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**Review**

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Review

# Parallel origins and functions of T cells and ILCs

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## Summary

Innate lymphoid cells (ILCs) are tissue resident cells that are triggered through a relatively broad spectrum of alarmins, inflammatory cues, neuropeptides, and hormones. Functionally, ILCs are akin to subsets of helper T cells and are characterized by a similar effector cytokine profile. They also share a dependency on many of the same essential transcription factors identified for the maintenance and survival of T cells. The key distinguishing factor between the ILC family and T cells is the lack of antigen-specific T cell receptor (TCR) on ILCs and, thus, they can be considered the "ultimate invariant T cells". ILCs, like T cells, orchestrate downstream effector inflammatory responses by adjusting the cytokine microenvironment in a fashion that promotes protection, health, and homeostasis at mucosal barrier sites. But also, like T cells, ILCs have recently been implicated in several pathological inflammatory disease states. This review focuses on the selective role of ILCs in the development of allergic airway inflammation (AAI) and fibrosis in the gut where a complex ILC interplay has been shown to either attenuate or worsen disease. Finally, we discuss new data on TCR gene rearrangements in subsets of ILCs that challenge the current dogma linking their origin to committed bone marrow progenitors and instead propose a thymic origin for at least some ILCs. In addition, we highlight how naturally occurring TCR rearrangements and the expression of major histocompatibility (MHC) molecules in ILCs provide a useful natural barcode for these cells and may prove instrumental in studying their origins and plasticity.

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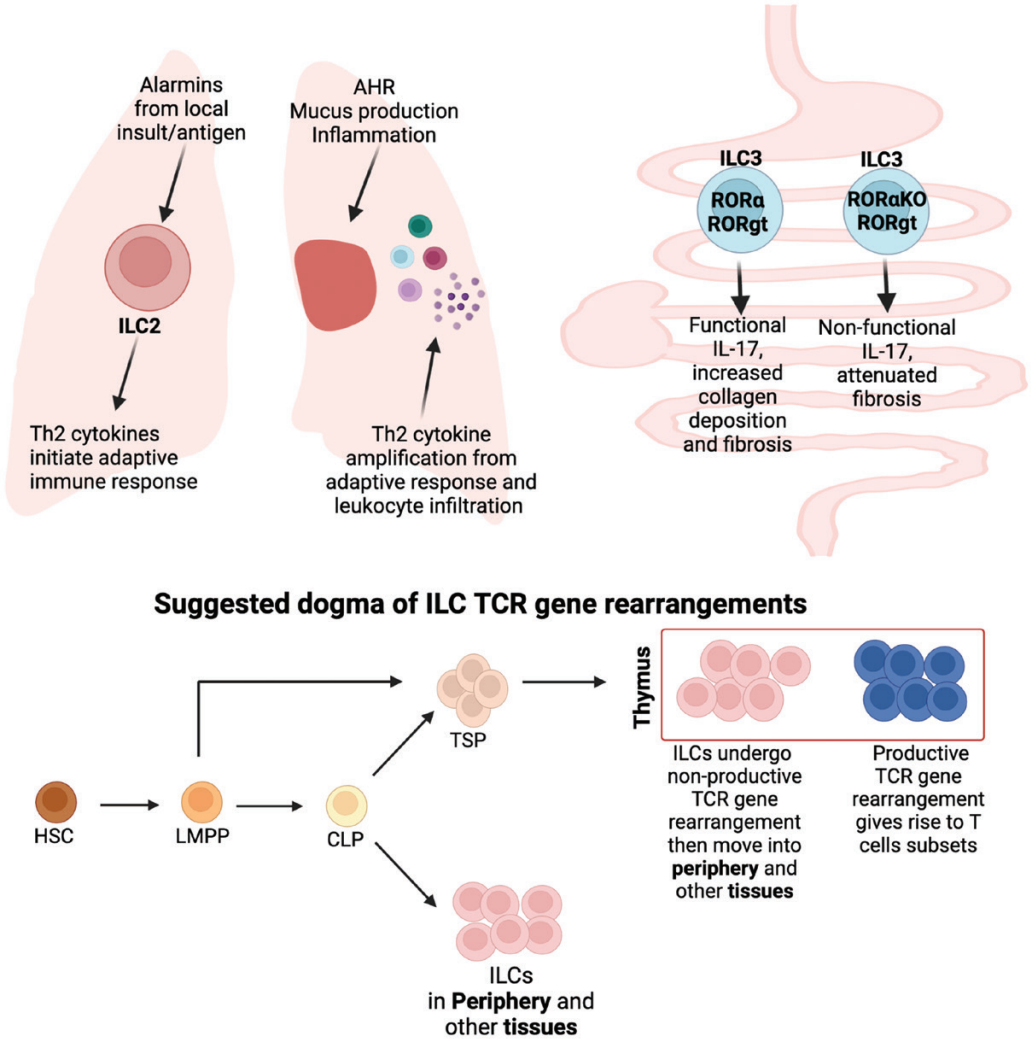
## Graphical Abstract




**Keywords:** Innate Lymphoid Cells (ILCs), Allergic Airway Inflammation (AAI), type 2 immunity, plasticity, TCR gene rearrangements, fibrosis

**Abbreviations:** AAI: allergic airway inflammation; AHR: airway hyperresponsiveness; AR: allergic rhinitis; BMT: bone marrow transfer; CD: Crohn's disease; CLP: common lymphoid progenitor; HSC: hematopoietic stem cell; IBD: irritable bowl syndrome; ILCP: innate lymphoid cell precursor; ILCS: innate lymphoid cells; INFγ: interferon gamma; LMPP: lymphoid-primed multipotent progenitor; LTi: lymphoid tissue inducer; NK: natural killer; RAG: recombinant activating gene; RORα: retinoic acid-related orphan receptor alpha; RORγt: retinoic acid-related orphan receptor gamma; Stat6: signal transducer and activator of transcription 6; Tbet: T-box transcription factor; TCR: T-cell receptor; TGFβ: transforming growth factor beta; Th1: T-helper 1/type 1; Th17: T-helper 17/type 3; Th2: T-helper 2/type 2; TNF: tumor necrosis factor; TSLP: thymic stromal lymphopoietin; TSP: thymus-seeding progenitor.

## Introduction

### ILC overview

Innate lymphoid cells (ILCs) are a potent but rare family of recently discovered tissue resident lymphocytes known to play a major role in early host defense through the initial priming of immune responses, modulation of inflammation, and the regulation of tissue homeostasis and repair [1]. There are 5 major subtypes of ILCs: natural killer (NK) cells, ILC1, ILC2, lymphoid tissue inducer (LTi) cells, and ILC3, based on their transcription factor profiles, functional characteristics, and cytokine expression profile [1, 2].

Functionally, NK cells have been likened to cytotoxic $CD8^+$ T cells due to their efficacy in granzyme dependent cell killing while ILC1, 2, and 3 are more akin to $CD4^+$ Helper T(Th) cells based on their similar dependence on a shared repertoire of transcription factors and cytokine expression profiles (Table 1) [2]. However, the unifying features of ILCs that distinguish them from other hematopoietic lineages, and specifically T cells, is their lack of dependence on recombination activating genes (RAG) for their development and their failure to express lineage-specific cell surface proteins commonly used to selectively define cells of the B-cell, T-cell, monocytic, granulocytic, dendritic, and erythroid lineages [1–5].

While serving a parallel innate function to cytotoxic $CD8^+$ T cells in their ability to kill tumors and virus infected cells, NK cells are clearly distinct in their ability to kill in the absence of expression of an antigen-specific receptor or the recognition of antigens presented by MHC class 1 molecules on their target cells [3]. These differences distinguish them as components of the innate, rather than the adaptive, immune system [3, 4]. Similarly, ILC1s are functionally and developmentally analogous to Th1 cells in their dependence on the t-box transcription factor (T-bet) for their development and to generate a functional "helper" immune response against viruses and other intracellular pathogens through

**Table 1.** Innate lymphoid cell (ILC) subsets

| ILC subset | TFs | Stimuli | Cytokine | Function | Analogous T-cell subset |
|---|---|---|---|---|---|
| NK cell | Tbet, EOMES | IL-12, IL-15, IL-18 intracellular infections | TNFα, IFNγ perforin, granzyme | Viral immunity, cancer, chronic inflammation | $CD8^+$ T cells |
| ILC1 | Tbet | IL-12, IL-15, IL-18 | TNFα, IFNγ | Viral immunity, intracellular pathogens, chronic inflammation | Th1 cells |
| ILC2 | RORα GATA3 | IL-25, IL-33, TSLP, large parasite, tissue damage | IL-4, IL-5, IL-13, IL-9, Areg | Helminths, asthma, allergic disease, tissue repair | Th2 cells |
| LTI | RORγt | IL-1β, IL-23 TGFβ extracellular infections | IL-17, IL-22 | Lymphoid tissue development | Undetermined |
| ILC3 | RORγt | IL-1β, IL-23 TGFβ extracellular infections | IL-17, IL-22 | Extracellular bacteria, tissue repair, chronic inflammation | Th17 cells |

The transcription factors (TFs), activation stimuli, effector cytokines, and functions are summarized.

the secretion of Th1-associated cytokines interferon gamma (IFNγ) and tumor necrosis factor alpha (TNFα) [2, 3]. ILC2s, in contrast, depend on the retinoic acid-receptor-related orphan nuclear receptor alpha (RORα) and GATA-binding protein 3 (GATA3) transcription factors (TFs) for development [2, 6]. Like Th2 cells they are involved in protection against helminths, allergic inflammation and tissue repair and tissue remodeling through the production of Th2-associated cytokines IL-4, IL-5, IL-9, and IL-13 [6]. Finally, LTi cells and ILC3s are more Th17-like and known for their ability to promote lymphoid organogenesis during development and antibacterial responses, respectively [2]. For their development and function LTi and ILC3s are critically dependent on the Th17 cell-linked transcription factor RORγt. Consistent with their developmental role in formation of secondary lymphoid structures, LTi cells express the key cytokines lymphotoxin alpha and beta (LT-α/ LT-β) as well as IL-17 and IL-22 [2]. ILC3s, in contrast, play key roles in gut homeostasis, antibacterial responses, chronic inflammation, remodeling, and repair through the production of IL-17A, IL-17F, and IL-22 [2, 6].

ILCs are most commonly found at mucosal barrier surfaces and respond rapidly to a variety of environmental stimuli to initiate appropriate immune responses prior to the initiation of an antigen-specific adaptive response [2, 3]. Despite this and due to their relatively recent discovery, the respective role of ILCs and T cells in immune responses has been controversial and it has been argued that ILCs may, in fact, be largely or fully redundant [7–9]. In this review, we highlight our own studies and those of others that delineate the selective role of ILCs in two disease contexts: airway allergy disease and Crohn's disease-like intestinal fibrosis. We discuss how the cytokine environment and ILC plasticity can influence disease pathology. Furthermore, we highlight recent evidence for a parallel pathway of ILC and T-cell development in the thymus and new strategies for the selective ILC lineage tracing and functional targeting of ILCs as tools for their study.

## ILCS in allergic disease

Allergic airway inflammation (AAI) is a chronic inflammatory disease whose prevalence has increased by 28% between 2001 and 2011 [10]. In 2007, the costs in lost productivity and hospitalizations associated with allergic asthma in the USA, alone, were estimated at $56 billion; a 6% increase [11]. Current treatments are largely non-targeted and include corticosteroids and long-acting β-agonists which only address disease symptoms but not the underlying immune dysregulation [12]. Hence, although emphasis has been placed on developing therapeutics that target downstream effectors of the allergic response, there is an urgent need to better understand the upstream pathways involved in the initiation of disease.

AAI is characterized by a type 2 (Th2) immune response and disease hallmarks include airway hyperresponsiveness (AHR), eosinophilic inflammation, mucus hypersecretion, and the production of the canonical Th2-associated cytokines IL-4, IL-5, and IL-13 [13]. Although $CD4^+$ T helper cells are the best-known sources of these cytokines, various innate cells, mast cells, eosinophils, basophils, and ILC2s have also been implicated in their production [14].

In the lungs, upon exposure to pathogens, chemicals and mechanical injury, airway epithelial cells, and mesenchymal precursors release alarmins IL-25, IL-33, and thymic stromal lymphopoietin (TSLP) and initiate a cascade that culminates in antigen-specific polarization and expansion of Th2 cells in the draining lymph nodes (Figure 1) [13]. It is known that within the lymph node, IL-4 acting through its receptor on naïve T cells activates a signal transducer and activator of transcription 6 (STAT6) leading to upregulation of the key Th2 transcription factor, GATA3 [14]. GATA3, in turn, acts as a master regulator and guide for Th2 differentiation and once specified, the resulting antigen-specific Th2 cells exit the draining lymph nodes, enter circulation through the thoracic duct lymph and are recruited to the sites of ongoing inflammation [14]. Despite this knowledge, the mechanisms underlying how allergens selectively prime Th2 responses during the first allergen sensitization and how innate effector cells (e.g. eosinophils and mast cells) are recruited to the lungs early and prior to adaptive responses, were formerly, unclear.

In studies of RAG deficient mice lacking T and B cells, ILC2s (formerly known as natural helper (NH) cells) were identified in different tissues: spleen, adipose tissue, lymph nodes, intestine, lungs, and nasal mucosa and were shown to be potent producers of IL-5 and IL-13 upon *in vitro* stimulation with

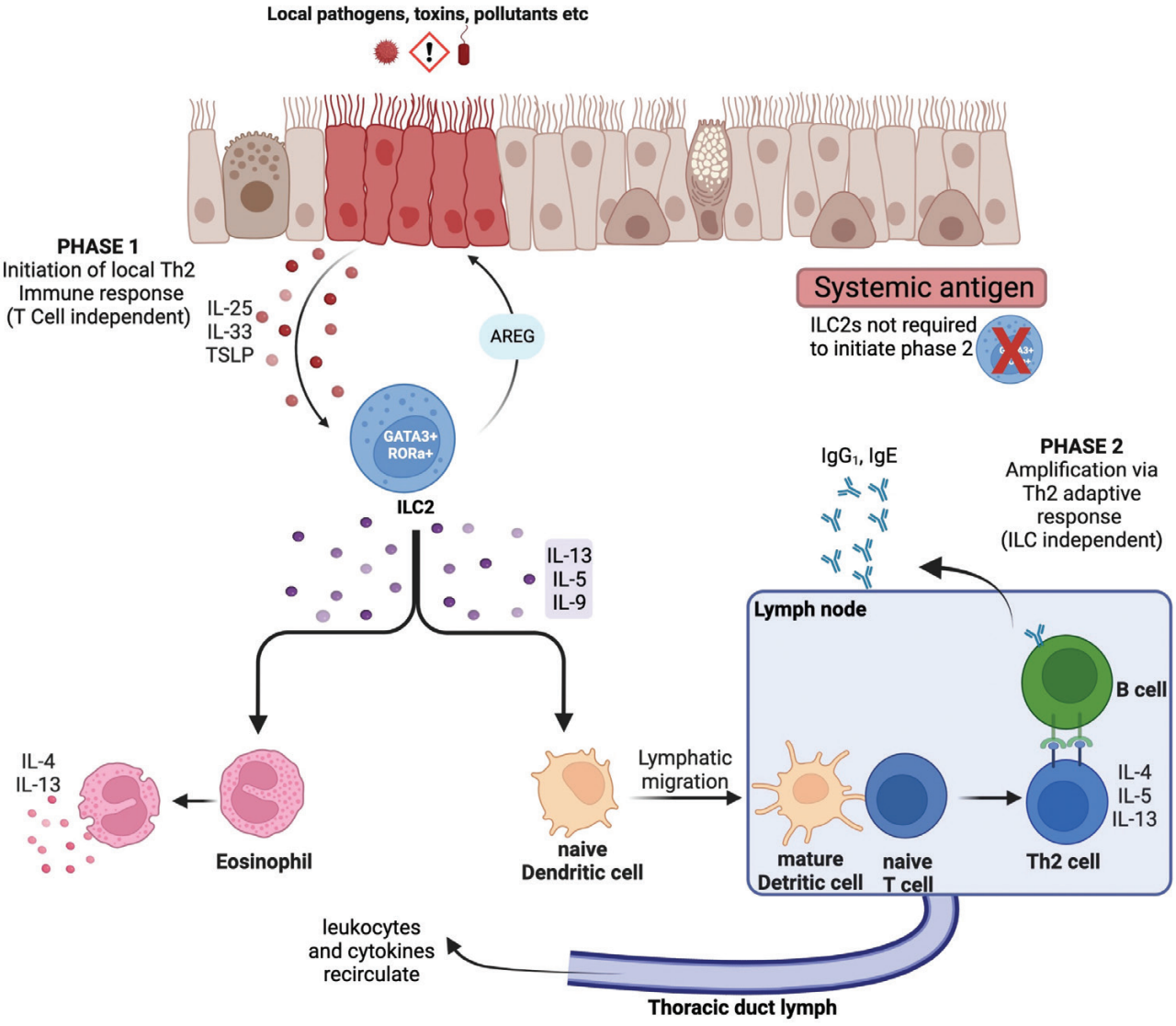



**Figure 1.** Upon local insult or antigen stimulation lung epithelial cells release alarmins IL-25, IL-33, and TSLP that activate tissue resident ILC2 leading to the production of Th2 cytokines IL-5, IL-9, and IL-13. These cytokines facilitate expansion and proliferation of naïve CD4⁺ T cell to Th2 cells, amplify the Th2 immune response, and facilitate leukocyte in filtration and other hallmarks of AAI. ILC2s are dispensable in response to a systemic antigen, TSLP is sufficient to generate a type 2 immune response [15]

IL-25 and *in vivo* with IL-33 or IL-25 [13, 16, 17]. However, only minuscule amounts of IL-4 were produced, particularly in the lungs [13, 17]. Regardless, the possibility that IL-5 and IL-13 producing ILC2s could still play a central role in the priming of an innate Th2 environment prior to polarization of CD4⁺ naive cells was clear as there are other IL-4 sources and evidence of IL-4-independent Th2 cell polarization through somewhat cryptic mechanisms [18].

To better characterize the role of ILC2s in adaptive Th2 cell responses, we examined the effects of ILC2 deficiency on Th2 cell responses to different allergens and route of administration [19–21]. Previously, we showed that ILC2s express the transcription factor RORα and that mice mutant for this factor ("staggerer" or $Rora^{sg/sg}$) are ILC2 deficient [20, 21]. Accordingly, we generated healthy ILC2-deficient mice by transplanting lethally irradiated Ly5.1 recipient mice with bone marrow (BM) from $Rora^{sg/sg}$ CD45.2 mice to produce hematopoietic chimeras [21]. These chimeras are preferred as the staggerer mutant mice exhibit a variety of neurological defects (a staggering gait, mild tremor, and hypotonia, etc.) and as a result these mice have a greatly shortened life span [22]. Based on surface markers expression (CD127, CD25, Sca1, cKit, CD45, and IL1RL1(ST2/IL-33R), we find that these $Rora^{sg/sg}$ chimeric mice have virtually no ILC2s in the lungs or intestines, but they have normal functional T cells [17, 20].

In a house dust mite (HDM)-induced asthma model, ILC2s were shown to be crucial for AAI [19]. When WT and $Rora^{sg/sg}$ BM transplanted (BMT) mice were intranasally exposed to HDM compared to WT BMT mice, $Rora^{sg/sg}$ BMT mice exhibited a striking decrease in all leukocyte subset infiltration, but especially eosinophils. Moreover, the production of serum IgE and lung transcripts of the Th2-associated cytokines IL-4 and IL-5 were potently attenuated, suggesting impaired development of downstream adaptive immune responses. The role of ILC2 as essential first responders was further supported by other studies in which papain, as a model protease allergen, was used to induce AAI and compared in WT mice, T- and B

cell-deficient *Rag1*$^{-/-}$ mice, and T, B, NK, and ILC-deficient *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ (RGC) mice [21]. Three days of intranasal papain administration induced robust leukocyte tissue infiltration (especially eosinophils) in the lungs, of *Rag1*$^{-/-}$ and WT mice but not in RGC mice. To further investigate the role of ILC2s in allergen-induced acute eosinophilic inflammation, ILC2s were depleted in *Rag1*$^{-/-}$ mice by a CD25 antibody injection leading to a significant decrease in allergen induced eosinophilia. Furthermore, in both studies [19, 21], the lack of ILC2s was linked to a significant reduction in lung histopathology, which correlated with a reduction in bronchoalveolar lavage (BAL) eosinophil numbers and mucus production by airway goblet cells. Importantly, these observations were reversed when ILC2s were transplanted into ILC2-deficient mice establishing ILC2s as critical for the development of allergic lung inflammation [21].

To investigate differences elicited by the route of priming, an OVA/alum model was also used since this model relies on intraperitoneal injections of antigen for systemic priming followed by a local intranasal airway challenge [19]. Strikingly, lung ILC2s were found to be completely dispensable in this model of AAI induction: WT BMT mice and ILC2-deficient *Rorα*$^{sg/sg}$ BMT mice had identical levels of airway leukocyte infiltration including normal eosinophil recruitment in the airways and lungs. Furthermore, serum IgE and IL-5 expression were comparable, suggesting that, with systemic priming, Th2 responses develop normally in the absence of ILC2s. These results have been independently confirmed in two more recent studies that each used elegant strategies to selectively dampen GATA3 expression in mature ILC2s; one via deletion of a functionally important ILC2-specific regulatory element in the GATA3 gene and the second using selective inactivation GATA3 in mature ILC2s via KLRG1-Cre-mediated deletion [23, 24]. Each strategy resulted in mice with reduced lung ILC2s, and both noted an important role for ILC2s in the downstream production of appropriately polarized Th2 cells but only in response to local signals in the draining lymph nodes rather than systemically. It is also noteworthy that the alarmin TSLP, generated during intraperitoneal priming with Ova/alum, could short circuit the need for ILC2s in the production of Th2 T cell responses [23].

Finally, to show that these responses were selective to Th2 polarization rather than broad-spectrum tissue-resident immunity WT BMT mice and ILC2-deficient *Rorα*$^{sg/sg}$ BMT mice were primed with Saccharopolyspora recti virgula, in a Th1/Th17-driven "Farmer's Lung" model of hypersensitivity pneumonitis (HP) [25]. Here we found that ILC2s were completely dispensable; no significant changes were observed in the production of antigen-specific $IgG_{2a}$, *Il17a* gene transcription or IL-17A production in restimulated lung cultures, suggesting that ILC2s are not important for Th1 and Th17 inflammatory responses. In summary, these data suggest that ILC2s have likely evolved to provide a rapid, tissue-resident, and appropriately polarized, response to pathogens while downstream antigen-specific T-cell responses are primed in the local lymph nodes [6].

## ILCs in fibrotic disease

Fibrosis is the pathological replacement of normal functioning healthy tissues with scar tissue due to excessive extracellular matrix deposition and is normally associated with chronic inflammation [26, 27]. Fibrotic tissue damage can result from a variety of different stimuli including mechanical injury, exposure to toxins, infection, autoimmune responses, etc. There are normally two distinct phases in the reparative response to injury: (1) an immediate, mesenchymal-driven production of fibroblasts and myofibroblasts to produce extracellular matrix and a fibrotic "scar" that limits the impact of the damage on healthy parenchymal tissue followed by (2) a subsequent regenerative response, where damaged cells and fibrotic scar are replaced by healthy cells of the same tissue leaving little-to-no evidence of the initial damage [26]. Though beneficial, if these two phases are not appropriately coordinated wider tissue fibrosis occurs leading to overall loss of tissue and organ function. Collagen deposition is one of the most widely recognized hallmarks of fibrosis and is triggered by various signals that activate the primary collagen producing cells, the myofibroblasts [27]. The etiology of many of the most widely recognized fibrotic diseases are parasitic, bacterial, and viral infections leading to chronic inflammation and recently studies have begun to highlight the role of ILCs in the development of fibrosis in different tissues [28–33].

As an example [29], in a pulmonary fibrosis mouse model, ILC2 deficient *Rorα*$^{sg/sg}$ BMT mice were found to have impaired development of fibrosis and Th2-associated cytokine production. Upon IL-25 intranasal administration *Rorα*$^{sg/sg}$ BMT mice had a significant decrease in lung collagen deposition, IL-13 and TGF-β production. Furthermore, the BAL fluid of patients with IPF were found to be significantly enriched in IL-25, IL-13, and TGF-β which, in the mouse model, were shown to correlate with worsened fibrosis. Collectively, these data suggest a central role for ILC2s in fibrotic responses and that targeting ILC2s may be beneficial for attenuating fibrosis development in the lungs. Intriguingly, in a separate mouse model [30], pulmonary fibrosis was found to be mediated by either an IL-13 dependent mechanism or an IL-13 independent mechanism that instead was driven by IL-17A and INFγ. Increased amounts of these Th17-associated cytokines correlated with more robust fibrosis suggesting a more complex ILC interplay in different disease states. In addition, and in contrast to a pathogenic role in fibrosis, ILC2 has been shown to be crucial for tissue repair through their production of amphiregulin [31, 32]. Similarly, ILC3s have been implicated in pathogenic or protective role in the gut and liver, respectively [33, 34]. In the context of gut pathogenicity, both ILC2s and ILC3s have been implicated in Crohn's disease (CD), a subtype of inflammatory bowel disease (IBD) that drives recurrent formation of gastrointestinal strictures that can only be treated through surgical intervention [34–37]. CD is primarily considered to be a Th17-associated disease as patients with CD show elevated levels of IL-17A and IL-22 [36]. In contrast, IL-13, a Th2-associated cytokine, has been implicated in the promotion of collagen deposition in a retrospective study of patients with CD [38].

To better clarify the role different ILCs play in driving excessive fibrosis in the gut, we attempted to use the ILC2-deficient *Rorα*$^{sg/sg}$ BMT mice to evaluate gut fibrosis in a *Salmonella*-induced model of intestinal inflammation that closely mimics the transmural fibrosis observed in severe CD patients (Figure 2) [34]. WT and *Rorα*$^{sg/sg}$ BMT mice were treated with streptomycin 24 h prior to oral infection with the highly attenuated Δ*aroA* strain of Salmonella. Pathogen burden and immunohistopathology in the spleen and ceca of these mice between days 0 and 35 were equivalent indicating an equal ability of these mice to clear the bacterial infection.

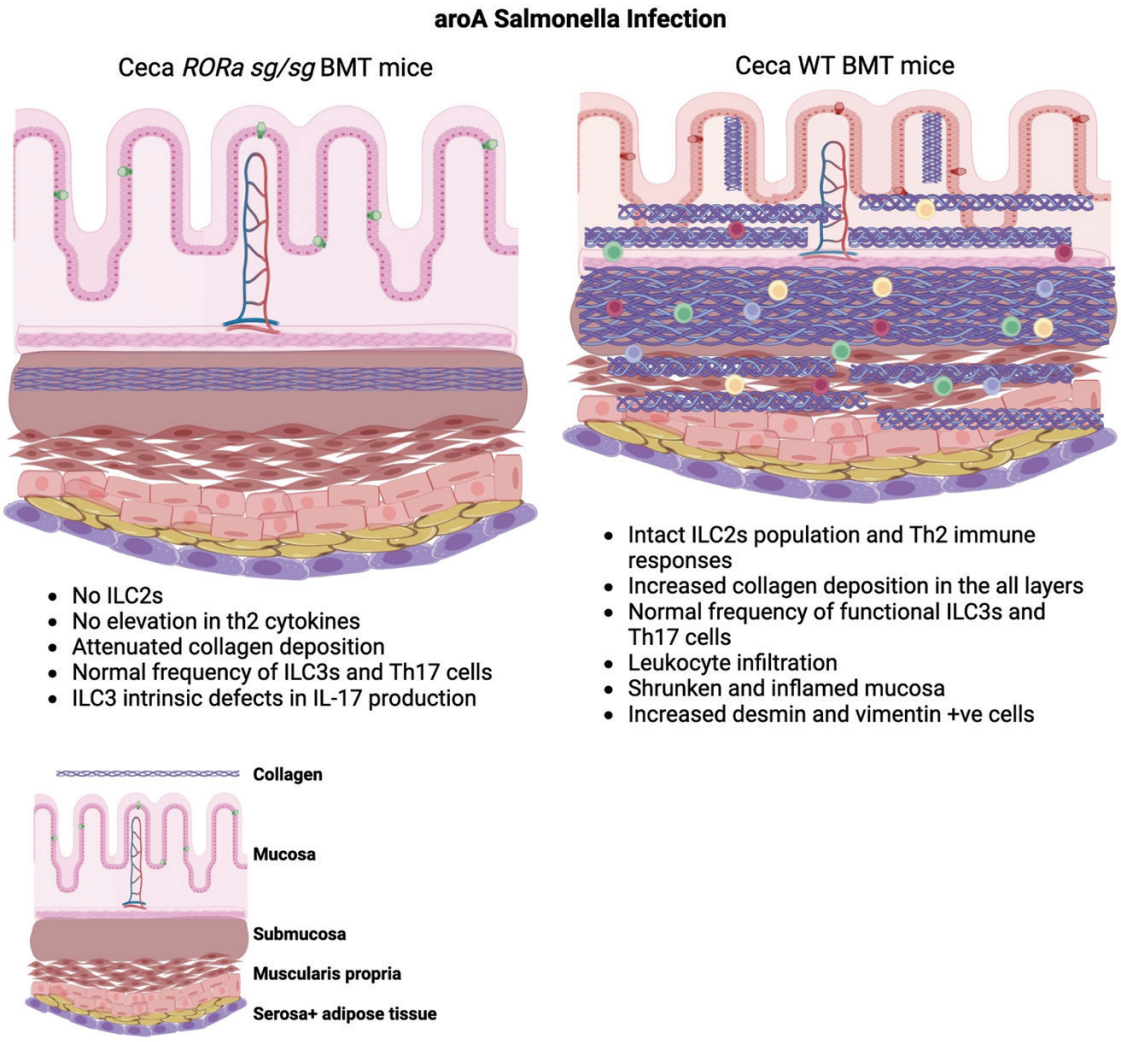



**Figure 2.** *Rorα*$^{sg/sg}$ BMT mice show attenuated fibrosis compared to WT BMT mice in a Salmonella model of gut fibrosis. Collagen deposition in submucosa of *Rorα*$^{sg/sg}$ BMT mice is significantly less and independent of Th2 immunity and instead is linked to IL-17 production by ILC3s [15]

Strikingly, however, 21 days after infection *Rorα*$^{sg/sg}$ BMT mice exhibited highly attenuated collagen deposition, reduced mucosal thickening and minimal formation of lymphoid cell aggregates. In addition, *Col1a2* and *Tgfb1* transcripts and TGF-β1protein were significantly decreased in the ceca of *Rorα*$^{sg/sg}$ BMT mice. Finally, common fibroblast markers vimentin and desmin were also reduced. Collectively, these data indicate that inactivation of RORα in *Rorα*$^{sg/sg}$ BMT mice leads to protection from fibrosis. Surprisingly, however, this appeared to be independent of Th2 immunity: we failed to detect elevated production of Th2 cytokines, IL-4, -5, or -13 at any stage of the disease in Salmonella infected mice. To further rule out the role of Th2 immunity in this model, we induced disease in eosinophil-deficient, ΔdblGATA mice and *STAT6*$^{-/-}$ mice. Neither strain exhibited significant differences in immunopathology and fibrosis-associated transcripts when compared to WT mice. In summary, although *Rorα*$^{sg/sg}$ BMT mice are clearly ILC2-deficient and protected from intestinal fibrosis in this model, we could find no evidence that this protection was related to type 2 immunity.

Although RORα is expressed in ILC2s and essential for their development, it is also expressed in mature ILC3s and Th17 cells where its function has remained more enigmatic [39, 40]. ILC3 and Th17 cells are critically dependent on the RORα-related transcription factor, RORγt, for their development and deletion of RORγt is known to lead to a severe deficit of LTi, ILC3s, and Th17 cells [39, 40]. But because these cells are present in relatively normal frequency in the RORα-deficient mice, it has largely been assumed that RORα is dispensable for their formation and function [39, 40]. To further investigate the selective role of RORα in ILC3 function, we measured the production of ILC3-associated cytokine transcripts, Il17a and Il22, and IL-17A and IL-22 protein in Salmonella-induced intestinal fibrosis [34]. Strikingly, we found highly elevated levels of both cytokines in cecal homogenates and mesenteric lymph nodes of WT BMT mice, but these were highly attenuated in *Rorα*$^{sg/sg}$ BMT mice, despite having comparable numbers of total ILC3 and Th17 cells. Furthermore, intracellular staining revealed a clear, cell-intrinsic defect in production of these cytokines arguing that RORα inactivation leads to a selective functional deficit in ILC3 and Th17 without major effectors on their frequency. To further evaluate the relative contributions of ILC3 versus Th17 cells to fibrosis, lethally irradiated mice were reconstituted with equal numbers of cells from *RAG*$^{-/-}$ mice combined with either WT or *Rorα*$^{sg/sg}$ cells. Because *Rag1*$^{-/-}$and

*Rorα*$^{sg/sg}$ BM cells mice would generate normal ILC3s (*Rag1*$^{-/-}$ donor derived) without altering the *Rorα*$^{sg/sg}$-derived T-cell compartment (derived exclusively from *Rorα*$^{sg/sg}$ BM) this served as an approach to selectively inactivate RORα in T cells but not ILC3. These mice exhibit no differences in *Salmonella* burden, immunopathology, immune infiltrates, collagen deposition, epithelial cell hyperplasia, and transcript levels of fibrosis associated factors, and support the idea that ILC3s are the primary cell-type driving intestinal fibrosis in this model. Finally, in WT mice, neutralizing antibodies to IL-17A/F were found to potently attenuate the epithelial remodeling and fibrotic scarring observed in the late phases of chronic *Salmonella* infection, suggesting that this model is highly IL-17A dependent. In summary, these data argue for a selective role of ILC3-derived IL-17 in driving severe intestinal fibrosis and suggest that targeting ILC3 would provide therapeutic benefit.

## ILC plasticity in allergic disease and fibrosis

In previous sections of this review, specific ILCs have been identified as the main drivers of pathogenicity or protection in the context of AAI and gut fibrosis. However, some may argue that this description is not as definite due to the phenomenon of ILC plasticity. Depending on the tissue and cytokine environment, ILC subsets exhibit the potential to switch phenotypes and behave like other ILC subsets while sometimes maintaining most of their defining phenotypic markers [41, 42]. For instance, in human gut ILC1s under the influence of IL-2, IL-23, and IL-1β were shown to become ILC3-like cells by upregulating RORγt and Th17-associated cytokine expression, a process which was accelerated in the presence of retinoic acid and could be reversed in a different cytokine microenvironment [43]. Further studies have also shown that human ILC3s and ILC2s can gain an ILC1-like phenotype upon exposure to IL-12, IL-1β, and under the regulation of T-bet [44–47]. Similar populations of ILC3-like ILC2s have been described in mouse models for allergic rhinitis (AR), a type of AAI and psoriasis in humans [48, 49].

In the study of minimal persistent inflammation (MPI), a subclinical inflammatory state that occurs before and after AR onset [50] a subset of ILC3-like ILC2 cells that was found to persist following type 2 allergic inflammation are proposed to be one of the drivers of MPI even though typically IL-17$^{+}$ ILC3s have been associated with MPI airway hyperresponsiveness [48]. The source of these IL-17$^{+}$ ILC2s which increase rapidly in AR and MPI had not been determined [48, 50]. The rapid increase in ILC3-like cells in inflamed versus control conditions eliminated proliferation and migration as possible sources. The main source of IL-17 was revealed to be IL-5$^{+}$, IL-17A$^{+}$ GATA3$^{+}$, RORγt$^{+}$, IL23R$^{+}$ ILC2s which upregulated RORγt in the presence of IL-23 but not TGF-β and were able to revert to a canonical ILC2 phenotype upon IL-4 exposure [48]. In the human psoriasis study, human dermal ILC2s were shown to produce IL-17 in the presence of IL-1β, IL-23, and TGF-β and this ILC3-like characteristic could also be reverted by IL-4 [49]. The ILC3-like phenotype was achieved through the inhibition of GATA3 and upregulation of RORγt in cKit$^{+}$ NKp44$^{-}$ CRTH2$^{-}$ ILC2s [49].

Considering these findings, it is logical to hypothesize that some pathology observed in the AAI and gut fibrosis studies [19, 21, 22] may not be due to a single *bona fide* ILC subtype but rather due to complex interaction between *bona fide* and plastic ILCs.

## ILC TCR gene rearrangement suggest they develop from thymic T-cell precursors

As highlighted in previous sections, ILCs are very important innate counterparts to T cells and show a remarkable degree of similarity in form and function. While the previous sections highlight their selective role in the development of inflammatory disease, a clearer understanding of their origin, lineage trajectory, and function is essential to future attempts to manipulate them with therapeutics for better health outcomes.

With regards to their origins, the presence of mature ILCs and ILC precursors (ILCP) in adult BM has led to speculation that ILCs and T cells share a common precursor in the form of common lymphoid progenitors (CLP) which then diverge to form more committed ILC precursors within the BM environment and early thymic progenitors which move to the thymus and subsequently become restricted to the T-cell lineage [51]. Using PLZF-GFP reporter mice, it was shown almost a decade ago that expression of this transcription factor can separate the two lineages (ILCs vs T cells) with PLZF$^{hi}$ cells being committed ILCP [52]. An extra-thymic origin of ILCs was further supported by the observation that these cells, like mature NK cells, develop normally in mice bearing genetic lesions that severely hinder T-cell development, including *Nude*, *Rag1*$^{-/-}$, and *Rag2*$^{-/-}$ mice [53]. These present observations merit attention as RAG expression has been shown to influence the functional fitness of NK cells. Specifically, a noteworthy finding is that NK cells lacking RAG expression exhibit a heightened state of activation under normal conditions and exhibit an impaired ability to proliferate in response to viral infection. This finding potentially extends to other innate lymphoid cell (ILC) subsets, thus highlighting the importance of RAG expression in regulating ILC responses [54].

Finally, adoptive transfer studies showed that the BM harbors ILC-lineage restricted progenitors that can give rise to mature, tissue resident ILCs [53]. Therefore, ILC and T-cell development were thought to be separate from one another with the BM being the principal source of ILCs and the thymus the principal organ of origin for T cells. This model became far more tenuous when we and others made the surprising observation that mature ILCs express high levels of TCR constant region transcripts [55, 56]. In our case, scRNA-seq analyses of highly purified cecal ILCs as well as purified ILC2s in lung revealed that all TCR constant region genes are expressed by various subsets of ILCs [55]. Because TCR gene rearrangements normally occur exclusively in cells developing in the thymic microenvironment, this observation naturally led us to test whether the observed transcripts were from fully rearranged TCR loci or merely sterile transcripts linked to high-level expression of T-cell transcription factors in peripheral ILCs. Using lung ILC2s as a clearly defined and easy to purify population (with minimal contamination), we conducted genomic PCR amplification and sequence analyses of all the different possible combinations of TCR-γ chain genes. Surprisingly, we found that WT ILC2s retain a high frequency of Vγ2-Jγ1 gene rearrangements. Subsequently, we investigated the nature of the TCR-gamma locus to determine its sequence clonality and functionality. Of note, ILC2s were found to have a lower diversity of TCR-γ clonal DNA

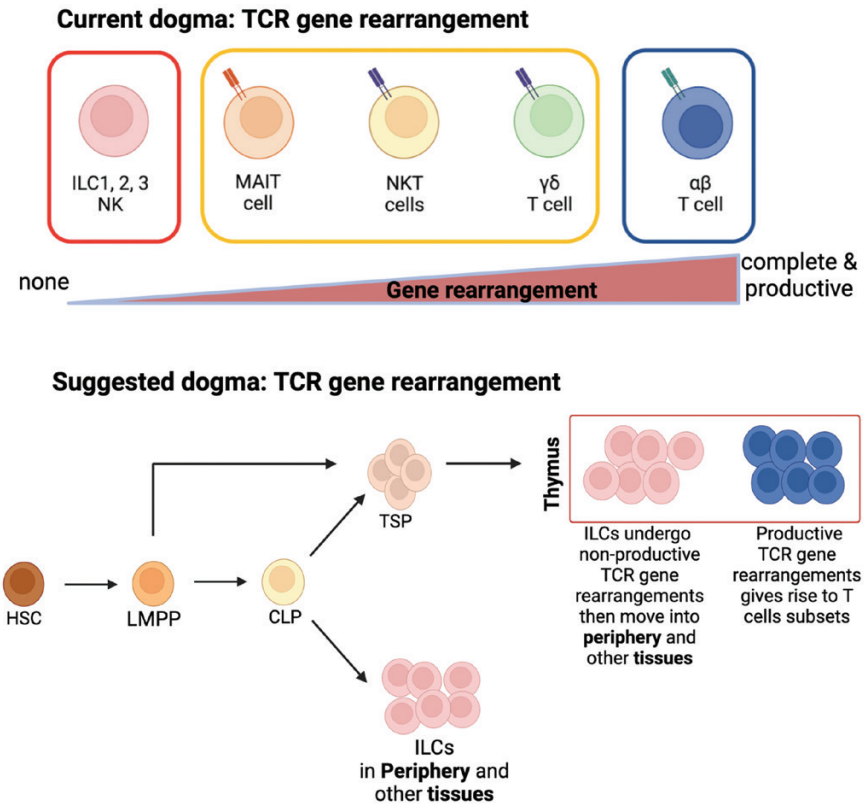



**Figure 3.** Current dogma of ILC development suggests that they develop from ILC precursors (ILCP) in the bone marrow and then move into the periphery. However, the presence of gene rearrangement in ILC2s suggests that at least a subset of ILCs originate from the thymus following non-productive TCR gene rearrangement. Hematopoietic stem cells (HSC), lymphoid-primed multipotent progenitor (LMPP), common lymphoid progenitor (CLP), and thymus-seeding progenitor (TSP) [15]

rearrangements relative to *bona fide* lung γδ T cells. Strikingly, when these were compared for productive rearrangements that could lead to functional, full-length proteins we were surprised to find that the vast majority of V(D)J rearrangements in ILC2s were non-functional due to premature stops or frame shifts. Therefore, even though ILC2s actively transcribe TCR genes and rearrange TCR gene loci, these rearrangements would never lead to expression of a functional cell surface TCR due to a failure to generate requisite productive gene rearrangements at both the γ and δ or α and β loci within single cells. It is important, to note that although these rearrangements provide incontrovertible evidence that at least a subset of ILCs develop within the thymus. It is also clear that TCR gene rearrangements within these cells are not an essential pre-requisite for their development: ILCs are abundantly present in RAG gene deficient mice, and, to date, there are no reports of functional deficits in ILCs that develop from these mice. Rather, these instead serve as indelible fate maps that reflect the fact that these cells were present within the thymus at the time when TCR gene rearrangements occurred. It is also noteworthy that previous studies have observed similar types of rearrangements, albeit in low frequencies, within subsets of NK/ILC-like cells [57].

Accordingly, based on the TCR rearrangement data, we now propose an alternative developmental pathway redefining the life history of ILCs from a more T-cell centric point of view. We note that functionally, phenotypically, and developmentally, NK cells and ILCs bear many of the key hallmarks of T cells (Figure 3) and, knowing that the thymus provides an ideal niche microenvironment for the development of these cells, it would make an ideal specialized niche for ILC development. Thus, we postulate that at least some, and possibly the majority, of adult ILCs could trace their origins to a transient residence within the thymic microenvironment.

To summarize, we postulate that early in development, NK/ILC/T cell precursors move to the thymus. Within this microenvironment, some will give rise to NK and ILCs directly while others will begin to rearrange their TCR loci. If the resulting rearrangements are productive, they will give rise to typical αβ and γδ T cells. But if they fail to make productive rearrangements, these early DN precursors may still retain the option to become ILCs and colonize peripheral tissues as long-lived tissue resident ILCs. Based on this model, one can think of the developmental pathway of ILCs and T cells as one and the same and that ILCs are a by-product of early T-cell development from T-cell progenitors that have failed to properly rearrange their TCR genes or receive adequate T-cell commitment signals.

## TCR gene rearrangements as nature's barcode

Regardless of the mechanistic details surrounding the stepwise restriction of T-cell fate and maturation within the thymus during neonatal and adult development [58] we note that the observed TCR gene rearrangements can readily serve as a natural barcode for tracing the fate of ILCs, T cells and potentially NK cells during development. As we and others have noted previously, γδ T cells are well known to undergo developmentally programmed temporal use of select Vγ gene

regions during neonatal ontogeny [59, 60]. Thus, we can use V-region expression or, more elegantly, genomic sequence junctional diversity as a way of lineage tracing the origins of ILCs during development. Mapping shared rearrangements in T cells, ILCs, and NK cells provide an additional method for divining their early commitment to respective fates and their longevity.

The incorporation of major histocompatibility complex (MHC) expression into our barcoding strategy may hold great potential for enhancing its efficacy, particularly considering the unique nature of each TCR which is designed to recognize-specific antigen peptides presented by MHC molecules. In light of the striking parallels that exist between ILCs and T cells, it is noteworthy that both cell types have been demonstrated to express MHC class II molecules in certain settings [61–63]. For example, it has been reported that CCR6$^+$ LTi-like ILC3s exhibit high levels of MHCII expression and are capable of delivering inhibitory signals to effector T cells [62]. However, the functional relevance of this remains a topic of ongoing debate, particularly in light of the emergence of new models of antigen presentation [64]. Future research focusing on ILC niches in the fetal thymus and their MHC expression profiles may serve as means of increasing our TCR rearrangement analysis as nature's barcode.

We also recognize, and have noted in this review, the pressing need for more selective ILC reporter animal strains to fate map, lineage-trace, and selectively delete genes in ILC subsets. When combined with natural VDJ-recombination barcoding, these novel strains will serve as ideal tools to resolve current controversies in the field and elucidate the physiological relevance of ILCs in health and disease, including the contribution of ILC plasticity, the independent large-scale recruitment of new ILC subsets from distal sites and the local, in situ, differentiation of uncommitted resident precursors [65].

## Conclusion

The discovery and study of ILCs in recent years have provided a better understanding of how the innate and adaptive immune system collaborate to orchestrate a substantial immune response against foreign antigens. However, overlap in cytokine repertoire and tissue microenvironment have led to the suggestions that ILC function may be redundant. In this review, we have highlight several situations in which ILCs play a unique and important role in disease progression and homeostasis. We have also highlighted the plastic nature of ILCs in different cytokine microenvironments. ILC plasticity may prove to be one of the more difficult confounding variables to control for in the manipulation of ILCs for therapeutic purposes and we propose that more work needs to be done to address the ambiguous nature of ILC phenotypes in different conditions. Another obstacle in the ILC field is defining their ontogeny to allow for fate mapping and lineage tracing studies. We have suggested a novel technique using TCR gene rearrangements and MHC expression on ILCs to tag these cells and facilitate further exploration of the different subsets for easier therapeutic manipulation. This barcoding technique once perfected we believe will provide more clarity and resolve significant ambiguities in ILC origin and study.

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## Conflict of interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Data availability

Data available from this publication.

## Author contributions

The authors confirm contribution to the paper as follows: conception and outline: Dr. Kelly McNagny, Sia Cecilia Jan-Abu; draft manuscript preparation: Dr. Kelly McNagny, Sia Cecilia Jan-Abu, and Ahmed Kabil. All authors reviewed and approved the final version of the manuscript.

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