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Development, Differentiation, and Diversity of Innate Lymphoid Cells

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Recent years have witnessed the discovery of an unprecedented complexity in innate lymphocyte lineages, now collectively referred to as innate lymphoid cells (ILCs). ILCs are preferentially located at barrier surfaces and are important for protection against pathogens and for the maintenance of organ homeostasis. Inappropriate activation of ILCs has been linked to the pathogenesis of inflammatory and autoimmune disorders. Recent evidence suggests that ILCs can be grouped into two separate lineages, cytotoxic ILCs represented by conventional natural killer (cNK) cells and cytokine-producing helper-like ILCs (i.e., ILC1s, ILC2s, ILC3s). We will focus here on current work in humans and mice that has identified core transcriptional circuitry required for the commitment of lymphoid progenitors to the ILC lineage. The striking similarities in transcriptional control of ILC and T cell lineages reveal important insights into the evolution of transcriptional programs required to protect multicellular organisms against infections and to fortify barrier surfaces.

Introduction

The last years have witnessed an unprecedented change in our understanding of innate lymphocyte lineages. It was previously believed that innate lymphocytes were represented by a single lymphoid lineage, namely natural killer (NK) cells, that, in many aspects, resembles cytotoxic T cells. However, it has become apparent that additional innate lymphocyte subsets exist that use transcriptional programs and display functions distinct from conventional NK (cNK) cells. All innate lymphocytes including cNK cells are now referred to as ILCs. In addition to cNK cells, three additional groups of ILCs are now being discriminated, ILC1s, ILC2s, and ILC3s. Strikingly, the transcriptional and effector programs of the various ILC populations resemble those of T helper subsets, suggesting that the underlying transcriptional circuitry is evolutionarily more ancient than previously appreciated (Tanriver and Diefenbach, 2014). Here, we will discuss our current view of developmental and transcriptional programs common to all ILC lineages and those required for specification of distinct ILC populations. These recent data provide a framework for our current view of two principal ILC lineages, cytotoxic or killer ILCs (i.e., cNK cells) and helper-like ILCs (i.e., ILC1s, ILC2s, ILC3s) (Figure 1). We will put a focus on recent progress in dissecting the ILC1 lineage and on common transcriptional programs controlling ILC specification.

Identification of ILC1s: More Than Just NK Cells?

ILC1s have only recently been better characterized and are now classified as an ILC group distinct of cNK cells that expresses and requires the transcription factor T-bet for lineage specification (Bernink et al., 2013; Daussy et al., 2014; Fuchs et al., 2013; Klose et al., 2014) (Figure 1; Tables 1, 2, and 3). The identification of bona fide ILC1s in mice was obscured by the fact that ILC1s were found to express NK cell receptors such as natural killer cell p46-related protein (NKp46) and NK1.1, which have served as an operative definition of NK cells. Early on, Di Santo and colleagues noticed that thymic NK cells in mice have a distinct phenotype; they are less cytotoxic but secrete more interferon-γ (IFN-γ) than splenic NK cells do (Table 2) (Vosshenrich et al., 2006). They proposed that the dichotomy between splenic NK cells and thymic NK cells in mice might parallel the division of $CD56^{lo}$ and $CD56^{hi}$ NK cell subsets in human blood (Caligiuri, 2008) (Table 1). Recent data from organ-resident "NK cells" indicated that the population of $NKp46^{+}NK1.1^{+}$ cells might in fact be heterogeneous and composed of various ILC lineages (Daussy et al., 2014; Fuchs et al., 2013; Gordon et al., 2012; Klose et al., 2014; Vosshenrich et al., 2006). Indeed, liver-resident $NKp46^{+}NK1.1^{+}$ cells can be separated into a VLA2 $(CD49b)^{+}$ population expressing the T-box transcription factors Eomes and T-bet and into a $VLA2^{-}TRAIL^{+}IL\text{-}7R\alpha^{lo}$ population that expressed T-bet, but not Eomes (Daussy et al., 2014; Gordon et al., 2012; Peng et al., 2013; Takeda et al., 2001). $VLA2^{+}TRAIL^{-}$ cells likely represent cNK cells in that they are cytotoxic, require Eomes for development, and express class I major histocompatibility complex (MHC)-specific inhibitory receptors (i.e., Ly49 receptors, NKG2A). $VLA2^{-}TRAIL^{+}NKp46^{+}NK1.1^{+}$ cells did not express Eomes but strictly required T-bet for their development (Gordon et al., 2012). It has been controversial whether $VLA2^{-}TRAIL^{+}$ cells constitute immature cNK cells (Gordon et al., 2012; Takeda et al., 2005) or a distinct ILC lineage (Daussy et al., 2014; Peng et al., 2013; Sojka et al., 2014). In the intestine,

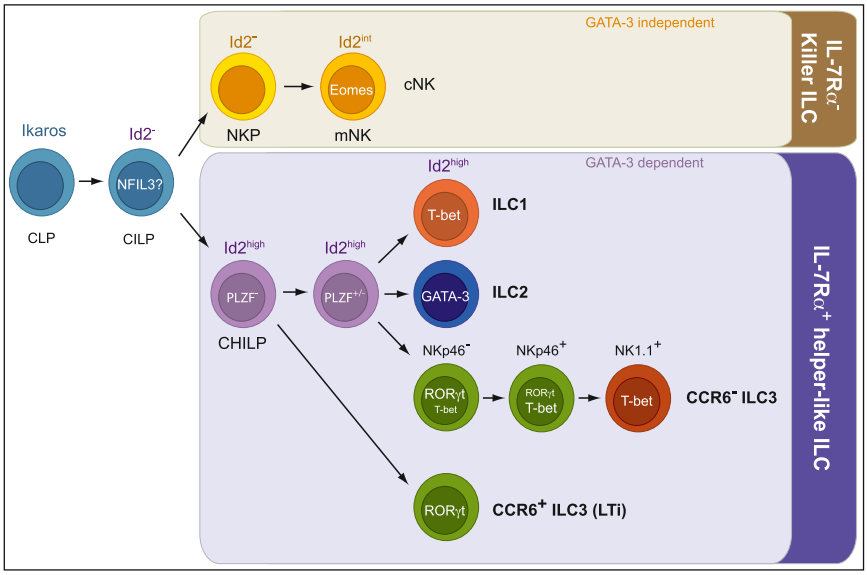

Figure 1. Refined Lineage Map for the Development of ILC Lineages

All lymphoid lineages are the progeny of the CLP. After the branchpoint with the B and T lineages, an ILC-restricted progenitor might exist (CILP). Downstream of the CILP, two main ILC lineages can be discriminated—killer ILCs and helper-like ILCs. Killer ILCs are represented by cNK cells and helper-like ILCs are composed of the various cytokine-producing ILC subsets (i.e., ILC1s, ILC2s, ILC3s). Whereas helper-like ILCs express IL-7Rα and require GATA-3 for differentiation, killer ILCs do not express IL-7Rα and are normally represented in GATA-3-deficient mice. All helper-like ILCs (but not killer ILCs) differentiate from the Id2$^+$ CHILP. A PLZF$^+$ CHILP population has been identified that has more restricted differentiation potential. Whether PLZF$^-$ CHILP are the precursors of PLZF$^+$ CHILP remains to be experimentally addressed. CLP, common lymphoid progenitor; CILP, common ILC progenitor; CHILP, common helper-like ILC progenitor; NKP, cNK-restricted progenitor.

distinction between the various subsets of NKp46$^+$NK1.1$^+$ cells was even more complex because NKp46$^+$ ILC3s were recognized as well (Cella et al., 2009; Cupedo et al., 2009; Luci et al., 2009; Sanos et al., 2009; Satoh-Takayama et al., 2008). Within the intraepithelial space of the intestine, an ILC1 subset was identified that was phenotypically distinct from cNK cells and required T-bet and nuclear factor, interleukin-3 (IL-3) regulated (NFIL3, also known as E4BP4) for differentiation (Tables 2 and 3) (Fuchs et al., 2013). Genetic reporter systems for lineage-defining transcription factors allowed to identify intestinal ILC1s as an ILC lineage separate from cNK cells (expressing an *Eomes* reporter) and NKp46-expressing ILC3s (expressing a *Rorc* reporter). Intestinal ILC1s produced copious amounts of IFN-γ in response to IL-12 and provided innate protection against the intracellular parasite *Toxoplasma gondii* (Klose et al., 2014).

Identification of ILC2s

IL-25, IL-33, and thymic stromal lymphopoietin (TSLP), all of which are epithelial cell-derived cytokines, regulate type 2 innate immune responses against helminths and pathophysiology of airway allergens (Koyasu and Moro, 2012). The existence of lineage-negative innate immune cells producing type 2 cytokines was first reported by Fort et al. demonstrating that IL-25 administration induced production of IL-5 and IL-13 in *Rag2*$^{-/-}$ mice that lack all B and T cells (Fort et al., 2001). It was later shown that a non-B non-T c-Kit$^+$ FcεR1$^-$ (non-mast cell) population, which appears during the initial stages of helminth infection, is capable of producing IL-4, IL-5, and IL-13 in response to IL-25 (Fallon et al., 2006; Humphreys et al., 2008; Hurst et al., 2002; Voehringer et al., 2006). The identity of such innate effector cells had been obscure until 2010 when natural helper cells (Moro et al., 2010) and nuocytes (Neill et al., 2010), which are now known as ILC2s, were identified. ILC2s, which produce large amounts of IL-5 and IL-13 in response to IL-25 or IL-33, were identified in mesenteric fat-associated lymphoid clusters (FALC) in naive mice as natural helper cells (Moro et al., 2010) and in mesenteric lymph nodes of mice administered with IL-25 or IL-33 as nuocytes (Neill et al., 2010). ILC2s were later shown to be present in other tissues such as lung, intestinal lamina propria, bone marrow, liver, and skin (Furusawa et al., 2013; Halim et al., 2012a; Hoyler et al., 2012; Kabata et al., 2013; McHedlidze et al., 2013; Roediger et al., 2013; Salimi et al., 2013). In addition to type 2 cytokines, ILC2s produce amphiregulin and support the recovery of epithelial barrier integrity after tissue damage (Monticelli et al., 2011). Recent studies have identified a role of ILC2s in the initiation of type 2 adaptive immune responses through class II MHC and cytokine-mediated activation of T helper 2 (Th2) cells (Halim et al., 2014; Oliphant et al., 2014). Another type 2 innate cell population, MPP^{type2} induced by IL-25 administration was also reported in 2010 (Saenz et al., 2010). MPP^{type2} cells, however, differ from ILC2s in that they express neither IL-7 receptor (IL-7R) nor IL-33R and possess the potential to differentiate into myeloid cells (Saenz et al., 2013; Saenz et al., 2010). IL-5 and IL-13 produced by ILC2s are critical for innate protection against helminth and nematode infections (Koyasu et al., 2010).

ILC3s: Lymphoid Tissue-Inducer Cells and More

The first ILC subset to be characterized were retinoic acid-related orphan receptor γ t (RORγt)-expressing ILC3 in human tonsils (Cella et al., 2009; Cupedo et al., 2009) and the lamina propria of the intestine (in both human and mice) (Cella et al., 2009; Cupedo et al., 2009; Luci et al., 2009; Sanos et al., 2009; Satoh-Takayama et al., 2008), a subpopulation of which expressed cell surface markers also found on NK cells (e.g., NKp46, NKG2D, NKp44, or CD56). Intestinal ILC3s require RORγt for lineage specification and, consequently, mice genetically lacking RORγt have no ILC3s (Table 3). A fetal liver-derived innate lymphocyte subset, termed lymphoid tissue (LTi) inducer cells, has been identified in humans and mice that also depends on RORγt for development (Adachi et al., 1997; Cupedo et al., 2009; Kurebayashi et al., 2000; Mebius et al., 1997; Sun et al., 2000). It is now believed that LTi cells constitute a subpopulation of ILC3s (Cupedo et al., 2009). While

Table 1. Phenotype of Human IFN-γ-Producing ILC Populations

	CD56	NKp44	CD94	KIR	CD103	CD160	VLA1 CD49a	VLA2 CD49b	CD127
Blood CD56^{hi} NK cells	++	–	+	low/–	–	–	–	+	+
Blood CD56^{lo} NK cells	+	–	low/+	+	–	–	–	+	–
Tonsil intraepithelial ILC1s	+	+	+	low/–	+	+	+	n.d.	–
Intestinal intraepithelial ILC1s	+/–	+	–	n.d.	+	+	+	n.d.	–
Tonsil and intestinal converted ILC3s	–	–	–	–	–	–	–	–	+

n.d., not determined.

LTi cells are CCR6^{hi} ILC3, another ILC3 subset expresses only low amounts of CCR6 (Klose et al., 2013). Such CCR6$^{-/lo}$ ILC3s rapidly proliferate after birth, express the transcription factor T-bet, and could upregulate NKp46 (Figure 1). One significant finding was that ILC3s express type 17 cytokines such as IL-22 and IL-17A (Cella et al., 2009; Cupedo et al., 2009; Sanos et al., 2009; Takatori et al., 2009). IL-22 produced by ILC3s is absolutely required for immunity to attaching-and-effacing bacterial infections such as those with *Citrobacter rodentium* (Sonnenberg et al., 2011; Zheng et al., 2008). These lines of findings have revealed unprecedented complexity in innate lymphocyte lineages. In the following, we will first review recent data that have revealed core transcriptional hubs required for the development of various groups of ILCs. Then, we will highlight our current understanding of the molecular programs controlling the specification of distinct ILC lineages. Finally, we will discuss some of the future outstanding questions that are likely to move the field forward.

Common Developmental Programs for ILC Fate

Given that ILCs and adaptive immune system lymphocytes all derive from the common lymphoid progenitor (CLP) (Cherrier et al., 2012; Klose et al., 2014; Moro et al., 2010; Possot et al., 2011; Yang et al., 2011), the question arose whether an ILC lineage-restricted progenitor for some or all ILC populations exists that is downstream of the CLP. The existence of such common innate lymphoid progenitor (CILP) or ILC progenitor (ILCP) was predicted because mice lacking expression of the transcriptional regulator inhibitor of DNA binding 2 (Id2) lack all ILC lineages, whereas B and T cell development was largely unperturbed (Cherrier et al., 2012; Moro et al., 2010; Satoh-Takayama et al., 2010; Yokota et al., 1999). Id2 is a member of the helix-loop-helix (HLH) family of proteins that have important functions in immune cell-fate decisions (Kee, 2009; Murre, 2005). Id2 heterodimerizes with E proteins that function as transcriptional activators or repressors. Four mammalian E proteins have been characterized (E2A, HEB, E12, and E47) that bind to specific DNA sequences called E-box sites. Although Id2 cannot bind to chromatin itself (due to the lack of a basic HLH domain), it heterodimerizes with E proteins and sequesters them away from chromatin, thereby controlling the pool of E proteins available for DNA binding (Benezra et al., 1990). Id2 is expressed in high amounts in all ILC lineages (Carotta et al., 2011; Hoyler et al., 2012), whereas naive T cells and B cells either express only very low amounts of Id2 or are Id2-negative. Interestingly, B cell lineage specification requires the transcription factor early B cell factor 1 (EBF1) (Lin and Grosschedl, 1995), which is a potent repressor of Id2 (Treiber et al., 2010). Current concepts indicate that repression of Id2 is required to establish appropriate concentrations of E2A, which are permissive for the development of B cells (O'Riordan and Grosschedl, 1999). Deletion of EBF1 in B cell progenitors (late pro-B cells) led to derepression of Id2 and Notch and reprogrammed committed B cell progenitors into ILC and T cells (Nechanitzky et al., 2013). These data suggest that high level Id2 expression is required to establish ILC fate. While Id2 is now recognized as an important knot within the transcriptional network establishing ILC fate, the stage-specific and Id2 controlled transcriptional targets required for ILC specification and/or maintenance are an important goal for future research.

Id2 and ILC Fate

Recently, Id2 reporter mice (Rawlins et al., 2009) were employed to identify Id2$^+$ lymphoid progenitors in fetal liver and in the bone marrow of adult mice that were characterized by the expression of IL-7Rα and intermediate levels of c-Kit and Sca-1. Although these cells expressed markers also found on the CLP such as 2B4 and CD27, they were distinct from the CLP as they expressed Id2 and integrin $\alpha_4\beta_7$ (not expressed by CLP) but did not express Flt3 and CD93 (expressed by CLP). In addition, Lin$^-$Id2$^+$IL-7Rα$^+\alpha_4\beta_7^+$Flt3$^-$CD25$^-$ cells did not express any of the lineage-defining transcription factors of ILC populations (i.e., RORγt, Eomes, T-bet) suggesting that this population is not specified to any of the ILC lineages (Klose et al., 2014). After transfer into alymphoid mice or on the clonal level in vitro, a single Lin$^-$Id2$^+$IL-7Rα$^+\alpha_4\beta_7^+$Flt3$^-$CD25$^-$ cell had the potential to differentiate into ILC2s, ILC3s (including CCR6$^+$ LTi-like cells), and a peculiar non-NK (i.e., Eomes$^-$T-bet$^+$) ILC1 population. Thus, on the clonal level, Lin$^-$Id2$^+$IL-7Rα$^+\alpha_4\beta_7^+$Flt3$^-$CD25$^-$ cells gave rise to all ILC lineages, but not to cNK cells, suggesting that all cytokine-producing, helper-like ILCs share a common progenitor and that cNK cells branch off earlier from a putative CILP (Figure 1). Given its restricted helper-like ILC potential, the Id2$^+$ common progenitor has been dubbed CHILP, common helper-like ILC progenitor.

Promyelocytic Leukemia Zinc Finger Is Transiently Expressed in Helper-like ILC Progenitors

Bendelac and colleagues found that ILCs do not express the transcription factor promyelocytic leukemia zinc finger (PLZF), which controls the formation of an innate-like program in CD1d-restricted T cells (also referred to as iNKT cells) (Kovalovsky et al., 2008; Savage et al., 2008). However, lineage tracing for expression of *Zbtb16*, the gene encoding PLZF, revealed that all helper-like ILCs were prominently labeled, whereas cNK cells and LTi cells did not express PLZF during lineage specification (Constantinides et al., 2014). Accordingly, *Zbtb16*-deficient

Table 2. Phenotype of Mouse cNK Cells, ILC1s, and other IFN-γ-Producing NKp46$^+$ ILCs

	NKp46	NK1.1	VLA2 CD49b	VLA1 CD49a	TRAIL	CD160	CD103	CD127	CD69	Ly49
Splenic cNK	+	+	+	–	–	–	–	subset	–	+
Liver cNK	+	+	+	–	–	–	–	–	–	+
Intestinal LP cNK	+	+	low	subset	low	+	n.d.	–	+	+
Salivary Gland NK	+	+	+	+	+	–	+	–	+	+
Uterine NK	+	+	–	+	+	–	?	low	+	+
Intraepithelial ILC1s	+	+	+	+	+	+	–	low	+	?
Intestinal LP ILC1s	+	+	–	+	low	+	?	+	+	–
Liver ILC1s	+	+	–	+	+	+	–	subset	+	(subset)
Intestinal LP ex-RORγt$^+$ ILC3s	+	+	–	+	n.d.	+	n.d.	low	+	–
Thymic NK cells	+	+	low	n.d.	n.d.	n.d.	n.d.	high	+	(subset)

n.d., not determined.

mice showed normal development of cNK cells and LTi cells, whereas ILC2s and liver ILC1s were mildly reduced (Table 3). While NKp46$^+$ ILC3s were positive in a *Zbtb16* fate map, they did not require PLZF for differentiation and/or maintenance. These data suggest that PLZF is not strictly required for ILC development. Interestingly, within Lin$^-$IL-7Rα$^+$c-Kit$^+$α$_4$β$_7^+$ cells in fetal liver and bone marrow, a population of PLZF$^+$ progenitors could be discerned. Adoptive transfer experiments and clonal differentiation assays revealed that PLZF$^+$ Lin$^-$IL-7Rα$^+$c-Kit$^+$α$_4$β$_7^+$ cells gave rise to ILC1s, ILC2s, and CCR6$^{-/lo}$ ILC3s, but not to cNK cells or CD4$^+$ ILC3s (i.e., LTi cells). The more restricted differentiation potential of PLZF$^+$ Lin$^-$IL-7Rα$^+$c-Kit$^+$α$_4$β$_7^+$ cells as compared to the CHILP might be explained by the fact that only a subpopulation of CHILP expressed PLZF (Klose et al., 2014), suggesting that PLZF$^+$ CHILP are the progeny of PLZF$^-$ CHILP and have a more restricted differentiation potential. Furthermore, clonal analyses showed that only less than 10% of the PLZF$^+$ progenitor population had potential to generate three ILC subsets and more than 50% of clones generated only a single ILC type, raising the possibility that these progenitor populations are a mixture of bona fide CHILP and already committed cells (Constantinides et al., 2014). Future work will need to reveal how PLZF and Id2 restrict the lymphoid differentiation program to the ILC lineage.

Helper-like ILCs Require the Transcription Factor GATA-3 for Development

Both of the above reports (Constantinides et al., 2014; Klose et al., 2014) might indicate that cNK cells develop independently of CHILP populations, suggesting that NK cell progenitors branch off earlier during ILC development (Figure 1). Indeed, cNK cells seem to upregulate Id2 only after lineage specification because the recently identified refined NK cell progenitor (Fathman et al., 2011) is largely Id2$^-$ (Klose et al., 2014), which is consistent with previous reports (Boos et al., 2007). The view that ILCs might be subdivided into two major lineages (i.e., killer ILCs and helper-like ILCs) is supported by another set of data revealing that GATA binding protein-3 (GATA-3) plays important roles for the development of helper-like ILCs, but not for that of cNK cells. Genetic deletion of *Gata3* in all hematopoietic cells resulted in a lack of all IL-7Rα-expressing helper-like ILCs, whereas the differentiation of cNK cells was largely normal (Samson et al., 2003; Vosshenrich et al., 2006; Yagi et al., 2014). Very similar data were obtained using fetal liver chimeras generated with *Gata3*-deficient hematopoietic progenitors (Serafini et al., 2014). It is interesting that helper-like ILCs, but not cytotoxic cNK cells, are *Gata3*-dependent, revealing another interesting analogy to T cell lineages. Previous data demonstrated that deletion of *Gata3* in developing T cells diminished helper T cell development, but not that of cytotoxic CD8 T cells (Zhu et al., 2004). It should be noted that the roles of GATA-3 for ILC development and function are complex and highly stage-specific. Early deletion of *Gata3* in all hematopoietic cells perturbs development of all helper-like ILC lineages, whereas deletion of *Gata3* downstream of the CHILP such as in all *Id2*-expressing cells or in *Ncr1*-expressing cells leads to a selective block in ILC2 (Hoyler et al., 2012; Yagi et al., 2014) or ILC1 development (Klose et al., 2014), respectively, whereas the differentiation of other ILC subsets was rather normal (Table 3).

NFIL3, a Central Transcription Factor for All ILCs?

Very recently, the transcription factor NFIL3 (also known as E4BP4) has been implicated in the differentiation of various ILC lineages. Previous work had identified NFIL3 to be required for the differentiation of cNK cells (Gascoyne et al., 2009; Kamizono et al., 2009). Now a broader role of NFIL3 for the differentiation of various ILC lineages is becoming apparent (Geiger et al., 2014; Seillet et al., 2014b). NFIL3-deficient mice have reduced numbers of ILC2s and ILC3s (including LTi cells), leading to profound defects in immunity to *C. rodentium* infection (requiring IL-22-producing ILC3s) or in papain-induced allergies (requiring ILC2s) (Seillet et al., 2014b). NFIL3-deficient mice lacked the CHILP population indicating that *Nfil3* might be already needed for the generation of early ILC progenitors most likely at the stage of CILP given that both cytotoxic and helper-like ILC are affected by *Nfil3* deficiency (Figure 1; Table 3). However, this model needs to be revisited in the light of previous data showing that the NK precursor is unaffected in *Nfil3*$^{-/-}$ mice and that mice lacking NFIL3 still have lymph nodes indicating that ILC3 development cannot be entirely blocked (Gascoyne et al., 2009; Kamizono et al., 2009). Future studies will need to define on a molecular level how NFIL3 controls or contributes to ILC fate.

Table 3. Transcription Factors and Cytokines Required for Mouse ILC Development and Survival

	Id2	NFIL3	GATA-3 (*Vav*-Cre or chimeras)	GATA-3 (*Id2*-CreERt2)	GATA-3 (*Ncr1*-Cre)	PLZF	Eomes	T-bet	RORγt	IL-7R	IL-15
Splenic cNK	↓↓↓	↓↓↓	→	n.d.	n.d.	n.d.	↓↓↓	→ or↓↓	→	↑↑	↓↓
Liver VLA2$^+$ cNK	n.d.	↓↓↓	n.d.	n.d.	n.d.	→	↓↓↓	→	n.d.	→	↓↓
Liver VLA2$^-$ ILC1	n.d.	→[a] or ↓↓[b]	n.d.	n.d.	n.d.	↓↓	→	↓↓↓	n.d.	n.d.	↓↓
Intestinal LP cNK	n.d.	↓↓↓	n.d.	n.d.	→	n.d.	↓↓↓	→	→	↑↑	↓↓
Intestinal LP ILC1	n.d.	↓↓↓	n.d.	n.d.	↓↓↓	n.d.	→	↓↓↓	→	→	↓↓
Intestinal LP ex-RORγt$^+$ ILC3	n.d.	↓↓↓	n.d.	n.d.	→	n.d.	→	↓↓↓	↓↓↓	↓↓	↓↓
Intraepithelial ILC1	↓↓↓	↓↓↓	↑↑	n.d.	n.d.	→	n.d.	↓↓↓	→	n.d.	↓↓
Uterine VLA2$^+$ cNK	n.d.	↓↓↓	n.d.	n.d.	n.d.	n.d.	↓↓↓	→	n.d.	n.d.	↓↓
Uterine VLA2$^-$ ILC1	n.d.	→	n.d.	n.d.	n.d.	n.d.	→	→	n.d.	n.d.	↓↓
Salivary gland NKp46$^+$	n.d.	→	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	↓↓
Thymic NKp46$^+$	→ (?)	↓↓	↓↓↓	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	↓↓	→
ILC2 (LP, visceral fat, lung)	↓↓↓	↓↓	↓↓↓	↓↓↓	n.d.	↓↓	n.d.	n.d.	→	↓↓	→
Intestinal LP CCR6$^+$ ILC3	↓↓↓	↓↓	↓↓↓	→	n.d.	→	→	→	↓↓↓	↓↓	→
Intestinal LP CCR6$^{-/lo}$ ILC3	↓↓↓	↓↓	↓↓↓	→	n.d.	→	→	NKp46$^-$ → or ↑↑ NKp46$^+$ ↓↓↓	↓↓↓	NKp46$^-$ ↓↓ NKp46$^+$ ↓↓	NKp46$^-$ → NKp46$^+$ →

n.d., not determined; ↓↓↓, population strictly requires factor for development; ↓↓, population is reduced; →, no change in population size; ↑↑, population is increased.

[a]Seillet et al. (2014a) and Sojka et al. (2014) have reported virtually normal numbers of liver VLA2$^-$ ILC1 in *Nfil3*$^{-/-}$ mice.

[b]Kamizono et al. (2009) and Crotta et al. (2014) have reported reduced of liver VLA2$^-$ ILC1 in *Nfil3*$^{-/-}$ mice.

Notch and ILC Development

Notch signals play a complex role in ILC development, and it has recently become apparent that they are a major common denominator for ILC lineage differentiation. The CHILP gives rise to ILC1s, ILC2s, and ILC3s in in vitro cultures with OP9 feeder cells expressing the Notch ligand delta-like 1 (DL1) (OP9-DL1) (Klose et al., 2014). In line with these findings, ILC1s, as well as NKp46$^+$ ILC3s, were severely reduced in mice lacking the Notch signaling adaptor RBP/J (Lee et al., 2012). Moreover, ILC2 development in vitro and in vivo required Notch signals (Wong et al., 2012; Yang et al., 2013). ILC3s also require Notch for development, although its necessity seems to differ depending on the origin of the precursor used (fetal versus adult) (Cherrier et al., 2012; Possot et al., 2011). It was suggested that ILC3 differentiation is supported by intermittent provision of Notch ligands, whereas it is hindered in constant presence of Notch signaling, adding an additional layer of complexity to Notch signaling in developing ILCs (Cherrier et al., 2012). Beyond the initial development, ILC3 differentiation and acquisition of T-bet and NKp46 requires Notch signals, corroborating the previous findings in RBP/J-deficient animals (Rankin et al., 2013). Notch signaling drives the development of both ILCs and T cells; however in humans, the signal strength through Notch regulates the fate of progenitor cells and directs lineage commitment (Gentek et al., 2013). Moreover, the Notch signaling pathway probably differs in ILCs and T cells because Hes-1, a target of the Notch signaling pathway, is dispensable for ILC2s, but not for T cell differentiation (Yang et al., 2013). Downstream of Notch, TCF-1 encoded by *Tcf7* is induced and directly triggers upregulation of *Il7ra* gene expression in ILC2s (Mielke et al., 2013; Yang et al., 2013). The role of Notch also extends to ILC function because the Notch-TCF-1 axis positively regulates the expression of receptors for IL-2, IL-25, and IL-33 through induction of GATA-3 and is required for IL-22 production by ILC3s (Mielke et al., 2013; Yang et al., 2013). However, spatiotemporal control of Notch signals for ILC fate is not well understood at the moment. The work summarized has provided a great deal of information on transcriptional programs affecting common ILC fate in the mouse model. Analogous precursors in humans still remain to be identified.

Differentiation of ILC1s

The general challenge when dealing with ILC1 subsets was their phenotypic resemblance (e.g., NKp46$^+$, NK1.1$^+$, T-bet$^+$) making it difficult to discriminate subsets and to assign them to separate lineages. Only recently, through the use of genetic reporter systems faithfully reporting the expression of lineage-specifying transcription factors, some progress has been made in separating ILC1 populations from cNK cells. Differentiation of cNK cells depends on sequential steps controlled by the transcription factors Id2, NFIL3, and Eomes. The development and maintenance of cNK cells require IL-15 signaling through IL-2Rβ (CD122) and the cytokine receptor common γ chain (γ_c). However, thymic NK cells develop through a distinct developmental pathway that involves the transcription factor GATA-3 and signaling through the IL-7 receptor (CD127), and they produce IFN-γ and granulocyte macrophage colony-stimulating factor (GM-CSF) in much higher amounts than cNK cells (Ribeiro et al., 2010; Vosshenrich et al., 2006) (Table 3). IFN-γ-producing

ILCs in the oral and intestinal epithelium of both humans and mice (intraepithelial ILC1s) are equipped with a unique set of integrins and adhesion molecules such as VLA-1, CD160, and CD103 (in humans), manifest strong transforming growth factor β (TGF-β) imprinting and do not entirely rely on IL-15 signaling (Fuchs et al., 2013) (Tables 1 and 2).

The human oral and intestinal mucosa were found to harbor yet another subset of IFN-γ-producing ILCs that express the receptor for IL-7 and lack NK cell markers and lytic enzymes (Bernink et al., 2013). These cells were also called ILC1s and were proposed to arise from the conversion of RORγt$^+$ ILC3s under the influence of IL-12 during inflammation. Supporting this, human ILC3s can differentiate into RORγt$^-$ IFN-γ-producing cells in vitro when cultured in IL-2 (Cella et al., 2010; Hughes et al., 2010; Hughes et al., 2009). Moreover, fate-mapping experiments using *Rorc*-Cre mice and ILC3 transfer experiments showed that some IFN-γ-producing ILCs do, indeed, originate from RORγt$^+$ ILC3s (Klose et al., 2014; Vonarbourg et al., 2010). Together, these studies have suggested that a certain percentage of IFN-γ-producing ILCs might stem from ILC3s.

Because the development of cNK cells required Eomes, and T-bet was required for functional maturation and bone-marrow egress (Gordon et al., 2012; Jenne et al., 2009; Townsend et al., 2004), attention has focused on these two transcription factors. Interestingly, TRAIL$^+$VLA-1(CD49a)$^+$VLA-2$^-$ liver NK cells are T-bet and IL-15-dependent, but Eomes-independent (Daussy et al., 2014; Sojka et al., 2014), consistent with a distinct developmental pathway. Indeed, TRAIL$^+$VLA-2$^-$ liver NK cells did not express Eomes but strictly required T-bet for their development (Daussy et al., 2014; Gordon et al., 2012). Uterine NK cells are both T-bet and Eomes independent (Sojka et al., 2014), perhaps reflecting redundancy of these transcription factors. This might also be the case for salivary gland NK cells, because they express high amounts of both T-bet and Eomes (Cortez et al., 2014).

NFIL3 promotes the development of bone-marrow-derived NK cells from CLP in the steady state (Gascoyne et al., 2009; Kamizono et al., 2009; Male et al., 2012). Recent studies have shown that *Nfil3* is required for the formation of splenic cNK cells, as well as thymic NK cells, possibly by directly regulating the expression of downstream transcription factors such as Eomes and Id2 (Male et al., 2014; Seillet et al., 2014a). In contrast, liver VLA-2$^-$Eomes$^-$ NK cells (Sojka et al., 2014) and salivary gland VLA-2$^+$Eomes$^+$ NK cells (Cortez et al., 2014) seem to develop independently of NFIL3. It should be noted, however, that the impact of NFIL3 seems to be affected by the environment and might vary due to infection, inflammation, and perhaps housing conditions. For example, although NFIL3 is required for the development of conventional NK cells in the steady state, NFIL3 is dispensable for NK cells that express the MCMV-specific receptor Ly49H during MCMV infection (Firth et al., 2013). This variability might explain seemingly contradictory results regarding the impact of NFIL3 on the development of thymic NK cells (Crotta et al., 2014; Seillet et al., 2014a) and liver VLA-1$^+$ NK cells (Sojka et al., 2014). Therefore, dependence on NFIL3 is most likely insufficient to unequivocally define a cell lineage (Table 3).

While cNK cells develop from Id2$^-$ CLP, intestinal ILC1s and VLA-1$^+$ liver NK cells develop from Id2$^+$PLZF$^+$ CHILP (Constantinides et al., 2014; Klose et al., 2014). Thus, these studies establish liver VLA-1$^+$ NK cells and intestinal ILC1s as a separate ILC1 lineage, distinct from cNK cells. It will be interesting to see whether uterine and salivary gland NK cells also arise from CHILP or are more related to cNK cells. In addition to originating from discrete precursors, cNK cells and ILC1s might also differ in the timing and site of development. For example, liver ILC1s might develop early on during embryogenesis from the fetal liver and replicate in situ to maintain stable numbers.

Differentiation of ILC2s

Beyond Id2, IL-7, and the cytokine receptor common γ chain (Cao et al., 1995; Moro et al., 2010; Satoh-Takayama et al., 2010; Yokota et al., 1999), differentiation of ILC2s requires the transcription factors GATA-3, RORα, and TCF-1, as well as Notch signaling (Furusawa et al., 2013; Gentek et al., 2013; Hoyler et al., 2012; Klein Wolterink et al., 2013; Mielke et al., 2013; Moro et al., 2010; Wong et al., 2012; Yang et al., 2013). GATA-3 has broader roles in ILC development because deletion of *Gata3* in all hematopoietic cells using *Vav*-Cre (Yagi et al., 2014) or in fetal liver chimeras (Serafini et al., 2014) revealed that GATA-3 is critical for the differentiation of all helper-like ILC subsets, but not for cNK cells. Deletion of the *Gata3* gene at a later time point using *Id2*$^{CreERt2/+}$ mice resulted in the impaired differentiation of ILC2s, but not ILC3s (Hoyler et al., 2012). Similarly, when *Rosa26*$^{CreERt2/+}$ x *Gata3*$^{flox/flox}$ bone-marrow cells were transplanted into lethally irradiated mice and 4-hydroxytamoxifen was administered into the mice 1 day after the bone-marrow transplantation, differentiation of ILC2s, but not of ILC3s, was strongly suppressed (Furusawa et al., 2013; Yagi et al., 2014). Transgenic overexpression of GATA-3 increased ILC2 numbers concomitant with higher expression of IL-33R (Klein Wolterink et al., 2013). These results suggest that GATA-3 plays important roles in multiple steps of ILC differentiation and/or survival but high-level GATA-3 expression in lineage-specified ILC is specifically required for ILC2 maintenance (Table 3). GATA-3 is also required for the differentiation of human ILC2 (Mjösberg et al., 2012). Future research needs to provide a molecular understanding of the GATA-3-directed gene expression networks in the various ILC subsets and in progenitor populations.

RORα, a member of retinoic-acid-receptor-related orphan nuclear receptor, is highly expressed in ILC2s (Moro et al., 2010; Wong et al., 2012). ILC2 differentiation was severely impaired in a natural *Rora* mutant (*Rora*$^{sg/sg}$ mouse) without affecting other ILC subsets (Halim et al., 2012b; Wong et al., 2012), indicating that RORα is a transcription factor specifically required for ILC2 differentiation. Small numbers of ILC2 cells are present in the mesentery of *Rora*$^{sg/sg}$ mice and those residual cells are able to produce IL-5 and IL-13 in response to IL-33 (Furusawa et al., 2013), indicating that RORα is dispensable for cytokine production induced by IL-33 signals. Unlike RORα, GATA-3 is essential for the expression of IL-5 and IL-13 in mature ILC2s (Furusawa et al., 2013; Hoyler et al., 2012; Klein Wolterink et al., 2013; Liang et al., 2012; Yagi et al., 2014).

In addition to factors involved in the differentiation of ILC2s, other factors play roles in differentiation and functional maturation of ILC2s. Vitamin A and its metabolites derived from food control the balance between ILC2s and ILC3s in the intestine as discussed later (Spencer et al., 2014; van de Pavert et al.,

2014). Functional specification of ILC2s requires Gfi1 because the lack of the *Gfi1* gene resulted in the loss of GATA-3 expression and coexpression of IL-13 and IL-17 (Spooner et al., 2013). Gfi1 directly activated the expression of *Il1rl1* and *Il17rb* genes encoding receptors for IL-33 and IL-25, respectively. On the other hand, Gfi1 suppressed the characteristics of ILC3s through the suppression of the *Sox4-Rorc* axis required for the expression of *Il17a* gene (Spooner et al., 2013). Interestingly, work with human ILC3s had indicated that they can become producers of IL-5 and IL-13 when stimulated with TLR2 ligands (Crellin et al., 2010a). These results suggest potential plasticity between ILC2s and ILC3s under certain environmental conditions, which remains to be elucidated. Other outstanding questions are whether class II MHC expression is limited to ILC2s (Oliphant et al., 2014) and ILC3s (Hepworth et al., 2013) and how such class II MHC expression fits with our current understanding of transcriptional regulation of ILCs and of ILCs being part of the lymphoid branch of hematopoietic cells.

Differentiation of ILC3s

Group 3 ILCs contain various populations of RORγt-expressing ILCs that play important roles in lymphoid organogenesis and that are a substantial innate source of "type 17 cytokines" involved in protecting mucosal barriers against extracellular bacterial and fungal infections (Diefenbach, 2013). The common denominator of the various ILC3 populations is their dependency on the transcription factor RORγt, which specifies and defines the ILC3 lineage. RORγt expression has not been reported in other ILC lineages. *Rorc(gt)*, an alternative transcript of the *Rorc* gene, was initially found to be expressed in all $CD4^+CD8^+$ thymocytes, and overexpression of RORγt protected such DP thymocytes against activation-induced cell death (He et al., 1998). Surprisingly, genetic deletion of the *Rorc* gene had a mild T cell phenotype but these mice lacked all peripheral lymph nodes and Peyer's patches (Eberl et al., 2004; Kurebayashi et al., 2000; Sun et al., 2000). Lack of lymphoid organ development in RORγt-deficient animals was linked to the absence of LTi cells. LTi cells were first identified as innate lymphocytes in lymph node and Peyer's patch anlagen of E13.5–E15.5 mice (Adachi et al., 1998; Adachi et al., 1997; Mebius et al., 1997; Yoshida et al., 2002). Interestingly, a phenotypically related and RORγt-dependent lymphoid population was found to be required in the small intestine to coordinate formation of dispersed lymphoid clusters found in the lamina propria of the intestine known as cryptopatches and isolated lymphoid follicles (Eberl, 2005; Eberl and Littman, 2004; Hamada et al., 2002; Kanamori et al., 1996). ILC3s can be differentiated from $Lin^-IL\text{-}7R\alpha^+Flt3^-\alpha_4\beta_7^+$ fetal liver progenitors that sequentially acquired Id2, CXCR6, and RORγt expression (Cherrier et al., 2012; Possot et al., 2011).

Although a role of LTi cells as organizers of lymphoid organ development is well documented, only recently did it become clear that they are substantially represented in the intestinal lamina propria of adult mice where they produce copious amounts of IL-22 and IL-17A and contribute to immunity to infections and the pathogenesis of inflammatory diseases (Buonocore et al., 2010; Cella et al., 2009; Cupedo et al., 2009; Luci et al., 2009; Powell et al., 2012; Sanos et al., 2009; Satoh-Takayama et al., 2008; Takatori et al., 2009; Vonarbourg et al., 2010). More recently, it was shown that ILC3s also produce GM-CSF, which established important crosstalk with GM-CSF-responsive mononuclear phagocytes that regulate oral tolerance (Mortha et al., 2014). These intestinal "LTi-like cells" expressed surface receptors often found on NK cells such as NKp46, NKp44, and CD56 raised questions about the relationship between $NKp46^+$ ILC3s and NK cells. Compelling evidence from fate-map studies has been provided showing that $NKp46^+$ ILC3s develop independent of the NK cell lineage and are the progeny of $NKp46^-$ ILC3s (Crellin et al., 2010b; Klose et al., 2014; Satoh-Takayama et al., 2010; Sawa et al., 2010; Vonarbourg et al., 2010).

The existence of $NKp46^-$ and $NKp46^+$ ILC3s raised the question of whether these are independent ILC lineages or rather differentiation or activation products of the same lineage. Two reports have found that ILC3s can be subdivided into $CCR6^+IL\text{-}7R\alpha^{hi}Kit^{hi}$ and $CCR6^{-/lo}IL\text{-}7R\alpha^{lo}Kit^{lo}$ cells that might constitute separate ILC3 lineages, both of which require RORγt for development (Klose et al., 2013; Sawa et al., 2010). It has now become clear that $CCR6^+$ and $CCR6^{-/lo}$ ILC3s follow distinct transcriptional programs. $CCR6^{hi}$ ILC3 seed the intestine early during fetal development and seem to be long-lived and slowly cycling cells (Eberl and Littman, 2004; Hanash et al., 2012; Sawa et al., 2010). In contrast, $CCR6^{-/lo}$ ILC3s account only for a small fraction of ILC3s at birth but vigorously expand during the first 4 weeks after birth (Klose et al., 2013). Expansion of $CCR6^{-/lo}$ ILC3 was dependent on the expression of the ligand activated transcription factor aryl hydrocarbon receptor (AhR). Mice genetically lacking AhR expression in all cells or in ILC3s had a largely unaltered compartment of $CCR6^+$ ILC3 whereas $CCR6^{-/lo}$ ILC3s were diminished (Kiss et al., 2011; Klose et al., 2013; Lee et al., 2012; Qiu et al., 2012). AhR-independent development of $CCR6^+$ ILC3s with LTi function was also documented by the fact that fetally forming lymphoid organs (i.e., lymph nodes, Peyer's patches) were normal in AhR-deficient mice. Curiously, cryptopatches and ILF were absent in AhR-deficient mice, suggesting that $CCR6^{-/lo}$ ILC3s have either nonredundant LTi function or that AhR regulates crucial LTi effector molecules in $CCR6^+$ ILC3s. The reduced maintenance of $CCR6^{-/lo}$ ILC3 in AhR-deficient mice might be explained by a role of AhR in regulating expression of the receptor tyrosine kinase c-Kit because AhR directly bound to the c-Kit promoter and controlled transcription of the *Kit* gene (Kiss et al., 2011). AhR is a sensor of various small molecules that confer transcriptional activity to the AhR (McIntosh et al., 2010). Interestingly, nutrient-derived AhR ligands such as glucobrassicins contained in vegetables of the *Brassicaceae* family (e.g., broccoli, Brussels sprouts) were recently identified to have an important role in driving postnatal expansion of $CCR6^{-/lo}$ ILC3s (Kiss et al., 2011) and intraepithelial γδ T cells (Li et al., 2011), demonstrating a broad role of plant-derived phytochemicals in controlling development and maintenance of immune system components at barrier surfaces.

The majority of $NKp46^-CCR6^{-/lo}$ ILC3s coexpressed T-bet (Klose et al., 2013; Rankin et al., 2013; Sciumé et al., 2012). While plastic ("ex-$ROR\gamma t^+$") ILC3s can be found in humans (Bernink et al., 2013), it remains to be seen whether T-bet-expressing $CCR6^{-/lo}$ ILC3s exist in humans. Coexpression of T-bet and RORγt was a remarkable finding because it was believed that lineage-specifying transcription factors such as T-bet and RORγt might be mutually exclusive in expression. T-bet was not required for the differentiation or maintenance of

NKp46^{-}CCR6$^{-/lo}$ ILC3s but controlled differentiation of NKp46^{-}CCR6$^{-/lo}$ ILC3s to NKp46^{+}CCR6$^{-/lo}$ ILC3s (Klose et al., 2013; Rankin et al., 2013). In addition, an increasing T-bet gradient in CCR6$^{-/lo}$ ILC3s directed a transcriptional program that allowed for functional and phenotypic plasticity (Figure 1). For example, T-bet controlled IFN-γ production in NKp46^{+}CCR6$^{-/lo}$ ILC3s that contributed to early immunity to *Salmonella enterica* infection. When inappropriately stimulated, NKp46^{+}CCR6$^{-/lo}$ ILC3s contributed to intestinal inflammatory diseases in mice and humans (Bernink et al., 2013; Vonarbourg et al., 2010). T-bet expression in CCR6$^{-/lo}$ ILC3s was independent of IL-12 signaling but was promoted by IL-23 (Klose et al., 2013), revealing an interesting parallel to the signals driving plasticity of Th17 cells in a mouse model of multiple sclerosis (Hirota et al., 2011).

Recent data revealed an important role for maternal vitamin A metabolites in the differentiation and maintenance of fetal CCR6^{+} ILC3 with LTi function. Mice deficient of retinoic acid receptor (RAR)α expression in hematopoietic cells had reduced numbers of fetal CCR6^{+} ILC3s due to inefficient upregulation of the *Rorc(gt)* transcript of the *Rorc* gene (van de Pavert et al., 2014). Reduced ILC3 numbers led to the formation of substantially smaller lymphoid organs, ultimately reducing the fitness of adult mice to virus infections (van de Pavert et al., 2014). Similar findings were reported for adult mice under conditions of vitamin A malnutrition that presented with reduced numbers of IL-22-producing ILC3s (Spencer et al., 2014). Vitamin A depravation of adult mice led to impaired immunity to attaching-and-effacing infections with *C. rodentium*. Whereas ILC3 numbers and function were reduced under conditions of retinoic acid deficiency, ILC2s were increased in a compensatory manner resulting in enhanced immunity to nematode infection (Spencer et al., 2014). These data and the data from AhR-deficient mice establish an important trajectory by which nutrients instruct differentiation and maintenance of mucosal ILC subsets.

Conclusions and Perspectives

The discovery of new ILC lineages has entirely redefined our hematopoietic lineage maps (Figure 1). It is now clear that the diversity of ILCs is much higher than previously appreciated. The description of ILC-restricted progenitors (i.e., CHILP) has now opened opportunities to examine the cues that drive differentiation of the various helper-like ILC populations. While the similarities between helper-like ILCs and T helper cell subsets are striking, it is already clear that the cytokine signals required for T helper fate decisions are dispensable for the differentiation into the various helper-like ILC populations (Guo et al., 2014; Klose et al., 2013; Liang et al., 2012). Thus, one of the important future goals will be to identify the signals that drive differentiation of the CHILP. Although a naive CD4 T cell can be differentiated into any Th effector state depending on the cytokine environment provided, it should be considered that ILC fate might be determined through a timed release of progenitors during ontogeny into an environment that allows for the preferential differentiation into a certain ILC fate. Precedent for such temporal organization of lymphoid-fate decisions are the various waves of distinct γδ T cell subsets leaving the thymus before birth. Such future lines of research might also reveal an evolutionary perspective on how such transcriptional programs controlling lymphoid fate have emerged. In addition, although ILC2s seem to be a relatively stable subset compared to ILC1s and ILC3s, there is still a possibility of plasticity between ILC2s and other ILC subsets (Crellin et al., 2010a; Spooner et al., 2013). Given the emerging roles of transcriptional and functional plasticity of lymphocyte subsets for the pathogenesis of inflammatory diseases (Hirota et al., 2011; Vonarbourg et al., 2010), the molecular cues stabilizing ILC fates and those driving plasticity should be identified because they might reveal therapeutic targets for such debilitating diseases. Thus, future research needs to identify the signals and the spatiotemporal organization of helper-like ILC development.

Research into ILC lineages has also revealed that the population of NKp46^{+} cells, previously addressed as "NK cells," is more complex than appreciated. For example, the population of NKp46^{+} cells in the lamina propria of the intestine is composed of at least three distinct ILC populations (Klose et al., 2014). The unique contributions of these various NKp46^{+} ILC subsets to immune responses remain to be determined. In that context, it is interesting to note that cNK cells might be a lineage distinct from helper-like ILCs. It will be informative to position the branching point of the cytotoxic and helper-like ILC lineages on hematopoietic lineage maps. This will be complemented by analyzing the core transcriptional program determining ILC fate. Id2 has already been discovered as one central hub for ILC fate and NFIL3 and TOX might be other candidates. The DNA binding factor TOX is an interesting candidate because *Tox*$^{-/-}$ mice lack NK cell and ILC3 development (Aliahmad et al., 2012; Geiger et al., 2014).

Finally, lymphoid cells use immune-recognition receptors for development and to interact with infected or damaged cells. It remains unclear whether immunoreceptors expressed by ILCs are required for their function and/or development. Mice lacking the *Ncr1* gene and consequently not expressing NKp46 showed normal development and function of ILC3s documented by their resistance to *C. rodentium* infection (Satoh-Takayama et al., 2009). Consecutive work documented a role for activating immunoreceptors for ILC function. Human ILC3s from tonsils and colon could be activated for TNF release when triggered by the NKp44 receptor (Glatzer et al., 2013). In mice, liver-resident ILC1s responded to engagement of various activating immunoreceptors (i.e., NKp46, NKG2D, NK1.1) with the production of TNF, IFN-γ, and CCL3 (Daussy et al., 2014). Interestingly, liver-resident ILC1s with an "NK cell phenotype" display antigen-specific memory that does not require recombining receptors (O'Leary et al., 2006; Paust et al., 2010). The phenotype of such memory "NK cells" might indicate that they are an ILC1 subset. It will be highly informative to gain a more molecular understanding of how ILCs discriminate between "self" and "non-self" and how previous activation might introduce a memory-like program into innate lymphocytes.

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