NK cells and type 1 innate lymphoid cells: partners in host defense

Hergen Spits[1,2], Jochem H Bernink[1,2] & Lewis Lanier[3]

Innate lymphoid cells (ILCs) are effectors and regulators of innate immunity and tissue modeling and repair. Researchers have identified subsets of ILCs with differing functional activities, capacities to produce cytokines and transcription factors required for development and function. Natural killer (NK) cells represent the prototypical member of the ILC family. Together with ILC1s, NK cells constitute group 1 ILCs, which are characterized by their capacity to produce interferon-γ and their functional dependence on the transcription factor T-bet. NK cells and ILC1s are developmentally distinct but share so many features that they are difficult to distinguish, particularly under conditions of infection and inflammation. Here we review current knowledge of NK cells and the various ILC1 subsets.

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NK cells, lymphoid tissue inducer (LTi) cells and ILCs are distinct subsets of lymphocytes that, like T and B cells, arise from a common lymphoid progenitor (CLP) cell. They are distinguished from B and T cells by their ability to differentiate into mature effector cells without a thymus and without recombination-activating gene or activation-induced cytidine deaminase activity. As a consequence, these ILCs lack antigen receptors encoded by rearranged genes, such as the T cell antigen receptor (TCR) and immunoglobulins. The prototypic members of this family of innate lymphocytes are NK cells and fetal LTi cells, which mediate innate immune responses against pathogens and cancer and are required for the formation of secondary lymphoid organs, respectively. Despite these very different functions, NK and LTi cells have been recognized to have a common origin because they depend on the transcriptional repressor Id2 and the common γ-chain of receptors for the cytokines IL-2, IL-7, IL-15 and others. Other ILC types that share the dependence on Id2 and common γ-chain have been identified. Analysis of the cytokine secretion profiles and transcription factor requirements revealed remarkable similarity between these innate lymphocytes and T cell subsets[1], which led to the proposal to classify them into group 1 ILCs (comprising ILC1s and NK cells) or group 2 or 3 ILCs (ILC2s or ILC3s, respectively)[2]. Group 1 ILCs were tentatively defined as cells that produce interferon-γ (IFN-γ) and are dependent on the transcription factor T-bet for their function and development, similarly to how type 1 helper T (T_H1) cells are defined. Because NK cells produce large amounts of IFN-γ, they were classified as group 1 ILCs, although they are not strictly dependent on T-bet (encoded by *Tbx21*) for their development[3].

[1]Department of Cell Biology, Academic Medical Center at the University of Amsterdam, Amsterdam, the Netherlands. [2]Department of Experimental Immunology, Academic Medical Center at the University of Amsterdam, Amsterdam, the Netherlands. [3]Department of Microbiology and Immunology, University of California, San Francisco, San Francisco, California, USA. Correspondence should be addressed to H.S. (hergen.spits@amc.uva.nl).

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A nagging problem is that although NK cells and ILC1s can be phenotypically distinguished in the liver of naive mice, the distinction is much more difficult to make in other tissues and in mice that have inflammation or infection, because the markers used to identify these cells are modulated during immune responses. Further adding to the confusion is the high plasticity of ILCs. ILC2s and ILC3s have been shown to acquire features of ILC1s in infected mice and humans. Here we review current knowledge of NK cells and ILC1s.

Properties of NK cells

Historically, NK cells are identified by the cell surface phenotype $CD3^-CD56^+$ (and, more recently, $NKp46^+$) in humans (**Table 1**) and $CD3^-NK1.1^+$ (and, more recently, $CD49b^+$ or $NKp46^+$) in mice[4] (**Table 2**). Subsets of human peripheral blood NK cells have been identified on the basis of differential expression of CD56 and CD16 (refs. 5,6). At steady state, $CD56^{lo}CD16^+$ NK cells possess more cytolytic activity than $CD56^{hi}CD16^-$ NK cells[5,6], whereas $CD56^{hi}CD16^-$ NK cells are more responsive to stimulation by inflammatory cytokines such as IL-12, but they rapidly acquire potent lytic function after stimulation[6,7]. $CD56^{hi}CD16^-$ NK cells are thought to be immature precursors to $CD56^{lo}CD16^+$ mature NK cells[8], although some studies have suggested that the two are separate lineages[9]. After activation, cell surface expression of CD56 and CD16 can be modulated, making the $CD56^{hi}$ and $CD56^{lo}$ designations relevant only in analysis of blood from healthy individuals. For example, mature NK cells lacking CD56 are expanded in HIV-infected individuals[10–12], and CD56 is expressed on a subset of myeloid cells[13], which confounds identification of NK cells unless additional markers, such as CD7 (expressed on lymphocytes but not myeloid cells) are used to discriminate lymphoid from myeloid cells[14]. A unique subset of NK cells, also identified as $CD3^-CD56^+$ lymphocytes, has been identified in human uterine tissues, but these cells are distinguished from peripheral blood NK cells by the expression of CD9 (ref. 15). When assayed *ex vivo*, these decidual NK cells have low lytic activity but are characterized by secretion of cytokines and chemokines that may shape the decidual

Table 1 Cell expression of human surface antigens, granzyme, perforin, T-bet and Eomes

	Liver					
	Blood				Lung and intestine	
Marker	ILC1[a]	CD56^{hi} NK	CD56^{lo} NK	Hepatic NK	Intraepithelial NK[b]	Ex-ILC2s or ex-ILC3s
CD4	±	–	–	–	–	–
CD16	–	–	+	–	nd	–
CD27	+	±	±	nd	–	+
CD49a	nd	–	–	+	+	nd
CD49b	nd	+	+	–	–	nd
CD56	–	+	+	+	+	–
CD69	–	–	–	+	+	+
CD94	–	+	±	–	+	–
CD103	–	–	–	–	+	–
CD127	+	–	–	–	–	+
CD161	nd	+	±	±	–	+
NKG2D	nd	+	+	+	+	nd
NKp30	nd	+	+	+	+	nd
NKp44	nd	–	–	–	+	–
NKP46	nd	+	+	+	+	+
Inhibitory KIRs (2DL, 3DL)	nd	–	±	+	±	nd
Activating KIRs (2DS, 3DS)	nd	–	±	–	nd	nd
T-bet	+	+	+	+	+	+
Eomes	–	+	+	–	+	–
TRAIL	nd	–	–	nd	–	nd
Granzyme	–	±	+	±	+	–
Perforin	–	–	+	–	+	–
Refs.	43,44	9,33	9,33	22	23,41	23,40,55

Expression of a number of cell surface antigens and the transcription factors T-bet and Eomes on subsets of human ILC1s, peripheral blood NK cells and tissue-resident NK cells.
[a]These cells are designated ILC1s because of their expression of T-bet and capacity to produce IFN-γ and lack of TCRαβ and TCRγδ. However, these cells express many T cell antigens, such as cytoplasmic CD3ε, CD4, CD5 and CD28, and it has yet to be confirmed that they are not T cells that have downregulated and degraded TCRs. [b]The intraepithelial cells have been designated $CD103^+$ ILC1s (refs. 23,41), but it is more likely that they are NK cells because they express CD94, Eomes, perforin and granzyme. nd, not determined; ± denotes that only some of the cells are positive.

vasculature during pregnancy. Similarly, NK cells, as well as ILC1s, ILC2s and ILC3s, are found in the uterine tissues of mice[16].

Subsets of mouse NK cells have been identified on the basis of differential expression of CD27 and CD11b, with mature NK cells losing expression of CD27 and gaining CD11b[17,18]. In mice and humans, NKp46 can be expressed on subsets of ILC3s, in addition to ILC1s, and in mice NK1.1 can also be expressed on ILC1s and some ILC3s. Many of the other cell surface receptors initially described on NK cells, such as NKp44, NKG2D and Ly49, have been reported on subsets of ILC1s and ILC3s. In mice, NK cells express CD49b but lack CD49a, a marker that has been associated with ILC1s (also referred to as tissue-resident NK cells)[19]. However, CD49a is induced on NK cells *in vivo* after viral infection[20], and CD49b expression can be downregulated on proliferating NK cells *in vitro*[21], although whether this occurs in some circumstances *in vivo* is not known. In humans, CD49a is expressed on some NK cells in the liver but not in peripheral blood[22].

CD127 is expressed on ILCs but is absent on mature NK cells in mice and humans[23,24], and eomesodermin (Eomes) is expressed by mature NK cells but not ILC1s[25,26]. However, progenitors of mouse NK cells express CD127 (ref. 27), and it is unclear when during maturation immature NK cells lose expression of CD127. Similarly, whether Eomes can be downregulated in mature NK cells in some tissues or infections has not been addressed in fate-mapping reporter mouse studies. At steady state, the majority of human mature NK cells can be identified as $CD3^-CD127^-CD7^+CD56^+$ (or $NKp46^+$) $T\text{-}bet^+Eomes^+$ lymphocytes, and mature mouse NK cells as $CD3^-CD127^-NK1.1^+$(or $NKp46^+$)$T\text{-}bet^+Eomes^+$ lymphocytes. However, in humans all $CD56^{hi}CD16^-$ NK cells express Eomes, but a fraction of peripheral blood $CD3^-CD56^{lo}CD16^+$ NK cells, which are well-characterized, highly cytotoxic NK cells, have low expression or lack Eomes[28]; therefore, the presence or absence of Eomes expression is insufficient to discriminate between NK cells and ILC1s. At present, there are no markers that can unambiguously discriminate NK cells and ILC1s in human or mouse tissues and during infection or inflammation.

Properties of ILC1s

Originally ILC1s were distinguished from NK cells by the absence of cytotoxic activities, but some data indicate that ILC1 subsets can also mediate some cytotoxic activities, although to a lesser extent than NK cells. The description of thymic NK cells[24] was the first indication of the existence of an IFN-γ-producing innate lymphocyte distinct from NK cells. In contrast to NK cells in the spleen, thymic NK cells express CD127 and are dependent on the transcription factor GATA-3. Because subsequent studies demonstrated that all ILC subsets express CD127 and are dependent on GATA-3, thymic NK cells may now be classified as ILC1s (ref. 24). More recent findings have revealed that NK cells and ILC1s differ in phenotype and dependence on T-bet and Eomes. Three ILC1 subsets, all expressing NKp46 and NK1.1, have been described in mice (**Table 2**). In addition, a fourth subset sharing features with both NK cells and ILC1s was described in salivary gland (**Table 2**). Trafficking studies using parabiotic mice have provided evidence that at steady state and in the absence of infection, ILC1s are mostly tissue resident, whereas NK cells recirculate[19,29].

Whereas fate-mapping studies in mice have demonstrated that NK cells never express RORγt during their development, a subset of mouse $CD127^+$ ILC1s lose expression of RORγt, upregulate expression of T-bet and several NK cell markers (such as NK1.1 and NKp46), and acquire the ability to secrete IFN-γ[30,31] (**Tables 1** and **2**). These $CD127^+$ ILC1s are also referred to as ex-ILC3s. The problem is that ILC1s, which develop independently of RORγt, can be unambiguously distinguished from ex-ILC3s only by using RORγt fate mapping in mice. It is currently not possible to distinguish ex-ILC3s from RORγt-independent ILC1s in wild-type mice or in humans. ILC1s express and depend on T-bet for their development but do not require Eomes, which is necessary for the development of mature NK cells, as shown in mice[25,26]. At steady state, $CD49a^+CD49b^-Eomes^-$ ILC1s are not precursors of $Eomes^+$ NK cells, as they do not develop into $Eomes^+$ NK cells when adoptively transferred into congenically marked recipients[25].

What complicates a clear definition of ILC1s are emerging data indicating that ILC1s and NK cells in different tissues have overlapping but different phenotypes and functions (**Table 1**). Intestinal and hepatic ILC1s are $CD49a^+CD49b^-$, produce high amounts of IFN-γ and are $Eomes^-$ and less cytotoxic than NK cells[32]. These hepatic cells have been referred to in the literature as ILC1s or NK cells by different investigators[25,32]. Hepatic ILC1 cytotoxicity is mediated by the cytokine TRAIL rather than perforin, which characterizes NK cell–mediated cytotoxicity, although in human both activated $CD56^{hi}CD16^-$ and $CD56^{lo}CD16^+$ NK cells can express TRAIL[33], and in mice, TRAIL can be induced on NK cells by interferons[34]. Another

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Table 2 Expression of mouse surface antigens, granzyme, perforin T-bet and Eomes by cells from various tissues

						Salivary gland
				Liver		
		Spleen				
	Intestine					
Marker	Intraepithelial NK	CD27⁺CD11b⁻ NK	CD27⁻CD11b⁺ NK	Ex–ILC2 or ex-ILC3	Hepatic NK or ILC1	Salivary gland ILC1
CD11b	–	–	+	–	–	±
CD27	–	+	–	–	+	–
CD49a	nd	–	–	+	+	nd
CD49b	–	+	+	–	–	+
CD69	+	–	–	±	+	+
CD103	nd	–	–	–	nd	±
CD127	–	–	–	+	+	–
CD160	+	–	–	+	+	nd
KLRG1	–	±	±	–	–	–
NKG2D	+	+	+	+	+	+
NK1.1	+	+	+	+	+	+
NKp46	+	+	+	+	+	+
Ly49A	nd	±	±	–	+	–
Ly49C/I	nd	±	±	–	±	–
Ly49D	nd	±	±	nd	–	+
Ly49G2	nd	±	±	–	±	±
Ly49H	nd	±	±	–	–	±
Eomes	+	+	+	–	–	+
T-bet	+	+	+	+	+	+
TRAIL	–	–	–	nd	+	+
Granzyme	+	–	+	–	±	–
Perforin	+	–	+	–	–	–
Refs.	41	32,35	36	30–32,41	25,32,36	35

Expression of cell surface antigens, T-bet and Eomes on subsets of mouse ILC1s, NK cells and NK cells from different tissues. nd, not determined; ± denotes that only some of the cells are positive.

population, found in salivary glands, is similar to the liver-resident ILC1s in that it expresses CD49a and kills target cells using TRAIL[35]; but, in contrast to liver ILC1s, these cells express Eomes and CD49b, which are both expressed by NK cells, and they produce very low levels of IFN-γ[35]. These salivary gland cells have been referred to as salivary gland ILC1s and salivary gland NK cells. Development of salivary gland cells and tissue-resident NK cells in liver, skin, uterus and kidney is independent of the transcription factor Nfil3 (refs. 35,36), which is required for development of NK cells at steady state; however, NK cells can bypass Nfil3 and mature during infection with mouse cytomegalovirus[37]. More recently, cells with a phenotype similar to that of salivary gland ILC1s (or NK cells) have been found in mouse breast and prostate tumors[38]. These cells, described as ILC1-like, express CD49a and CD103, are tissue resident and have a transcriptional profile that overlaps with but is not identical to that of NKp46⁺NK1.1⁺CD127⁺ ILC1s or NK cells. Like salivary gland ILC1s or NK cells, these ILC1-like cells require IL-15 but not Nfil3 for development, and they produce low amounts of IFN-γ and TNF but express high amounts of TRAIL, granzyme B and perforin[38]. Given these results, it is unclear whether these cells in endocrine tissues, salivary gland, breast and prostate represent a distinct cell type from ILC1s and NK cells or instead reflect the action of factors present in these tissue environments that modulate the phenotype of ILC1s and NK cells.

A comprehensive analysis of gene expression of mouse ILC1 and NK cell populations has been reported[39]. Three different NKp46⁺NK1.1⁺ NK cell subsets (CD127⁻ cells in spleen and small intestine and CD49b⁺TRAIL⁻ cells in liver) and four distinct NKp46⁺NK1.1⁺ ILC1 subsets (CD127⁺ cells in spleen and lamina propria and intraepithelial cells in the small intestine) were analyzed. The gene expression of these cells was compared with that of ILC2s and of NKp46⁻ and NKp46⁺ ILC3s. The mRNA profiles of ILC1s and NK cells showed considerable overlap with each other and with T-bet-dependent NKp46⁺ ILC3s, whereas those of NKp46⁻CD4⁻ ILC3s and LTi-like ILC3s (RORγt⁺NKp46⁻CD4⁺) were clearly distinct. As expected, T-bet was most highly expressed in the ILC1 and NK cell populations, and Eomes was most highly expressed in NK cells, but these distinctions were not absolute. Intraepithelial ILC1s shared a transcriptional profile with NK cells (for example, expressed Eomes), and intestinal ILC1s showed germline expression of variable gene segments of *Trgv2* and *Tcrg-V3*. Contamination of the intraepithelial ILC1 population with T cells or NK cells might account for these results. Taken together these results indicate that, although some ILC1 and NK cell populations in mouse are derived from different precursors, these two populations have highly overlapping gene expression profiles. Obviously, the similarity of the phenotypes of NK cells and ILC1s raises the possibility that these overlapping gene expression profiles are due to mutual contamination of the sorted ILC1s and NK cells.

The first studies reporting human ILC1s described two different populations of IFN-γ-producing ILC1s[40,41]. One expressed high amounts of CD127 and CD161 but lacked CD56, CD94, granzyme B and perforin, which are expressed by mature NK cells[40]. These CD127⁺ ILC1s express T-bet but lack Eomes and reside in the lamina propria[23]. Another population that shares features of NK cells and expresses CD56 and only very low amounts of CD127 was found in tonsils and the intraepithelial space in the intestine[41]. These cells express CD103, the integrin α_E subunit that associates with integrin β_7, which interacts with E-cadherin[42], to promote interaction with epithelial cells. The CD103⁺ cells in the tonsils express CD94, which is only poorly expressed by intestinal intraepithelial CD103⁺ cells[41]. Because these CD103⁺ cells express Eomes and perforin, it is possible that they represent a subset of NK cells rather than ILC1s.

Human CD127⁺CRTH2⁻CD117⁻NKp44⁻CD56⁻ cells have been found and these are distinct from ILC2s, which express the prostaglandin D2 receptor CRTH2, and distinct from ILC3s, which express CD117 and NKp44. CD127⁺CRTH2⁻CD117⁻ ILCs express cytoplasmic CD3 and a number of other T cell markers including CD4, CD8, CD27 and CD28, but do not express αβ TCR or γδ TCR in the cytoplasm and no CD3–TCR complex on the cell surface[43]. Because these cells express T-bet and the chemokine receptor CXCR3 (which are also expressed on T_H1 cells) but lack Eomes, they were considered to be ILC1s. Single-cell RNA-seq analysis of human CD127⁺ ILC1s isolated from noninflamed tonsils revealed that some of these cells express T cell–associated genes including those encoding CD5 and CD6 (ref. 44). In addition, several transcripts encoding variable regions of the TCR were found. Surprisingly, single-cell RNA-seq analysis also revealed a low frequency of T-bet expression, which raises the question whether these cells should be considered ILC1s, which were originally defined as T-bet⁺ IFN-γ-producing cells. In summary, a considerable proportion

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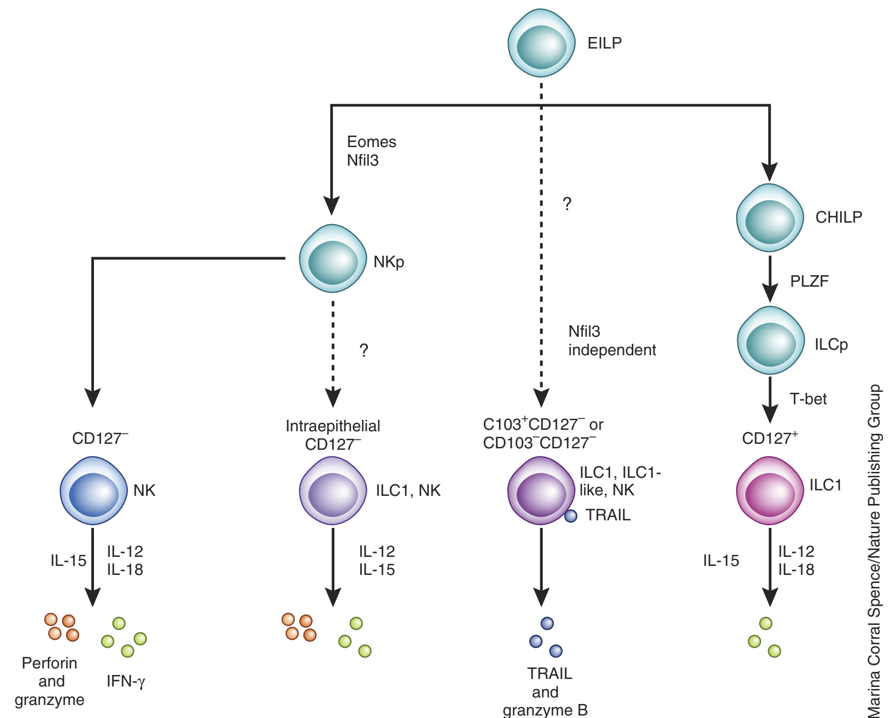

Figure 1 Development of NK cells and ILCs under steady state conditions. ILCs and NK cells develop from an EILP. Downstream of EILPs are NK precursors and CHILPs, which give rise to all ILC subsets, including ILC1s. A PLZF$^+$ ILC precursor (ILCp) with a more restricted developmental potential lies downstream of the CHILPs. The developmental path of ILC1s and NK cells found in salivary gland and breast and prostate tumors is not known. In contrast to development of NK cells and ILCs, development of salivary gland cells is independent of Nfil3, suggesting that these cells do not derive from CHILPs or NK progenitors (NKp). It is unknown whether intraepithelial ILC1s, which share many features with NK cells, develop from NKp or from CHILPs.

of CD127$^+$CRTH2$^-$CD117$^-$NKp44$^-$ ILCs with low expression of T-bet in human peripheral blood and noninflamed tonsils that lack markers for myeloid cells and B cells appear to be related to T cells. Although these cells produce IFN-γ after activation *in vitro*, they may not be ILC1s. Further analysis is required to ascertain whether these cells are truly ILCs or, alternatively, T cells that have downregulated and degraded the TCR. Analysis of TCR rearrangement must be done to ensure these cytoplasmic CD3$^+$ cells are ILCs, which would not have productively rearranged TCR genes.

A population of CD56^{hi}CD49a$^+$T-bet$^+$Eomes$^-$ cells that share properties with NK cells and ILC1s have been described in human liver. These cells lack CD127 and RORγt but express perforin and other markers of NK cells[22]. It was concluded that these cells represented bona fide NK cells, but the lack of definitive criteria to discriminate NK cells from ILC1s leave open the lineage of these cells.

NK cell and ILC1 development

Recent studies have provided detailed insights into mechanisms of ILC development and the intermediate developmental stages, which are discussed in a review by Zook and Kee in this issue[45]. Here we summarize briefly the knowledge about ILC1 and NK development that is relevant to the distinction between NK cells and ILC1s. Studies in mouse models to define the development of ILC populations combined with fate mapping have provided evidence for the existence of group 1 ILCs that are distinct from NK cells, ILC2s and ILC3s. CLP cells differentiate into an early innate lymphoid precursor (EILP) positive for the transcription factor TCF1, which gives rise to both NK cells and ILCs[46] (**Fig. 1**). An Id2$^+$ common helper-like innate lymphoid precursor (CHILP) downstream of EILP is able to differentiate into all ILC subsets, including a CD127$^+$ ILC1 population, but not into NK cells[32]. In mice, the CD127$^+$ ILC1 subset is dependent on T-bet but independent of Eomes and therefore distinct from mature NK cells. In the intestine these ILC1s can be distinguished from NK cells by their lack of expression of Eomes. Intriguingly, intestinal CD127$^+$ ILC1s are dependent on IL-15 but not IL-7; thus, with respect to cytokine dependency, the ILC1s are more similar to NK cells than to other ILCs, which are dependent on IL-7 (ref. 32). The Id2$^+$ CHILP cells express CD127 and integrin $\alpha_4\beta_7$, as do CLP cells, but they lack the receptor Flt3 and CD93, unlike CLP cells. These cells are also heterogeneous, as about 50% of them express the transcription factor PLZF. The PLZF$^+$ cells are downstream of PLZF$^-$ID2$^+$ CHILPs, as PLZF$^+$ cells give rise to ILC1s, ILC2s and ILC3s but not LTi cells[47]. ILC1s do not express PLZF, but studies using PLZF fate-mapping reporter mice have shown that ILC1s (but not NK cells) arise from progenitor cells that expressed PLZF at an earlier stage of development[48]. An analysis of the tissue distribution of the PLZF fate-mapped ILC1s revealed that these cells are present in the intestinal epithelium and liver[48].

In humans the NK cell developmental pathway can be separated from that of CD127$^+$ ILC populations, including ILC1s. A NK cell–restricted precursor was found in fetal tissues, neonatal cord, adult blood and in tissues that have the phenotype CD34$^+$CD38$^+$CD123$^-$CD45RA$^+$CD7$^+$CD10$^+$CD127$^-$ but lack markers for mature B cells, T cells and myeloid cells (and are therefore denoted lineage-negative)[49]. *In vitro*, these precursor NK cells are capable of differentiating into T-bet$^+$Eomes$^+$ NK cells but not into CD127$^+$ ILCs. A study identified a CD34^{lo} population of cells that express RORγt and are committed precursors for ILC3s[50]. These cells are probably downstream of CLP cells. Because the developmental pathways of human ILC2s and ILC1s are currently unknown, it is difficult to define the equivalents of the mouse ILC1s identified in fate-mapping studies.

The observation that mouse ILC1s but not NK cells can be fate mapped by PLZF allowed a comparison of the developmental pathway of ILC1 and NK cells. Tracking intermediate cellular stages of PLZF$^+$ fate-mapped ILC1s and PLZF$^-$ fate-mapped NK cells revealed that ILC1s and NK cells follow a similar developmental path[48]. Intermediate stages of ILC1 development had identical phenotypes as previously defined intermediate stages of NK cell development, such as pre-NK cell precursors[48]. Previously defined immature NK cells, which expressed CD127 and CD49a and lacked Eomes, may represent ILC1s rather than NK cells. The parallel sequence of developmental stages of NK cell and ILC1 development makes it impossible to distinguish these pathways in wild-type mice.

ILC2- and ILC3-derived ILC1s

Although studies using fate-mapping mice have provided evidence for the existence of ILC1s distinct from other ILC subsets, it is now becoming clear that ILC2s and ILC3s can transdifferentiate into ILC1s

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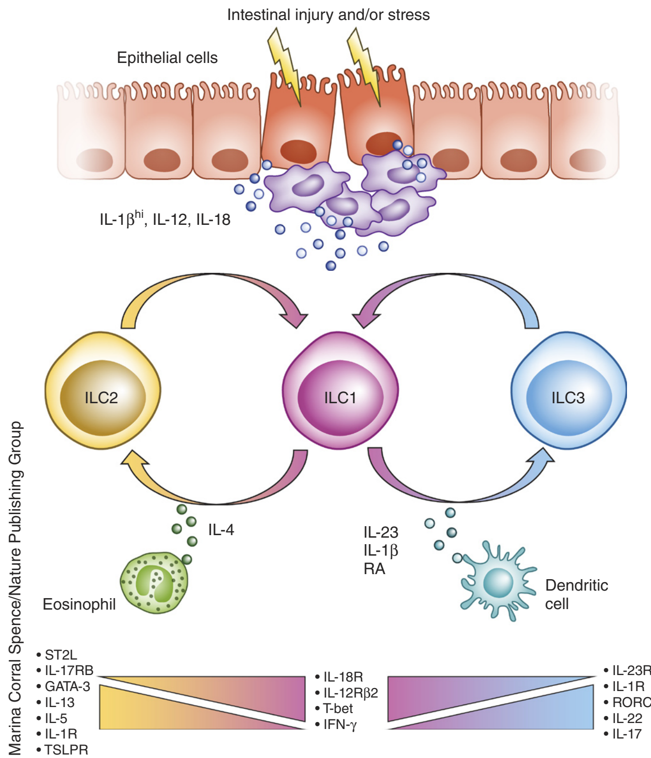

Figure 2 Plasticity of ILC2s and ILC3s. IL-12 can drive transdifferentiation of ILC2s and ILC3s to ILC1s in mice and humans. These transdifferentiated ILC1s can, in turn, reverse fate under the influence of IL-4 and IL-23, respectively.

by downregulating GATA-3 and RORγt, respectively, and upregulating T-bet while acquiring the capacity to produce IFN-γ (**Fig. 2**). The first evidence for ILC plasticity came from studies showing heterogeneous cytokine secretion by ILC3s and by cloned lines of these cells that depended on the mode of activation[51–53]. Detailed analysis of cytokines that triggered human ILC3 activation revealed that whereas activation in the presence of IL-7 resulted in high IL-22 production, IL-2 reduced IL-22 and IL-17 secretion and triggered IFN-γ production, which was enhanced after restimulation in the presence of IL-1β[52]. Genetic lineage-tracking experiments in mice confirmed the importance of IL-7 to maintenance of ILC3 identity but also showed that IL-12 and IL-15 induce downregulation of RORγt and upregulation of T-bet in ILC3s[30,31]. These studies also demonstrated that NKp46$^-$ ILC3s could develop into NKp46$^+$ ILC3s. Because development of NKp46$^+$ ILC3s depends on T-bet, transdifferentiation of NKp46$^-$ ILC3s via NKp46$^+$ ILC3s to NKp46$^+$RORγt$^-$ ex-ILC3s is a T-bet-dependent process. Human ILC3s are also capable of transdifferentiation into ex-ILC3 ILC1s. Exposure of human ILC3s to IL-12 results in downregulation of RORγt, upregulation of T-bet and IFN-γ production, which is enhanced in the presence of IL-18 and/or IL-1β[23,40].

ILC2s can also transdifferentiate toward CD127$^+$ ILC1s[54–57]. IL-33 and TSLP are strong activators of ILC2s in both humans and mice. Paradoxically, activation by IL-33 and TSLP not only induces production of the ILC2 signature cytokines IL-5 and IL-33 but also renders ILC2s responsive toward T_H1 signals by upregulating the IL-12 and IL-18 receptors and simultaneously downregulating the IL-33 receptor ST2L. Recent studies have shown that IL-1β is a potent activator of ILC2s and regulates ILC2 plasticity[54,55,57]. Strong IL-1β signaling triggers the upregulation of the IL-12 receptor, whereas low-dose IL-1β signaling favors responsiveness to type 2 cytokines in mouse ILC2s[55]. IL-12 is likely to be a key factor in transdifferentiation of ILC2s into ILC1s, as it downregulates GATA-3 and upregulates T-bet in humans and mice. This transition is characterized by acquisition of transcriptional accessibility of the *Ifng* locus, whereas accessibility of the *Il5* and *Il13* loci is maintained[55]. In addition, ILC2s from patients with biallelic mutations in *IL12RB1*, which encodes an IL-12 receptor chain, lacked ILC1s and did not acquire the capacity to produce IFN-γ[56], further emphasizing the importance of IL-12 in transdifferentiation of ILC2s into ILC1s.

Transdifferentiation of human and mouse ILC3s into ex-ILC3 ILC1s is reversible, as CD127$^+$ ILC1s convert to ILC3s in the presence of IL-1β and IL-23, a process accelerated by retinoic acid[23], whereas ILC2-derived ILC1s revert to ILC2s in the presence of IL-4 (ref. 57). *In vitro* experiments suggest that myeloid cells interacting with ILCs regulate transdifferentiation. For example, IL-12-producing CD14$^+$ intestinal dendritic cells (DCs) induce ILC3s into ex-ILC3 ILC1 conversion, whereas retinoic acid and IL-23-producing CD14$^-$ DCs induce the reverse transdifferentiation of ex-ILC3 ILC1s into ILC3s[23]. It has yet to be determined which myeloid cell type stimulates ILC2s to ILC1 differentiation, but IL-4-producing eosinophils may be responsible for the reversal process. It is tempting to hypothesize that ILC2s and ILC3s transdifferentiate into ILC1s under proinflammatory conditions while maintaining the potential to revert once the inflammation is resolved.

Notwithstanding the importance in host defense, ILC plasticity adds another layer of complexity to the control of mucosal homeostasis, and it is likely that this process needs to be tightly regulated, as imbalances may contribute to pathology. Indeed, many mouse studies indicate that a chronic inflammatory environment results in accumulation of IFN-γ-producing ILC2- and ILC3-derived ILC1s, some of which completely lose their ILC2 and ILC3 characteristics and propagate intestinal immunopathology. In humans the increased frequency of inflammatory ILC1s inversely correlates with the frequency of ILC3s in inflamed intestinal resection specimens from people with Crohn's disease[40,58] and of ILC2s in bronchial resection specimens from people with chronic obstructive pulmonary disease (COPD)[57]. Whether reprogramming of ILCs occurs exclusively at the site of infection, involves the recruitment of cells from the periphery that are subsequently transdifferentiated *in situ* or both is not yet resolved. However, studies with parabiotic mice have demonstrated that mouse ILCs are usually tissue-resident cells, which are maintained and expanded locally under homeostatic conditions and after an inflammatory insult[29]. These studies support the idea that changes in frequencies of ILC1s in inflamed tissues of people with Crohn's disease and in COPD are caused mainly by local transdifferentiation of ILC3s and ILC2s, respectively.

ILC1s and NK cells in infectious disease

Analysis of the innate response against microorganisms in mice lacking T-bet have provided information on the role of ILC1s in infectious diseases. CD127$^+$ intestinal ILC1s protect mice against *Toxoplasma gondii* because T-bet-deficient mice, which lack ILC1s but have normal NK cell numbers, are unable to control *T. gondii* infection[32]. On the other hand, T-bet-deficient NK cells are functionally impaired in their migration[59], thus a role for NK cells in control of *T. gondii* cannot be excluded. T-bet-dependent ILC1s are critically involved in the host defense against acute *Clostridium difficile* infections, but the origin of these ILC1s could not be determined[60]. In a study using RORγt fate-mapping mice, T-bet-dependent ILC1s were essential for

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protection of the epithelial barrier against *Salmonella enterica*[31] and were shown to be derived from RORγt+ ILC3s. These data indicate that it is not possible to ascribe a particular effect to NK1.1+NKp46+ ILC1s, which include RORγt-independent- and ILC3-drived ILC1s, without using fate-mapping reporter mice.

All ILC subsets have been implicated in inflammatory disease in a variety of models. ILCs that produce IFN-γ and IL-17A, which are probably a mixture of ILC1s and ILC3s, are involved in *Helicobacter hepaticus*–induced colitis[61]. However, T-bet-deficient ILCs develop colitis in *Tbx21*$^{-/-}$*Rag2*$^{-/-}$ mice, indicating that ILC1s or NK cells can be protective against colitis in some models[62]. In humans, increased proportions of ILC1s have been observed in inflamed tissues in a variety of inflammatory autoimmune diseases, including Crohn's disease[40] and COPD[57], suggesting an involvement of these cells in the pathology. In these cases it is hard to determine whether the ILC1s in inflammatory tissues are derived from ILC3s or from a pathway independent of ILC3s. More detailed analyses, particularly on the level of single cells, should be done to help resolve these questions.

ILC1s and NK cells in cancer

The role of NK cells in cancer has been studied extensively, and NK cells have been implicated in cancer immune surveillance[63]. However, studies implicating NK cells in antitumor responses were conducted before ILC1s were identified, and there are no definitive means to selectively deplete NK cells and ILC1s in experimental models. NKp46+ ILC3s have been found to be essential for a protective response against B16 melanoma cells in an IL-12-dependent manner[64]. Rejection of these tumor cells is dependent on RORγt and therefore not mediated by NK cells. Because IL-12 is a strong inducer of ILC3-to-ILC1 conversion, it is possible that the effector cells in this model are ex-ILC3s, but this was not investigated.

ILCs may also be involved in immunosurveillance. An ILC1 or ILC1-like population with similarities to salivary gland ILC1s or NK cells (described above) and innate TCRαβ+ and TCRγδ+ T cells that share a common phenotype (NK1.1+CD49a+CD103+) with NK cells and ILC1s were shown to be involved in surveillance of certain oncogene-induced breast and prostate mouse tumors[38], although the relative contributions of the ILCs and the innate T cells in the antitumor response were not evaluated. Both the ILC-1-like and the innate T cells were dependent on IL-15, and deletion of IL-15 accelerated growth of the tumors, whereas overexpression of IL-15 delayed it. Given the now recognized heterogeneity of the ILC populations, the role of these cells and NK cells in infectious diseases and cancer should be revisited.

CONCLUSIONS

The productive rearrangement and expression of genes encoding TCR and immunoglobulin provides for the definition of lymphocytes in the T and B cell lineages, respectively. Under steady state conditions, mature NK cells and ILC1s can be distinguished in mice by a combination of markers. In mice, both cell types express NK1.1 and NKp46, and cells that lack CD127 but express Eomes, perforin, Ly49 and CD49b are probably NK cells. Cells that express CD127, CD49a and T-bet but not Eomes or CD49b and are weakly cytotoxic are likely to be ILC1s. However, NK cells and ILCs have no unique and stable markers that can be used to unambiguously distinguish them in different tissue environments or states of differentiation or activation, particularly in infections and inflammatory diseases. Moreover, a number of cell types share features with both NK cells and ILC1s but are nonetheless different; for example, some are unable to produce IFN-γ. Unlike ILC2s and ILC3s, NK cells and ILC1s do not depend on IL-7 for their development. Progenitors of NK cells express CD127 at early stages of development; CD127 expression is absent on mature NK cells but is retained on ILC1s. At steady state, NK cells and some ILC1s require IL-15 for maturation, but this can be overcome during certain infections or in some tissues. Similarly, Nfil3 is required for development of mature ILCs and NK cells; however, during infection and in some tissues this may be bypassed. Eomes is generally considered necessary for NK cell but not ILC1 maturation; however, some mature NK cells lack Eomes. Reciprocally, some ILC1s have been reported to express Eomes. A hallmark of ILC3 development is RORγt, but during infection or inflammation ILC3s can silence expression of RORγt and acquire T-bet and IFN-γ production. Similarly, ILC2s may acquire characteristics of ILC1s in some conditions. As is being recognized in the T helper and T regulatory cell subsets, ILCs may be 'chameleons' that adapt to their environmental cues. As a result of this plasticity, a full spectrum of transitional phenotypes may be displayed by ILCs and NK cells; this can frustrate immunologists attempting to characterize them but is essential to generate appropriate and optimal responses against pathogens and ensure barrier integrity in mucosal tissues. Further single-cell analysis of the epigenetic and transcriptional properties of NK cells and ILC1s may advance understanding of these ILCs and their relationships and help provide markers and tools to distinguish these cell types in a more decisive way.

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COMPETING FINANCIAL INTERESTS
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