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ILC2s — resident lymphocytes pre-adapted to a specific tissue or migratory effectors that adapt to where they move?

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A cardinal feature of the T-cell adaptive immune system is the antigen-dependent activation of naïve T cells in secondary lymphoid sites, followed by the migration of the resultant effector cells through the efferent lymph to the blood and then into a peripheral tissue site of infection or tumor growth. In contrast, the current view of innate lymphocytes (ILCs), the innate counterparts of T cells, is that they are tissue-resident cells, adapted to their specific environments during development and performing their effector functions locally upon cytokine stimulation. Here we present recent findings that challenge the latter as defining the properties of ILCs, at least ILC2s. Our studies show that IL-25, administrated experimentally or generated in response to helminth infection, triggers local proliferation and activation of intestinal ILC2s that are the precursors to inflammatory ILC2 (iILC2) cells. These cells downregulate CD69 expression, upregulate S1P receptors and move across the villus lymphatic endothelium in an S1P-depndent manner. They subsequently enter the blood stream, through which they traffic to distant organs such as the liver and lung. In the lung, these iILC2 cells play a crucial role in host defense during the pulmonary stage of helminth infection. In the later stage of infection, a fraction of the iILC2 cells phenotypically convert into lung-resident natural ILC2 (nILC2)-like cells while another fraction homes back to their original location in the small intestine. These data support the view that ILC2s possess properties considered characteristic of adaptive T lymphocytes, namely local activation and distant effector function, but in response to alarm cytokines instead of specific antigen. These findings also raise questions about whether other ILC subsets show similar trafficking potential when suitably challenged, the extent to which such cells show plasticity in adapting to new tissue environments beyond the course of early development, and the relative roles of organ-resident versus migratory ILCs in host defense.

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The adaptive T cell paradigm

The adaptive T cell system must deal with three key issues in generating and executing its host protective functions — *identity*, *kinetics*, *and topography* [1]. *Identity* means recognizing the presence of a foreign invader, which we now understand can be not just peptides bound to classical MHC class I and class II molecules, but other components such as vitamin-like bacterial products presented to mucosal associated invariant T (MAIT) cells or lipids presented by CD1 to (natural killer T) NKT cell [2,3], all of which are recognized by clonally distributed receptors formed by somatic recombination and expressed by small numbers of cells in the naïve repertoire. *Kinetics* refers to the two types of temporal issues that confront the adaptive immune system. Evolution has selected a specific solution to the issue of getting rare cells to find unpredictable ligands in relatively variable locations around the body — it generates a large pool of circulating naïve T cells that traffic into and out of secondary lymphoid tissues throughout the body. The rate at which they move through a given lymphoid node or other secondary lymphoid site must be slow enough to give the cells time to find a rare antigen presenting cell — missing such an informative presenting cell would markedly retard or diminish the extent of the T cell effector response to the detriment of the host. But spending too long in one lymphoid site while doing such scanning can delay movement or another site draining the actual site of an infection, with similar negative effects of the host response. This trafficking problem is a macro scale issue to which evolution has obviously found a solution [4]. The second kinetic issue is the duration of interaction between other cells within these lymphoid site — this must be long enough to transmit essential membrane or secreted signals but not so long as

to prevent other required interactions or necessary migration [5], which is linked to the third issue of *topography*. Again, this operate sat two levels — within lymphoid sites the relevant T cell and antigen presenting cell populations need to be organized to promote within a suitable time frame what would otherwise be vanishingly rare interactions and interaction-enhancing micro-anatomical features of lymph nodes have been reported for both innate defense components [6] and cells involved in adaptive responses [7,8]. At the larger scale, once T cells are activated with a lymph node or other secondary lymphoid site, they must leave that location and traffic via lymph and blood to the parenchymal site of infection or tumor growth where they can orchestrate a suitable host defensive response. This latter aspect of adaptive T cell immunity means that a complex system for first keeping T cells within a lymphoid site to ensure that activation and differentiation proceed effectively must be followed by regulated exit from that site and entry into a totally distinct tissue environment [9].

The prevailing ILC paradigm

In contrast to the scenario just laid out for adaptive T cells, a large body of observations has given rise to a very distinct view of ILCs, especially non-NK helper-like ILCs. ILCs develop from common lymphoid progenitors (CLPs), and these progenitors first populate various tissues during the mid to late stages of fetal development or at the perinatal stage and then undergo further maturation along with the development of the relevant tissue [10]. Mature ILCs can be found widely distributed in both lymphoid and non-lymphoid tissues, especially barrier and mucosal surfaces, but different ILC subsets show clear differences in proportional representation among tissues [11]. ILC1s are mainly found in spleen and liver where they play a dominant role in tumor and viral infection surveillance. ILC3s are mostly concentrated in the gut, playing a critical role in resistance to pathogenic bacteria and in epithelium integrity. ILC2s localize in most barrier tissues including gut, lung and skin, where they modulate allergic responses and contribute to protection against helminth infections. ILC2 cells also play a critical role in thermogenesis through a process termed 'beiging' of adipose tissue. Therefore, the current concept for ILC development is that different ILC subsets are strategically localized in particular tissues in a manner that relates to their roles in maintaining normal tissue function and in contributing to protection against a large but specific constellation of pathogens. A more comprehensive review of ILC development and tissue specification can be found in a recent paper by J. Santo *et al.* [12].

Each group of ILCs expresses a particular lineage-specific transcription factor that promotes a distinct gene expression profile, which supports a selective capacity for cytokine production that enables their effector function in supporting host defense. But even within the same group, ILCs exhibit functional and phenotypic heterogeneity [13–16]. For instance, ILC2s in different tissues exhibit distinct cytokine responsiveness; ILC2s in fat-associated lymphoid clusters and in the lung primarily respond to IL-33 and intestinal ILC2s primarily respond to IL-25, while skin ILC2s also respond to thymic stromal lymphopoietin (TSLP) [17–23]. In this regard, ILCs can be analogized to embryologically derived tissue resident macrophages that transcriptional profiling shows possess tissue-adapted gene expression patterns that distinguish such cells present in different organs even though they appear to derive from the same precursors [24]. Further, under certain conditions circulating monocytes can 'replace' the tissue resident macrophages when the latter are lost due to pathologic process. The new macrophages take on most, though not all, of the specific gene expression patterns of the tissue in which they find themselves [25].

This dominant role of local environment has been combined with data from parabiosis experiments that (with an exception to be highlighted below) show little if any exchange of helper-like ILCs between partners in the steady state [26–28]. The resulting view has been that ILCs are tissue-resident effectors that are locally activated and that mediate their function where they reside, in marked distinction to the characteristic migratory nature of adaptive T cell immunity.

Changing the ILC paradigm

A few years ago, Bill Paul and one of us (YH) published a study looking at the effects of IL-25 administration or helminth infection on ILC2s [29]. In contrast to the usual view of ILC2s expressing ST2, the receptor for IL-33, in the lung, they defined a subset of ILC2s that expressed the receptor for IL-25 and did not express ST2. After IL-25 exposure, the ILC2s in the lung, liver and other peripheral sites were characterized by high expression of KLRG1 and production of type 2 cytokines, defining a new subset termed inflammatory ILC2 or iILC2. iILC2 have significant plasticity with the ability to become IL-17-producing ILC3-like cells when exposed to the proper cytokines or during fungal infection. With time after IL-25 exposure, some of the iILC2s in the lung gained ST2 expression and lost IL-25 receptor expression, coming to look phenotypically like the ILC2s naturally residing in the lung (nILC2s).

Follow-on experiments provided data that became harder and harder to reconcile with the existing tissue resident paradigm of ILCs [27]. First, prolonged parabiosis showed that there was a small but clear exchange of intestinal and mesenteric ILC2s between the paired hosts, sometime reaching 10% of the recovered cells. Second, adoptive transfer of genetically marked cells from the lung (the presumed location of the iILC2 precursor cells), bone marrow (the putative source of early precursor cells that could circulate before maturation), and small intestine (chosen because of this being

another major site of helminth infection and having tuft cells already shown to make IL-25 and promote ILC2 activity against worm infection) [30–32], gave very unexpected results after the animals were treated with IL-25. It was the small intestinal cell pool, and not the others, that gave rise to nearly all the iILC2s detected in the lung. This latter finding prompted studies using antibody labeling for intravascular cells to see if iILC2s generated by IL-25 treatment or infection would be found in the blood circulation, which they were, and additional parabiosis experiments, showing substantial exchange of iILC2s between hosts after cytokine treatment or infection.

These findings pointed to a need to reconsider the concept that ILC2 are strictly tissue-resident cells and also raised the question of how they moved between the small bowel and lungs or liver. Imaging studies showed that in IL-25-treated animals, a lamina propria resident pool of resting $CD69^+$ ILC2s proliferated, as indicated by Ki-67 positivity, became blasts and high for KLRG1 expression, reduced CD69 expression and strikingly, entered the central lymphatic in the small intestinal villi (the lacteal). This provided a clear pathway to the blood circulation and hence, from there to the liver or lungs.

Such images of activated lymphocytes traversing a lymphatic barrier to enter the efferent lymph and then the blood circulation seemed to parallel exactly the migratory behavior of adaptive T cells [33]. Since the latter was well characterized to involve changes in the activity and expression of receptors for the ligand sphingosine-1-phosphate (S1P), and in particular, the capacity for S1P gradient sensing promoted in part by a decline in post-activation CD69 expression [34], as also seen with the iILC2s, we postulated that the ILC2s might use a similar molecular pathway to control their trafficking. This was readily tested by treating the IL-25-exposed animals with the drug FTY720, which inhibits the S1P chemotaxis pathway [35,36]. As we predicted, this treatment blocked egress of the lamina propria ILC2s into the central lymphatic and prevented their appearance in distal sites, with just a small 'leakage' into the draining mesenteric lymph nodes. Evidence that this migration was critical for effective anti-helminth protection in the lungs came from experiments in which RAG deficient mice lacking a normal Th2 response were treated with FTY720 and infected with helminths. This proved lethal to the animals and the lethality could be reversed by transferring iILC2s isolated from IL-25-exposed mice directly into the bloodstream of the infected RAG KO animals, bypassing the trans-lymphatic blockage created by the FTY720 treatment.

A new view of ILC function

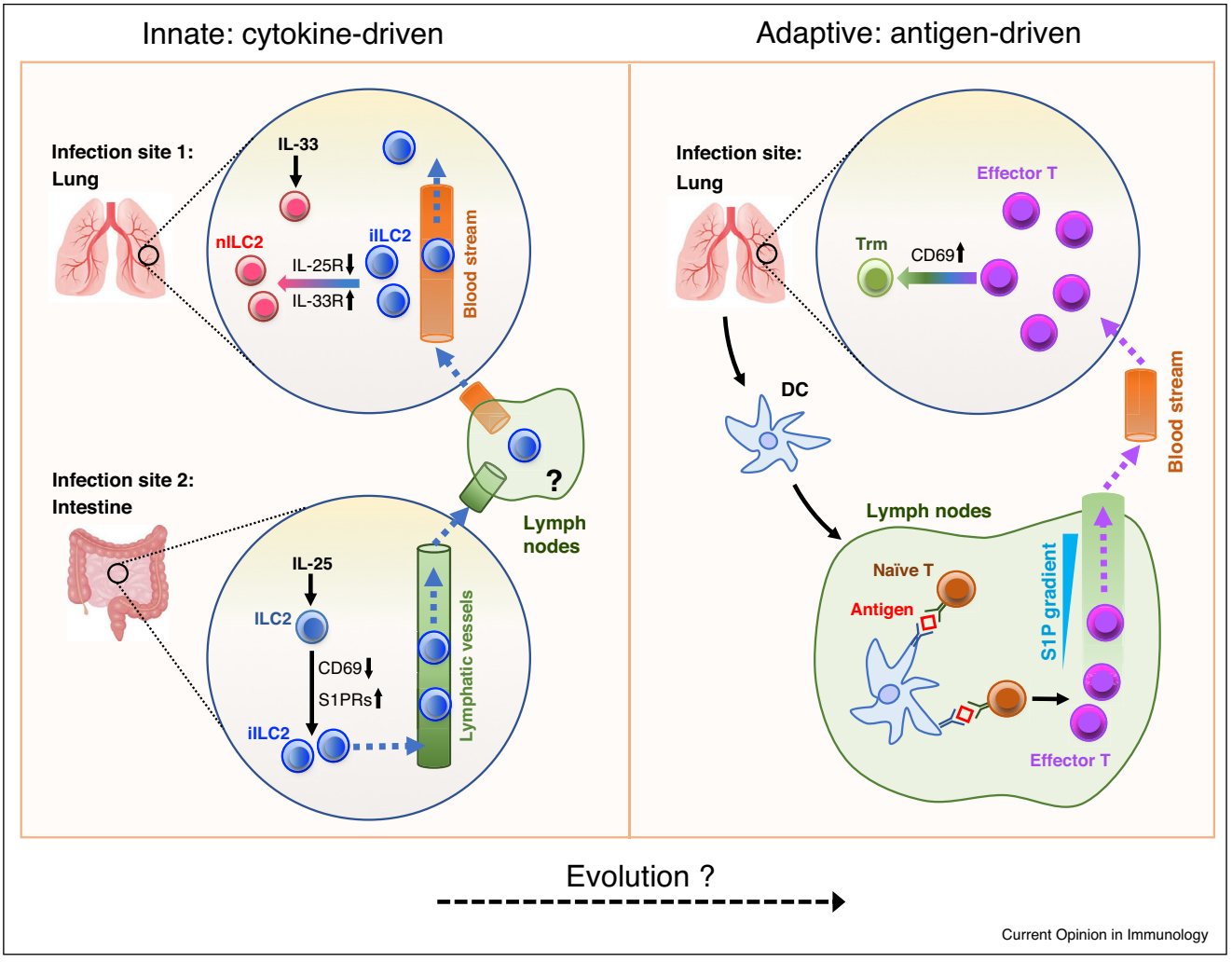
Given these new findings, we propose that a revision to the tissue resident model of ILC function is called for. For ILC2s at a minimum, an important aspect of their behavior is the capacity to be activated in one site, in the case of our studies the small bowel, and mediate crucial anti-pathogen effector functions and as well as tissue preserving cytokine production (e.g. amphiregulin) in a distinct organ site after migration through the lymphatics and blood circulation (Figure 1).

These data do not deny the established evidence that ILCs adopt phenotypes dictated by their local tissue environment [11,37]. However, it seems that such features are plastic and when an ILC population migrates to a new site, it can sense the new tissue cues and change its state to adapt to this new location. This is fully consistent with the evidence that small bowel derived iILC2s become nearly indistinguishable from lung nILC2 after some time of residence in pulmonary tissue [27,29]. This model can thus completely reconcile evidence for a high degree of tissue specialization with the evidence we have accumulated for lymphocyte migration between tissue sites. Tissue-specific adaptation of iILC2 following migration parallels the generation of T resident memory (Trm) cells. The molecular basis for arresting Trm, involving the transcription loss of S1pr1 and expression of CD69 [38,39], may also apply to the innate equivalent, namely iILC2 adaption to peripheral tissues.

An important paper in the field looking for evidence of migration of ILCs detected only marginal (5–10%) but not substantial exchange of ILC2s between parabiotic hosts during helminth infection [26], in contrast to the data we have obtained in our studies. One possible explanation for this different outcome lies in our preliminary data indicating that microbial signals contribute to the IL-25-driven migratory response of iILC2. The earlier study that did not detect substantial migration involved animals maintained on antibiotics when in parabiosis. If this is the correct explanation for the different sets of observations, it suggests that there is not just an effect of intrinsic tissue cues on ILC2 behavior, but either a direct response to microbial factors or an indirect effect of microbial signals on other types of lymphoid or non-lymphoid cells that stimulates production of signals essential to the effector responses of the resident ILC population. This potential impact of microbial factors might also offer an explanation for the difference in ILC representation in the blood of mice versus humans. While ILCs are barely detected in blood in the steady state in mice, human ILC subsets, particularly ILC2s, and ILC progenitors have been observed in the blood circulation [40,41]. This could be due to the fact that lab animals are housed in a 'specific-pathogen free' facility whereas human beings live in natural environment with a more diverse exposure to microbes.

While our data pertain directly only to the migration of gut ILC2s, we think it is important to raise some concerns

Figure 1



The migratory responses of innate and adaptive lymphocytes.
During helminth infection, intestinal lamina propria resident ILC2s respond to IL-25 cytokine to proliferate, downregulate CD69 expression, and upregulate S1PR expression, which allows these activated cells, termed iILC2s, enter the central lymphatics of intestinal villi and subsequently the blood stream. These circulating iILC2s accumulate in the lung, which is another site of helminth infection, and contribute to protective immunity and tissue repair. In the later stage of infection, a fraction of iILC2s adapts the local tissue environment and converts to lung-resident nILC2-like cells. This cytokine-driven migratory response of ILC2 parallels antigen-driven adaptive T cell migration, and the tissue adaption is also comparable to the process of effector T cells converting to tissue-resident memory T (Trm) cells. Whether adaptive lymphocytes grafted the migratory mechanism from innate lymphocytes during the evolution is an important question to be addressed.

about whether the existing data against migration of other ILC subsets is as strong as it is considered to be. Beyond the technical issues just raised about how prior studies looking for circulation of ILCs were conducted [26], it is quite clear from our experiments that substantial migration is only seen in the face of a strong activating stimulus. Because there is a constant but low degree of such stimulation by the bacteria in the gut, this can account for the small but detectable steady-state exchange of ILC2s seen in parabiosis studies above a near zero background for ILC3. But such analyses have not been conducted after infection with pathogens known to elicit ILC3 activation and to involve such infection at disparate sites. A series of such studies is currently underway to test the hypothesis that ILC can be mobilized just as adaptive T cells are following activation, though in the ILC case by cytokines and not antigen, and that they then patrol the body looking for suitable sites for accumulation based on local tissue inflammation.

It is also important to consider the parabiosis model itself. Circulatory conjunction in such animals is mediated only by skin vessels [42]. While these serve as adequate conduits for exchange between the hosts of cells with

prolonged blood residence such as neutrophils, monocytes, and naïve or resting memory lymphocytes, it has not been tested whether cells with short circulatory residence time effectively exchange. In cell biology, the principle is well established that a receptor moving between different cell compartments accumulates where the slowest transit step occurs and can show extremely low numbers in compartments where transit time is very rapid [43]. A well-known example is the transferrin receptor, which rapidly leaves the cell surface to enter early endosomes and then resides there for substantial times [44]; this leads to a very biased distribution of the receptor between cell surface membrane and intracellular vesicles, a ratio of 0.68–5.6 in healthy populations. For ILCs, if they leave a tissue site and then rapidly re-enter the tissue after a very brief time in the circulation, they will be very infrequent in the blood in the steady state and the short residence time makes trafficking through the entire circulatory tree unlikely. Thus, few will enter the shared localized skin anastomoses involved in parabiosis and hence, very little if any exchange will be seen, especially over modest time frames. It is essential that additional studies be conducted in which putative tissue resident cells, whether tissue-resident memory T (Trm) cells or ILCs, are placed directly in the blood of one of the parabiotic pair and assays performed to see if any of these cells can be detected in the partner, for example, using sensitive genomic tools that can detect very rare cells such as metastasizing tumor cells [45]. By comparing cells that are known to show good equilibration in parabiotic pairs that have been co-transferred with the Trm or ILCs and conducting a time series experiment, one can ascertain whether there is an efficiency difference with which cells known to be in the bloodstream actually move to a second host.

Concluding remarks

In closing, it is interesting to think at the evolutionary level about the shared pattern and indeed, molecular mechanism of migration control seen with adaptive T cells and ILC2s. There is a debate at present about whether ILC evolved from adaptive cells [46], or as would be more expected, the innate lymphocytes provided a platform that evolved into the adaptive system by the engraftment of the RAG recombination system onto the ILC backbone to produce cells with clonal receptors sensitive to antigen (Figure 1). This latter process would have presumably been accompanied by the emergence of discrete secondary lymphoid tissue sites and then the well-known multi-step process of intranodal activation, lymphatic egress, blood circulation, and peripheral tissue entry that is a defining characteristic of the adaptive T cell system. But rather than this being a unique evolutionary development emerging with the adaptive system, it might very well represent the appropriation of this existing mechanism from ILCs. Of course, while such speculations are intellectually appealing, the bottom line message is that the present adaptive T cell and ILC systems not only share transcription factors, early progenitors and effector cytokines, but also migratory and tissue plasticity behaviors of critical importance to host defense.

Conflict of interest statement

Nothing declared.

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