Published in final edited form as:

Nat Immunol. 2016 June 21; 17(7): 783–789. doi:10.1038/ni.3484.

Innate lymphoid cell function in the context of adaptive immunity

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Abstract

Innate lymphoid cells (ILCs) are a family of innate immune cells that have diverse functions during homeostasis and disease. Subsets of ILCs have phenotypes that mirror polarized T helper subsets in their expression of core transcription factors and effector cytokines. Given the similarities between these two classes of lymphocytes, it is important to understand which functions of ILCs are specialized and which are redundant in comparison to T cells. Here, we discuss genetic mouse models that have been used to dissect the contributions of ILCs versus T cells, and review our current understanding of the specialized *in vivo* functions of ILCs.

In 1986, Mosmann and Coffman identified T helper 1 (T_H1) and T helper 2 (T_H2) subsets by demonstrating that CD4⁺ T cell clones can be divided into two groups with distinct patterns of cytokine expression¹. These findings, together with the discovery of T helper 17 (T_H17) cells almost two decades later, formed the helper T cell paradigm, a concept which functionally defined subsets of polarized CD4⁺ T cells by the specific cytokines they produce. Remarkably, a similar paradigm has recently emerged from within the innate immune compartment with the discovery of a new group of lymphocytes called innate lymphoid cells (ILCs). ILCs are a functionally diverse but developmentally related family of innate lymphocytes that have phenotypes that resemble those of polarized T cell subsets. Specific cell types within this lymphocyte class have been recognized for decades; the first identified ILC, the conventional natural killer (cNK) cell, was discovered over 40 years ago², while the lymphoid tissue inducer (LTi) cell was described in neonatal mouse lymph nodes in 1992³. In more recent years, additional cell types within this family have been identified^{4, 5, 6, 7, 8, 9, 10}, generating increased interest in ILCs and their functions during homeostasis and inflammation.

With the recognition that innate lymphoid populations have striking similarities to polarized CD4⁺ T cell subsets, the ILC family was divided into three groups that parallel T_H1, T_H2, and T_H17 cells. Group 1 ILCs (ILC1s) are analogous to T_H1 cells, as they express the transcription factor T-bet and produce interferon- γ (IFN- γ). ILC1s include Eomes⁻ IL-7R⁺ ILC1s as well as Eomes⁺ IL-7R⁻ cNK cells, although cNK cells arise from a divergent developmental pathway and are perhaps more analogous to CD8⁺ cytotoxic T cells because they produce high amounts of granzymes and perforin. Eomes⁻ and Eomes⁺ ILC1s represent two extremes of a broad spectrum of ILC1 phenotypes that were previously attributed to NK

populations. These cells, which exhibit varying cytolytic activities and contrasting requirements for Eomes and other transcription factors such as T-bet and Nfil3, include intestinal intraepithelial ILC1s, salivary gland ILC1s, and uterine ILC1s^{11, 12, 13, 14}. Group 2 ILCs (ILC2s) are analogous to T_H2 cells in that they express high amounts of the transcription factor GATA-3, and produce interleukin 5 (IL-5), IL-9, and IL-13 during both helminth infection and allergic inflammation^{4, 5, 6}. These cells are subdivided based on responsiveness to the epithelial-derived cytokines IL-33 and IL-25¹⁵. Finally, group 3 ILC3s (ILC3s) express the transcription factor ROR γ t, an isoform of the gene *Rorc*¹⁶. These cells produce IL-22 and/or IL-17A during extracellular fungal and bacterial infection, and are analogous to T_H17 cells. ILC3s include LT α i cells that are required for secondary lymphoid tissue development in the fetus¹⁷, as well as CCR6⁺ LT α i-like cells, NKp46⁺ ILC3s, and CCR6⁻NKp46⁻ ILC3s in adult mice. In humans, ILC3s isolated from tonsils express NKp44⁸.

Although ILCs and helper T cells have similar effector programs, these cells are regulated in distinct ways. Naïve CD4⁺ T cell activation requires priming by major histocompatibility complex (MHC)-peptide-T cell receptor (TCR) interactions and costimulatory signals; both are provided by direct contact with an antigen-presenting cell. In contrast, ILCs do not require specific antigen or costimulation, and are instead activated by an expanding list of soluble mediators that include proinflammatory cytokines, alarmins, lipids, and hormones released by stromal, epithelial, and myeloid populations. ILCs and CD4⁺ T cells also require different amounts of time to mount effector responses. T cell priming takes place over several days in lymphoid organs as these cells undergo activation and clonal expansion, acquire effector functions, and produce the surface molecules required to migrate to nonlymphoid tissues. By comparison, most ILCs take up permanent residence in nonlymphoid tissues during the steady state¹⁸, and are able to produce substantial amounts of cytokines upon stimulation without further differentiation. Thus, ILCs are physically and functionally poised to be immediate sources of effector cytokines in peripheral tissues, placing these cells among the ranks of other rapidly responding tissue resident lymphocytes such as iNKT cells, $\gamma\delta$ T cells, and tissue resident memory cells (T_{RM}) (Fig.1). The limited activation requirements of ILCs allow them to serve as first responders during the early stages of a primary immune response, as well as produce cytokines in response to subtle perturbations during homeostasis.

Given the similar effector programs of ILCs and T cells, an obvious question is: what are the unique contributions of ILCs *in vivo*? To distinguish the contributions of ILCs from those of T cells, the functions of ILCs are frequently interrogated in T cell-deficient mice. For example, a common strategy is to compare the phenotypes of Rag-deficient mice, which lack adaptive lymphocytes but have intact ILCs, with mice deficient in both Rag and *Il2rg* (the common gamma chain, or γ_c), which lack both adaptive lymphocytes and ILCs. As a result, there remains a major gap in our knowledge surrounding the activities of ILCs under physiological settings. Studying ILCs in the context of an intact T cell compartment will be required to (1) identify functions of ILCs that are distinct from those performed by T cells, and to (2) determine how ILCs communicate with T cells during an immune response. Additionally, the shared and distinct regulatory elements that govern lineage determination and function in ILCs and T cells need to be explored. Expansion within these areas of

research will be of particular importance if investigators propose to selectively modulate ILC function in patients to improve disease outcomes.

Mouse models for assessing ILC function: considering adaptive immunity

Early studies characterizing ILCs with Rag-deficient and Rag- and *Il2rg*-doubly deficient mice demonstrated that these cells were not of the T cell lineage, but were lymphocytes that require γ_c cytokines to develop. Since then, Rag-deficient mice have been essential for showing that cytokines derived from ILCs can promote microbial killing, granulocyte migration, epithelial barrier integrity, smooth muscle contraction, and mucus production in the context of T cell immunodeficiency. However, there are potential caveats regarding ILCs in Rag-deficient mice. ILC2s and ILC3s in Rag-deficient mice are elevated in number in tissue, and can produce increased amounts of cytokine both at rest and after stimulation *in vitro*^{19, 20, 21, 22}. These changes likely result from an excess of γ_c cytokines that arises in the absence of T cells. IL-7, which promotes proliferation, survival, and changes in fate decisions in ILCs, is normally limited by T cell consumption in wild-type mice, but is significantly increased in the serum of Rag-deficient mice²³. These observations should be considered when assessing the functional contributions of ILCs with this system.

Moreover, although ILCs do not express rearranged antigen-specific receptors, a proportion of cNK cells, ILC2s, and ILC3s express Rag genes during development and are altered by Rag-deficiency in a cell-intrinsic manner^{24, 25}. Rag-deficient ILCs have increased histone H2AX phosphorylation, a marker for double stranded DNA break events, and *Rag2*^{-/-} cNK cells exhibit a reduced response to radiation-induced DNA damage. Rag-deficient cNK cells also have reduced cellular fitness, with *Rag2*^{-/-} cNK cells displaying increased apoptosis during the steady state and diminished survival after murine cytomegalovirus (MCMV) infection. The cell-intrinsic effects of Rag-deficiency on cellular fitness in ILC2s and ILC3s are unknown.

A second commonly employed strategy to study ILC function relies on the depletion of ILCs using antibodies against Thy1. Most ILCs and all T cells express high amounts of cell surface Thy1, and ILCs are thus largely eliminated by administering a depleting Thy1 antibody into Rag-deficient mice²⁶. In some studies, chimeric mice containing T cells that express a Thy1 variant not recognized by depleting antibodies have been used in order to deplete the ILC compartment without affecting T cells²⁷. With the Thy1 depletion system, variable efficiencies of cell depletion have been reported in blood and tissue, potentially due to the method of antibody administration^{28, 29}. An important consideration with these studies is that Thy1 expression is not restricted to lymphocytes, but is also expressed by several other cell types including fibroblasts, endothelial cells, neurons, basophils, and dendritic cells. The effects of Thy1 antibody treatment on these cell types have not been fully explored.

Importantly, the use of these models has provided critical information on ILC biology, and has moved the field forward to a point where researchers can start testing how ILCs function in the presence of adaptive immunity using more advanced tools. So far, the overlapping genetic requirements of T cells and ILCs have made it difficult to manipulate innate

lymphoid populations in mice without simultaneously altering T cell populations. As of yet, no single transcription factor has been shown to be uniquely required by ILCs but not other immune cells for their development or function. Genes necessary for normal ILC precursor specification (such as *Id2*, *Tcf7*, *Gata3*, *Tox*, and *Nfil3*) and genes required for ILC subset maturation (such as *Bcl11b*, *Tbx21*, and *Rorc*(γ t)) are required either during thymocyte development or by polarized T cells for their functions. Similarly, strategies for generating ILC-specific Cre-recombinase mice have been complicated by the shortage of positive, exclusive lineage markers for ILCs. This is a particular limitation for ILC2s and ILC3s, which are identified in part by the absence of cell surface markers for other lineages, including T cells.

In general, two strategies for genetically targeting ILCs have been used to divide the innate and adaptive lymphoid compartments: (1) using Cre-lox systems to target two genes expressed by ILCs that in combination, are minimally expressed or required by T cells, and (2) driving expression of a floxed diphtheria toxin receptor (DTR) allele in both T cells and ILCs, then “rescuing” T cells from DTR expression through T cell-specific expression of Cre recombinase. Some examples of effective Cre recombinase lines that have been used to study ILC subsets, but that are not necessarily specific to ILCs, include transgenic *Ncr1*-Cre mice^{30, 31, 32}, which drive Cre expression in all subsets of group 1 ILCs as well as NKp46⁺ ILC3s; knock-in *IL5*-Cre mice³³, which target ILC2s but also an IL-5-expressing subset of T_H2 cells; and transgenic *Rorc*(γ t)-Cre mice¹⁶, which drive Cre expression in ILC3s, as well as in T cells during thymopoiesis. These mice have been crossed to several floxed alleles of cytokines and transcription factors expressed by ILCs. Additional selected mouse models that have been used to study ILCs are listed (Table 1).

The discovery of additional ILC-specific genes should allow for more targeted manipulation of ILCs at different stages during development and maturation. Such genes are starting to be uncovered through the Immunological Genome (ImmGen) Project, which has provided gene expression analyses of intestinal ILC subsets³⁴. Additional work directly comparing gene expression and regulation in analogous effector T cell and ILC subsets (for example, IL-5-expressing T_H2 cells versus ILC2s), will be important for generating tools to distinguish contributions by these populations³⁵. Gene expression in ILC subsets should also be compared to T_{RM} cells. These subsets of memory T cells, following expansion and differentiation, migrate into nonlymphoid tissues where they reside without recirculating in blood³⁶. ILCs are likely to share more core transcriptional signatures with T_{RM} than with effector T cell subsets based on genes associated with tissue residency and activation.

Unique *in vivo* activities of ILCs versus T cells in mice

Several unique functions of murine ILCs have been described during fetal and neonatal development, under steady state conditions in adults, and after irradiation. At this point, there is less known about the non-redundant functions of ILCs during an immune response, although ILCs have been shown to regulate epithelial cells, T cells, and myeloid populations during infection. Here, we discuss selected examples of unique activities of ILCs, while additional functions of ILCs are discussed in the companion reviews in this issue.

ILCs in gestation and neonatal life

Secondary lymphoid tissue organogenesis occurs in the fetus and is dependent on a specialized subset of ILC3, the LT α cell¹⁷. During fetal development, LT α cells and their precursors develop in the fetal liver and migrate to peripheral tissues, where they undergo further maturation and induce lymph node and Peyer's patch development via lymphotoxin- $\alpha_1\beta_2$ (LT $\alpha_1\beta_2$)³⁷. Interactions between LT $\alpha_1\beta_2$ ⁺ LT α cells and lymphotoxin- β receptor (LT β R)⁺ stromal cells (also known as stromal organizer cells) initiate development by inducing stromal production of adhesion molecules and chemokines, including VCAM-1, the CXCR5 ligand CXCL13, and the CCR7 ligands CCL19 and CCL21. These factors initiate a positive feedback loop by locally recruiting additional LT α cells and ILC precursor populations to the developing lymphoid organ. The absence of LT α cells (as in *Rorc*^{-/-} mice), lymphotoxin signaling (as in *Lta*^{-/-}, *Ltb*^{-/-} or *Ltr*^{-/-} mice), or the chemokine receptors CXCR5 and CCR7 lead to severe deficiencies or complete abrogation of secondary lymphoid tissue development.

The postnatal development of tertiary lymphoid organs in the gut is similarly driven by ILC3s. NKp46⁺ LT α -like ILC3s induce the development of cryptopatches (CPs) and isolated lymphoid follicles (ILFs) through activation of LT β R signaling^{38, 39}. However, unlike secondary lymphoid tissue organogenesis, tertiary lymphoid tissue development in the gut requires additional signals from the environment. Peptidoglycan from intestinal microbes drives the development of ILFs in a NOD1-dependent manner⁴⁰. In addition, the aryl hydrocarbon receptor (AHR), a sensor of diet-, microbe- and endogenously-derived planar aromatic hydrocarbons, is required for normal expansion of adult ILC3s in the small intestine^{41, 42, 43}. Thus, tertiary lymphoid structures are absent in AHR-deficient mice, while secondary lymphoid tissue development proceeds normally in the absence of AHR.

During pregnancy, ILC1s have been implicated in promoting vascular changes in the uterus. In humans, uterine spiral arteries undergo remodeling during pregnancy to increase blood and nutrient flow to the fetus and placenta. In mice, the equivalent vascular structures are the major decidual arteries, which undergo remodeling during pregnancy in an IFN- γ -dependent manner⁴⁴. Data strongly suggest that ILC1s are required for this process. Remodeling of murine uterine vasculature requires lymphocytes and IL-15, whereas T cells are not required^{13, 44, 45, 46}. Importantly, mice with *Nfil3*-deficient lymphocytes exhibit defects in uterine vascular remodeling^{13, 47}. *Nfil3*-deficient mice lack uterine Eomes⁺CD49b⁺CD49a⁻ cNK cells, and have reduced numbers of a second uterine ILC1 subset that expresses CD49a¹³, suggesting that these populations are important for normal vascular remodeling. Additional studies will be required to determine the specific contributions of ILC1 subsets and the mechanisms behind uterine vascular remodeling.

Constitutive production of cytokine

ILC2s actively secrete type 2 cytokines in the absence of infection. In naïve mice, ILC2s produce the eosinophil growth factor IL-5 in the heart, kidney, lung, muscle, skin, gut, and uterus³³. IL-5 secretion follows circadian rhythms regulated by food intake, with serum IL-5 and blood eosinophil levels elevated at the beginning of the light cycle, and reduced at the

beginning of the dark cycle, as well as after fasting. Although the effects of circadian IL-5 secretion and blood eosinophil levels on host physiology are unclear, the induction of this pathway following food intake suggests that they might have roles in regulating dietary metabolism. Indeed, increased adiposity has been observed in eosinophil-deficient mice⁴⁸.

Recently, ILC2s have been shown to regulate the development of a specialized gut epithelial lineage, the tuft (or brush) cell^{49, 50, 51}. Tuft cells, which previously had no known function, have been implicated as sentinel cells that detect parasitic worms. This epithelial population is a unique producer of IL-25, an IL-17 family cytokine that activates ILC2s. During the steady state, intestinal ILC2s produce IL-13, which acts on the epithelial stem cell compartment at the base of the crypt-villus axis to promote a basal level of tuft cell development. In turn, tuft cells produce IL-25 at a level that maintains steady-state ILC2 activation. During infection with worms, this regulatory circuit between tuft cells and ILC2s is enhanced in a manner dependent on the tuft cell chemosensory signaling molecule *Trpm5*⁵⁰, leading to tuft cell and goblet cell hyperplasia, and thereby promoting worm expulsion^{49, 50, 51}. Thus, ILC2s have unique functions in the pathways that detect the initial presence of parasitic worms in the gut.

ILC3s also actively produce effector cytokines in the steady state. In unchallenged mice, ILC3s are the major source of IL-22, a cytokine that induces the proliferation and survival of intestinal epithelial cells (IECs). ILC3-derived IL-22 also induces the production of the anti-microbial peptide Reg3 γ and α (1,2)-fucosylation of IECs, which contribute to the maintenance of homeostatic interactions with gut microbiota^{10, 52}. Intestinal NKp46⁻ ILC3s are located at the bases of intestinal crypts in cryptopatches, while NKp46⁺ ILC3s are widely distributed throughout the lamina propria. Thus, it is possible that these subsets may provide IL-22 to epithelial cells at different locations along the crypt-villus axis. It is not clear whether basal IL-22 production by ILC3s is induced by microbiota, since there are conflicting reports of whether intestinal flora induces or represses IL-22 production by ILC3s^{7, 10, 19, 41}. Interestingly, gut epithelial IL-25 negatively regulates ILC3s, and IL-25 expression by the epithelium is reported to increase with age¹⁹. Future studies should shed light on how aging correlates with changes in ILC populations in the gut.

Finally, it has been proposed that NKp46⁺ and NKp46⁻ ILC3s in the gut constitutively produce GM-CSF in response to IL-1 β secreted by macrophages⁵³. In the absence of GM-CSF, dendritic cell and macrophage populations are reduced in the colon. Additionally, dendritic cells from *Csf2*^{-/-} mice have blunted TGF- β production, and macrophages from these same mice produce less IL-10 than wild-type mice. Thus, ILC3s may contribute to the maintenance of normal cellularity and myeloid functionality in the gut lamina propria under steady-state conditions through GM-CSF production.

Radioresistance

ILC3s have been observed to persist in mice exposed to lethal doses of gamma-radiation. The radioresistance of these cells is presumably due to their slow cell division rate⁵⁴, which reduces the fraction of the population in radiosensitive phases of the cell cycle, and may

allow more time for DNA repair to take place. In comparison, T cells are extremely radiosensitive, and are greatly reduced three days post-irradiation.

Radioresistant ILC3s have been shown to protect mice from tissue damage induced by irradiation as well as allogeneic responses during graft versus host disease (GVHD). In mice and humans, irradiation induces proliferating epithelial cells in intestinal crypts to undergo apoptosis⁵⁵. This disrupts crypt-villus organization, and can create breaches in the intestinal epithelial barrier. Regeneration of gut epithelium requires *Lgr5*⁺ intestinal stem cells (ISCs), which reside at the bases of crypts and give rise to all lineages of gut epithelial cells^{55, 56}. During GVHD, IL-22 derived from radioresistant ILC3s protects this ISC pool. GVHD can be experimentally induced by transplanting irradiated mice with bone marrow and T cells from MHC mismatched mice, or from mice that have mismatched minor histocompatibility antigens (MHAs). Mice that lack IL-22 have increased gut pathology, more severe loss of ISCs, and reduced survival in this model⁵⁷.

Similarly, thymic regeneration after irradiation is enhanced by IL-22 secreted by radioresistant ILC3s. In the thymus, irradiation induces loss of thymocytes as well as the loss of cortical and medullary thymic epithelial cells (cTECs and mTECs), which are required for positive and negative T cell selection. IL-22 expression is increased after irradiation, and enhances the regeneration of both cTECs and mTECs⁵⁸, restoring normal thymopoiesis. These studies have important implications for the roles of ILCs in tissue regeneration and protection against GVHD in patients undergoing bone marrow transplantation.

Regulation of T cells

Both ILC2s and ILC3s have been reported to express MHC class II⁵⁹. Because TECs, dendritic cells, macrophages, and B cells require MHC class II to present antigen to T cells, it has been hypothesized that ILCs may also directly interact with T cells with this molecule. Multiple groups have shown that, unlike professional antigen presenting cells, MHC class II-expressing ILC3s do not induce the expansion of T cells in culture^{60, 61}. Instead, *Rorc*(γ t)-Cre $\times$ *H2-Abi*^{fl/fl} mice, which have MHC class II deleted from both ILC3s and T cells, have increased T_H17 responses in the gut^{60, 61}. There have been conflicting reports on whether this phenotype is linked with intestinal inflammation. Two groups found no gut inflammation in *Rorc*(γ t)-Cre $\times$ *H2-Abi*^{fl/fl} mice^{60, 62}, while another group found that these mice develop spontaneous intestinal inflammation and rectal prolapse⁶¹. These differences may be due to the variances in microbial communities across mouse facilities. Although the mechanisms behind the increased T_H17 responses in *Rorc*(γ t)-Cre $\times$ *H2-Abi*^{fl/fl} mice are not well understood, it has been proposed that ILC3s directly induce cell death of microbe-specific CD4⁺ T cells via mechanisms similar to those that promote negative selection of T cells in the thymus⁶³. ILC3s migrate from the intestine to interfollicular regions of the draining mesenteric lymph nodes in a CCR7-dependent manner, and the impact of this trafficking on T cells is unclear⁶⁴. Outside of the gut, loss of MHC class II expression by ILC3s was reported to lead to reduced T cell responses in the spleen in an OT-II adoptive transfer model⁴³, suggesting that the functions of MHC class II in ILC3s may be different across tissues. In ILC2s, MHC class II expression is partly dependent on trogocytosis⁶⁵.

Similar to ILC3s, ILC2s do not induce T cell proliferation *in vitro*, although ILC2s may enhance T_H2 polarization in T cells stimulated with plate-bound anti-CD3 and anti-CD28^{65, 66}. Instead, MHC class II-expressing ILC2s have been proposed to induce T cells to produce IL-2, which feeds back on ILC2s to promote activation⁶⁵.

ILCs also regulate T cells indirectly by modulating macrophage and dendritic cell function. During a primary response, IL-13 produced by ILC2s promote dendritic cell migration from the lung to the draining lymph node, where these dendritic cells prime naïve T cells⁶⁷. During a secondary response, IL-13 secreted by ILC2s induce IRF4⁺CD11b⁺CD103⁻ dendritic cells in the lung and skin to produce CCL17, a ligand to the T_H2-associated chemokine receptor CCR4⁶⁸. In the absence of IL-13 or ILC2s during a recall response, CCL17 is not expressed in the lung, and the accumulation of memory T_H2 cells in the lung is reduced. Likewise, ILC3-derived GM-CSF production in the gut induces macrophages and dendritic cells to produce TGF- β and IL-10, which promote regulatory T (T_{reg}) cell expansion⁵³.

Overlapping roles of murine T cells and ILCs during infection

The pathogen *Citrobacter rodentium* (*C. rodentium*), a model for enteropathic *Escherichia coli* (*E. coli*), has been used extensively to demonstrate that IL-22 has protective roles during intestinal inflammation. Both T cells and ILC3s produce IL-22 in this model. In studies using *Rag2*^{-/-} mice, ILC3s have been shown to be important for controlling infection. Infected *Rag2*^{-/-} mice have a survival advantage over *Rag2*^{-/-}*Il2rg*^{-/-} mice⁷, and depletion of IL-22 in *Rag2*^{-/-} mice decreases survival⁶⁹. Additionally, mice that lack IL-23, a cytokine that induces ILC3s to secrete IL-22, rapidly succumb to infection after inoculation with standard experimental doses of bacteria⁶⁹. However, studies using mice with intact T cells have indicated that ILC3s may have redundant roles in this model. Mice infected with low doses of *C. rodentium* exhibit survival that is independent of IL-23, and thus, independent of ILC3s⁷⁰. Instead, IL-22-expressing CD4⁺ T cells are crucial for survival at low inoculums. Notably, mice lacking functional NKp46⁺ ILC3s exhibit no differences in survival or weight loss compared to wild-type mice after infection with standard inoculums^{71, 72}, indicating that NKp46⁺ ILC3s are not required for host protection against *C. rodentium*. However, mice deficient in NKp46⁺ ILC3s develop ulceration and bleeding in cecums during infection, indicating that these cells have other specialized functions in this model that still need to be characterized⁷².

Comparing ILCs and T cells in human

Recently, genome-wide comparisons between transcriptomes and epigenomes in human ILC and helper T cell subsets have been performed⁷³. In cells isolated from inflamed pediatric tonsils, active enhancers in T cells are enriched for AP1 and IRF binding motifs, while active enhancers in ILCs are enriched for Runx and ETS binding motifs. The different transcription factors that bind these motifs likely reflect differences in T cell and ILC activation pathways (i.e. by TCR signaling versus cytokine receptor signaling). ILC3s and T_H17 cells also exhibit super-enhancers within IL-22 and IL-17 loci, respectively, reflecting the different capacities of ILC3s and T_H17 cells to produce these cytokines. Transcriptional

profiling of human ILCs conducted at the single cell level using RNA-seq recently demonstrated that there is further heterogeneity in Lin[−]CD127⁺ cells defined as ILC3s based on transcriptional clustering and expression of *RORC*⁷⁴. Thus, single cell comparisons between human T cells and ILCs may provide additional ontogenic and functional insights.

Concluding remarks

The overlapping and unique functions of ILCs and T cells are still being defined in mouse and human. The development of more specific genetic tools to study ILCs in mice will be crucial to identify specialized activities of these cells during disease states. Expansion within this area, and in research characterizing regulatory pathways in these cells, will be essential to identify therapeutic interventions for human diseases that act either selectively on ILCs or collectively on lymphocytes.

Acknowledgements

The authors would like to thank M. Robinette, M. Patnode, M. Cella, and G. Oltz for critical reading of the manuscript. We also thank J. O'Shea for helpful discussion. This work was supported by the NIH grants AI095542, DE021255, and DK103039, and by the Kenneth Rainin Foundation. J.K.B received support from the grant T32 HL731737 and is a Cancer Research Institute Irvington Postdoctoral Fellow.

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Fig 1. ILCs, innate-like T cells, and conventional T cells are functionally compartmentalized based on tissue residency and activation pathways

ILCs, iNKT cells, $\gamma\delta$ T cells, and T_{RM} cells are tissue resident populations that can rapidly produce cytokines at sites of infection. In contrast, primary conventional T cell responses take place over several days as these cells clonally expand and differentiate in secondary lymphoid organs, and then migrate to nonlymphoid tissues. ILCs are mainly activated by an array of soluble mediators, while the activation of iNKT cells, $\gamma\delta$ T cells, and conventional T cells primarily involves direct interactions with TCR ligands presented by other cell types.

Table 1

Genetic strategies for targeting ILCs

Genetically engineered allele(s)	Ref	Category	ILC subset(s) targeted	T cells targeted (Effects on lymphoid tissue development are also noted)
<i>Ncr1</i> -Cre	30, 31, 32	Conditional	ILC1s and NKp46 ⁺ ILC3s	None known
<i>Ncr1</i> -Cre x <i>Mcl1</i> ^{fl/fl}	72, 75	Conditional deletion of <i>Mcl1</i> in NKp46 ⁺ cells	NK cells and NKp46 ⁺ ILC3s; effects on other ILC1s are not reported	None known
<i>Ncr1</i> -Cre x Rosa-DTA	72	Conditional expression of diphtheria toxin by NKp46 ⁺ cells	ILC1s and NKp46 ⁺ ILC3s	None known
<i>Ncr1</i> -Cre x <i>Tgfr2</i> ^{fl/fl}	76	Conditional deletion of TGF- β receptor in NKp46 ⁺ cells	NKp46 ⁺ salivary gland ILC1s as well as other <i>Tgfr2</i> -dependent ILC1s	None known
<i>Il5</i> -Cre	33	Conditional, knock-in	IL-5 ⁺ ILC2s	Cre is expressed by IL-5-producing T _H 2 cells
<i>Cd4</i> -Cre x ICOS ^{flox} DTR ^{flox}	65	The diphtheria toxin receptor (DTR) is expressed by ICOS ⁺ non-T cells	ILC2s and other CD4 ⁺ ILCs that express ICOS	T cells are rescued from DTR expression at the double positive stage in the thymus
<i>Rora</i> ^{sg/sg}	77, 78	Knockout	ILC2s	Unknown whether all T _H 2 subsets are intact
<i>Rorc</i> (yt)-Cre	79	Conditional	ILC3s	Cre is expressed by T cells during thymopoiesis
<i>Rorc</i> (yt) ^{-/-}	16	Knockout	ILC3s	T cells lack ROR γ t, leading to increased thymocyte apoptosis. Almost all secondary and tertiary lymphoid organs are absent.
<i>Rorc</i> (yt)-Cre x <i>Ahr</i> ^{fl/fl}	71	Conditional deletion of <i>Ahr</i> in <i>Rorc</i> (yt)-expressing cells	NKp46 ⁺ ILC3s and LT α i-like cells are greatly diminished	Loss of the aryl hydrocarbon receptor (AHR) in all T cells. ILFs and CPs are absent.
<i>Ncr1</i> -Cre x <i>Rorc</i> (yt) ^{fl/fl}	71	Conditional deletion of <i>Rorc</i> (yt) in NKp46 ⁺ cells	NKp46 ⁺ ILC3s	None known
<i>Il7r</i> -Cre	80	Conditional, knock-in	ILC1s, ILC2s, and ILC3s	Cre is expressed by T cells
<i>Zbtb16</i> -Cre	81	Conditional	ILC1s, ILC2s, and ILC3s are targeted, but not LT α i cells. Lower proportions of NK cells express <i>Zbtb16</i> .	Cre is expressed by NK T cells and MAIT cells. Also, some background expression occurs before the hematopoietic stem cell (HSC) stage.
<i>Nfil3</i> ^{-/-}	11, 82, 83, 84, 85, 86	Knockout	The development of cNK cells, ILC1s, ILC2s, and ILC3s is defective. <i>Nfil3</i> -independent ILC1 populations persist in	Effects on T _H 17 cells development reported.

Genetically engineered allele(s)	Ref	Category	ILC subset(s) targeted	T cells targeted (Effects on lymphoid tissue development are also noted)
			the salivary gland and uterus.	

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