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Cellular pathways in the development of human and murine innate lymphoid cells

Steven D. Scoville[1], **Aharon G. Freud**[2,*], **Michael A. Caligiuri**[3,*]

[1]Department of Surgery, The Ohio State University, Columbus OH

[2]Department of Pathology, The Ohio State University, Columbus OH

[3]Department of Hematology, City of Hope National Medical Center, Los Angeles CA

Abstract

Innate lymphoid cells (ILCs) are critical to effective immune surveillance against pathogens, have malignant counterparts, and contribute to disease. Thus, it is important to understand ILC development. All ILCs are derived from the common lymphoid progenitor cell; however, the exact mechanisms and signals that initiate their divergence from T cells, B cells and one and other are incompletely understood. Evidence now supports a stepwise developmental process that includes distinct cellular intermediates, progressively narrowed differentiation, and some plasticity. While the current models of human and murine ILC development share many similarities, they also include some distinct differences. Together these findings have established a working dynamic model of ILC development.

Introduction

Innate lymphoid cells (ILCs) are an increasingly important component of both normal immunity and pathologic processes. Collectively, the ILC family is composed of developmentally related but functionally distinct groups of cells similar to the relationships between T cell subtypes. Mature ILCs are categorized into three groups based on their immunophenotypes such that Group 1 ILCs include non-cytotoxic ILC1s that express Tbx21 (Tbet) transcription factor (TF), as well as cytotoxic natural killer (NK) cells that also express Eomesodermin (Eomes), and both produce interferon (IFN)-γ; Group 2 ILCs consist of ILC2s that produce interleukin (IL)-5 and −13 and express GATA-3 and RAR-related orphan receptor (ROR)α TFs; and Group 3 ILCs include NK cell receptor $(NCR)^{+}$ and NCR^{-} ILC3s and lymphoid tissue inducer (LTi) cells that produce IL-22 and/or IL-17 and express RORγt and the aryl hydrocarbon receptor (AHR) TFs [1]. Physiologically, ILCs play significant roles in anti-viral, bacterial and helminth infections; they are involved in metabolic regulation of fat; and they provide immunity to cancer [2]. Their roles in disease processes continue to expand and thus our ability to understand the regulation of their

*Corresponding Authors: mcaligiuri@coh.org; aharon.freud@osumc.edu.

development and activation pathways will provide novel avenues toward disease prevention and treatment.

Developmentally, all ILCs derive from the common lymphoid progenitor (CLP), which is capable of generating all lymphocyte subsets including ILCs as well as B and T cells. This relationship was first established when mice lacking *Ikaros* or the common gamma chain were found to be deficient in NK, B and T cells[3,4]. Furthermore, isolation of the multipotent CLP in mice and in humans was definitive proof that these innate and adaptive cells were derived through the same lymphoid pathway [5,6]. Although there is an abundance of similarity between mouse and human ILC development, there is evidence for some key differences in their precursor populations and pathways. Therefore, in this review we discuss mouse and human ILC development separately.

Murine ILC development

Discoveries related to murine ILC development have been augmented by robust genetic tools and in vitro clonal culture systems (Table 1) that have assisted in the identification of individual progenitors, associated TFs, and signals required for development downstream of the CLP (Figure 1). Two distinct progenitors downstream of the murine CLP have been identified, each with restricted ILC potential. These include the α-lymphoid precursor (αLP) and the early innate lymphoid progenitor (EILP) that along with the other downstream progenitors are most prevalent in murine BM [7]. The αLP was first discovered in part through the Nfil3 knockout models that demonstrated a loss of a specific $CXCR6^{+}\alpha_4\beta_7{}^{+}CD127^{+}$ cell subset (i.e., the αLP subset), in addition to the loss of the EILP and the absence of all mature ILCs, including NK cells [8]. The EILP, identified as Lin^{-} $Thy1.2^{-}CD127^{-}\alpha_4\beta_7{}^{+}$ most notably differs from the αLP in that it lacks expression of CD127, a common marker of all most ILCs, but otherwise appears to have an identical lineage potential [9]. Identification of the EILP required labeling of the *Tcf7* gene, an HMG box-containing TF, with a GFP-reporter and mice deficient in this gene lacked all ILC populations. When purified and cultured the EILP gives rise to a more restricted progenitor known as the common helper ILC progenitor (CHILP), that is functionally distinct in that it lacks NK cell developmental potential yet maintains helper ILC (ILC1, ILC2, ILC3 and LTi) potential [10]. The exact relationship and importance of the αLP and the EILP, was recently explored by Harly *et al.* They utilized an αLP armed with $Tcf7^{EGFP}$ in addition to fate mapped $CD127^{YFP}$ to show that GFP^{+} EILPs are indeed CD127 fate map positive, suggesting they originate from the $CD127^{+}$ αLP that subsequently express *Tcf7* [11]. These data suggest that the $CD127^{+}$ αLP gives rise to the $CD127^{-}$ EILP, that in turn gives rise to the $CD127^{+}$ CHILP (Figure 1). An additional advancement in early murine ILC development was the discovery that these multipotent ILC progenitor cells begin to acquire mature ILC fates at this early development stage. Seillet *et al.* recently performed extensive expression analysis on the CLP, αLP, and ILC2 progenitor (ILC2P) and found that certain subsets of the αLP express TFs associated with some mature ILC progenitors suggesting that lineage decisions, known as lineage priming, are occurring at this early stage [7,12]. Therefore, by some yet-to-be-defined mechanism, TFs that define terminal ILCs are being expressed early in development and impacting the lineage potential of that particular cell.

The CHILP, identified as Lin$^-$Id2$^+$CD127$^+$CD25$^-\alpha_4\beta_7^+$, was first discovered through its high levels of *Id2* expression. *Id2* is a helix-loop-helix TF that has been shown to suppress the E2A TF essential for B and T cell development [13,14]. Furthermore, the CHILP can be further separated based on expression of the *Plzf* TF. The *Plzf*$^+$ subset loses its ability to produce LTi's compared to the *Plzf*$^-$ subset [10,15]. Subsequent work has shown that the *Plzf*$^+$ CHILP, also referred to as the ILC precursor (ILCp) can be identified through expression of the cell surface marker Pd-1 [16]. The identification of these latter intermediates suggests that downstream of the αLP and EILP, differentiation of helper ILCs is independent of NK development. Consistent with this, the previously described NK1.1$^-$ CD122$^+$ NK progenitor (NKP) population [17] is absent in Tcf-1 knockout mice, but still exists in models where helper ILC lineages are disrupted such as seen in Id2 and Tox deficient mice [9,14,18]. Collectively, these published data support a widely accepted and established model of murine ILC development in which the NK cell developmental pathway diverges early from the helper ILC developmental pathways (Figure 1). Nonetheless, we note that few NK1.1$^+$CD49a$^-$CD49b$^+$ NK cells were reportedly produced by the putative more committed helper ILCPs in both the Constantinides *et al.* and Yu *et al* studies [15,16]. Therefore, additional studies are likely warranted to investigate the pathways of ILC development in mice.

Human ILC Development

The study of human ILC development is often limited by the lack of comparable genetic and other tools that are available in animal models of lymphoid development. Nonetheless, substantial progress has been made in understanding human ILC development, sometimes inspired by and/or validated by murine studies. As was mentioned earlier murine and human development share the CLP, identified in humans as Lin$^-$CD34$^+$CD45RA$^+$CD10$^+$CD117$^-$ and as the starting point for ILC differentiation [5]. A useful framework for investigating human ILC development stems from a pre-existing model of human NK cell development based on the identification and characterization of human NK cell developmental intermediates (NKDIs) prior to the discovery of non-NK helper ILCs [22]. According to the human NK cell development model, NKDIs differentiate at least within secondary lymphoid tissues, including tonsils and lymph nodes, through functionally distinct stages based on the differential expression of multiple surface markers and TFs [23]. The most immature NKDI in this pathway, originally described as "stage 1" and "stage 2" cells by Freud et al [24], are now defined as early tonsillar progenitors (ETP) within the context of non-NK ILCs, and are identified as Lin$^-$CD34$^+$CD10$^+$CD117$^-$ (ETP1) and Lin$^-$CD34$^+$CD10$^-$CD117$^+$ (ETP2), respectively. Both populations were originally found to be multipotent and to also give rise to T cells and dendritic cells (DCs) under supportive experimental conditions *in vitro* in bulk [24]. These data raised the possibility that each progenitor cell may be multipotent or that clonally-restricted subsets exist within each ETP population. In light of this, a recent report by Renoux *et al.* described a CD34$^+$CD45RA$^+$CD10$^+$CD7$^+$CD38$^+$CD123$^-$CD127$^-$ putatively committed NKP population [25]. Notably, however, the immunophenotype of this population overlaps with ETP1s [24], and in a later study by Chen *et al.* the NKPs described by Renoux *et al* were found to be multipotent in bulk *in vitro* cultures [25, 28]. Montaldo *et al.*, described an RORγt$^+$ population that was sorted ex vivo with the same phenotype

(CD34$^+$CD117$^+$) as the ETP2, showing these cells preferentially gave rise to ILC3s in vitro under the conditions tested (Table 2) [26]. The authors of that study concluded that tonsillar CD34$^+$CD117$^+$ cells represent lineage restricted ILC3 progenitors; however, evaluation for ILC1, ILC2, T cell, or DC potential was not reported in the study, and other reports have found this population to be multipotent [24,29]. More recently, the ETP2 was found to be heterogeneous with respect to expression of the IL-1 receptor 1 (IL-1R1). The ETP2 IL-1R1$^-$ population was found to be multipotent, although more primed for ILC development compared to the ETP1, as it gave rise to proportionally more ILCs than T cells and DCs. The ETP2 IL-1R1$^+$ population however lacks all non-ILC potential and was shown to only give rise to ILCs *in vitro* and *in vivo* in the conditions tested (Table 2). Therefore, the ETP2 IL-1R1$^+$ cell population is developmentally the earliest known committed common innate lymphoid progenitor (CILP) that has been discovered in humans.

The most recent developmental work has been within the CD34$^-$ fraction of ILC progenitors that are downstream of the CILP. One particularly relevant finding was by Lim *et al.* who identified a Lin$^-$CD34$^-$CD7$^+$CD127$^+$CD117$^+$ population of ILCPs in cord blood, peripheral blood, fetal liver and other human tissues that could only generate all ILCs [27]. Building on these prior intermediates, Chen *et al.* recently discovered a CD56$^+$ subset of CD34$^-$CD117$^+$ ILCPs with more restricted potential for Group 1 and 3 ILCs in that it could generate NK cells and ILC3s but not ILC2s; here we refer to this subset as the restricted ILCP (rILCP) [28]. In this report the authors also demonstrated that CILPs could generate ILCPs and rILCPs and furthermore that ILCPs could generate rILCPs, thus supporting a more refined model for human ILC development in secondary lymphoid tissues (Figure 2). Finally, evidence for lineage priming may also occur in early human lymphoid developmental intermediates as clonal work from ETPs, CILPs, ILCPs, and rILCPs has demonstrated bias towards specific ILC lineages [27,29].

Updates on differentiation and identification of human and murine mature ILCs

When initially proposed, each ILC group was neatly classified based on the basis of TF expression, immunophenotypic markers, and functional abilities. Work since that time has continued to refine these initial models to accommodate the most recent findings. As such, our understanding of ILCs is proving to be dynamic, and plasticity among ILC populations is becoming increasingly apparent. Indeed, through the years consistent distinctions have been found within ILC subsets once thought to be homogenous populations. Examples of this include identification of natural cytotoxicity receptor (NCR) expression or the lack thereof in ILC3 phenotypes (NKp44 in humans and NKp46 in mice) [30–33]; the identification of human CD103$^+$ intraepithelial (ie) ILC1s that express perforin and granzyme and are cytotoxic [34]; and the plasticity of ILC1 and ILC2s in generating ILC3s and vice versa [35–37]. In addition, Simoni et al. utilized mass cytometry (cytometry by time of flight or CyTOF) to investigate both normal and diseased tissues from human cord blood, tonsils, colon, omentum, and lung [38]. This comprehensive approach showed that ILC2s and ILC3s cannot alone be identified by GATA-3 and RORγt expression, respectively, as these TFs are not exclusive to those subsets, verifying previous studies

[27,29,39,40]. This is not to say that TFs are not enriched within populations, but more to say that ILCs cannot therefore be identified solely based on the qualitative existence of certain TFs. Collectively, these studies capture the dynamic field of ILC development and biology.

Finally, a multitude of studies and commentaries surround the identity and existence of the ILC1 population. Challenging the existence of ILC1s in humans, Simoni et al, could not isolate ILC1-like cells similar to those described in other studies but found this population to consist of multiple lineages including T cells, $CD34^+$ progenitors, DCs, ILC3, and NK cells [38]. This finding raises an important issue of how to define NK cells and ILC1s, especially as there has yet to be an identifying surface marker or TF that specifically distinguishes ILC1s from NK cells in humans or in mice [28,41–45]. Furthermore, and in support of ILC1 existence and importance in humans, Gao *et al.* have shown that ILC1s can derive from NK cells through immune-suppressive mechanisms that are operative in the tumor microenvironment [46]. Further, the NK cell phenotype can be altered following activation, such as has been documented with CD16 [47,48]. Additional work must be completed to determine how human ILC1s represent a distinct entity and if so, under what conditions.

Conclusions

A wealth of knowledge has been accumulated in recent years regarding the intricacies of both mouse and human ILC development. From identifying specific multipotent progenitor populations as well as lineage restricted precursor populations, to refining the identities of novel subsets of mature ILCs, the field is continuing to expand the knowledge surrounding ILC biology. One exciting aspect of this continued effort is the development of new methods with which to investigate these issues such as gene editing [49] and the recent development by Lopez-Lastra et al whereby human lymphoid progenitors can develop into each ILC subset in mice [50]. These efforts should further our understanding of ILC development, which in turn will provide insight for the prevention and treatment of human disease.

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*special interest

**outstanding interest

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Highlights

- ILCs develop stepwise through intermediates with increasingly restricted potential
- Murine studies have shown that NK cells develop separately from helper ILCs
- Human ILCs have a more common pathway for ILCs that includes NK cells
- ILCs have plasticity and are able to convert to other ILC populations

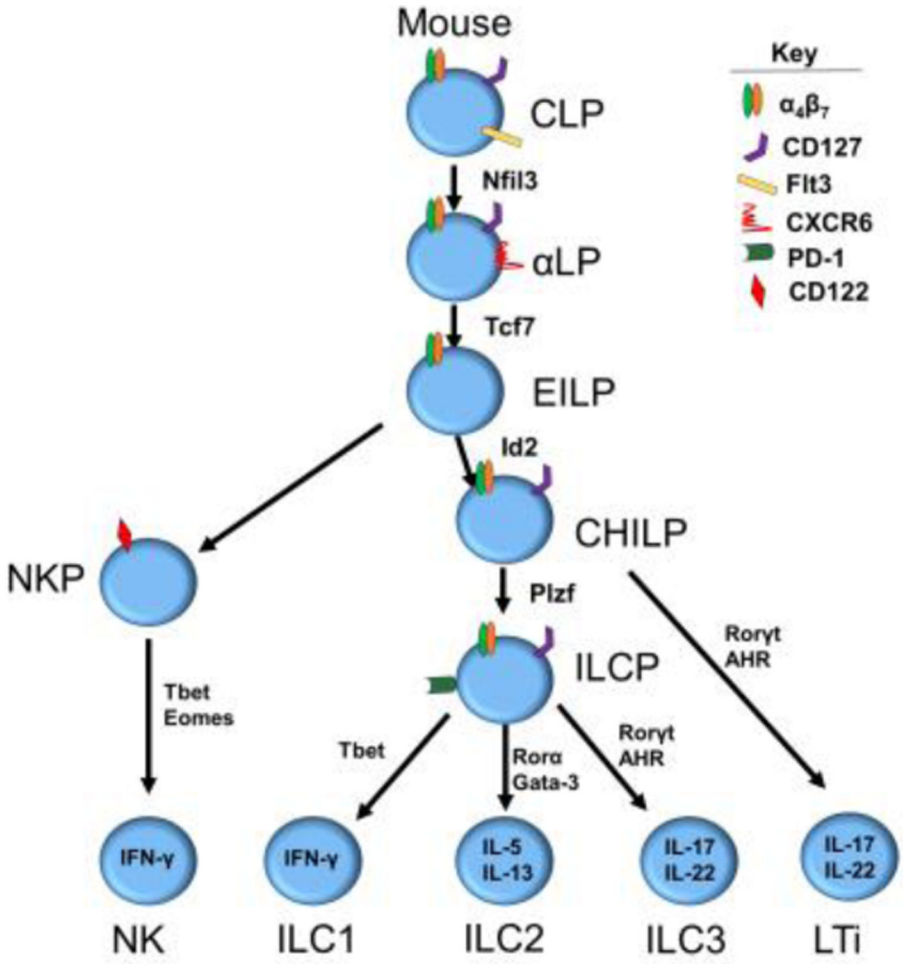

Figure 1.
Current pathways of murine ILC development

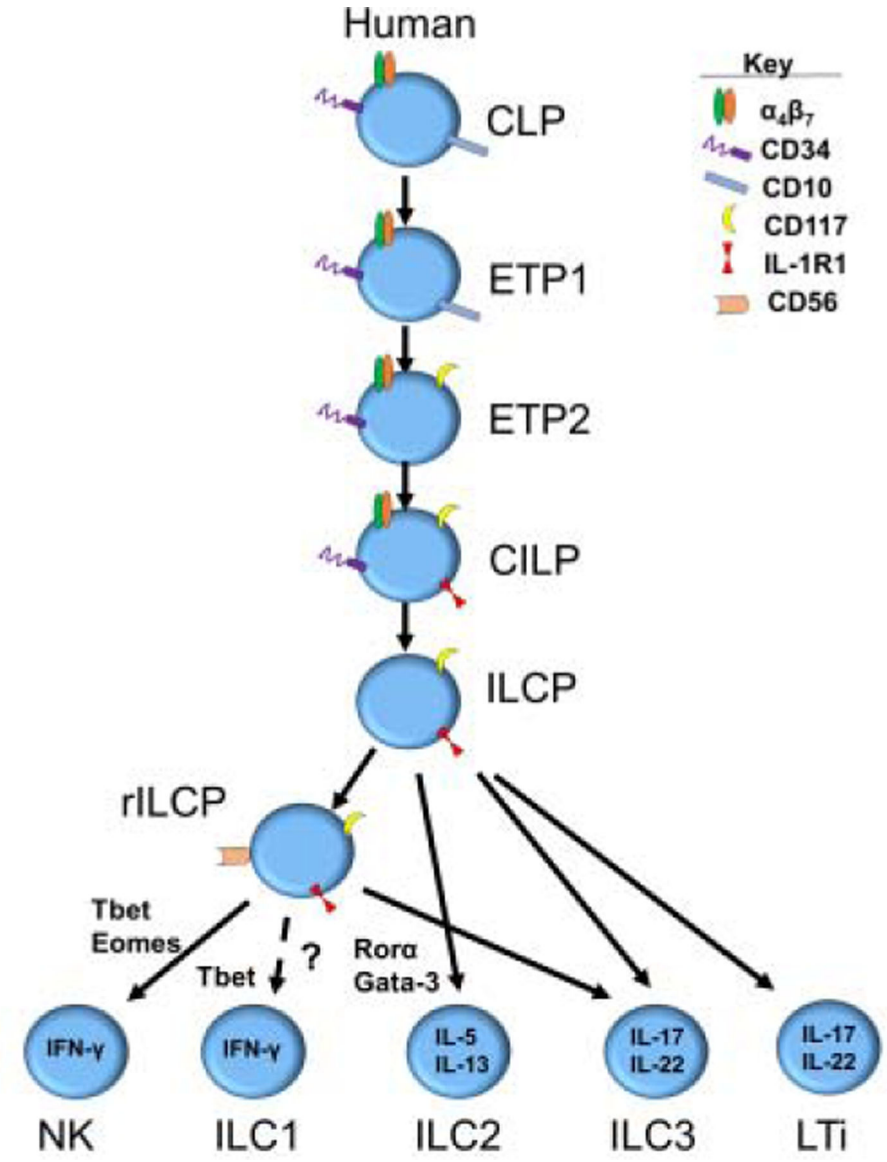

Figure 2.
Current pathways of human ILC development

Table 1:

Murine ILC progenitors *in vitro* analysis.

This table represents insight into the single cell *in vitro* culture conditions used in mice to discover lineage restriction and subsequent results.

Cell	Identification (Lin$^-$)	Progenitor Location	Transcription factors	Stromal Cell use	Length of culture (days)	Culture Cytokines	NK cell	ILC1	ILC2	ILC3	Refs
αLP	CXCR6$^+$α4β7$^+$CD127$^+$	BM	Tox, Nfil3	OP9-DL1	21	KL, IL-7	NK1.1$^+$Eomes$^+$Tbet$^+$	NK1.1$^+$Eomes$^-$Tbet$^+$	CD127$^+$Gata3$^+$Rorγt$^-$	CD127$^+$Gata3$^-$Rorγt$^+$	8
EILP	Thy1.2$^-$α4β7$^+$CD127$^-$	BM	Tox, Nfil3, Tcf7	OP9	10	KL, IL-7, IL-2	NK1.1$^+$Gata3$^-$NKp46$^+$DX5$^+$	NK1.1$^+$Gata3$^-$NKp46$^+$DX5$^-$	NK1.1$^-$Gata3$^+$	NK1.1$^-$Gata3$^-$NKp46$^-$Rorγt$^+$	9
CHILP	CD117$^+$α4β7$^+$CD127$^+$CD25$^-$Id2$^+$	BM	Tox, Tcf7, Id2	OP9-DL1	14	KL, IL-7	NK1.1$^+$Eomes$^+$**	NKp46$^+$Tbet$^+$Rorγt$^-$	NKp46$^-$Gata3$^+$Rorγt$^-$	NKp46$^{+/-}$Rorγt$^+$	10
ILCP	CD127$^+$α4β7$^+$Plzf$^+$	BM	Tox, Tcf7, Id2, Plzf	OP9, OP9-DL1	10–12	KL, IL-7	NK1.1$^+$CD49a$^-$CD49b$^+$**	NK1.1$^+$Bcl11b$^-$	NK1.1$^-$Bcl11b$^+$	NK1.1$^-$Bc11b$^-$Rorγt$^+$	15, 16

** indicates population was not tested for in single cell cultures

Table 2:

Human ILC progenitors *in vitro* analysis.

This table represents insight into the single cell *in vitro* culture conditions used in humans to determine lineage restriction and subsequent results.

Cell	Identification (Lin^-)	Transcription factors	Stromal cell use	Length of culture (days)	Cytokines	NK cell	ILC1	ILC2	ILC3	Refs
ETP1	$CD34^+CD117^-CD94^-CD10^+$	Tox	OP9-DL1	28	IL-3, IL-7, KL, FL, IL-15	$CD94^+IFN\text{-}\gamma^+$	$CD94^-$ $CD161^+IFN\text{-}\gamma^+$	$CD94^-$ $CD161^+IL\text{-}13^+$	$CD94^-$ $CD161^+IL\text{-}22^+$	22, 29
ETP2	$CD34^+CD117^+IL\text{-}1R1^-CD94^-$	Tox, Id2, Nfil3	OP9-DL1	28	IL-3, IL-7,KL, FL, IL-15	$CD94^+IFN\text{-}\gamma^+$	$CD94^-$ $CD161^+IFN\text{-}\gamma^+$	$CD94^-$ $CD161^+IL\text{-}13^+$	$CD94^-$ $CD161^+IL\text{-}22^+$	22, 29
CILP	$CD34^+CD117^+IL\text{-}1R1^+CD94^-$	Tox, Id2, Nfil3, AHR, Rorγt	OP9-DL1	28	IL-3, IL-7, KL, FL, IL-15	$CD94^+IFN\text{-}\gamma^+$	$CD94^-$ $CD161^+IFN\text{-}\gamma^+$	$CD94^-$ $CD161^+IL\text{-}13^+$	$CD94^-$ $CD161^+IL\text{-}22^+$	22, 29
ILCP	$CD34^-CD117^+IL\text{-}1R1^+CD94^-$	Tox, Tcf7, Id2, Plzf	OP9, OP9-DL4	14–18	IL-2, IL-7, IL-1β, IL-23	$CD7^+CD94^+Eomes^+$*	$CD7^+CD94^-$ $Eomes^-IFN\text{-}\gamma^+$*	$CD7^+CD94^-$ $IL\text{-}13^+$	$CD94^-$ $CD7^+IL\text{-}22^+$and/or $CD7^+IL\text{-}17A^+$	27
rILCP	$CD34^-CD117^+IL\text{-}1R1^+CD56^+$	Tcf7, Plzf	OP9-DL1	28	IL-2, IL-7, KL, FL	$CD94^+$	**	$CD94^-CD294^+$	CD94-NKp44$^+$	28

* Indicates populations were not distinguished from one another in single cell cultures

** Indicates population was not detected or tested for during this study