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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. NF κ B 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, PGD₂) 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 NF κ B- 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 NF κ B and AP-1 via Act1. 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 NF κ B[42] and signals via its receptor TSLPR combined with IL-7R α 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 PGD₂,

CRTH2 [13]. PGD₂ 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 β 2-adrenergic receptor (β 2AR) 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, PGI₂ 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, RORα⁺ 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/repairative 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 NF κ B-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 β 2-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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References:

** Special Interest

*** Outstanding Interest

1. Pawankar R, Canonica GW, Holgate ST, RF Lockey - World Allergy Organization, 2011: White book on allergy 2011–2012 executive summary.

2. Fort MM, Cheung J, Yen D, Li J, Zurawski SM, Lo S, Menon S, Clifford T, Hunte B, Lesley R, et al.: IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. *Immunity* 2001, 15:985–995. [PubMed: 11754819]
3. Hurst SD, Muchamuel T, Gorman DM, Gilbert JM, Clifford T, Kwan S, Menon S, Seymour B, Jackson C, Kung TT, et al.: New IL-17 family members promote Th1 or Th2 responses in the lung: in vivo function of the novel cytokine IL-25. *J Immunol* 2002, 169:443–453. [PubMed: 12077275]
4. Moro K, Yamada T, Tanabe M, Takeuchi T, Ikawa T, Kawamoto H, Furusawa J-I, Ohtani M, Fujii H, Koyasu S: Innate production of T(H)2 cytokines by adipose tissue-associated c-Kit(+)Sca-1(+) lymphoid cells. *Nature* 2010, 463:540–544. [PubMed: 20023630]
5. Neill DR, Wong SH, Bellosi A, Flynn RJ, Daly M, Langford TKA, Bucks C, Kane CM, Fallon PG, Pannell R, et al.: Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature* 2010, 464:1367–1370. [PubMed: 20200518]
6. Price AE, Liang H-E, Sullivan BM, Reinhardt RL, Eisley CJ, Erle DJ, Locksley RM: Systemically dispersed innate IL-13-expressing cells in type 2 immunity. *Proc. Natl. Acad. Sci. U.S.A.* 2010, 107:11489–11494. [PubMed: 20534524]
7. Gasteiger G, Fan X, Dikiy S, Lee SY, Rudensky AY: Tissue residency of innate lymphoid cells in lymphoid and nonlymphoid organs. *Science* 2015, 350:981–985. [PubMed: 26472762] **This paper uses parabiotic mice to demonstrate that most ILC2s are tissue-resident cells that self-renew locally during homeostasis as well as acute helminth infection.
8. Huang Y, Mao K, Chen X, Sun M-A, Kawabe T, Li W, Usher N, Zhu J, Urban JF, Paul WE, et al.: SIP-dependent interorgan trafficking of group 2 innate lymphoid cells supports host defense. *Science* 2018, 359:114–119. [PubMed: 29302015] ***This paper finds that inflammatory ILC2s induced by IL-25 or helminth infection can recirculate from the SI to the lung in a SIP-dependent manner.
9. Lim AI, Li Y, Lopez-Lastra S, Stadhouders R, Paul F, Casrouge A, Serafini N, Puel A, Bustamante J, Surace L, et al.: Systemic Human ILC Precursors Provide a Substrate for Tissue ILC Differentiation. *Cell* 2017, 168:1086–1100.e10. [PubMed: 28283063]
10. Nussbaum JC, Van Dyken SJ, Moltke von J, Cheng LE, Mohapatra A, Molofsky AB, Thornton EE, Krummel MF, Chawla A, Liang H-E, et al.: Type 2 innate lymphoid cells control eosinophil homeostasis. *Nature* 2013, 502:245–248. [PubMed: 24037376]
11. Wilhelm C, Hirota K, Stieglitz B, Van Snick J, Tolaini M, Lahl K, Sparwasser T, Helmby H, Stockinger B: An IL-9 fate reporter demonstrates the induction of an innate IL-9 response in lung inflammation. *Nat. Immunol.* 2011, 12:1071–1077. [PubMed: 21983833]
12. Monticelli LA, Sonnenberg GF, Abt MC, Alenghat T, Ziegler CGK, Doering TA, Angelosanto JM, Laidlaw BJ, Yang CY, Sathaliyawala T, et al.: Innate lymphoid cells promote lung-tissue homeostasis after infection with influenza virus. *Nat. Immunol.* 2011, 12:1045–1054. [PubMed: 21946417]
13. Mjösberg JM, Trifari S, Crellin NK, Peters CP, van Drunen CM, Piet B, Fokkens WJ, Cupedo T, Spits H: Human IL-25- and IL-33-responsive type 2 innate lymphoid cells are defined by expression of CCR4 and CD161. *Nat. Immunol.* 2011, 12:1055–1062. [PubMed: 21909091]
14. Tamari M, Tanaka S, Hirota T: Genome-wide association studies of allergic diseases. *Allergol Int* 2013, 62:21–28.
15. Torgerson DG, Ampleford EJ, Chiu GY, Gauderman WJ, Gignoux CR, Graves PE, Himes BE, Levin AM, Mathias RA, Hancock DB, et al.: Meta-analysis of genome-wide association studies of asthma in ethnically diverse North American populations. *Nat. Genet.* 2011, 43:887–892. [PubMed: 21804549]
16. Stadhouders R, Li BWS, de Bruijn MJW, Gomez A, Rao TN, Fehling HJ, van IJcken WFJ, Lim AI, Di Santo JP, Graf T, et al.: Epigenome analysis links gene regulatory elements in group 2 innate lymphocytes to asthma susceptibility. *J. Allergy Clin. Immunol.* 2018, doi: 10.1016/j.jaci.2017.12.1006.
17. Smith SG, Chen R, Kjarsgaard M, Huang C, Oliveria J-P, O'Byrne PM, Gauvreau GM, Boulet L-P, Lemiere C, Martin J, et al.: Increased numbers of activated group 2 innate lymphoid cells in the airways of patients with severe asthma and persistent airway eosinophilia. *J. Allergy Clin. Immunol.* 2016, 137:75–86.e8. [PubMed: 26194544]

18. Christianson CA, Goplen NP, Zafar I, Irvin C, Good JT, Rollins DR, Gorenz B, Liu W, Gorska MM, Chu H, et al.: Persistence of asthma requires multiple feedback circuits involving type 2 innate lymphoid cells and IL-33. *J. Allergy Clin. Immunol.* 2015, 136:59–68.e14. [PubMed: 25617223]
19. Salimi M, Barlow JL, Saunders SP, Xue L, Gutowska-Owsiak D, Wang X, Huang L-C, Johnson D, Scanlon ST, McKenzie ANJ, et al.: A role for IL-25 and IL-33-driven type- 2 innate lymphoid cells in atopic dermatitis. *J Exp Med* 2013, 210:2939–2950. [PubMed: 24323357]
20. Kim BS, Siracusa MC, Saenz SA, Noti M, Monticelli LA, Sonnenberg GF, Hepworth MR, Van Voorhees AS, Comeau MR, Artis D: TSLP elicits IL-33-independent innate lymphoid cell responses to promote skin inflammation. *Sci TranslMed* 2013, 5:170ra16–170ra16.
21. Doherty TA, Baum R, Newbury RO, Yang T, Dohil R, Aquino M, Doshi A, Walford HH, Kurten RC, Broide DH, et al.: Group 2 innate lymphocytes (ILC2) are enriched in active eosinophilic esophagitis. *J. Allergy Clin. Immunol.* 2015, 136:792–794.e3. [PubMed: 26233928]
22. Van Dyken SJ, Nussbaum JC, Lee J, Molofsky AB, Liang H-E, Pollack JL, Gate RE, Haliburton GE, Ye CJ, Marson A, et al.: A tissue checkpoint regulates type 2 immunity. *Nat. Immunol.* 2016, 17:1381–1387. [PubMed: 27749840] ***This paper demonstrates striking transcriptional and functional similarities between tissue Th2 cells and ILC2s, that Th2 cells can differentiate independently of ILC2s, and that both populations are dependent on tissue-derived ‘epithelial’ cytokines for terminal differentiation.
23. Bartemes KR, Iijima K, Kobayashi T, Kephart GM, McKenzie AN, Kita H: IL-33- responsive lineage- CD25+ CD44(hi) lymphoid cells mediate innate type 2 immunity and allergic inflammation in the lungs. *J Immunol* 2012, 188:1503–1513. [PubMed: 22198948]
24. Halim TYF, Krauss RH, Sun AC, Takei F: Lung natural helper cells are a critical source of Th2 cell-type cytokines in protease allergen-induced airway inflammation. *Immunity* 2012, 36:451–463. [PubMed: 22425247]
25. Mjösberg J, Bernink J, Golebski K, Karrich JJ, Peters CP, Blom B, Velde te AA, Fokkens WJ, van Drunen CM, Spits H: The transcription factor GATA3 is essential for the function of human type 2 innate lymphoid cells. *Immunity* 2012, 37:649–659. [PubMed: 23063330]
26. Moltke von J, O’Leary CE, Barrett NA, Kanaoka Y, Austen KF, Locksley RM: Leukotrienes provide an NFAT-dependent signal that synergizes with IL-33 to activate ILC2s. *J Exp Med* 2017, 214:27–37. [PubMed: 28011865] **This paper recognizes leukotriene signaling as a non-redundant signal that initiates and amplifies ILC2- driven type 2 inflammation in the lung via activation of NFAT
27. ***Klose CSN, Mahlakoiv T, Moeller JB, Rankin LC, Flamar A-L, Kabata H, Monticelli LA, Moriyama S, Putzel GG, Rakhilin N, et al.: The neuropeptide neuromedin U stimulates innate lymphoid cells and type 2 inflammation. *Nature* 2017, 549:282–286. [PubMed: 28869965]
28. ***Cardoso V, Chesne J, Ribeiro H, Garcia-Cassani B, Carvalho T, Bouchery T, Shah K, Barbosa-Morais NL, Harris N, Veiga-Fernandes H: Neuronal regulation of type 2 innate lymphoid cells via neuromedin U. *Nature* 2017, 549:277–281. [PubMed: 28869974]
29. Wallrapp A, Riesenfeld SJ, Burkett PR, Abdulnour R-EE, Nyman J, Dionne D, Hofree M, Cuoco MS, Rodman C, Farouq D, et al.: The neuropeptide NMU amplifies ILC2- driven allergic lung inflammation. *Nature* 2017, 549:351–356. [PubMed: 28902842] ***These three papers report that ILC2s can be activated by the cholinergic neuropeptide NMU to promote type 2 responses in the lung and intestine.
30. Sui P, Wiesner DL, Xu J, Zhang Y, Lee J, Van Dyken S, Lashua A, Yu C, Klein BS, Locksley RM, et al.: Pulmonary neuroendocrine cells amplify allergic asthma responses. *Science* 2018, 127:eaan8546. **This paper identifies pulmonary neuroendocrine cells in the lung as a novel amplifier of ILC2-driven allergic inflammation via production of CGRP.
31. Yu X, Pappu R, Ramirez-Carrozzi V, Ota N, Caplazi P, Zhang J, Yan D, Xu M, Lee WP, Grogan JL: TNF superfamily member TL1A elicits type 2 innate lymphoid cells at mucosal barriers. *Mucosal Immunol* 2014, 7:730–740. [PubMed: 24220298]
32. Barlow JL, Peel S, Fox J, Panova V, Hardman CS, Camelo A, Bucks C, Wu X, Kane CM, Neill DR, et al.: IL-33 is more potent than IL-25 in provoking IL-13-producing nuocytes (type 2 innate lymphoid cells) and airway contraction. *J. Allergy Clin. Immunol.* 2013, 132:933–941. [PubMed: 23810766]

33. Molofsky AB, Nussbaum JC, Liang H-E, Van Dyken SJ, Cheng LE, Mohapatra A, Chawla A, Locksley RM: Innate lymphoid type 2 cells sustain visceral adipose tissue eosinophils and alternatively activated macrophages. *J Exp Med* 2013, 210:535–549. [PubMed: 23420878]
34. Dalmas E, Lehmann FM, Dror E, Wueest S, Thienel C, Borsigova M, Stawiski M, Traunecker E, Lucchini FC, Dapito DH, et al.: Interleukin-33-Activated Islet-Resident Innate Lymphoid Cells Promote Insulin Secretion through Myeloid Cell Retinoic Acid Production. *Immunity* 2017, 47:928–942.e7. [PubMed: 29166590]
35. Oboki K, Ohno T, Kajiwarra N, Arae K, Morita H, Ishii A, Nambu A, Abe T, Kiyonari H, Matsumoto K, et al.: IL-33 is a crucial amplifier of innate rather than acquired immunity. *Proc. Natl. Acad. Sci. U.S.A.* 2010, 107:18581–18586. [PubMed: 20937871]
36. Van Dyken SJ, Mohapatra A, Nussbaum JC, Molofsky AB, Thornton EE, Ziegler SF, McKenzie ANJ, Krummel MF, Liang H-E, Locksley RM: Chitin activates parallel immune modules that direct distinct inflammatory responses via innate lymphoid type 2 and $\gamma\delta$ T cells. *Immunity* 2014, 40:414–424. [PubMed: 24631157]
37. Lefran9ais E, Duval A, Mirey E, Roga S, Espinosa E, Cayrol C, Girard J-P: Central domain of IL-33 is cleaved by mast cell proteases for potent activation of group-2 innate lymphoid cells. *Proc. Natl. Acad. Sci. U.S.A.* 2014, 111:15502–15507. [PubMed: 25313073]
38. Cayrol C, Duval A, Schmitt P, Roga S, Camus M, Stella A, Burlet-Schiltz O, Gonzalez-de-Peredo A, Girard J-P: Environmental allergens induce allergic inflammation through proteolytic maturation of IL-33. *Nat. Immunol.* 2018, 19:375–385. [PubMed: 29556000] ***This paper is the first to report that allergen proteases cleaves and activates full-length IL-33 to increase its biological activity and induce allergic inflammation.
39. ***Howitt MR, Lavoie S, Michaud M, Blum AM, Tran SV, Weinstock JV, Gallini CA, Redding K, Margolskee RF, Osborne LC, et al.: Tuft cells, taste-chemosensory cells, orchestrate parasite type 2 immunity in the gut. *Science* 2016, 351:1329–1333. [PubMed: 26847546]
40. ***Moltke von J, Ji M, Liang H-E, Locksley RM: Tuft-cell-derived IL-25 regulates an intestinal ILC2-epithelial response circuit. *Nature* 2016, 529:221–225. [PubMed: 26675736]
41. Gerbe F, Sidot E, Smyth DJ, Ohmoto M, Matsumoto I, Dardalhon V, Cesses P, Garnier L, Pouzolles M, Brulin B, et al.: Intestinal epithelial tuft cells initiate type 2 mucosal immunity to helminth parasites. *Nature* 2016, 529:226–230. [PubMed: 26762460] ***These three papers identified epithelial tuft cells as the major, critical producers of IL-25, acting with ILC2 to promote intestinal type 2 immunity.
42. Takai T: TSLP expression: cellular sources, triggers, and regulatory mechanisms. *AllergolInt* 2012, 61:3–17.
43. Wojno EDT, Monticelli LA, Tran SV, Alenghat T, Osborne LC, Thome JJ, Willis C, Budelsky A, Farber DL, Artis D: The prostaglandin D2 receptor CRTH2 regulates accumulation of group 2 innate lymphoid cells in the inflamed lung. *Mucosal Immunol* 2015, 8:1313–1323. [PubMed: 25850654]
44. Barnig C, Cernadas M, Dutilleul S, Liu X, Perrella MA, Kazani S, Wechsler ME, Israel E, Levy BD: Lipoxin A4 regulates natural killer cell and type 2 innate lymphoid cell activation in asthma. *Sci TranslMed* 2013, 5:174ra26–174ra26.
45. Lund SJ, Portillo A, Cavagnero K, Baum RE, Naji LH, Badrani JH, Mehta A, Croft M, Broide DH, Doherty TA: Leukotriene C4 Potentiates IL-33-Induced Group 2 Innate Lymphoid Cell Activation and Lung Inflammation. *J Immunol* 2017, 199:1096–1104. [PubMed: 28667163]
46. Verma AK, Manohar M, Upparahalli Venkateshaiah S, Mishra A: Neuroendocrine cells derived chemokine vasoactive intestinal polypeptide (VIP) in allergic diseases. *Cytokine Growth Factor Rev.* 2017, 38:37–48. [PubMed: 28964637]
47. Moriyama S, Brestoff JR, Flamar A-L, Moeller JB, Klose CSN, Rankin LC, Yudanin NA, Monticelli LA, Putzel GG, Rodewald H-R, et al.: β 2-adrenergic receptor-mediated negative regulation of group 2 innate lymphoid cell responses. *Science* 2018, 359:1056–1061. [PubMed: 29496881] ***This paper reports the negative regulation of ILC2 by catecholamines, a neuronally-derived signal in the context of type 2 responses.
48. Maazi H, Banie H, Aleman Muench GR, Patel N, Wang B, Sankaranarayanan I, Bhargava V, Sato T, Lewis G, Cesaroni M, et al.: Activated plasmacytoid dendritic cells regulate type 2 innate

- lymphoid cell-mediated airway hyperreactivity. *J. Allergy Clin. Immunol.* 2018, 141:893–905.e6. [PubMed: 28579374]
49. Molofsky AB, Van Gool F, Liang H-E, Van Dyken SJ, Nussbaum JC, Lee J, Bluestone JA, Locksley RM: Interleukin-33 and Interferon- γ Counter-Regulate Group 2 Innate Lymphoid Cell Activation during Immune Perturbation. *Immunity* 2015, 43:161–174. [PubMed: 26092469]
 50. Moro K, Kabata H, Tanabe M, Koga S, Takeno N, Mochizuki M, Fukunaga K, Asano K, Betsuyaku T, Koyasu S: Interferon and IL-27 antagonize the function of group 2 innate lymphoid cells and type 2 innate immune responses. *Nat. Immunol.* 2016, 17:76–86. [PubMed: 26595888]
 51. Bi J, Cui L, Yu G, Yang X, Chen Y, Wan X: NK Cells Alleviate Lung Inflammation by Negatively Regulating Group 2 Innate Lymphoid Cells. *J Immunol* 2017, 198:3336–3344. [PubMed: 28275135]
 52. Han M, Hong JY, Jaipalli S, Rajput C, Lei J, Hinde JL, Chen Q, Hershenson NM, Bentley JK, Hershenson MB: IFN- γ Blocks Development of an Asthma Phenotype in Rhinovirus-Infected Baby Mice by Inhibiting Type 2 Innate Lymphoid Cells. *Am. J. Respir. Cell Mol. Biol.* 2017, 56:242–251.
 53. Laffont S, Blanquart E, Savignac M, Cenac C, Laverny G, Metzger D, Girard J-P, Belz GT, Pelletier L, Seillet C, et al.: Androgen signaling negatively controls group 2 innate lymphoid cells. *J Exp Med* 2017, 214:1581–1592. [PubMed: 28484078]
 54. Cephus J-Y, Stier MT, Fuseini H, Yung JA, Toki S, Bloodworth MH, Zhou W, Goleniewska K, Zhang J, Garon SL, et al.: Testosterone Attenuates Group 2 Innate Lymphoid Cell-Mediated Airway Inflammation. *Cell Rep* 2017, 21:2487–2499. [PubMed: 29186686]
 55. Krishnamoorthy N, Burkett PR, Dalli J, Abdunour R-EE, Colas R, Ramon S, Phipps RP, Petasis NA, Kuchroo VK, Serhan CN, et al.: Cutting edge: maresin-1 engages regulatory T cells to limit type 2 innate lymphoid cell activation and promote resolution of lung inflammation. *J Immunol* 2015, 194:863–867. [PubMed: 25539814]
 56. Zhou W, Toki S, Zhang J, Goleniewska K, Newcomb DC, Cephus JY, Dulek DE, Bloodworth MH, Stier MT, Polosuhkin V, et al.: Prostaglandin I₂ Signaling and Inhibition of Group 2 Innate Lymphoid Cell Responses. *Am. J. Respir. Crit. Care Med.* 193:31–42. [PubMed: 26378386]
 57. Taylor S, Huang Y, Mallett G, Stathopoulou C, Felizardo TC, Sun M-A, Martin EL, Zhu N, Woodward EL, Elias MS, et al.: PD-1 regulates KLRG1+group 2 innate lymphoid cells. *J Exp Med* 2017, 214:1663–1678. [PubMed: 28490441]
 58. Saluzzo S, Gorki A-D, Rana BMJ, Martins R, Scanlon S, Starkl P, Lakovits K, Hladik A, Korosec A, Sharif O, et al.: First-Breath-Induced Type 2 Pathways Shape the Lung Immune Environment. *Cell Rep* 2017, 18:1893–1905. [PubMed: 28228256]
 59. de Kleeer IM, Kool M, de Bruijn MJW, Willart M, van Moorleghem J, Schuijs MJ, Plantinga M, Beyaert R, Hams E, Fallon PG, et al.: Perinatal Activation of the Interleukin-33 Pathway Promotes Type 2 Immunity in the Developing Lung. *Immunity* 2016, 45:1285–1298. [PubMed: 27939673]
- **These two papers demonstrate that ILC2s are part of a postnatal type 2 developmental wave in the lung, which is sensitive to external signals and may increase susceptibility to allergic inflammation later in life.
60. Steer CA, Martinez-Gonzalez I, Ghaedi M, Allinger P, Matha L, Takei F: Group 2 innate lymphoid cell activation in the neonatal lung drives type 2 immunity and allergen sensitization. *J. Allergy Clin. Immunol.* 2017, 140:593–595.e3. [PubMed: 28216436]
 61. Cheon IS, Son YM, Jiang L, Goplen NP, Kaplan MH, Limper AH, Kita H, Paczesny S, Prakash YS, Tepper R, et al.: Neonatal hyperoxia promotes asthma-like features through IL-33-dependent ILC2 responses. *J. Allergy Clin. Immunol.* 2017, doi:10.1016/j.jaci.2017.11.025.
 62. Halim TYF, Steer CA, Matha L, Gold MJ, Martinez-Gonzalez I, McNagny KM, McKenzie ANJ, Takei F: Group 2 innate lymphoid cells are critical for the initiation of adaptive T helper 2 cell-mediated allergic lung inflammation. *Immunity* 2014, 40:425–435. [PubMed: 24613091]
 63. Morita H, Arae K, Unno H, Miyauchi K, Toyama S, Nambu A, Oboki K, Ohno T, Motomura K, Matsuda A, et al.: An Interleukin-33-Mast Cell-Interleukin-2 Axis Suppresses Papain-Induced Allergic Inflammation by Promoting Regulatory T Cell Numbers. *Immunity* 2015, 43:175–186. [PubMed: 26200013]

64. Malhotra N, Leyva-Castillo JM, Jadhav U, Barreiro O, Kam C, O'Neill NK, Meylan F, Chambon P, Andrian Von UH, Siegel RM, et al.: ROR α -expressing T regulatory cells restrain allergic skin inflammation. *Sci Immunol* 2018, 3:eaa06923. [PubMed: 29500225]
65. Rigas D, Lewis G, Aron JL, Wang B, Banie H, Sankaranarayanan I, Galle-Treger L, Maazi H, Lo R, Freeman GJ, et al.: Type 2 innate lymphoid cell suppression by regulatory T cells attenuates airway hyperreactivity and requires inducible T-cell costimulator-inducible T-cell costimulator ligand interaction. *J. Allergy Clin. Immunol.* 2017, 139:1468–1477.e2. [PubMed: 27717665]
66. Brestoff JR, Kim BS, Saenz SA, Stine RR, Monticelli LA, Sonnenberg GF, Thome JJ, Farber DL, Lutfy K, Seale P, et al.: Group 2 innate lymphoid cells promote beiging of white adipose tissue and limit obesity. *Nature* 2015, 519:242–246. [PubMed: 25533952]
67. Lee M-W, Odegaard JI, Mukundan L, Qiu Y, Molofsky AB, Nussbaum JC, Yun K, Locksley RM, Chawla A: Activated type 2 innate lymphoid cells regulate beige fat biogenesis. *Cell* 2015, 160:74–87. [PubMed: 25543153]
68. Gadani SP, Smirnov I, Smith AT, Overall CC, Kipnis J: Characterization of meningeal type 2 innate lymphocytes and their response to CNS injury. *J Exp Med* 2017, 214:285–296. [PubMed: 27994070] **This paper identifies and characterizes ILC2s in the brain meninges, and describes a functional role for ILC2s following CNS injury.
69. McHedlidze T, Waldner M, Zopf S, Walker J, Rankin AL, Schuchmann M, Voehringer D, McKenzie ANJ, Neurath MF, Pflanz S, et al.: Interleukin-33-dependent innate lymphoid cells mediate hepatic fibrosis. *Immunity* 2013, 39:357–371. [PubMed: 23954132]
70. Bartemes K, Chen C-C, Iijima K, Drake L, Kita H: IL-33-Responsive Group 2 Innate Lymphoid Cells Are Regulated by Female Sex Hormones in the Uterus. *J Immunol* 200:229–236. [PubMed: 29133293]
71. Doisne J-M, Balmas E, Boulenuar S, Gaynor LM, Kieckbusch J, Gardner L, Hawkes DA, Barbara CF, Sharkey AM, Brady HJM, et al.: Composition, Development, and Function of Uterine Innate Lymphoid Cells. *J Immunol* 2015, 195:3937–3945. [PubMed: 26371244]
72. Vachier I, Chavis C, Majori M, Farce M, Bousquet J, Godard P, Chanez P: Effects of glucocorticoids on endogenous and transcellular metabolism of eicosanoids in asthma. *J. Allergy Clin. Immunol.* 2001, 107:824–831. [PubMed: 11344349]
73. Barnes PJ: Targeting cytokines to treat asthma and chronic obstructive pulmonary disease. *Nat. Rev. Immunol.* 2018, doi:10.1038/s41577-018-0006-6.