in patients with CRSwNP who have intolerance to inhibitors of cyclooxygenase-1.[30,57] These reports suggest that RANK-L and lipid mediators may be key activators and that TSLP is a costimulator to control ILC2-mediated type 2 inflammation in CRSwNP.

Although IL-33 may not be elevated,[56] IL-33 is present in NPs and IL-33 can act in synergy with other elevated factors including lipid mediators and TSLP. This implies the possibility that IL-33 together with TSLP and/or lipid mediators may play a role in type 2 inflammation in CRSwNP. This possibility may also be true in allergic rhinitis. Furthermore, Kohanski et al[12] recently found that IL-25 is expressed in a minor subset of epithelial cells called SCCs and these cells are increased in NPs. This may suggest that IL-25 plays a role in the activation of ILC2s in a limited area of NPs. Further studies require examination of the direct role of IL-25 and IL-33 in CRSwNP and allergic rhinitis.

ILC2s in Asthma

Asthma is a heterogeneous lower airway inflammatory disease and is frequently divided into two phenotypes: type 2 high (eosinophilic) asthma and type 2 low (neutrophilic) asthma. Many groups have clearly shown that ILC2s are increased in blood and sputum in patients with asthma, especially in type 2 high severe eosinophilic asthma.[27,58-60] Smith et al[59] also found that IL-5$^+$IL-13$^+$ ILC2s are elevated in the sputum of patients with uncontrolled asthma. In the case of ILC2 activators, genome-wide association studies showed that IL-33, IL-33R, and TSLP are strongly associated with asthma.[61-63] Several groups showed that IL-25, IL-33, and TSLP are elevated in patients with asthma and that they are all increased in the bronchial epithelium after inhalational allergen challenge in subjects with atopic asthma.[64-68] In addition, lipid mediators including LTC_4, LTD_4, and PGD_2 are well known to be involved in asthma and leukotriene receptor antagonists are used to treat asthma. These results suggest that ILC2s are highly elevated and activated in patients with asthma and that epithelial-derived cytokines and lipid mediators may play a key role in ILC2-mediated type 2 inflammation.

Inhibition of ILC2 Activation

Since ILC2s are highly elevated and activated in type 2 airway inflammatory diseases, the inhibition of ILC2 mediators and activators could be a therapeutic option for these diseases. Several monoclonal antibodies against ILC2 mediators including IL-5 (mepolizumab, reslizumab), IL-5Rα (benralizumab), IL-13 (lebrikizumab, tralokinumab), and IL-4Ra (dupilumab) have been developed and some of them are already approved for treatment of type 2 airway inflammatory diseases.[69] Furthermore, monoclonal antibodies and small-molecule antagonists against activation pathways of ILC2s including TSLP (tezepelumab), TSLPR (MK-8226), IL-33 (etokimab, REGN3500), IL-33R (MSTT1041A), and CRTH2 (AZD1981, fevipiprant) are in clinical trials for many type 2 inflammatory diseases (not limited to the airway). Although they are not currently used to target type 2 inflammatory diseases, monoclonal antibodies against other ILC2 activators including IL-1β (canakinumab), TNF (infliximab, adalimumab), and RANK-L (denosumab) as well as an IL-1R antagonist (IL-1RA) protein are commercially available for treatment of other diseases including rheumatoid arthritis and psoriasis. These biologics may have benefit in a subset of patients with type 2 inflammatory disease who present with elevation of these cytokines.

Activation of inhibitory pathways for ILC2s could represent other therapeutic options. Currently, a subset of cytokines (IL-10, IL-27, TGF-β, IFN-α, IFN-β, and IFN-γ), lipid mediators (PGE_2 and PGI_2), norepinephrine, and glucocorticoids are known to suppress ILC2 activation (Fig 2).[70]

Initial studies identified that regulatory T cells suppress the function of ILC2s via production of IL-10 and TGF-β in mice.[71,72] Subsequently, our group found that IL-10RA and TGFBR2 are expressed on human ILC2s, and IL-10 and TGF-β are able to suppress IL-33 plus TSLP-mediated production of IL-4, IL-5, and IL-13 in human ILC2s.[73] We also found that IL-33-induced IL-9 is inhibited by IL-10 whereas TGF-β further enhanced production of IL-9.[73] This indicates that the induction of IL-9 may be differently controlled in ILC2s compared with IL-4, IL-5, and IL-13, although NF-κB is involved in expression of all type 2 cytokines.

Several groups found receptors for type I IFN (IFNAR), type II IFN (IFNGR1), and IL-27 (IL-27RA) on ILC2s; and showed that their ligands IFN-α, IFN-β, IFN-γ, and IL-27 inhibit proliferation and production of type 2

cytokines in ILC2s in mice.[74-76] Importantly, STAT1-deficient mice lose these functions by IFN-β, IFN-γ, or IL-27, indicating that the STAT1 pathway is a negative regulator of ILC2s.

Two lipid mediators are known to inhibit ILC2 function. Human ILC2s express receptors for PGE_2 (EP2 and EP4) and PGI_2 (IP); and PGE_2 and PGI_2 inhibit the proliferation and production of type 2 cytokines in ILC2s.[77,78] Since PGE_2 and PGI_2 both activate adenylate cyclase and cyclic AMP (cAMP)/protein kinase A (PKA) pathways, these pathways may be additional negative regulators of ILC2s.[8]

Moriyama et al[79] found that murine ILC2s express the β_2-adrenergic receptor (ADRB2) and that administration of an ADRB2 agonist, salmeterol, inhibits ILC2-mediated airway type 2 inflammation in mice. An endogenous ligand of ADRB2, epinephrine, activates adenylate cyclase and cAMP pathways, suggesting that inhibitory pathways involving ADRB2 may be similar to PGE_2 and PGI_2.

Inhibition of ILC2s by Hormone and Glucocorticoids

The prevalence of asthma is known to be higher in women than in men. Several groups found significant elevation of ILC2s in women compared with men in both people with asthma and mouse asthma models, suggesting that sex hormones may directly influence ILC2s in patients with asthma.[80,81] They also found that the androgen receptor (AR) is expressed on ILC2s; and that a ligand of AR, 5α-dihydrotestosterone, which is a downstream product of the male sex hormone testosterone, inhibits IL-33- and *Alternaria* extract-induced airway type 2 inflammation and ILC2-mediated production of type 2 cytokines in mice.[80,81] These results suggest that testosterone may be responsible for sex influences in patients with asthma by suppressing ILC2s.

Walford et al[54] reported that the frequency of ILC2s in NPs is reduced by treatment with systemic glucocorticoid. Our group[73] showed that dexamethasone strongly suppresses the production of IL-5 and IL-13 in NP-derived ILC2s ex vivo. These results suggest that treatment with glucocorticoids is a therapeutic option to directly inhibit ILC2-mediated inflammation in human diseases. However, several groups reported that ILC2s in patients with asthma are resistant to glucocorticoids and that TSLP abrogates the inhibitory activity of glucocorticoids in ILC2s in patients with severe asthma.[66,82] In contrast, our study clearly showed that dexamethasone strongly suppresses IL-33-mediated production of type 2 cytokines in human ILC2s even in the presence of TSLP.[73] Future studies are required to clarify why TSLP induces steroid resistance in ILC2s but only in a subset of tissues and/or diseases.

Conclusion

Increasing evidence suggests that ILC2s and their activators are highly accumulated in human airway type 2 inflammatory diseases including allergic rhinitis, CRSwNP, and asthma; and that ILC2s significantly contribute to the production of type 2 cytokines including IL-5 and IL-13 in these diseases. Although there is almost no ILC2-targeting therapy currently, except treatment with glucocorticoids, mechanisms of activation and inhibition of ILC2s have become clearer in the past decade. In the future, this area of research may lead to the development of novel therapies for these diseases aimed at suppressing ILC2-mediated type 2 inflammation via blockages of ILC2 activators and stimulation of inhibitory pathways.

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