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All along the watchtower: Group 2 innate lymphoid cells in allergic immunity

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Abstract

Group 2 innate lymphoid cells (ILC2) are a subset of innate lymphocytes that responds to local, tissue-derived signals and initiates allergic immune responses. ILC2 activation promotes the recruitment of eosinophils, polarization of alternatively activated macrophages, and tissue-remodeling, processes associated with the 'weep and sweep' response to helminthic 'worm' colonization and infection. ILC2s also coordinate both physiologic and pathologic type 2 allergic immune responses, including promoting normal tissue development and remodeling and driving allergic pathology such as atopic dermatitis and allergic asthma. In this review we summarize recent advances in ILC2 biology, particularly focusing on how local cells and signals coordinately regulate ILC2s, how this may influence physiologic processes, and how ILC2 cooperate with adaptive Th2 cells to drive pathologic allergic inflammation.

Graphical Abstract

ILC2s are regulated by diverse signals in barrier and non-barrier sites. In response to tissue damage, helminths, or allergens, tissue-resident non-hematopoietic cells produce IL-25, IL-33, and TSLP that induce expansion and cytokine production by ILC2s, which can be further amplified by additional signals, including neuropeptides, leukotrienes and prostaglandins (PGs). Conversely, ILC2-activity is limited by signals classically associated to a type 1 immunity, such as type I and II interferons (IFNs), as well as other PGs and neurotransmitters. Activated ILC2s produce effector molecules that can influence the responses of innate effector cells, including polarization of alternatively activated macrophages (AAMs), eosinophil recruitment and dendritic cell (DC) migration, as well as epithelial and stromal cells by inducing mucus production, tissue-remodeling and repair. By integrating these inhibitory and activating signals from multiple sources, ILC2s (coordinate with Th2 cells), function as tissue-resident sentinels that rapidly responds to local changes.

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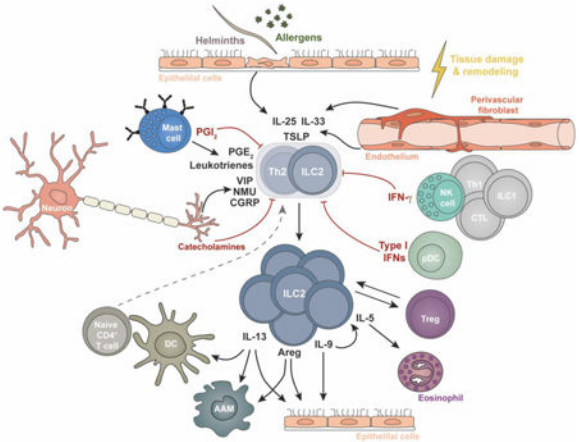

Introduction

In industrialized countries the prevalence of allergic disease has increased dramatically over the past 50 years, with 20–30% of the world's population suffering from an allergic disorder [1]. Allergic pathology such as asthma, allergic rhinitis and atopic dermatitis (AD) are driven by hyperactive type 2 immune responses at barrier tissues. Type 2 immunity is characterized by high levels of interleukin (IL)-4, IL-5, IL-9 and IL-13, cytokines traditionally associated with adaptive $CD4^+$ T helper (Th) 2 cells. However, the notion of Th2 cells as the sole coordinator of type 2 immunity was revised when group 2 innate lymphoid cells (ILC2) were identified as an innate cell with abundant production of type 2 cytokines [2–6].

ILC2s are strategically positioned to monitor and respond to local tissue changes and promote type 2 immunity in both barrier and non-barrier tissues. In contrast to $CD4^+$ Th2 cells, which require antigen presentation for their generation, ILC2s are developmentally positioned in tissues and predominantly replicate locally [7], although ILC2 have the ability to mobilize from one tissue to another with type 2 inflammation [8]. Indeed, ILC2 may develop from tissue resident ILC precursors, emphasizing that their terminal identity and function is responsive to local tissue cues [9]. ILC2s constitutively express IL-5 and promote eosinophil production and maintenance [10]. Multiple signals can enhance this tonic IL-5 signaling and further induce IL-9 and IL-13 production by ILC2s [4–6,11], stimulating goblet cell mucus production, smooth muscle cell contraction, eosinophil recruitment, and polarization of alternatively activated macrophages. ILC2s also promote epithelial repair via production of the epidermal growth factor receptor ligand amphiregulin (Areg) [12]. Together, ILC2 activation directs a tissue remodeling program that can have homeostatic/physiological and pathologic consequences. As such, ILC2s are a primary sentinel patrolling the 'watchtowers' of virtually all tissues, responding to tissue-derived signals to initiate type 2 immune responses, even in the absence of adaptive immune cells.

ILC2s in human allergic pathology

Multiple studies have linked allergic pathology to increased ILC2 activity in human tissues. The first identification of ILC2 accumulation in human disease were made in the nasal mucosa of patients with chronic rhinosinusitis (CRS) [13]. Genome-wide association studies (GWAS) have identified susceptibility loci in asthma, AD and/or allergic rhinitis in genetic regions associated with ILC2 activating signals (*TSLP*, *IL33*, *IL1RL1*), ILC2-produced cytokines (*IL4* and *IL13*), and ILC2-defining transcription factors (*GATA3*, *RORA*) [14,15]. Several of these susceptibility genes were also found to be epigenetically modified in a murine model of IL-33-driven airway inflammation, as well as *in vitro* stimulated human ILC2s [16]. Increased levels of cytokine producing ILC2s were identified in sputum and blood from patients with severe, as compared to mild, asthma [17]. IL-33 levels are increased in bronchiolar lavage fluid from asthmatic patients, negatively correlating to lung function, and associated with elevated ILC2s [18]. In AD, a chronic allergic skin disease, increased numbers of ILC2s are present in lesional skin from AD patients as compared to healthy controls [19,20], consistent with increased tissue-derived cytokines IL-25, IL-33 and TSLP [19]. The involvement of ILC2 in gastrointestinal allergic responses is less clear, although implied in eosinophilic esophagitis, where the percentage of ILC2s were significantly increased in patients with active disease [21]. These findings suggest that regulation of ILC2 is critical to human allergic disease. However, as tissue-resident Th2 cells resemble ILC2s transcriptionally and epigenetically [22], and also expand in human allergic disease, it is likely that these two populations cooperate to promote human allergic pathology.

Regulation of ILC2 activation in barrier tissues

The lack of both antigen-specific receptors and pattern recognition receptors, together with expression of a wide spectrum of receptors for tissue-derived signals, emphasizes the integrated role of ILC2s in tissue homeostasis and immunity. ILC2s expand in response to a wide spectrum of predominantly tissue-derived signals, including cytokines (IL-2, IL-7, IL-25, IL-33, TSLP, TL1A), eicosanoids (leukotrienes (LTs), prostaglandins (PGs)) and neuropeptides (neuromedin U (NMU), vasoactive intestinal peptide (VIP), calcitonin gene-related peptide (CGRP)) [2–6,10,13,23–31]. These signals can be broadly categorized based on the signaling pathways induced, with striking similarities to T cell activation. STAT5-mediated signaling (IL-2, IL-7, and TSLP) mainly appears to promote ILC2 development, tissue colonization, and survival. NFκB and AP-1 activating signals (IL-25, IL-33, TL1A) are essential for driving expansion and cytokine production by ILC2s in response to exogenous stimuli, but are dispensable for ILC2 development and survival. Short-acting signals such as neuropeptides and eicosanoids can rapidly induce the ILC2 response via induction of NFAT (LTs, NMU, PGD_2) or cyclic AMP (VIP, CGRP).

The ‘epithelial cytokines’:

IL-25, IL-33, and TSLP are well-described signals that activate ILC2. Although the epithelium is often designated as their source, and may represent a dominant source during conditions of barrier damage, endothelial cells, fibroblasts, and subsets of hematopoietic

cells also produce these cytokines. Thus, it remains to be determined which of these cells are the relevant source(s) for the activation of ILC2s in different tissues and inflammatory contexts. Notably, the signals that regulate the expression and release of these tissue-derived cytokines are largely unknown and could be critical regulators of type 2 immunity. Whereas IL-33 is primarily associated with ILC2 activation in the lung [32], adipose tissue (AT) [33], and pancreas [34], IL- 25 appears to be the main ILC2-activating signal in the small intestine (SI). TSLP is less well defined but has been associated with ILC2 activation in skin and nasal epithelium. This cytokine preference in activation likely reflects both the tissue-specific cellular sources of each cytokine, as well as tissue-specific exposure to external stimuli.

IL-33 binds to its receptor, IL-1 receptor-like 1 (IL1RL1, ST2), and signals via IL-1 receptor accessory protein 1 and MyD88 to activate NFκB- and AP-1-mediated transcription. In the lung, IL-33 signaling is one of the major pathways driving protease-induced allergic inflammation [35] via activation of ILC2s [23,24,36]. Although IL-33 is biologically active in its full length form, proteolytic cleavage by mast cells can increase its ability to activate ILC2s [37]. Several protease allergens potently cleave and activate full length IL-33, further highlighting the role for IL-33 in ILC2-driven allergic responses [38].

IL-25 (or IL-17E) binds and signals through a heterodimeric receptor (IL-25R) and activates NFKB and AP-1 via Actl. In the SI, epithelial tuft cells constitutively produce IL-25 and drive ILC2 expansion following helminth infection [39–41], while IL-13-producing ILC2s support tuft cell hyperplasia and worm expulsion. The intestinal ILC2-tuft cell circuit may also act as a physiologic loop to regulate intestinal secretory cell activity and remodeling, potentially impacting SI function beyond helminth infection and allergic disease.

TSLP expression is induced by NFκB[42] and signals via its receptor TSLPR combined with IL- 7Rα to induce STAT5 activation [25]. TSLP drives accumulation of ILC2s in a murine model of AD, independently of IL-33 or IL-25 signaling [20]. However, another group using the same model found that IL-33 and IL-25 predominantly promoted ILC2 accumulation and type 2 inflammation in the skin [19]. TSLP is expressed by nasal epithelial cells in response to various toll-like receptor ligands and increased in patients with CRS compared to healthy controls [25]. In combination with IL-2 and IL-33, TSLP is reported to enhance GATA3 expression and cytokine production by human ILC2s derived from nasal polyps [25].

Intriguingly, expansion of lung ILC2 following intranasal challenge with chitin polymers was independent of IL-33, IL-25 or TSLP [36]. Simultaneous deletion of all three could largely abrogate IL-5 and IL-13 production, whereas individual loss only partly reduced cytokine production by ILC2s, [36]. These data support two critical conclusions: 1) these tissue-derived cytokines are partially redundant with each other, and 2) other signals are responsible for ILC2 development and tissue colonization.

Lipid mediators:

Several eicosanoids are potent ILC2 activators, often in combination with the above described cytokines. Human ILC2s are identified by expression of the receptor for PGD_2,

CRTH2 [13]. PGD_2 signaling regulates late ILC2 accumulation in the lung in response to worm infection [43] and enhances IL-13 production in peripheral blood-derived ILC2s [44]. LTs, strongly implicated in allergic disease, were shown to support ILC2 cytokine production in the lung via NFAT-signaling [26]. IL-33 and LT signaling are non-redundant for worm-induced production of type 2 cytokines, but could synergistically enhance the activation of ILC2s by administration of both mediators [26,45].

Neurotransmitters:

Emerging evidence suggests crosstalk between ILC2 and the peripheral nervous system and neuroendocrine systems, particularly in the small intestine and lung. For example, type 2 cytokines have been shown to directly regulate peripheral neurons to mediate itch responses [Oetjen, Kim, Cell 2017]. Conversely, multiple neuropeptides have been shown to stimulate cytokine secretion by ILC2. These include VIP [10] and CGRP, which is produced by pulmonary neuroendocrine cells and can amplify allergic asthma [30,46]. Recently, three groups demonstrated that ILC2s express high levels of the receptor for the neuropeptide neuromedin U (NMU) [27–29], and that peripheral cholinergic neurons producing NMU rapidly amplify allergic inflammation in an ILC2 dependent manner in the gut and lung [28]. Opposing the ILC2-activating properties of NMU and VIP, adrenergic signaling via the β2-adrenergic receptor (β2AR) restricts ILC2 proliferation and responses in the SI and lung during allergic inflammation and worm infection [47]. These studies suggest the intriguing possibility that the tissue remodeling properties of ILC2 may be suppressed by sympathetic ‘fight or flight’ cues, and activated by parasympathetic signaling via cholinergic neurons.

Inhibitory signals:

ILC2s are poised to rapidly respond to activating tissue signals. Therefore, negative regulation of ILC2s is crucial to prevent pathologic type 2 inflammation. Cytokines associated with type 1 immunity, such as type I interferons (IFNs) [48], IFN-γ [49–52] and IL-27 [50] are dominant inhibitors of ILC2 activation. Androgen receptor (AR) signaling on ILC2s restricts their activation, and may account for the lower susceptibility to allergic airway inflammation in males [53,54]. Lipoxin A4, PGI_2 and maresin 1 are eicosanoids that have been suggested as pro-resolving mediators in severe asthma with direct suppressive effects on IL-13 production by ILC2s [44,55,56]. Similar to T cells, PD-1 signaling in ILC2s limits their expansion and cytokine production by reducing STAT5 activity [57].

ILC2s in development

ILC2s expand in the lung in the first two weeks of life, immediately followed by a modest contraction phase, reaching adult levels between 4–6 weeks after birth [58,59]. The early expansion of lung ILC2s has been reported to be partially driven by IL-33, which is induced in a subset of epithelial cells after birth, and is associated with the type 2-biased environment in the developing lung [58,60]. The immediate expression of IL-33 following birth was suggested to be induced by pressure changes associated with the “first breath”, demonstrated by induction of IL-33 in E19 lungs exposed to vacuum [58]. A recent study found that exposure of neonate and postnatal mice to hyperoxia increased TSLP and IL-33, ILC2 activation, airway hyper reactivity, mucus production and downstream type 2 airway

inflammation[61]. Together, these results suggest that excessive activation of ILC2s in the already type 2-biased developing lung may tip over into an increased sensitivity to allergic pathology later in life. However, while IL-33 is one signal reported to promote the ILC2-driven type 2 developmental wave, lack of IL-33, TSLP, and IL-25 does not prevent ILC2 tissue colonization [36], and therefore other signals must regulate ILC2 postnatal expansion even in the lung, as well as the SI, skin, AT, and elsewhere.

ILC2s and adaptive T-cell responses

The engagement of an adaptive immune response and generation of IgE is critical to allergic pathology, but the relative contributions of ILC2s and adaptive Th2 cells in allergic responses remain unclear. In a bone marrow (BM) transfer model, using *Rora*$^{-/-}$ BM to generate ILC2-deficient mice, the adaptive Th2 response to protease antigen was impaired. This was suggested to be due to a loss of ILC2-derived IL-13, which promoted dendritic cell migration to the draining lymph node [62]. In contrast, Th2 differentiation was intact in a ILC2-depletion model (deleting IL-5-producing cells on a *Rag1*$^{-/-}$ background) transferred with naïve T cells, [22], arguing for ILC2-independent Th2 differentiation in response to allergic challenge. Also, consistent with the similarities between tissue-Th2s and ILC2s, both subsets display severely impaired cytokine production in the absence of tissue-cytokines (IL-25, IL-33 and TSLP), while adaptive responses in the dLN, as well as IgE production, were fully intact [22]. Together, it appears that ILC2 can promote development of Th2 cells in certain contexts, but Th2 differentiation does not require ILC2s. Instead, terminal Th2 differentiation in tissues requires IL-25, IL-33, TSLP, and perhaps other tissue-signals, similar to ILC2s, and these populations likely cooperate to exert their tissue function [22].

Bi-directional interactions between ILC2s and T regulatory (Treg) cells have also been demonstrated. ILC2s co-localize with and support Treg accumulation in peripheral tissues in settings of high IL-33 levels and activated type 2 immunity [49], while Tregs can suppress ILC2 activation in the context of allergic inflammation [55,63,64]. In response to protease allergens, lung Tregs were supported by mast cell-derived IL-2 and reported to limit ILC2 cytokine production via IL-10 [63] and TGFβ signaling [55,65]. In contrast, RORa^{+} Tregs were demonstrated to limit ILC2-driven eosinophil accumulation in a model of allergic skin inflammation, independently of IL-10 and TGFβ, but via upregulation of the TL1A receptor DR3 [64]. Together, these results suggest a complex interplay between tissue ILC2, Th2, and Tregs to coordinate a balanced type 2 immune response.

ILC2 functions in non-barrier tissue homeostasis and repair

ILC2s promote metabolic function in AT [49,66,67], repair-responses in the brain meninges [68], pathologic inflammation in the liver [69], insulin production in the pancreas [34], and remodeling in the ovary and uterus [70,71]. Although IL-13 is one signal whereby ILC2 impact tissue physiology, additional mechanisms are poorly understood. The immediate tissue ‘niche’ of ILC2s appears to be conserved across many non-barrier tissues, suggesting possible conserved modules of physiologic regulation (Molofsky and colleagues,

unpublished). As most of these non-barrier tissues lack epithelial cells, stromal cells may play prominent roles in governing the function of ILC2s.

Conclusions and future directions

ILC2s integrate a spectrum of tissue-specific signals, functioning as a type 2-sentinel to produce a graded tissue response. ILC2s may act from their tissue 'watchtowers' to regulate the function of resident immune and stromal cells, as well as the composition and function of immune cells entering and leaving tissues. The relevant local sources and initiators of these ILC2-regulating signals remain largely undefined. While ILC2s are dominant activators of allergic inflammation, it is still unclear *how* and *when* they evolve from serving a balanced homeostatic/reparative function to promoting allergic pathology. Similarly, the definitive ILC2 versus Th2 contributions to allergic inflammation have been difficult to assign due to the functional similarities of ILC2 to tissue-resident Th2 cells, and resulting lack of ILC2 specific-deletion models. Nonetheless, as ILC2 and tissue Th2 cells appear to respond to similar signals, lessons learned from ILC2 biology could very well be applicable to tissue Th2 cells, although further studies are required to delineate these issues.

Many of the current treatment strategies of allergic disease target pathways involved in ILC2 regulation. Corticosteroids, used in both asthma and AD, are broadly immunosuppressive and target NFκB-dependent transcription of pro-inflammatory cytokines as well as generation of eicosanoids [72], two non-redundant pathways promoting ILC2 activation. Topical calcineurin inhibitors are used in AD and molecularly targets NFAT signaling (induced by LTs and NMU). Short-acting β2-agonists has a long term-history used as bronchodilators in asthma, but may also have direct suppressive effects on ILC2s [47]. Additionally, monoclonal antibodies against cytokines or cytokine receptors expressed by ILC2 (IL-5, IL-5Ra, IL-13), or directly activating ILC2 (IL-33, IL-33R, TSLP), are being developed or have come into recent clinical use as treatment for allergic disease [73]. As additional physiologic and developmental roles for ILC2 are defined and indicated as novel therapeutic targets, careful monitoring of the side-effects of type 2 blockade is warranted. Future studies on developmental and homeostatic roles for ILC2s will be of great importance to understand how allergic inflammation is triggered.

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** Special Interest

*** Outstanding Interest

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