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Lipid mediators as regulators of human ILC2 function in allergic diseases

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ABSTRACT

Group 2 innate lymphoid cells (ILC2) are specialized in type 2 immunity. ILC2 are activated early in immune responses and, despite their low abundance, are able to initiate and amplify allergic inflammation by orchestrating other type 2 immune cells. Based on recent discoveries, the spectrum of ILC2 regulating factors has been extended. It is now well established that not only epithelial cell-derived innate cytokines, but also bioactive lipids can regulate ILC2 activity and accumulation. Additionally, ILC2 appear to be susceptible to changes in the cytokine milieu and can acquire an ILC1-like phenotype due to a high degree of cellular plasticity. As ILC2 are fundamentally involved in the pathogenesis of type 2 diseases, they represent a promising therapeutic target for allergic airway and skin diseases.

In this review we summarize the current knowledge about ILC2 biology in the allergy context, with a particular focus on the emerging role of lipid mediators in regulating ILC2 function.

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1. Introduction

Group 2 innate lymphoid cells (ILC2) have recently emerged as critical players in the development of allergic inflammation. ILC2 belong to a family of cells depending on the transcription factor Id2 (inhibitor of DNA binding 2), but being independent of recombination activating gene 1 and 2 (RAG1/2). This family also comprises ILC1, ILC3 and NK cells [1–3]. ILCs are predominantly tissue-resident cells [4] lacking antigen-specific receptors such as the T and B cell receptors. Instead, ILCs rapidly respond to various environmental stimuli with cytokine production [5,6]. Human ILC2 display a distinct cytokine pattern upon stimulation, with preferential production of type 2 cytokines such as IL-4, IL-5, IL-9 and IL-13 similarly to Th2 cells, but ILC2 can also produce IL-6, IL-8 and IP10 [7]. As a consequence, ILC2 are capable of mounting an allergic immune response in the absence of Th2 cells [8]. ILC2 decode signals detected by epithelial and myeloid cells and convert these innate signals into an amplified immunological reaction against invading pathogens or allergens. The epithelial cell-derived factors include IL-33, thymic stromal lymphopoietin (TSLP) and IL-25 [9–11]. Additionally, IL-25 can be produced by eosinophils, mast

cells and Th2 cells [12]. These three innate cytokines are the best known activators of ILC2, however, in the absence of TSLP, IL-2 or IL-7 is additionally required for a potent ILC2 activation [7,13]. Furthermore, IL-1β [14,15] and the TNF superfamily cytokine TL1A [16] were shown as potent activators of ILC2 cytokine production (Fig. 1A).

Besides cytokines, lipid mediators are also able to regulate ILC2 function. In particular, leukotriene D₄ (LTD₄) and prostaglandin D₂ (PGD₂) appeared as potent activators of ILC2 [17–21]. As negative regulators of ILC2 function, other lipid mediators like the aspirin-triggered lipoxin A₄ (LXA₄) [18], PGI₂ [22] and indirectly maresin-1 [23] were demonstrated to attenuate ILC2 responses (Fig. 1A). Bioactive lipids have been known for long time for their crucial engagement in maintaining homeostasis and more importantly as potent mediators of acute inflammation [24]. The importance of lipid mediators is solidly established in the pathology of allergic inflammation [25]. There is a body of evidence describing lipid mediators as diagnostic markers and they are promising therapeutic targets in type 2 diseases [25,26]. In this mini-review we will give a short general update on ILC2 biology with focus on allergic diseases, and particularly provide a summary of the known roles of bioactive lipids in shaping ILC2 activity.

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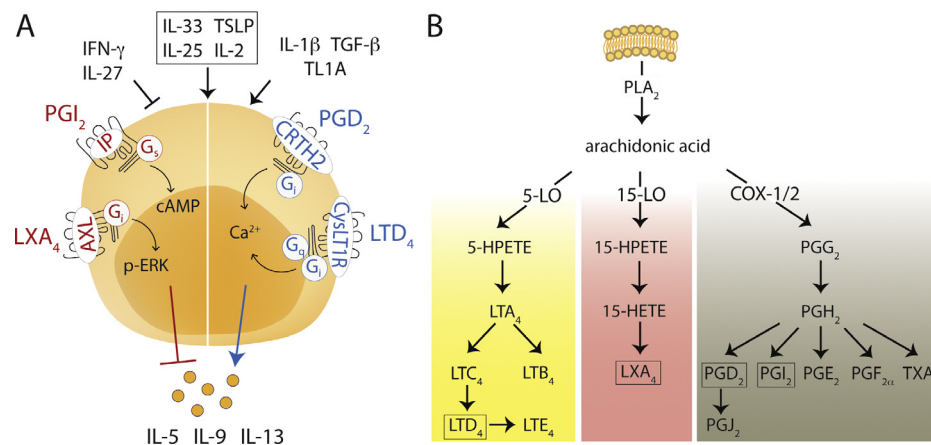


Fig. 1. Bioactive lipids regulate ILC2 function. (A) ILC2 produce type 2 cytokines; IL-5, IL-9 and IL-13 after stimulation by IL-33, IL-25, TSLP and IL-2 cytokines. Additionally, IL-1 β , TGF- β and TL1A stimulate ILC2 activity, while IFN- γ and IL-27 inhibit it. PGD₂ binds to CRTH2, a G_i-coupled receptor and LTD₄ activates CysLT1R, a G_i- or G_q-coupled receptor. Both lipid mediators, via inducing intracellular Ca²⁺ mobilization, trigger production of type 2 cytokines on their own and have synergistic effect with IL-25 plus IL-33. PGI₂ binds to IP, a G_s-coupled receptor and elevates cAMP levels intracellularly that results in blocking the IL-33-induced ILC2 cytokine production. Similarly, LXA₄ activates AXL, a G_i-coupled receptor on ILC2, and most likely via inducing ERK phosphorylation, it inhibits IL-33 plus IL-25- and PGD₂-stimulated IL-13 release. (B) Phospholipase A₂ (PLA₂) enzyme converts cell membrane phospholipids into arachidonic acid, which is the precursor of the 5-lipoxygenase (5-LO)-derived leukotrienes, the 15-LO-product lipoxins and the cyclooxygenase (COX)-1/2-metabolite prostaglandins.

2. ILC2 development and function

Based on mouse studies, ILC2 develop from the immature ILC2 precursor, which is originated from the common ILC progenitor (CHILP) in the bone marrow [27–29]. Although detailed knowledge about the origin of human ILC2 is still lacking, it was recently shown that all human ILC subsets in secondary lymphoid tissues could be generated from a ROR γ t⁺ progenitor [30]. For the proper development and function of ILC2 the transcription factors GATA3 [7,27], ROR α [29,31], TCF1 [32], GFI-1 [33], BCL11b [34,35] and ETS1 [36] were demonstrated to be indispensable. In mice, ILC2 are readily detected in the blood, intestine, lung, spleen, liver, adipose tissue, lymph nodes and skin [37–42]. In humans, ILC2 were first identified and characterized in the human fetal gut and in nasal polyps of patients with chronic rhinosinusitis. However, ILC2 have also been reported in the blood, lung parenchyma, bronchoalveolar lavage (BAL), sputum, thymus, tonsil and skin [18,43–46]. Mouse and human ILC2 are phenotypically very similar as they are lineage negative lymphocytes expressing the following combination of cell surface markers: CD45⁺CD127⁺CD161⁺CD117^{–/–}CD25⁺CRTH2⁺ST2^{+/–} [47]. Additionally, mouse ILC2 express Sca1, ICOS and KLRG1 [5].

The innate cytokines IL-33, IL-25 and TSLP are major activators of ILC2 function and they also induce ILC2 accumulation at sites of inflammation [48–50]. In mice, TGF- β 1 was recently demonstrated as an additional epithelial-derived cytokine critical for optimal ILC2 recruitment and activation in allergic asthma [51]. In response to stimulation, ILC2 release high amounts of IL-4, IL-5, IL-9 and IL-13, which initiate and amplify a type 2 immune response [6]. Furthermore, ILC2 can also be directly stimulated by IL-2 released from activated CD4⁺ T cells, via synergistic activation together with IL-33 [52,53]. Notably, ILC2 express MHCII and to some extent CD80 and CD86 that allow cellular interaction with CD4⁺ T cells. Thus, the ILC2-CD4⁺ T cell crosstalk is mutually beneficial and promotes the transition between innate to adaptive immunity [52–54].

In addition to soluble cytokines and the MHCII-TCR interaction, a number of receptor-ligand interactions have been shown to mediate ILC2 activation, including ICOS-ICOSL [55], TL1A-DR3 [16,56] OX40-OX40 [52] and NKp30-ligand B7-H6 [57]. As negative regulator of ILC2 function the KLRG1-E-cadherin interaction was demonstrated [58]. Furthermore, IFN- γ and IL-27 were shown to restrict ILC2 accumulation and activation [59–61].

3. ILC2 subsets and plasticity

Single-cell analysis has revealed significant transcriptional heterogeneity among human tonsillar ILC2 [62]. However, the functional consequences of such diversity in an inflammatory setting in the human remain to be determined. In the mouse lung, two functionally diverse ILC2 subsets were described [50]. One population was described as a tissue-resident natural (n)ILC2 expressing the IL-33 receptor ST2, while the other subset was characterized as an inflammatory (i)ILC2, accumulating during type 2 inflammation, preferentially responding to IL-25 and expressing IL-17RB and KLRG1A, but lacking ST2 [50].

In addition to displaying a certain degree of heterogeneity, ILC2 were recently shown to be functionally plastic, adapting to environmental cues. In particular, the IL-12/IL-12R-signaling axis was shown to skew human IL-13-expressing ILC2 towards an IFN- γ and T-bet-expressing ILC2 phenotype. Importantly, this IFN- γ ⁺IL-13⁺ ILC2 subset was also identified in the intestine of patients with Crohn's disease [63]. These findings were complemented by the description of IL-1 β as a critical factor potentiating IL-12 to switch ILC2 to an ILC1-like phenotype by upregulating T-bet and IL-12R in ILC2 [14,15]. Interestingly, the IL-12-induced skewing of ILC2 to ILC1 could be reversed by IL-4, indicating that these cells keep their plasticity [14]. The relevance of these in vitro findings was strengthened by a couple of clinical observations. Firstly, chronic obstructive pulmonary disease (COPD) was associated with an IL-12 signature and, by association, consequently ILC1 accumulation. Secondly, nasal polyps from chronic rhinosinusitis patients displayed an IL-4 signature, predicting ILC2 accumulation, which was indeed seen [14,64].

4. ILC2 in type 2 diseases

4.1. Allergic airway diseases

ILCs have been identified during homeostatic conditions in several types of upper and lower human airway tissues including nasal mucosa [14,43], lung parenchyma [14,43,44], BAL fluid [41] and sputum [46]. Histological examinations revealed that lung ILCs can be detected in small- and medium-sized airways where they, alike in the skin, seem to interact with mast cells [18]. However, this study identified ILCs as CD161⁺c-kit⁺tryptase[–] cells, which will

exclude all c-kit⁺ ILCs such as ILC1 and some ILC2. It is therefore of high relevance to establish where in the airways the different subpopulations of ILCs actually reside during both homeostatic and inflammatory conditions.

All hereto described ILC populations can be found in histologically healthy human airway tissue. This indicates that ILCs might be important for tissue homeostasis, e.g. via production of amphiregulin (Areg) [44]. However, ILCs seem to also play critical roles in type 2 mediated inflammation of the human airways. Elevated numbers of ILC2 have been detected in airway tissues obtained from patients with asthma [46,65], idiopathic pulmonary fibrosis [41], chronic rhinitis [66] and chronic rhinosinusitis with nasal polyps (CRSwNP) [14,43].

Indications of an involvement of ILCs in human asthma exist but are limited as of today. Bartemes et al. showed that the production of type 2 cytokines from circulating ILC2 was higher in allergic asthmatics as compared to healthy controls or allergic rhinitis patients [13], although, these findings were not confirmed by Barnig et al. [18]. Furthermore, ILC2 were shown to be enriched in asthmatic BAL [67] and in sputum samples of severe asthmatics where they were the major source of IL-5 and IL-13 [46]. Similar observations were made in severe pediatric asthma [65].

In CRSwNP, ILC2 are enriched as compared to non-affected nasal tissue, particularly in polyps characterized by high eosinophil infiltration [14,43]. Co-culture experiments revealed a bidirectional crosstalk between ILC2 and eosinophils, with ILC2 enhancing the survival of eosinophils via IL-5 production, and eosinophils promoting the activity of ILC2 via IL-4 production [14].

The signals driving ILC2 expansion in human airways are largely unknown but mouse studies have demonstrated several important mechanisms. In mouse models of OVA-, HDM-, papain- or *Alternaria alternata*-induced asthma, ILC2 are triggered by epithelial/myeloid-cell derived soluble factors including IL-25, IL-33, TSLP, TGF- β , and TL1A [8,16,29,51,68–70] as well as cell surface receptors such as ICOS ligand [55]. Hence, targeting such molecules, e.g. TSLP [71], might be a promising strategy to prevent ILC2 activation and the initiation of the asthmatic inflammation.

4.2. Allergic skin diseases

All subsets of ILCs have been identified in healthy human skin [45,58,72–74]. Skin ILCs reside predominantly in the dermis [45] probably in close proximity to the epidermis, although that has so far only been demonstrated in mice [42]. Two separate mechanisms for a role of ILC2 in skin homeostasis have been presented. Firstly, it was shown using intravital-multiphoton microscopy in mice that ILC2 patrol the dermis and form stable interactions with skin-resident mast cells resulting in inhibition of mast cell function via production of IL-13 [42]. Secondly, in humans, ILC2 were shown to express transcripts of the epidermal-growth factor related molecule amphiregulin (Areg) [58], playing an important role in wound healing in the lung [44]. Hence, it is possible that human ILC2 control homeostatic skin integrity through Areg production. Interestingly, the same group also demonstrated that ILC2 are homeostatically suppressed by surface expression of KLRG1, interacting with E-cadherin on dermal stromal cells. Loss-of-function mutations in the human filaggrin gene (*FLG*) strongly predisposes for development of atopic dermatitis (AD) through defects in skin barrier function, including reduced expression of E-cadherin. Indeed, ILC2 were enriched in suction blisters obtained from such patients as compared to patients displaying the *FLG* wild-type genotype [75].

In established AD, ILC2 are enriched in the dermis and produce the archetypical cytokines IL-4, IL-5, IL-13 and IL-6 [19,58,72]. As demonstrated in mice [58,72], human dermal ILC2 cytokine-production was driven by IL-25, IL-33 and TSLP [58] and also by

PGD₂ [19]. Interestingly, migration of human ILC2 was promoted by IL-33 [58] and PGD₂ [19]. Recently, a novel mechanism of ILC2 activation in AD was revealed through the discovery that human ILC2 expressing NKp30 can be activated by the tumor-associated surface molecule B7-H7, which is upregulated in AD skin [57].

Hence in summary, cytokines, lipid mediators and cell-surface receptors, which are upregulated in atopic dermatitis, promote the recruitment and activity of ILC2, which can further contribute to the inflammation via cytokine production.

5. Role of lipid mediators in ILC2 function

Lipids are generally involved in energy metabolism and formation of cellular membranes. However, there is a class of lipid molecules, termed bioactive lipids, which turned out to be key players in physiological mechanisms and cell signaling, both in humans and mice [76]. The most important bioactive lipids are the polyunsaturated fatty acid (PUFA)-derived eicosanoids, endocannabinoids and pro-resolving lipid mediators, and the group of sphingolipids [76]. Eicosanoids are formed from omega-6 PUFA and have been implicated in several inflammatory diseases and cancer [77,78]. In particular, arachidonic acid is the precursor of leukotrienes, which are lipoxygenase metabolites, and prostaglandins, which are cyclooxygenase metabolites [77] (Fig. 1B). Eicosanoids can be rapidly released by mast cells, macrophages, dendritic cells and eosinophils [24], as well as by endothelial and epithelial cells [79,80]. Eicosanoids are crucial players in type 2 inflammation of the airways [24,25] and the skin [81].

5.1. Leukotrienes (LTs)

LTs are 5-lipoxygenase (5-LO) products of arachidonic acid and include two groups; the chemoattractant LTB₄ and the cysteinyl (Cys)LTs: LTC₄, LTD₄ and LTE₄ [82] (Fig. 1B). In the pathophysiology of asthma and other allergic diseases CysLTs, produced by activated eosinophils, mast cells and alveolar macrophages, play a central role as potent inflammatory lipid mediators [83–85]. CysLTs are highly active bronchoconstrictors, and can promote airway smooth muscle hyperplasia and pulmonary edema formation [83]. Notably, CysLTs, in particular LTD₄, was demonstrated to amplify the antigen-specific Th2 cell-mediated pulmonary inflammation [86], and more recently, LTD₄ turned out to be a potent chemoattractant of Th2 cells [87]. Amongst CysLTs, LTD₄ has the highest affinity to the CysLT1R, which is a G_i- and G_q-coupled receptor, triggering intracellular Ca²⁺ mobilization and subsequently rapid production of IL-4, IL-5 and IL-13 in Th2 cells [87]. LTD₄ was the first lipid mediator described to regulate ILC2 function [17] (Fig. 1A). Mouse lung and bone marrow ILC2 were also shown to express CysLT1R at steady state as well as during *Alternaria* species challenge [17]. Furthermore, *Alternaria* species-induced endogenous LTD₄ promoted ILC2 accumulation in the lung in a CysLT1R-dependent fashion. LTD₄ stimulation of ILC2 resulted in enhanced proliferation and robust production of IL-4, IL-5 and IL-13 [17]. Later on Xue et al., demonstrated that also human ILC2 can respond to CysLTs [19].

5.2. Prostaglandins (PGs)

PGs are generated from arachidonic acid via the cyclooxygenase (COX)-1/2 pathways through two unstable intermediate forms; PGG₂ and PGH₂, and by subsequent specific PG synthase enzymes [77] (Fig. 1B). The key prostaglandin in driving type 2 inflammation is PGD₂, showing elevated levels in the airways of patients with severe asthma and allergic rhinitis [88–91]. PGD₂ is synthesized by hematopoietic PGD₂ synthase (HPGDS) predominantly in mast cells during allergic responses [92]. The PGD₂ receptor CRTH2

(also known as DP2) is selectively expressed by type 2 inflammatory cells like Th2 cells, eosinophils and basophils. Importantly, human ILC2 were identified based on CRTH2 expression [43]. CRTH2 is a G_i -coupled receptor similarly to other chemoattractant receptors. CRTH2 mediates activating signals by reducing intracellular cAMP levels and triggering Ca^{2+} mobilization [90]. Barnig et al. demonstrated that PGD₂ stimulated IL-13 production of human peripheral blood ILC2 via CRTH2, and PGD₂ displayed strong synergistic effects with IL-25 and IL-33 in the presence of IL-2 [18] (Fig. 1A). A subsequent study further established the importance of the PGD₂-CRTH2 pathway in direct stimulation of human peripheral blood and skin ILC2 migration as well as of production of IL-4, IL-5 and IL-13 [19]. Furthermore, PGD₂ was shown to potentiate the IL-25 plus IL-33-mediated ILC2 responses, which was partially due to the CRTH2-ligation-induced upregulation of IL-25 (ST2) and IL-33 (IL-17RA) receptors [19]. Notably, besides type 2 cytokines, PGD₂ also promoted production of other pro-inflammatory cytokines (IL-3, IL-8, IL-9, IL-21, GM-CSF and CSF-1), which was again further enhanced by IL-25 and IL-33 stimulation [19]. In addition, the stimulating effects of PGD₂ were mimicked by IgE-activated mast cell supernatants in skin ILC2 in a CRTH2-dependent manner [19]. The PGD₂-CRTH2 pathway was shown to be a conserved activator of ILC2, as mouse ILC2 function and pulmonary accumulation was also driven by PGD₂ during helminth-induced type 2 pulmonary inflammation [21]. Consistently, CRTH2-deficient ILC2 displayed reduced responsiveness. However, the lack of CRTH2 expression in genetically modified mice did not influence ILC2 development and frequencies in the lung at steady state [21]. Thus, PGD₂ plays a central role in ILC2 activation, which further enhances the importance of ILC2 in orchestrating innate and adaptive immunity.

Unlike PGD₂, another prostaglandin, PGI₂, was shown to inhibit human and mouse ILC2 [22]. The ligation of the PGI₂ receptor; IP being preferentially a G_s -coupled receptor, leads to an elevation of intracellular cAMP levels and subsequent activation of protein kinase A (PKA) [93]. Additionally, IP receptor can also be G_i - or G_q -coupled [90]. Murine studies suggest that the PGI₂-IP signaling attenuates allergic inflammation as IP-deficient mice had increased IL-4 and IL-5 levels in the lung, and enhanced airway leukocyte infiltration in a short-term OVA model [94]. Recently, the PGI₂ analog cicaprost was shown to reduce IL-33-stimulated production of IL-5 and IL-13 in mouse bone marrow-derived ILC2 [22] (Fig. 1A). IP ligation potently blocked ILC2 proliferation and at the same time induced apoptosis, which was most probably mediated via downregulation of Id2 expression [22]. Additionally, *Alternaria alternata*-challenged IP-deficient mice displayed higher IL-5- and IL-13-expressing ILC2 counts in the lung and enhanced infiltration of eosinophils. The ILC2-attenuating role of PGI₂ was also demonstrated in human peripheral blood ILC2 but the effect was more modest than described in the mouse [22]. Generally, PGI₂ analogs (iloprost, cicaprost, treprostinil) are used in humans for pulmonary hypertension therapy [25]. Yet, in an observational study of patients with mild asthma, iloprost did not exert changes in lung function or methacholine responsiveness [95]. Similarly, an oral PGI₂ analog (OP-41483) did not modulate FEV1 or bronchial responsiveness in stable asthmatics [96] questioning the therapeutic value of targeting PGI₂ in asthma.

5.3. Pro-resolving lipid mediators

5.3.1. Lipoxins (LXs)

While the early lipid mediators LTs and PGs are involved in amplifying acute inflammation, the subsequent release of lipoxins (LXs) promote resolution of the inflammation (Fig. 1B). This program of lipid mediator release is played in a sequential order and has been termed “lipid mediator class switching” that allows resolution of the inflammatory response [97]. LXs are arachi-

donic acid-derived lipid mediators, generated by the 15-LO enzyme in the inflamed lung by bronchial epithelial cells, macrophages and eosinophils [98]. LXA₄ binds to the rather unconventional ALX receptor that is also called formyl-peptide receptor type 2 (FPR2) as it conveys signals induced by lipid and protein or short peptide ligands depending on its dimerization status [99]. Based on the encountered ligand, ALX/FPR2 receptor can exert pro- or anti-inflammatory effects. However, LXA₄ mediates exclusively anti-inflammatory functions [100]. During severe asthma, LXA₄ synthesis and ALX/FPR2 receptor expression is downregulated [101,102]. Barnig et al. reported the LXA₄-AXL pathway as a potent anti-inflammatory regulator of human ILC2 activity [18]. LXA₄ prevented both PGD₂- and IL-25 plus IL-33-stimulated IL-13 production of human peripheral blood ILC2 [18] (Fig. 1A). Adding physiological relevance to these in vitro findings, a clinical study reported on a modest improvement of asthma following LXA₄ inhalation after LTC₄-induced airway obstruction in asthmatic patients [103]. More recently, topical application of a stable LXA₄ analog reduced severity and triggered recovery of infantile eczema in a small exploratory study [104].

5.3.2. Specialized pro-resolving lipid mediators (SPM)

SPMs are derived from omega-3 PUFAs and include structurally different bioactive lipids such as resolvins, protectins and maresins [105]. Very recently, maresin-1 (MaR1) was demonstrated to reduce allergic lung inflammation and IL-5, IL-13 expression of mouse ILC2, with simultaneous induction of amphiregulin [23]. This effect was however not due to a direct effect of MaR1 on ILC2, but involved the de novo generation of regulatory T cells (Tregs), which via TGF- β production suppressed ILC2 activity. This work revealed a multi-cellular regulatory network of MaR1-induced resolution of allergic inflammation in the lung, which resulted in limited ILC2 function [23].

Together, bioactive lipids emerge as potent regulators of ILC2 that is now supported by an increasing body of evidence. Activating lipid mediators are able to stimulate ILC2 function alone without priming with IL-25, IL-33 or TSLP. However, lipid mediators can have synergistic effects with these innate cytokines, suggesting engagement of distinct signaling pathways in ILC2 activation. Notably, anti-inflammatory lipid mediators do not distinguish between eicosanoids- or cytokine-induced ILC2 activation, but suppress ILC2 responsiveness. Hence, ILC2 can be exploited in multiple ways as therapeutic targets in allergic diseases.

6. Pharmacological targeting of ILC2

ILC2 act as a first line of defense as they are tissue-resident and antigen-independent cells that can be rapidly activated via various mechanisms instantly leading to initiation of an immune reaction. In addition, by releasing IL-5 and IL-13, ILC2 can further amplify allergic inflammation, thus ILC2 are key drivers of the pathophysiology in type 2 diseases.

Current guidelines for adult asthma therapy include inhaled or systemic corticosteroids for attenuating ongoing airway inflammation, and short- or long-acting bronchodilators as well as inhibitors of the leukotriene or IgE pathways [106]. This treatment regimen likely affects ILC2 function, as corticosteroids were recently shown to reduce ILC2 counts in eosinophilic nasal polyp after systemic corticosteroid treatment [107]. Furthermore, leukotriene receptor antagonists (montelukast, zafirlukast) can prevent ILC2 activation by inhibiting the CysLT1R [17].

The novel, currently investigated biological treatments for asthma therapy also frequently affect ILC2-activating- or ILC2-derived cytokines [108]. For instance the anti-TSLP antibody (AMG 157) attenuated allergen-induced bronchoconstriction and airway

inflammation with reduced levels of blood and sputum eosinophils in a double-blind, placebo-controlled study [71]. Furthermore, a humanized monoclonal antibody against the downstream ILC2 cytokine IL-5 (mepolizumab) significantly reduced asthma exacerbation of patients with severe asthma in two separate clinical studies [109,110]. IL-4 and IL-13 also represent attractive targets [108]. Humanized anti-IL-4 antibodies did not show beneficial effects in the therapy of severe asthmatics [108], while targeting IL-13 might be a promising approach [111]. Importantly, dupilumab, a novel fully human antibody that targets the IL-4R α , thereby inhibiting both IL-4 and IL-13 signaling, improved uncontrolled moderate-to-severe atopic dermatitis as well as persistent asthma [112,113].

Therapeutic interventions targeting lipid mediators could also significantly affect ILC2 function in allergic diseases. Selective CRTH2 antagonists are currently being evaluated for treating allergic diseases [114,115]. Two smaller clinical studies reported improved late asthmatic responses after allergen challenge and reduced asthma symptom scores [116,117]. Agonists of the AXL/FP2R receptor also represent a promising therapy for allergic diseases [103,104]. While stable analogs of IP receptor did not show high therapeutic value for asthmatics [95,96].

7. Conclusions and open questions

The relatively recently described ILC2 population appears as crucial player in type 2 inflammatory diseases such as airway and skin allergies. In humans and mice, ILC2 had been identified in most examined tissues during homeostasis. Under allergic inflammatory conditions, ILC2 were found to be accumulated in the airways and the skin; notably, ILC2 counts were also elevated systemically in peripheral blood. ILC2 are capable of rapid IL-5 and IL-13 production in response to several cytokines and lipid mediators known to be fundamentally involved in driving type 2 inflammation. A number of bioactive lipids have been already identified to potently affect ILC2 proliferation, accumulation and cytokine production; yet, the role of other relevant lipid mediators in ILC2 biology remains to be elucidated.

As we have recently gained more insights into ILC2-ILC1 plasticity, the obvious question arises, whether there are also conditions leading to ILC2-ILC3 plasticity, as recently reported in mice. Another relevant question is, whether the lipid mediators only affect ILC2 function or can also regulate ILC2 plasticity. In addition, further studies are required for investigating if ex-ILC2 retain responsiveness to bioactive lipids or lose expression of the respective receptors as they lose ability to produce type 2 cytokines. How lipid mediators control other aspects of ILC2 biology awaits further investigations and may provide additional clues in dissecting the role of ILC2 in type 2 immunity.

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