Immunity
Perspective

Why Innate Lymphoid Cells?

Maya E. Kotas[1,*] and Richard M. Locksley[1,*]
[1]HHMI and Department of Medicine, UCSF, 513 Parnassus Avenue, S1032B, San Francisco, CA 94143-0795, USA
*Correspondence: maya.kotas@ucsf.edu (M.E.K.), richard.locksley@ucsf.edu (R.M.L.)
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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., natural killer T [NKT] cells, γδ T cells, mucosal-associated invariant T [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 mucosal-associated invariant T (MAIT) cells, γδ T cells, intra-epithelial lymphocytes (IELs), and natural killer T (NKT) cells share features with ILCs (Fan and Rudensky, 2016; Godfrey et al., 2015), but here we will focus on helper ILCs while pointing out similarities with some of these other innate-like cells where appropriate.

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 that set them apart from adaptive lymphocytes, ILCs exhibit functional diversity that is nearly identical to T cells. In addition to conventional natural killer (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(Th)-1, Th2, and Th17 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 major histocompatibility complex (MHC) genes.

If ILCs are activated by many infectious challenges but are largely redundant or nonessential, 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 Th 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 CILPs, have been observed in cord blood, fetal liver, blood, and secondary lymphoid organs (Lim et al., 2017; Scoville et al., 2016). A human 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, or 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 macrophages 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., 2015; Gosselin et al., 2014; Mass et al., 2016; Soucie et al., 2016). As compared to adult HSCs, innate and innate-like lymphocytes in the mouse arise later during fetal liver hematopoiesis when HSC lineage fate is lymphoid-biased (Beaudin et al., 2016). ILCs in mice arise during a wave of liver-derived fetal hematopoiesis from embryonic day (E)13.5 to 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 interleukin (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 germ-free mice, suggesting mechanisms not linked with the microbiota (unpublished data). Single-cell RNA sequencing 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 being 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