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Why ILCs?

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Summary

Innate lymphoid cells (ILCs) are positioned in tissues perinatally, constitutively express receptors responsive to their organ microenvironments, and perform an arsenal of effector functions that overlap those of adaptive $CD4^+$ T cells. Based on knowledge regarding subsets of invariant-like lymphocytes (e.g.; γδ T cells, MAIT cells, etc) and fetally-derived macrophages, we hypothesize that immune cells established during the perinatal period—including, but not limited to ILCs—serve intimate roles in tissue that go beyond classical understanding of the immune system in microbial host defense. In this perspective, we propose mechanisms by which the establishment of ILCs and the tissue lymphoid niche during early development may have consequences much later in life. Although definitive answers require better tools, efforts to achieve deeper understanding of ILC biology across the mammalian lifespan have the potential to lift the veil on the unknown breadth of immune cell functions.

Introduction

Innate lymphoid cells (ILCs) are a recently-described subset of lymphocytes that reside in peripheral tissues and are particularly abundant at barrier surfaces. Whereas adaptive lymphocytes are most numerous in lymphoid organs—hence the derivation of the term “lympho-cyte”— ILCs are relatively rare in primary and secondary lymphoid tissues. Consequently, their existence has been overlooked for many years, as immunologists focused efforts on peripheral blood and lymphoid organs. However, it is now recognized that their positioning in peripheral tissues affords a strategic advantage for ILCs as early responders to tissue perturbation. Indeed, as a result of their location and effector phenotype, ILCs produce cytokines within hours of activation, in contrast to the days required for naive adaptive lymphocytes to be primed, expand, differentiate and enter tissues. Other innate and innate-like lymphocytes such as MAIT cells, γδ T cells, intra-epithelial lymphocytes (IELs), and NKT cells share features with ILCs (Fan and Rudensky, 2016; Godfrey et al., 2015), but we will focus on helper ILCs here while pointing out similarities with some of these other innate-like cells where appropriate.

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Unlike T and B cells, ILCs lack antigen-specific receptors and do not undergo genomic receptor rearrangements or clonal selection. They react to tissue perturbations independent of antigen stimulation, and polarization of their effector functions is a feature that largely arises during their development, rather than at the time of immunologic challenge. Despite these features which set them apart from adaptive lymphocytes, ILCs exhibit functional diversity that is nearly identical to T cells. In addition to conventional NK (NK) cells, which may be considered an innate counterpart to cytotoxic $CD8^+$ T cells, three major subsets of helper ILCs—called ILC1, ILC2, and ILC3 (Spits et al., 2013)—have been defined, corresponding to T helper-1 (Th1), Th2, and Th17 helper T cell subsets. Roles for ILCs in mice and humans have been described in inflammation and in response to intracellular pathogens, helminths, and extracellular bacteria or yeast, respectively (Ebbo et al., 2017; Klose and Artis, 2016), in unsurprising similarity to their T cell analogs.

Despite numerous investigations describing roles for ILCs in controlling pathogens and propagating diverse types of inflammation in mouse models, current literature reports a minority of infections or inflammatory syndromes in which ILCs are essential for host survival or stereotypic responses in the setting of an intact adaptive immune system (Bando and Colonna, 2016). In most mouse models, deficiency of ILCs may result in delayed clearance or kinetically altered development of adaptive immunity, but little impact on the eventual outcomes. Similarly, in humans, ILCs seem neither necessary nor sufficient for survival from infection. Patients with severe combined immunodeficiency (SCID) ultimately die of infectious complications in the absence of reconstitution of the adaptive immune system. Among SCID patients, those with *Rag1 or Rag2* mutations, which spare ILC development, appear as severely immunocompromised as those with mutations in *Il2rg* or *Jak3*, who lack ILCs as well as T and B cells. Cohorts of patients who receive hematopoietic stem cell (HSC) transplant without preceding conditioning chemoablation do not reconstitute ILCs after HSC transplant, yet show no increase in infectious complications after transplant as compared to patients who effectively reconstituted ILCs (Vély et al., 2016). Though taken with the caveat that studies of SCID patients have focused on blood ILCs (or their progenitors) rather than tissue residents, these afflictions of humans attest to the absolute requirements for adaptive immunity in sustaining détente with the microbial world: a fact further evidenced by the extensive polymorphism of MHC genes.

If ILCs are activated by many infectious challenges but largely redundant or non-essential, why do we have these cells? One probable but also insufficient explanation is the practical lack of tools to inducibly and selectively deplete or replete tissue ILCs, which has impaired the ability to discover their nonredundant functions in health and disease. Another potential (though trivial) explanation is that ILCs protected vertebrates from a group of pathogens that are no longer encountered, but which generated a critical bottleneck for survival at some point in evolution. Alternatively, these cells may be truly redundant, and innate cells, innate-like cells, and adaptive resident tissue memory cells together can substitute for their loss. More intriguing, however, is the possibility that they participate in processes hitherto unappreciated, which might represent a more fitting consideration in view of how long these cells were overlooked by immunologists. In positing this, we first point out characteristics of ILCs not shared by adaptive lymphocytes that might lead to hypotheses regarding their ultimate functions in tissue biology. Features specific to ILCs are their anatomical

positioning and activation in tissues during development—before adaptive immunity comes into play—and their constitutive mirroring of the effector functions that adaptive helper T cells later display during infectious challenge.

Positioning, expansion, homeostasis and replacement of ILCs

The origins of ILCs from lymphoid progenitors—initially in fetal liver and later in bone marrow—and the constellation of transcription factors that orchestrate their separation first from B and T lymphocytes, and subsequently, from NK cells and lymphoid tissue inducer (LTi) cells, have been categorized and summarized with ever-better reagents in mice (Constantinides et al., 2014; Juelke and Romagnani, 2016; Klose and Artis, 2016). In brief, common lymphoid precursors give rise to common innate lymphoid precursors (CILPs), which lack the ability to produce T and B cells; then to common helper-like ILC progenitors (CHILPs), which can give rise to ILC1s, ILC2s, ILC3s and LTi cells but not NK cells; and finally to ILC progenitors (ILCPs) that generate helper-like ILC1s, ILC2s, and ILC3s (Artis and Spits, 2015; Cherrier et al., 2012; Constantinides et al., 2014; Diefenbach et al., 2014; Klose et al., 2014; Seillet et al., 2014, 2016; Xu et al., 2015; Yu et al., 2014). ILC development in humans is less well characterized, but precursors capable of producing ILC1s, ILC2s, ILC3s, and NK cells - analogous to mouse common innate lymphoid progenitors, or CILPs, have been observed in cord blood, fetal liver, blood, and secondary lymphoid organs (Lim et al., 2017; Scoville et al., 2016). A human common helper-like ILC progenitor, or CHILP, has not yet been described and further work remains to fill in the trajectory for ILC development in humans.

Although the pathway for differentiation of ILCs in mice has largely been elucidated, the stage at which these precursors enter tissue and terminally differentiate - both during fetal development and adulthood - remains incompletely understood. Immediate ILC2 precursors (Seillet et al., 2016) (which may be ILC2s that have yet to display the activated phenotype imparted by tissue residence) are present in bone marrow, and could represent a source for seeding peripheral tissues. Alternatively, or in addition, multipotent progenitors may infiltrate peripheral tissues in utero, and subsequently differentiate, proliferate and repopulate those peripheral sites as needed. Such progenitors can be detected in the intestine in mice (Bando et al., 2015) and in multiple secondary lymphoid organs and blood in humans (Lim et al., 2017), but it is not yet clear whether all organs that house tissue ILCs retain local pools of precursor cells, nor from where and when these precursors originate.

Investigations of hematopoietic differentiation reveal unsuspected lineage-bias among pluripotent self-renewing HSCs (Carrelha et al., 2018; Laurenti and Göttgens, 2018). Primitive tissue-resident macrophage arise during early embryogenesis when yolk sac-derived precursors move into tissues—directly or via the fetal liver—and only later diversify gene expression to acquire tissue-specific functions and the capacity for self-renewal (Gomez Perdiguero et al., 2014; Gosselin et al., 2014; Mass et al., 2016; Soucie et al., 2016). Comparatively, innate and innate-like lymphocytes in the mouse arise later during fetal liver hematopoiesis when HSC lineage fate is lymphoid-biased as compared to adult HSCs (Beaudin et al., 2016). ILCs in mice arise during a wave of liver-derived fetal hematopoiesis from E13.5 – birth (Bando et al., 2015), contemporaneous with fetal monocytes, at which

time they become positioned in tissues through unknown developmental cues. At birth, driven by both endogenous and exogenous signals, these cells undergo marked tissue expansion and terminally differentiate to acquire mature effector function (Huang et al., 2018; de Kleer et al., 2016; Nussbaum et al., 2013). For ILC3s, expansion and acquisition of effector functions such as IL-17 and IL-22 are affected markedly by the microbiota and dietary ligands (Gury-BenAri et al., 2016). Comparatively, in our own experiments, expansion and acquisition of IL-5 and IL-13 capacity by ILC2s occurs normally in germfree mice, suggesting mechanisms not linked with the microbiota (unpublished). Single-cell RNA-seq methods have also shown effects of the microbiota on ILC1s and ILC3s that are much more impactful than on ILC2s (Gury-BenAri et al., 2016), corroborating different inputs that become integrated to drive terminal maturation of ILCs in tissues during the critical period between birth and weaning.

After initial seeding, expansion, and maturation, ILCs show little hematogenous redistribution to other tissues under homeostatic conditions (Gasteiger et al., 2015; Moro et al., 2016), and can be characterized as tissue-resident (Fan and Rudensky, 2016). Such biology requires that tissue ILCs are either exceptionally long-lived, or are repopulated *in situ* through the lifetime of the host. Indeed, under steady-state conditions, tissue ILC2s have a low rate of proliferation, and retain label without dilution over many weeks (Gasteiger et al., 2015; Nussbaum et al., 2013). During states of inflammation in which tissue ILC pools are greatly expanded, parabiosis experiments have shown at most a modest increase in hematogenous recruitment of ILCs, and the majority of cells appear to have expanded from local tissue pools (Gasteiger et al., 2015; Moro et al., 2016). Presumably the rate of replacement is augmented by vacancies in the tissue niche caused by the presence of inflammatory stimuli or the ablation of resident populations—as occurs with other tissue-resident leukocytes such as macrophages (Epelman et al., 2014; Guilliams and Scott, 2017)—while constrained by the maximal niche size, which is likely established during development. Assuming that the developmental biology of ILCs in humans and mice is similar, the occasional need to refresh or repopulate tissue ILC pools likely explains the presence of circulating precursors in human blood (Lim et al., 2017). It should be noted that the parabiosis experiments are not exhaustive in examining all mouse tissues, and it remains possible that some tissues do not house a long-lived, locally replenishing population, but rather depend entirely on circulating precursors. Rigorous experiments that establish factors that may affect the propensity for dissemination of putative ILC precursors between parabionts, such as the blood ‘dwell’ times and efficiency of exchange through parabiotic anastomoses, have also not been performed. Of note, a recent study has suggested that under some conditions, ILCs derived from one tissue may be capable of reaching another (Huang et al., 2018). However, the precise origin of these migratory cells and their importance has not yet been determined.

In sum, although gaps remain, we suggest the following model for ILC development (Fig. 1). Following the dissemination of primitive yolk sac-derived macrophages and in close concordance with fetal liver monocyte dissemination, ILC precursors seed peripheral tissues. During a second wave that extends from just before birth through weaning, they locally expand and gradually complete their development through acquisition of lineage-specific cytokine outputs (as discussed below) and tissue-specific phenotypes in response to cues

from niche stromal cells (Koga et al., 2018). Thereafter, they are sustained by a combination of local division, differentiation from locally deposited precursors, and varying amounts of re-population from bone marrow cells (or potentially other tissues) that differentiate in adult life. The contributions of these various pathways to ILC populations and their maintenance likely differ between tissues and in response to different perturbations incurred during development, growth, and inflammation. The lifespan and fate of these cells requires further study, and their intimate relationships with the tissues in which they live will likely further inform our understanding of their ultimate function.

Three canonical outputs

ILC nomenclature and division into three groups was proposed “based on the cytokines that they can produce and the transcription factors that regulate their development and function” (Spits et al., 2013). As mentioned, these precisely mirror the canonical T helper subsets, Th1, Th2 and Th17 cells. Additional Th subsets, such as T follicular helper (Tfh) and regulatory T (Treg) cells, communicate primarily with other adaptive lymphocytes rather than with tissue cells, and are not further discussed in this context. The finding that ILC groups overlap with the outputs of adaptive helper T cells seems unlikely to be coincidental, and suggests shared functions by which these three outputs are sufficient to meet the needs of diverse tissues and the whole organism. Of note, the populations of innate-like T cells that express relatively invariant T cell receptors, such as some γδ T cells, NKT cells, MAIT cells, etc., can also generally be grouped into one or more of these three canonical functions (Fan and Rudensky, 2016; Godfrey et al., 2015). Such convergence warrants a closer consideration of the appearances of these outputs in evolution and the consequences of their absence.

Each of the canonical cytokine families is deeply rooted in the vertebrate lineage. Of these, the interleukin-17 family, the canonical effector of ILC3s, is most evolutionarily ancient, with orthologs in invertebrate deuterostomes and even nematodes (Han et al., 2015; Huang et al., 2015). The sea urchin has 30 IL-17-like genes and some are regulated in epithelial and immune cells at the intestinal barrier in response to bacterial injury (Buckley et al., 2017), akin to roles for IL-17 family cytokines in higher organisms including humans. Patients with defects in IL-17A signaling or auto-antibodies to IL-17A are unable to maintain environmental *Candida albicans* in the yeast form on body surfaces, and suffer from mucocutaneous candidiasis; cutaneous *Staphylococcus aureus* infections can also be problematic (Li et al., 2017). Intriguingly, as noted below, rare bi-allelic loss-of-function mutations in *RORC* have both impaired IL-17-mediated immunity and IFN-γ-mediated immunity, and present with both mucocutaneous candidiasis and environmental *Mycobacteria* infection (Okada et al., 2015). One might hypothesize that these pathways help to corral common environmental organisms that, when constrained, promote optimal immunologic tone for healthy tissues. This is akin to keystone organisms like segmented filamentous bacteria, which have important effects on maturation of the adaptive immune system in the mouse (Ivanov et al., 2009). The IL-17 family cytokines may also play roles outside of barrier tissues. In *C. elegans*, IL-17 signaling in interneurons regulates oxygen sensing critical for behavior (Chen et al., 2017). Recent findings of neurologic abnormalities in mice driven by elevated IL-17 during pregnancy demonstrate interactions with developing

neurons that extend to vertebrates (Choi et al., 2016) and build on the proposed pathologic role for subsets of IL-17-expressing T cells in multiple sclerosis (Hu et al., 2017).

Though hints of the relevant signaling pathways are seen earlier in evolution, the entire program for interferon family cytokines, including pattern recognition receptors, adapters, genes for interferon and related family members (e.g.; IL-22, see below), cytokine receptors and inflammatory target genes, are present in cartilaginous and bony fish, and typically characterized by a single type 2 IFN-γ-like gene (Secombes and Zou, 2017). Although present in the earliest jawed vertebrates, no IFN-γ gene has been found in jawless fish (e.g.; lamprey, hagfish) (Secombes and Zou, 2017), despite the presence of a variable lymphocyte receptor generated by combinatorial assembly, thus associating the appearance of the gene with *Rag1* or *Rag2*-mediated diversity used by adaptive lymphocytes (Agrawal et al., 1998). Within the immune system, interferon responses are particularly important for control of intracellular bacteria, protozoa, and viruses. Outside of the immune system, evidence has implicated IFN-γ in neural networks regulating social behavior in rodents, fish and flies, hinting at integration of this pathway more deeply into neural circuitry and social interaction (Filiano et al., 2016). Despite the wide-ranging roles for IFN-γ in mouse infectious and inflammatory models, humans deficient in components of the IL-12- and IFN-γ-pathway have a relatively restricted phenotype, characterized by inability to contain environmentally ubiquitous *Mycobacteria*. Adults with auto-antibodies to IFN-γ can present similarly (Browne et al., 2012).

Like IFN-γ, the type 2 cytokine locus shared by *IL5*, *IL13*, and *IL4* is present throughout fish, including the cartilaginous elephant shark, and land vertebrates, but is not evident in jawless organisms like the lamprey and hagfish, and thus also tracks with the acquisition of the *Rag1* and *Rag2* genes. Limited experiments suggest this locus performs similar activities in fish as in mammalian type 2 immune cell biology (Wang et al., 2016; Yamaguchi et al., 2015). Interest in ILC2 cytokines has been driven by effects on metabolism (Brestoff et al., 2015; Lee et al., 2015), tissue repair (Heredia et al., 2013; Li et al., 2014; Monticelli et al., 2011), and integration with central and peripheral nervous system (Cardoso et al., 2017; Gadani et al., 2017; Ibiza et al., 2016; Klose et al., 2017; Nussbaum et al., 2013; Sui et al., 2018) in diverse model systems that have together suggested fundamental roles in tissue and systemic homeostasis. We posit that the ability of ILC2s to impact tissue homeostasis is enabled by their localization within geographically limited stromal niches (Koga et al., 2018), and their ability to integrate tissue signals and restore homeostasis by directly stimulating tissue and recruiting circulating cells (Gadani et al., 2017; Nussbaum et al., 2013). Indeed, key circuits by which ILCs react to and modify epithelial barriers in order to affect tissue health and vertebrate behavior have been described (Van Dyken et al., 2014; Li et al., 2014; von Moltke et al., 2015; Monticelli et al., 2011, 2015; Oetjen et al., 2017). As noted below, tissue resident Th2 cells likely perform similar functions. Pathologic states of allergy and atopy represent dysfunctional responses driven by unregulated activation of these pathways and influenced by genetic, environmental, and possibly developmental aberrations, and are proposed to represent mechanisms prompting elimination of toxins or flight from exposure to environmental threats (Palm et al., 2012). Various type 2 cell-derived cytokines have been deleted from the mouse genome with relatively little impact, but natural genetic deletions of these pathways in humans remain undescribed.

Output integration and tissue states: Acute, Alert, and Restorative

We find it informative to consider the three canonical outputs of effector lymphocytes in the context of downstream targets and their temporal coordination (Fig. 2). In general, ILC3s and the IL-17-mediated pathway (including, more broadly, Th17 cells, many γδ T cells, etc.) regulate acute neutrophil accumulation and barrier reinforcement, and have been implicated in responses to extracellular bacteria and fungi. ILC1s, NK cells and the IFN-γ-mediated pathway (including, more broadly, $CD8^+$ T cells, Th1 cells, NK cells, NKT1 cells, etc.) contribute to an alert immunologic state by setting heightened cellular surveillance through augmentation of antigen-processing and -presentation on MHC molecules. Activation of this program is important in sustaining dormancy of latent DNA viruses and in providing heterologous protection against previously unencountered pathogens in an anticipatory manner (Barton et al., 2007). This pathway is constitutively engaged in homeostatic maintenance of peripheral tissue myeloid cells, particularly macrophages and dendritic cells, to sustain immune tone, including in humans (Nirschl et al., 2017). ILC2s and the IL-13-mediated pathway (including, more broadly, Th2 cells, NKT2 cells, etc.) contribute to restorative functions that intersect in incompletely defined ways with regenerative, neural, and metabolic pathways across multiple tissues to facilitate systemic homeostasis, as noted above. For the purposes of this perspective, we consider these general pathways as promoting dynamically interrelated tissue states designated Acute (induced by Type 3), Alert (induced by Type 1), and Restorative (induced by Type 2), that reflect these temporally integrated effects on tissues at a given time.

Although we here propose ‘Alert’ to describe the stably enhanced immune status of tissue, we note the use of ‘Trained’ (Netea et al., 2016) in reference to inherited epigenetic alterations of myeloid cells in response to inflammatory signals, including IFNγ. We use Alert to describe the tonic status of all tissues, inclusive of other immune and stromal cells, that may be affected by signals from lymphocytes. The Acute state represents a rapidly deployed metastable reaction to disruption of tissue function, whereas the Alert and Restorative states tend to be more stable states characterized by the concordant appearance of specialized effector outputs and adaptive immunity in vertebrate evolution. We envision the latter two states working in concert, such that energetic commitment to the Alert program is supported by systemic adjustments integrated by the Restorative program. Although activation of the Acute, Alert, and Restorative tissue states by the three canonical immune outputs is best described in the context of immunity, we believe that these pathways are activated continuously by sterile perturbations, such as metabolites (intracellular and extracellular), nutrients, hypoxia, abrasion, stretch, or membrane disruption, in order to maintain tissue health. Such a view further informs the observation that immune cells such as ILCs utilize these same essential pathways and yet are insufficient for defense against pathogenic microbes. Presumably, the degree of perturbation achieved by rapidly dividing organisms, such as viruses, bacteria, and fungi, with the capacity for genomic mutability, has necessitated the evolutionary scaffolding of adaptive immune cells onto the pre-existing innate pathways in order to sustain tissue homeostasis.

While acknowledging the over-simplification of lumping more elaborate systems of related cytokines into one of three groups, the re-iteration of these three canonical programs and

their employment as enforcers of Acute, Alert and Restorative programs by both ILCs and adaptive helper T cells, suggests that from an immunologic perspective, these three general programs are the core components necessary to sustain normal healthy tissues. Thus, though gradations of these functional outputs have been revealed by increasingly precise single-cell analytics, we feel that viewing immunologic function through the lens of these three essential outputs is a useful conceptual starting point. As above, we note the requirements for specialized adaptive cells – Tfh and Treg cells – to communicate with B cells and self-reactive T cells, but focus on the innate programs here.

The terms ‘Acute,’ ‘Alert,’ and ‘Restorative’ are not intended to imply that there is never acuity to Type 1 or Type 2 cell-associated responses, nor that tissue protective or reparative effectors can only be produced by the Type 2 cell-associated response. Eosinophilia, for instance, can be a rapid phenomenon that precedes tissue remodeling induced by Type 2 cell-associated cytokines. In the gut, IL-22, an effector cytokine that is part of the Type 3 panoply, plays an important role in epithelial homeostasis and tissue protection: effects that seem more consistent with an ‘alert’ or ‘restorative’ role. Intestinal IL-22-producing NK $^{+}$ILC3s (‘NK22s’) arise post-birth from NK-negative ILC3s in response to diet and microbiota (van de Pavert and Vivier, 2016; Vonarbourg et al., 2010). Notably, over time and in concert with stabilization of the diet and flora, a proportion of these cells acquire a spectrum of T-bet expression and therein gain the capacity to produce IFNγ, consistent with dynamic regulation of the Alert state (Klose et al., 2013). Intriguingly, IFNγ and IL-22 are evolutionarily related cytokines that reside at the same locus.

In considering relationships between these immune modules, we emphasize additional dynamic aspects of these canonical effector pathways. First, as discussed above, fate-mapping and other approaches have shown that, ‘ex-ILC3s’ or ‘ex-Th17 cells’ that previously expressed RORγt can, over time, become T-bet-positive ILC1s (Bernink et al., 2015; Vonarbourg et al., 2010), or, in the case of adaptive cells, Th1 cells (Hirota et al., 2011). This may serve as a mechanism by which Acute inflammation induces tissue transition towards an Alert state. Second, ILC2s have the capacity, with appropriate signals, to activate RORγt-dependent IL-17 expression (Huang et al., 2014) and promote the Acute tissue state, or activate T-bet-dependent IFN-γ expression (Bal et al., 2016; Lim et al., 2016; Silver et al., 2016) and promote the Alert tissue state. Thus, under conditions of tissue stress (such as exceeding metabolic thresholds necessary for healthy tissue function), Type 2 cell effectors can transform to promote Acute or Alert tissue states and restore homeostasis. Further research is necessary to uncover precise signals that drive these transitions *in vivo*.

Changing of the guard

The complement of tissue resident lymphocytes changes with the age of the organism and over progressive antigenic challenges. Specifically, we surmise that adaptive T cells progressively displace ILCs in response to pathogen-driven perturbations and during their natural senescence over life (Fig. 3). Examples of viral-specific CD8^{+} T cells replacing skin-resident γδ DETC after herpes infection illustrate the principles for replacement of innate, developmentally-regulated, lymphocytes by adaptive cells that bring the added capacity for antigen-specific recognition (Zaid et al., 2014). Recent studies in mice have also suggested a

replacement strategy by which ILCs orchestrate the initial wave of bowel colonization by the microbiota but adaptive T cells move in to ultimately adjudicate tissue homeostasis (Mao et al., 2018).

Given their striking similarity in form and function, we further hypothesize that tissue resident adaptive cells, which are critical for protective immunity, re-create the developmental pathways of their innate counterparts in order to establish tissue residency. In so doing, they likely use an overlapping array of signals to home, take up stable residence, and establish the transcriptional machinery necessary for longterm self-renewal in each specific host tissue. In short, we hypothesize that adaptive cells replace ILCs over the lifespan, achieve the capacity for activation by either innate or TCR-dependent signals, and rely on tissue occupancy in niches established during development where long-term residence, turnover, replacement and activation are accomplished. As such, roles for ILCs in tissue homeostasis are likely assumed by resident adaptive T cells, which take on proportionately larger roles in these functions with increasing age.

Embryonic establishment of the immunologic niche

If, as we surmise, ILCs largely reside in, replicate, and become activated in tissues in response to local perturbations, the initial positioning and activation of these cells in proportionate numbers becomes important. First, the relative ratios of one Group to another may impact the effector outputs on the tissue, with the potential to cause lasting effects on physiology (Fig 3). For example, an initial over-representation of ILC1s or ILC3s relative to ILC2s may favor the development of inflammatory immunopathology. The prototypical example to support a role in tissue patterning is that of LTi cells, without which secondary lymphoid organs are underdeveloped and the adaptive lymphoid niche is dysfunctional (van de Pavert et al., 2014). Because LTi cells stimulate mesenchymal cells to create the lymphoid niche, the size of the lymph node (and niche for adaptive lymphocytes) is directly dependent on the size of the seeding LTi population (van de Pavert and Vivier, 2016). Although LTi cells are unique in form and function, we hypothesize that other ILCs may serve unknown roles contributing to the health of peripheral tissues. Of note, interventions in pregnancy by nutrient deprivation (van de Pavert et al., 2014) or inflammatory stimuli (Beaudin et al., 2016) can alter the populations of innate lymphoid cells, such that animals challenged later in life have altered immune responses to challenge.

Second, the local renewal that follows initial seeding may lead to stable imprinting of tissue-specific functions. Such signals may define the receptivity of tissue resident ILCs to various activating signals, such as tissue-specific growth factors, hormones, cytokines, or neurotransmitters (Cortez et al., 2016). Work in our lab and others, for example, has shown that ILC2s in intestine constitutively express the receptor for IL-25, whereas those in the lung do not (Huang et al., 2018). While the mechanism underlying this diversity is unknown, it dramatically impacts the pathways by which these cells respond to tissue perturbation. In fact, there is precedent for tissue-resident cells adopting tissue-specific gene signatures to align them with the tissues where they reside. For example, both Treg cells and macrophages that reside in white adipose tissue express the transcription factor PPARγ, presumably to

interpret metabolic signals that inform their interactions within the specific tissue (Cipolletta et al., 2012; Odegaard et al., 2007).

Finally, we posit that interactions of ILCs with tissue niches are likely to involve bi-directional signaling aligned with their effector outputs. Thus, *establishment* of niches (presumably, sites where survival signals like IL-7, IL-15, and thymic stromal lymphopoietin (TSLP) are generated by specialized stromal cells) may be influenced by the timing, types and numbers of ILCs populating the tissue during development and perinatally, when these cells dramatically expand and activate their effector functions; such specialized mesenchymal niche cells are beginning to be identified (Koga et al., 2018). In other words, the perinatal production of effector cytokines by early tissue immigrants may communicate to the stroma the provisional requirements impacting the residency of future tissue lymphocytes later in life, akin to the effects of LTi cells in establishing Peyer's patch size (van de Pavert et al., 2014) or ILC3s in establishing intestinal cryptopatches and lymphoid follicles (van de Pavert and Vivier, 2016; Savage et al., 2017). Through this mechanism, the niche becomes itself sculpted for the long-term support and maintenance of stable pools of lymphocytes that work with resident macrophages and recruited myeloid cells to maintain organ function through life.

The big picture – or is there one?

So, again, why ILCs? Based on these speculative models, we suggest a number of ways by which ILC biology could be critical to human health, and yet have been missed – similar to the ways in which the cells themselves have remained obscure for so many years (Fig. 3). First, as discussed above, the numbers and types of ILCs positioned in tissues before birth and the perinatal period may establish the balance between Acute, Alert, and Restorative functions in tissue. Such patterns could affect the propensity towards development of early childhood illnesses as diverse as prolonged respiratory viral infections, asthma, or eczema, either directly or through secondary effects on the maturing microbiota and the development of tolerance for food antigens, and manifest by aberrant type 2 immune responses.

In addition, ILC-dependent alterations of lymphocyte niches in developing tissues may play out later in life by affecting the subsequent repopulation by resident adaptive T cells. Failure to engraft or sustain adaptive T cells in these faulty niches may propagate an imbalance between the three canonical outputs. Through this mechanism, quantitative or qualitative (i.e.; ratios of ILC1, ILC2, and ILC3 cells) alterations of the output from developmentally-programmed ILCs might alter the residence or function of adaptive T cells years or decades hence. These imbalances in tissue resident T cells may drive pathologic outcomes that no longer integrate appropriately with threshholds for tissue homeostasis, thereby compromising tissue function. Such a mechanism could underpin various auto-inflammatory and autoimmune diseases of young adulthood, often associated with aberrant IL-17-associated pathology, but would not be attributed to fundamental aberrations in ILC biology. Based on these considerations and epidemiologic arguments akin to the 'hygiene hypothesis', we suggest that aberrancies related to ILC differentiation and activation during the developmental and perinatal periods may influence the prevalence of diseases occurring later in life which only become manifest at the time of dysregulated adaptive T cell

responses, dysbiosis, and other consequences of faults in tissue homeostasis. Such a model of a tissue lymphoid niche, developmentally established with the help of ILCs, and subsequently reinforcing a persistent immunologic mileu, could explain why cytokine-blocking treatments used to treat chronic inflammatory diseases of adulthood often require chronic and even lifelong administration. Moreover, modulating the tissue seeding of ILCs —and their communication with the surrounding tissue—during early development may effectively alleviate the burden of T-cell-mediated inflammatory diseases of adulthood.

Even more intriguing is the possibility that quality control normally imposed on tissues by resident lymphoid cells may suffer deficits affecting longevity or cell fitness that might not be apparent until late in life. Tissue resilience necessitates smooth transition of stem-like cells from dormant to active states that are affected by the metabolic and inflammatory status (Keyes and Fuchs, 2018), and likely to be affected by aberrancies of immune cell occupancy. Imbalances created by alterations of normal ILC differentiation during development and propagated by adaptive T cells throughout life could impact stem cell dormancy, leading to senescence or fibrosis, or drive promiscuous entry into cell cycle, increasing risks for mutation and oncogenic transformation (Hernandez et al., 2018). An improved understanding of the developmental roles of ILCs on tissue differentiation and function during the critical early years of life will be essential to assess how these might play out in terms of pathologic states that appears decades later: a temporal dissociation that obscures the underlying processes. Additionally, concerted understanding of the role of resident T cells, both canonical and innate-like, in tissue homeostasis apart from anti-microbial defense may reveal unsuspected activities for lymphoid cells in maintaining wellness and longevity.

We suspect that integration of ILC biology into homeostatic pathways active in multiple tissues on a daily basis has obscured many aspects of their important role in filling the niche between development, the perinatal period, and the maturation of adaptive immunity. Investigation of these roles will require more refined reagents to fate-map ILCs and their adaptive counterparts, to selectively and inducibly delete or replete these populations, and to characterize the mechanisms by which they communicate with other cells and thereby modulate tissue functions. We foresee increasing interest in immune cell functions that are outside the more canonical roles assigned to host defense, and that might be targeted with an aim towards improving human health. The expanding ability to access and study human tissues has already impacted appreciation of the increasingly sophisticated developmental and transcriptional trajectories of ILCs. We predict continuing active research interests across the scientific community will elucidate the processes we lay out in this simplified and speculative Perspective, and help answer the essential question: Why ILCs?

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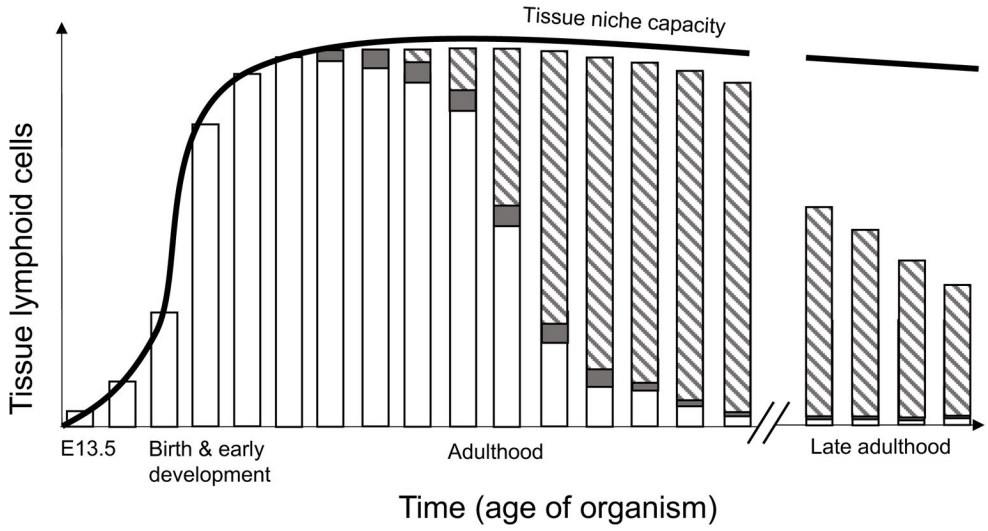

Figure 1. Positioning, expansion, homeostasis and replacement of ILCs

ILCs are initially generated and seed tissues during fetal development. A second wave of immigration and a dramatic expansion of tissue ILC pools occurs around the time of birth. Concurrently, both fetal and perinatally-seeded ILCs (white bars) develop their effector phenotypes under the control of tissue, diet, and microbiota-derived factors. During adulthood, ILCs regenerate predominantly through local renewal, although a minor contribution from circulating precursors (grey bars) can contribute to tissue pools. This contribution may be amplified if the local pools are depleted or under conditions of chronic tissue stress. As adulthood proceeds, the tissue lymphoid niche becomes progressively occupied by adaptive lymphocytes (hashed bars), which may assume the functions previously performed by ILCs (and other innate or innate-like lymphocytes). Eventually, with aging of the organism, tissue lymphoid populations may contract or may senesce, leaving the niche incompletely filled, and potentially leading to reduced function and/or impaired immunity.

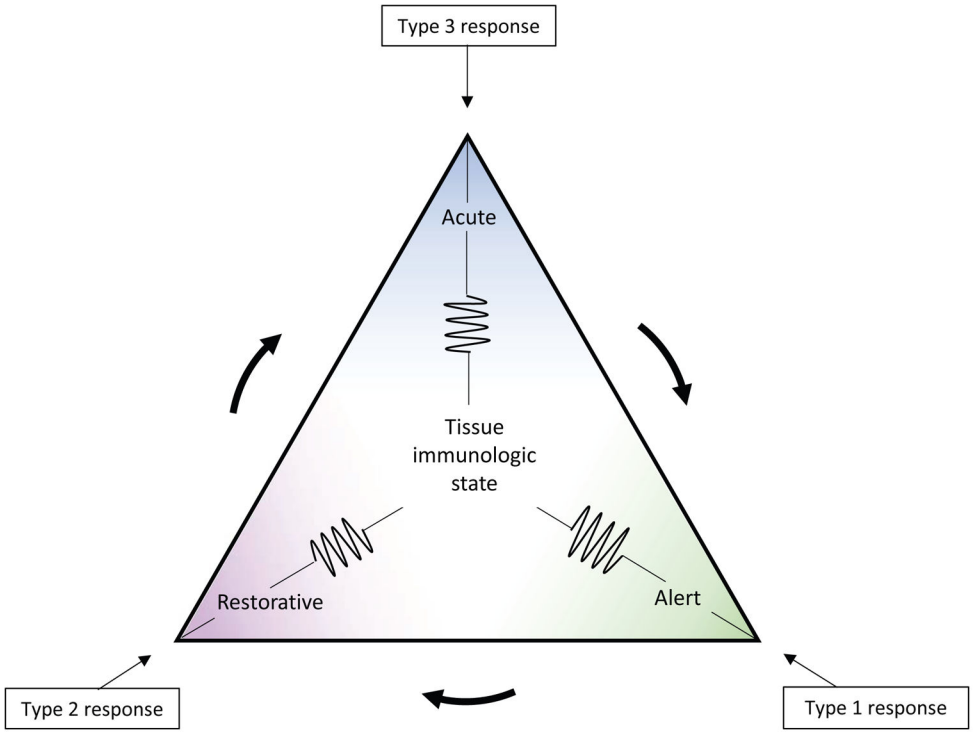

Figure 2. Three canonical outputs promote Acute, Alert and Restorative tissue states

The major outputs of the immune system—type 1, type 2, and type 3—promote 3 downstream tissue states: Acute, Alert, and Restorative. The Acute response, orchestrated by ILC3s, Th17 cells, and γδ T cells through elaboration of IL-17, promotes neutrophil accumulation and barrier reinforcement, and is a metastable state. The Alert state, orchestrated by ILC1s, NK cells, Th1 cells and others through IFN-γ, elevates the immunologic tone and heightens surveillance and tissue resistance. The Restorative state, coordinated by IL-4 and IL-13 from ILC2s, Th2 cells, etc., integrates with the tissue stroma and promotes tissue repair and stabilization of metabolic pathways. Compared to the Acute response, which is the most evolutionarily ancient but reactive and metastable, the Alert and Restorative states are more stable. These functions can each wax or wane depending on the (patho) physiologic context in order to promote the optimal tissue immunologic tone necessary to maintain tissue homeostasis. The states are normally constrained and balanced by one another such that a temporary perturbation—for example, a bacterial infection that requires an acute neutrophilic response—will ultimately be resolved by the Alert and Restorative responses (represented here by matched springs). If the balance of these functions is incorrectly established during development (one of the springs is wound too tight), the tissue state will oscillate around an altered and imbalanced equilibrium, and be susceptible to disease.

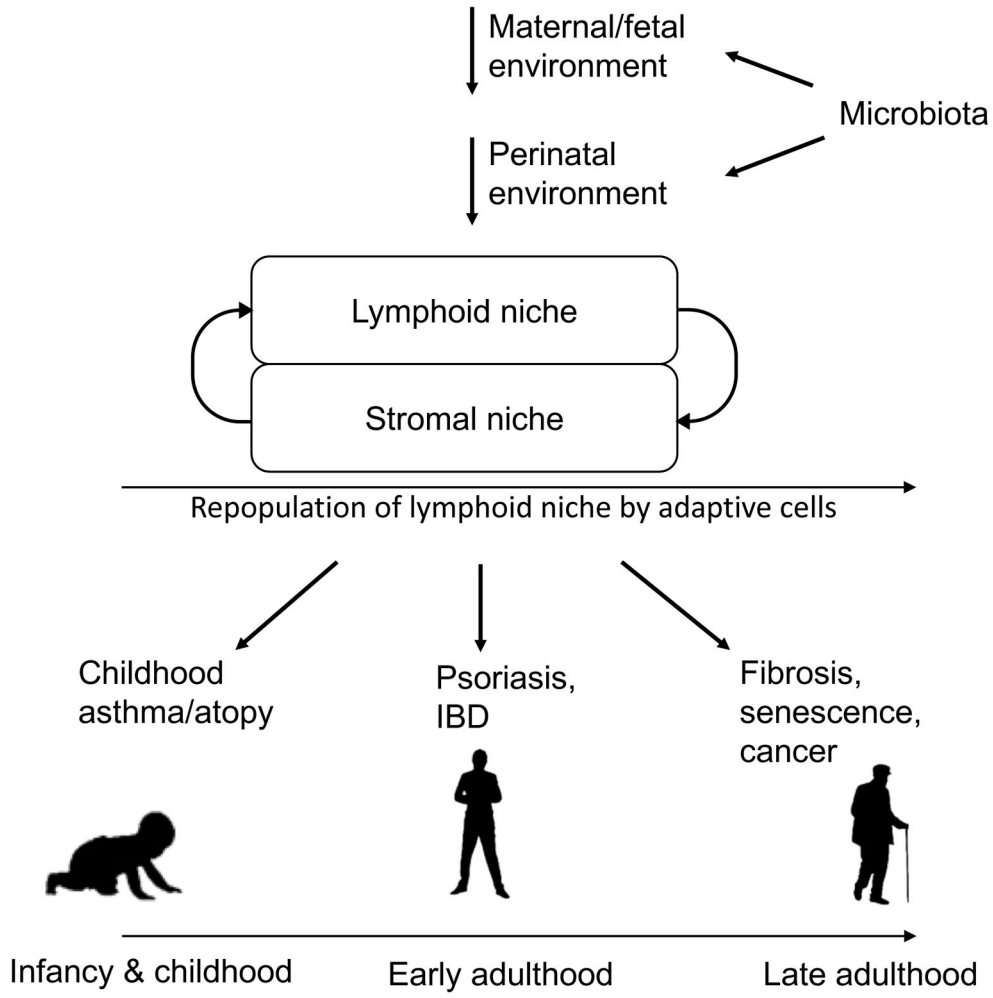

Figure 3. Embryonic patterning may impart long-term tissue effects

During development, the maternal-fetal environment, and subsequently the perinatal environment, determine the balance of tissue ILCs and other tissue-resident cells. As the tissue develops and grows, signals produced by these cells help establish a tissue niche that will support the lymphoid compartment over the lifetime of the organism. In addition, these signals from ILCs and other tissue-resident immune cells may impact other features of the stromal compartment, such as cell turnover, and stem cell protection. Therefore, early positioning of lymphoid cells helps define both the lymphoid niche and the tissue stromal niche. Disruptions in the normal developmental patterning and trajectory can lead to pathology at many stages of life. Initial ILC patterning that persists in infancy may yield atopy or asthma, for instance, if ILC2s are overrepresented. Later in life, adaptive lymphocytes assume the responsibilities of ILCs and take over the lymphoid niche. If the lymphoid niche established by fetal ILCs favors type 3 responses, for example, this may contribute to inflammatory conditions. Finally, lifelong crosstalk between the fetally-patterned immune niche and the stroma may promote normal tissue maintenance. When imbalanced, stem cells may exhaust or parenchymal cells may undergo gradual oncogenic or

mesenchymal transformation, leading to diseases of old age, such as fibrosis, senescence, and cancer.