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CURRENT OPINION

Type-2 innate lymphoid cells in human allergic disease

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Purpose of review
Recent decades have seen allergic diseases become endemic in a number of developed countries. Understanding the inflammatory processes that dictate these allergic responses is therefore important.

Recent findings
Critical to many allergic responses is the inappropriate release of the type-2 immune-regulatory cytokines: interleukin-4, interleukin-5, interleukin-9, and interleukin-13. The study of these inflammatory mediators has led directly to the development of two new asthma treatments: anti-interleukin-5 and anti-interleukin-13. Until recently, T helper 2 cells were considered to be the major cellular source of type-2 cytokines; however, a paradigm shift occurred with the discovery of a novel population, type-2 innate lymphoid cells (ILC2s), that can produce huge levels of type-2 cytokines and are sufficient to induce allergy in mice. This discovery raises interesting questions about how innate and adaptive type-2 immunity might interact to induce relapsing and remitting episodes of allergy in patients.

Summary
It is essential that alongside the mechanistic investigation using model organisms, the roles of ILC2s in human disease be explored. Here, we discuss how ILC2 traits, discovered in mouse models, have informed research in humans and how newly identified human ILC2 pathways might provide potential therapeutic benefits in the future.

Keywords
allergy, IL-13, IL-25, IL-33, innate lymphoid cell

INTRODUCTION

The type-2 cytokines, interleukin (IL)-4, IL-5, IL-9, and IL-13, are the major drivers of allergic asthma and studies [1–4], in both mice and humans, have indicated that they regulate many inflammatory processes, including bronchoconstriction, mucus production, eosinophilia, and immunoglobulin E class switching by B cells. This understanding has been essential for the development of two new potential asthma treatments, lebrikizumab (anti-IL-13) and mepolizumab (anti-IL-5) [5,6]. The primary source of type-2 cytokines was initially considered to be adaptive T helper 2 (Th2) cells, and early human studies demonstrated that these cells were present during allergic asthma [7]. However, an elegant study by Voehringer *et al.* [8] using mice gave an initial indication that although $CD4^+$ T cells were essential for the experimental allergic response, the production of IL-4 and IL-13 by these cells was not, suggesting the existence of another important cytokine source. Subsequent landmark publications characterized these innate type-2 cytokine producing cells, which were variably called nuocytes, innate helper 2 cells, and natural helper cells, and are now termed type-2 innate lymphoid cells (ILC2s) [9–14]. Further ILC populations have also been described and a new nomenclature has evolved to define these populations alongside ILC2s, which also includes natural killer (NK), lymphoid tissue inducer cells, ILC1s, and ILC3s (Fig. 1) [12]. ILC2s are defined by their responsiveness to IL-25 [13–15], IL-33 [16,17], and thymic stromal lymphopoietin (TSLP) [18,19▪▪,20▪]; lack of mature lineage markers (CD3, CD4, CD8,

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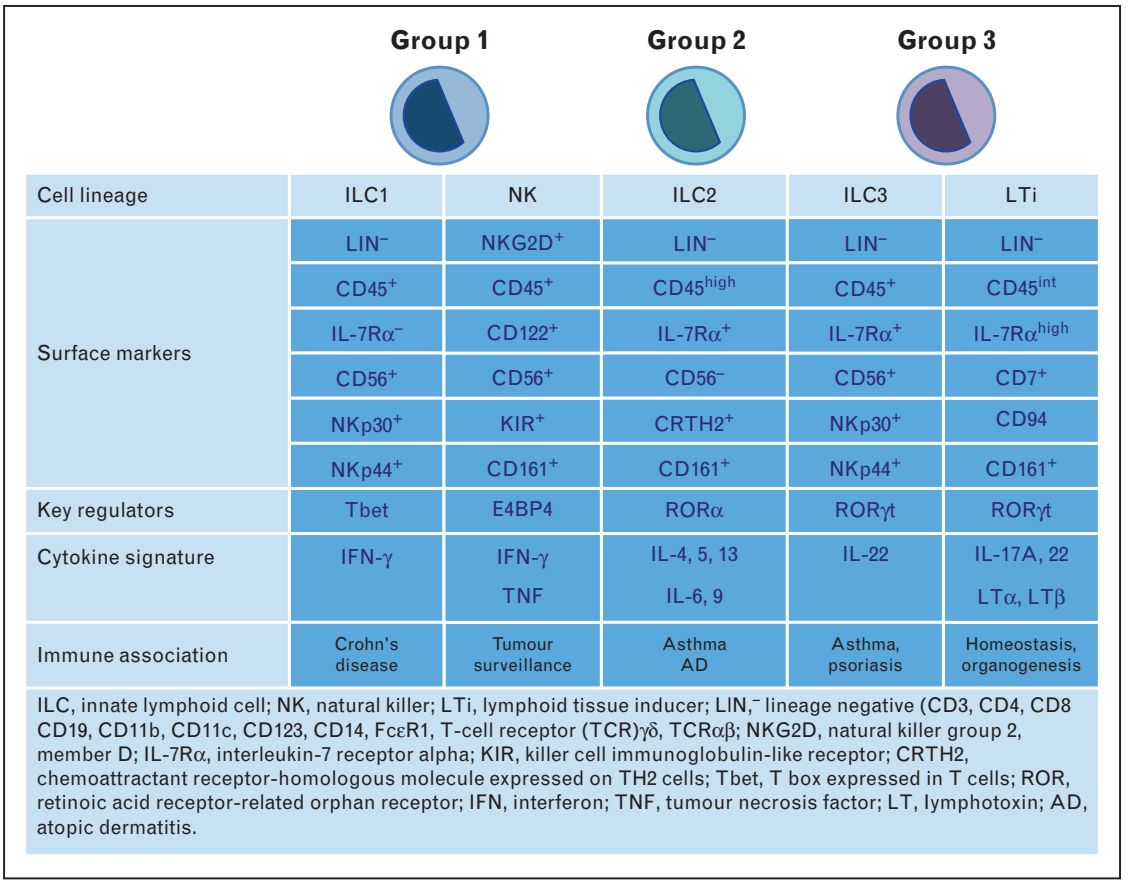
KEY POINTS

- Experimental allergic models in mice have identified an innate lymphoid cell (ILC2) that contributes an important source of type-2 cytokines during a type-2 immune response.
- Human ILC2s can be found in the gut, lung, skin and blood, and are defined as lineage$^-$CD127$^+$CRTH2$^+$ cells.
- The frequency of ILC2s is significantly increased in lesional skin taken from atopic dermatitis patients.

CD19, CD11b, CD11c, FcεR1, Gr1, and NK1.1); and by their expression of a number of lymphoid lineage-associated cell-surface markers [CD127, inducible T-cell co-stimulator (ICOS), ST2, IL-25 receptor (IL-17BR), CD25, CD117, killer cell lectin-like receptor G1 (KLRG1)] and cytokines (IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13). Experiments with retinoid-related orphan receptor α (*Rora*)$^{sg/sg}$ [21], GATA-binding protein 3 (*Gata3*)$^{fl/fl}$ [22,23], and *Tcf7*$^{-/-}$ [24] mice have also shown these transcription factors are essential for ILC2 differentiation and maturation. Functional studies [25–29,30▪] have further identified ILC2s as necessary for type-2 inflammation and airways hyperreactivity in a number of different experimental models. Importantly, following the discovery of ILC2s in mice, they have also been identified in humans. As in mice, human ILC2s lack mature lineage markers (lineage, lin$^-$) and express IL-13 in response to IL-25 and IL-2 [31]. However, these cells additionally express CD161 (C-type lectin receptor) and CRTH2 (prostaglandin D2 receptor). Human ILC2s were found at fetal and adult mucosal surfaces, including the lung and gut, and were also enriched in nasal polyps taken from patients with chronic rhinosinusitis. The potential contribution of ILC2s to allergic asthma is also highlighted by the reassessment of a genome-wide association study [32] of 10 365 asthmatic patients and 16 110 healthy controls, which showed a number of single-nucleotide polymorphisms in ILC2-related genes to be associated with asthma, including *RORA*, *IL13*, *IL1RL1* (IL33 receptor), and *IL33*. Thus, early mouse



	Group 1		Group 2	Group 3	
Cell lineage	ILC1	NK	ILC2	ILC3	LTi
Surface markers	LIN$^-$	NKG2D$^+$	LIN$^-$	LIN$^-$	LIN$^-$
	CD45$^+$	CD45$^+$	CD45^{high}	CD45$^+$	CD45^{int}
	IL-7Rα$^-$	CD122$^+$	IL-7Rα$^+$	IL-7Rα$^+$	IL-7Rα^{high}
	CD56$^+$	CD56$^+$	CD56$^-$	CD56$^+$	CD7$^+$
	NKp30$^+$	KIR$^+$	CRTH2$^+$	NKp30$^+$	CD94
	NKp44$^+$	CD161$^+$	CD161$^+$	NKp44$^+$	CD161$^+$
Key regulators	Tbet	E4BP4	RORα	RORγt	RORγt
Cytokine signature	IFN-γ	IFN-γ TNF	IL-4, 5, 13 IL-6, 9	IL-22	IL-17A, 22 LTα, LTβ
Immune association	Crohn's disease	Tumour surveillance	Asthma AD	Asthma, psoriasis	Homeostasis, organogenesis

ILC, innate lymphoid cell; NK, natural killer; LTi, lymphoid tissue inducer; LIN,$^-$ lineage negative (CD3, CD4, CD8 CD19, CD11b, CD11c, CD123, CD14, FcεR1, T-cell receptor (TCR)γδ, TCRαβ; NKG2D, natural killer group 2, member D; IL-7Rα, interleukin-7 receptor alpha; KIR, killer cell immunoglobulin-like receptor; CRTH2, chemoattractant receptor-homologous molecule expressed on TH2 cells; Tbet, T box expressed in T cells; ROR, retinoic acid receptor-related orphan receptor; IFN, interferon; TNF, tumour necrosis factor; LT, lymphotoxin; AD, atopic dermatitis.

FIGURE 1. A nomenclature for the ILCs. Innate cells from the lymphoid lineage, that is, those that do not express T-cell receptor (TCR), have now been classified as group 1 ILCs containing ILC1s and NK cells, group 2 ILCs, and group 3 ILCs containing ILC3s and LTi cells. They are all defined by their cell-surface marker expression, transcriptional regulation, and cytokine signature. Early studies have also begun to define what types of immune response each cell type is associated with.

and human studies have led to a good understanding of ILC2 phenotype and function. In order to realize the potential therapeutic benefits of studying ILC2 biology, more human studies will be required. This review aims to summarize the more recent research on human ILC2 biology.

EVIDENCE FOR THE EXISTENCE OF INNATE LYMPHOID 2 CELLS IN HUMAN DISEASE

Following on from the initial discovery of ILC2s in human nasal polyps, the lung, and the gut, a number of publications have also now identified similar ILC2 populations (lineage$^-$CD127$^+$CRTH2$^+$) in naïve peripheral blood [31,33]. The expression of the transcription factors GATA3 and ETS-1 (E26 avian leukaemia oncogene 1, 5′ domain) was noted and an analogous GATA3$^+$ETS-1$^+$ ILC2 population was found in the mouse lung after challenge with *Alternaria alternata* [34]. Along with IL-7Rα (CD127), the expression of ETS-1 and GATA3, which have previously been shown to be necessary for IL-2 and IL-13 expression in T cells, respectively [35,36], implies that human ILC2s, like mouse ILC2s are of lymphoid origin. Notably, overexpression of GATA3 in human ILC2s *in vitro* induced *IL1RL1* and *TSLPR* (TSLP receptor) expression and increased IL-4, IL-5, IL-13, and granulocyte/monocyte-colony stimulating factor (GM-CSF) production compared with controls [37]. GATA3 silencing also reduced IL-13 production, *in vitro*, suggesting that its modulation in ILC2s is important for type-2 cytokine release. Several mouse studies [23,38] have also shown GATA3 to be essential for a bone-marrow-derived ILC2 progenitor (Lin$^-$Sca1^{high}Id2^{high}GATA3^{high}) and subsequent population of peripheral tissues with mature ILC2s, with chimaeric mice receiving GATA3-deficient bone marrow unable to expand ILC2s. Although GATA3 is functionally important in mature ILC2s, human ILC2 progenitor cells have yet to be identified. GATA3 is also essential for the development of ILC1s and ILC3s [39,40].

Investigations in mice and humans have indicated that ILC2s represent a small fraction of lymphocytes in the blood, but can be found at greater frequency at mucosal barriers where they can release type-2 cytokines at the initiation of an ongoing immune response [9,10]. Thus, the most informative insight into human ILC2 function will likely necessitate the study of these cells at the site of disease. Indeed, the most recently published studies have focussed on skin punch biopsies from atopic dermatitis sufferers and healthy controls. Skin ILC2s were first described as lin$^-$CD25$^+$ST2$^+$ and were found to be enriched in skin from atopic dermatitis patients [19▪▪]. In addition to the 'signature' ILC2 markers (CD25, CD117, ICOS, CD161, CRTH2, and GATA3) [41▪▪], further studies have shown the expression of chemokine (C–C motif) receptor (CCR)4, CCR10, and *AMPHIREGULIN* (*AREG*) and *RORA* gene expression on ILC2s in normal skin [20▪]. CCR4 and CCR10 are both expressed on T cells and are important for skin and lung homing [42,43▪]. Their ligands, TARC (thymus and activation-regulated chemokine, CCL17) and CTACK (cutaneous T-cell attracting chemokine, CCL27), respectively, are essential for the distribution of T cells during immune responses. Thus, the expression of these chemokine receptors on ILC2s suggests that they are also capable of trafficking to the lung as well as the skin, and implies that they are influenced by similar chemotactic signals as T cells.

Interestingly, Salimi *et al.* also provided data to suggest differences in skin versus blood ILC2 number and phenotype. In healthy controls and atopic dermatitis patients, ILC2s were significantly more frequent in the skin than in the blood, with *GATA3* and *RORA* also being more highly expressed. The phenotype of skin ILC2s also seemed to change in the allergic disease state. Total ILC2 percentages were increased in atopic dermatitis patients versus healthy controls, as was cell-surface expression of IL-33R, IL-17BR, TSLPR, and KLRG1. This implies that in atopic dermatitis patients, the ILC2s in the skin are much more receptive to IL-33, IL-25, TSLP, and interactions with keratinocytes via E-cadherin and KLRG1, perhaps having an 'inflammatory phenotype' which might lead to increased type-2 cytokine production. Although KLRG1 (also expressed on NK cells) has been used as a marker of GATA3^{high}, type-2 cytokine-expressing mouse ILC2s [23], its function is unknown. However, treatment of human skin ILC2s *in vitro* with E-cadherin downregulated type-2 cytokine production and cell proliferation, this is noteworthy as a lack of E-cadherin on keratinocytes is associated with lesional skin in atopic dermatitis [20▪,44]. Thus, engagement of KLRG1 on ILC2s by E-cadherin on keratinocytes may serve to inhibit type-2 cytokine production and suggests a breakdown in this process in atopic dermatitis. Other phenotypic differences between 'naïve' and atopic dermatitis type ILC2s were not reported, and further experiments comparing these two populations, either by RNA sequencing techniques or by flow cytometry, may be valuable for determining ILC2 drug targets. Significantly, this study also demonstrated that ILC2s form part of the inflammatory infiltrate induced in response to allergen. SSC^{low}CD45$^+$Lin$^-$CD127$^+$CD25$^+$CRTH2$^+$ ILC2s infiltrated into the blisters raised on human skin where house dust mite had been administered

intraepidermally to allergic individuals, and their presence correlated with increased IL-4, IL-5, and IL-13 in blister serum [20▪]. Thus, ILC2s are present in the skin of atopic individuals and are capable of contributing to the type-2 cytokine response, known to drive allergic disease.

Human ILC3s have also been found in nonlesional and lesional psoriatic skin [45,46]. Although ILC2s and ILC3s appear to demarcate atopic dermatitis and psoriasis, the situation in asthma is potentially more complex as asthma exists as a spectrum of disease states. This is an important and largely unexplored area, and stratifying the cellular immune response in different asthma and allergy groups will be necessary if targeted monoclonal antibody therapy is to be successful. It is therefore interesting to speculate whether different ILC populations may be involved in different types of allergic disease, perhaps dependent on allergen, genetics, or environment. Mouse models using ragweed protein, ovalbumin, *A. alternata*, or papain suggest ILC2 pathways are sufficient to drive allergic lung responses [28,29,47]. However, obesity is a nonallergic risk factor often associated with asthma, especially in nonatopic adults that show steroid resistance. Recent data suggest that mice fed a high-fat diet (HFD) developed increased airways hyperreactivity, dependent upon IL-17A-expressing ILC3s [48▪▪]. These ILC3s were sensitive to the increased levels of IL-1β released by M1 macrophages in the lungs of obese mice, and blockade of the IL-1β signalling pathway using IL-1 receptor antagonist Anakinra was sufficient to prevent airways hyperreactivity. ILC3-like cells were also identified in the bronchoalveolar lavage of asthmatic patients. Larger patient cohorts will be necessary to determine whether there is a correlation between ILC3 numbers and specific asthma subtypes.

MODIFIERS OF TYPE-2 INNATE LYMPHOID CELL BIOLOGY

Although antibodies to block the effector cytokines IL-5 and IL-13 exist, the cytokines that regulate the growth and type-2 cytokine expression by ILC2s may also represent attractive upstream targets for preventing type-2 disease. A wealth of studies [49▪,50▪] using mice support a role for IL-25, IL-33, and TSLP in the initiation of ILC2 responses, with more molecular modifiers being reported recently in mice (Fig. 2).

Cytokines

As with earlier publications [31], the recent study [20▪] investigating skin ILC2s demonstrated the ability of human ILC2s *in vitro* to produce large amounts of IL-4, IL-5, IL-6, and IL-13. This type-2 cytokine production occurred in response to IL-33, but not in response to IL-25 or TSLP, although both these cytokines were able to act synergistically with IL-33 to increase cytokine production even further. Interestingly, IL-33 and, to a lesser extent, TSLP were also found to be chemotactic for skin ILC2s. These data are in contrast to the original finding by Mjosberg *et al.* [31], which showed ILC2s taken from fetal gut or peripheral blood could produce IL-13 in response to IL-2 in combination with either IL-25 or IL-33. It is possible that ILC2s show site-specific responses in humans, with IL-33 being more potent for skin ILC2 cytokine production. Indeed, mouse studies suggest varying roles for IL-25 and IL-33 between, and even within, tissue sites. Infection of wildtype, *Il17br*$^{-/-}$ (IL-25R), and *Il1rl1*$^{-/-}$ (IL-33R) mice with *Nippostrongylus brasiliensis* suggests that IL-25 is the necessary cytokine for driving a successful type-2 response in the gut [9]. However, using the same mouse strains in the experimental ovalbumin or ragweed pollen allergy models shows that it is the IL-33 pathway that is dominant in promoting airways hyperreactivity and contraction [47]. Significantly, some aspects of the type-2 inflammatory response, for example, ILC2 expansion and eosinophilia, required both IL-25 and IL-33. Similarly, an anti-IL-25-blocking antibody has been shown to reduce the aspects of experimental chronic lung allergy [51▪]. Continuing studies [19▪▪,20▪,52▪] with IL-25, IL-33, and TSLP pathway-deficient mouse lines in the calcipotriol-induced dermatitis model and an inhaled-chitin model suggest that all three cytokine pathways may contribute to the type-2 inflammatory response in the skin and lung, respectively, with *Il17br*$^{-/-}$ and *Il1rl1*$^{-/-}$ showing the most significant defects in ear swelling and inflammatory infiltrate. Thus, the role of the ILC2 initiating cytokines could differ depending on the tissue site or disease state, making the investigation of this question in humans key if monoclonal antibody technology is to be used to develop therapeutic antagonists to treat specific disease. Equally, it suggests that targeting just one of these cytokines for therapy may not be able to treat all types of allergic disease. Encouragingly, however, a recent study [53▪▪] looked at the effect of intravenous anti-TSLP treatment (AMG 157) prior to allergen challenge in mild asthmatic patients. The data imply that neutralization of TSLP can reverse both early and late allergic responses, including FEV1, sputum and blood eosinophilia, and exhaled nitric oxide.

TNF-like ligand 1A (TL1A, TNFSF15) was first discovered as a co-stimulatory cytokine, along with

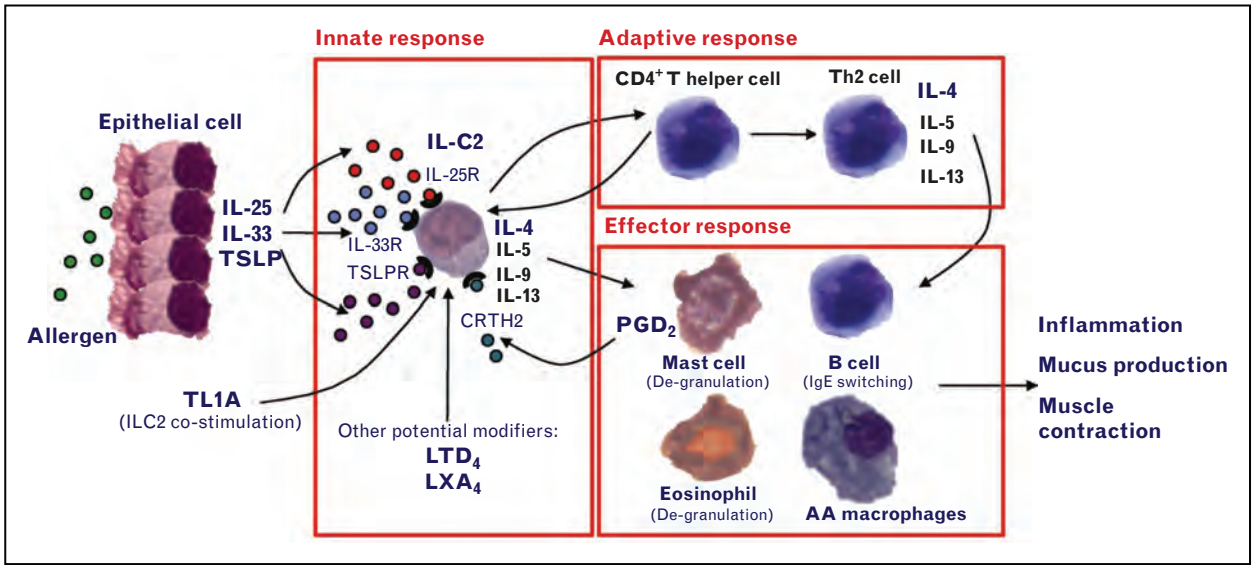


FIGURE 2. Soluble modifiers of ILC2 effector functions during allergy. Following allergen challenge at the mucosal surface release of IL-25, IL-33 and TSLP have been shown to influence ILC2 expansion and the production of the archetypal type-2 cytokines: IL-4, IL-5, and IL-13. Recently, other soluble factors such as TL1A and PGD2 have also been implicated in ILC2 activation. AA, alternatively activated; CRTH2, prostaglandin D2 receptor; IL, interleukin; LTD4, leukotriene D4; LXA4, lipoxin A4; R, receptor; Th, T helper; TSLP, thymic stromal lymphopoietin; TL1A, TNF-like ligand 1A.

other family members like OX40L, that promote T-cell activation [54]. Although T cells can develop and expand normally without TL1A, perhaps via other costimulatory pathways such as CD28 stimulation, it is thought to be important at the site of tissue inflammation for cytokine production [55]. Both mouse and human ILC2s were recently found to express the TL1A receptor (DR3) [56▪,57▪]. In *Il13*$^{eGFP/+}$ reporter mice, in which eGFP acts as a surrogate marker for IL-13 gene expression, TL1A alone was able to induce IL-13 in ILC2s *in vitro*, but not *in vivo*, whereas another study reported efficacy both *in vitro* and *in vivo* [56▪,57▪]. In both publications, however, TL1A was able to act synergistically with IL-25 and IL-33 to increase IL-13 production from ILC2s suggesting that, as with T cells, it may be an enhancer or co-stimulator of the ILC2 response. In human peripheral blood ILC2s, TL1A was unable to induce IL-5 and IL-13 production alone; however, cytokine production was significantly increased when ILC2s were treated with TL1A and IL-25 or IL-33, compared with just IL-25 or IL-33 alone [56▪].

Prostaglandins and leukotrienes

Although it is not known whether all human ILC2s express detectable levels of CRTH2 (prostaglandin D2 receptor), or whether they require this molecule for their function, it is now used routinely to identify ILC2s in human tissues [31]. CRTH2 is expressed on many other cell types, including Th2 cells, basophils, and eosinophils, which all form part of the inflammatory infiltrate observed in allergic disease [58–60]. It is therefore a candidate for potential new therapeutic treatments. Current data would, therefore, suggest that ILC2 pathways might also be susceptible to treatment with CRTH2 antagonists [61].

In-vitro treatment with prostaglandin D2 (PGD2) has been shown to induce chemotaxis and IL-13 production by human ILC2s from the peripheral blood of both allergic and nonallergic individuals, which could be prevented with a CRTH2 antagonist (BWA245C) [62▪,63]. Chemotaxis and IL-4, IL-5, and IL-13 production in response to PGD2 were also shown *in vitro* for skin-derived ILC2s and could be blocked with another CRTH2 antagonist (TM30089) [41▪▪]. Additionally, Xue *et al.* [41▪▪] showed that the ILC2 migration caused by PGD2 is significantly higher than following IL-33 treatment and that PGD2 caused skin ILC2s to secrete a number of other cytokines, including IL-8, IL-9, and GM-CSF. Co-culturing skin ILC2s with mast cell supernatant also induced type-2 cytokine production and adding the CRTH2 antagonist was able to block this, suggestive of a potential functional link between the mast cell de-granulation response and ILC2 activation and infiltration.

Few other details exist about the expression of other prostaglandin and leukotriene receptors on human ILC2s. However, cysteinyl leukotriene receptor 1 (CysLTR1) has recently been identified on the surface of mouse ILC2s taken from the lung and bone marrow [64]. The in-vitro addition of the CysLTR1 ligand leukotriene D4 (LTD4) was also able to drive IL-4, IL-5, and IL-13 production from lung ILC2s, and the CysLTR1 antagonist, montelukast, blocked this response. LTD4 also exacerbated ILC2-induced inflammation *in vivo* when given in combination with *A. alternata*.

The eicosanoid-like molecule lipoxin A4 (LXA4) might also influence ILC2 function in humans [63]. Human ILC2s from peripheral blood express the LXA4 receptor (ALX/FPR2) and culture with the anti-inflammatory molecule LXA4 inhibited their type-2 cytokine production in response to PGD2. This inhibition could itself be reversed by further addition of an ALX/FPR2 antagonist (WRW4) into the culture. This is the first report of one of these reactive species being able to dampen ILC2 responses and highlights the interesting possibility that particular eicosanoid family members might act not only to promote ILC2 responses, but also to regulate ILC2-driven immune responses.

CONCLUSION

It is now clear that ILC2s are present in numerous human tissues including peripheral blood, skin, lung, and gut in both healthy and disease states, and can contribute high levels of type-2 cytokine production following allergen exposure. This is not to say that Th2 cells are not important mediators of allergic disease, but must now also be examined in the context of ILC2s. Indeed, there appears to be co-dependency of T cells and ILC2s in the transition from innate to adaptive type-2 immunity and we are just starting to understand some of the signals involved [9,65▪▪,66▪▪]. How these interactions translate into the human system awaits further investigation and may offer additional therapeutic targets in allergic disease.

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Conflicts of interest

There are no conflicts of interest.

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- ▪▪ of outstanding interest

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