RESEARCH HIGHLIGHT

Building early defenses

Yi Ding¹✉ and Avinash Bhandoola¹✉

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Cell Research (2021) 31:1041–1042; <https://doi.org/10.1038/s41422-021-00535-4>

Innate lymphoid cells (ILCs) are considered as innate equivalents of T cells. Liu et al. used single-cell RNA sequencing to determine the proportions of ILCs in human fetal tissues, and additionally identified novel progenitor subsets undergoing ILC lineage specification in fetal liver.

Innate lymphoid cells (ILCs) possess similar functions to T cells but lack adaptive antigen receptors. They are enriched at barrier surfaces, and play critical roles in early defense against pathogens prior to expansion of T cells. They also function in maintenance of tissue homeostasis, promoting tissue repair and regulating tissue inflammation.¹

ILCs comprise lymphoid tissue inducer cells (LTi cells) which are critical for development of secondary lymphoid organs; helper ILCs which include ILC1s, ILC2s and ILC3s, secreting cytokines like CD4 T cells; and NK cells which are cytotoxic ILCs functionally similar to CD8 T cells.¹ ILCs are transcriptionally similar to T cells, and ILCs and T cells both derive from lymphoid progenitors. Transcription factors such as TCF-1 (encoded by *Tcf7*) are required early in development of both ILCs and T cells. However, T cell development takes place in the thymus, whereas development of ILCs is independent of the thymus.

ILC precursors (ILCPs) have been identified in mice.^{2,3} Upstream of ILCPs, early innate lymphoid progenitors (EILPs) that possess lineage potential for NK cells as well as dendritic cells are also identified (Fig. 1a).⁴ While a map of ILC development in mouse is beginning to be assembled, ILC development in human is much less well understood.

The distribution of postnatal human ILCs has been characterized in both lymphoid and non-lymphoid tissues, but the tissue distribution of human ILCs at fetal stages is still unclear.^{5,6} In addition, ILCPs are identified to be present in multiple postnatal tissues including peripheral blood, lung and tonsil, and are also present in fetal liver.⁷ Human ILCPs give rise to helper ILCs (ILC1, ILC2 and ILC3) and NK cells, but lack T cell and B cell lineage potential in vitro and in vivo, and thus are functionally similar to mouse ILCPs.⁷ Human common lymphoid progenitors (CLPs) are present within Lin[−]CD34⁺ hematopoietic stem progenitor cells (HSPCs) (Fig. 1b). However, intermediate developmental stages between committed ILC progenitors and HSPCs corresponding to mouse EILPs are unknown.

In a study published in *Cell Research*, Liu et al. used single-cell RNA sequencing (scRNA-seq) and flow cytometry to analyze ILCs and Lin[−]CD34⁺ progenitors in human fetal tissues including liver, intestine, thymus, spleen, skin and lung from 8 to 12 post-conception weeks (PCW).⁸ They found the major helper ILC subset was ILC3 in all tissues. Frequencies of ILC2s and putative ILC1s

were highest in thymus, and the frequency of helper ILCs in thymus was greatly reduced from 8 to 12 PCW. The authors isolated ILCs and Lin[−]CD34⁺ progenitors from 8–12 PCW embryos for scRNA-seq analysis. They identified two lymphoid progenitor clusters (LP1 and LP2) with high expression level of IL3RA. Computational analyses (Partition-based graph abstraction and STEMNET prediction) placed LP1 downstream of HSPCs and LP2 was likely downstream of LP1. Both LP1 and LP2 appeared to be closely related to ILCP.

The authors further assessed the in vitro lineage potential of Lin[−]CD34⁺CD127⁺IL3RA⁺ cells (which should include some LP1 and LP2 progenitor populations) and compared their lineage potential in vitro with ILCPs from fetal liver.⁸ As expected, fetal ILCPs gave rise to all helper ILC subsets. Newly identified IL3RA⁺ progenitors possessed lineage potential for NK cells, myeloid cells but very limited potential for B cells. IL3RA⁺ progenitors generated ILC1s and ILC2s as well, although not as efficiently as ILCPs. However, unlike ILCPs, IL3RA⁺ progenitors did not generate ILC3s. It is possible that IL3RA⁺ progenitors are a heterogeneous population, containing a range of progenitors with distinct lineage potentials, but lacking ILC3 lineage potential. An interesting possibility is that specialized progenitors seed tissues to generate the appropriate sets of ILCs populating that tissue. Overall, the results indicate that IL3RA⁺ lymphoid progenitors are an ILC lineage-specified population but lacking ILC3 lineage potential (Fig. 1b).

Liu et al. further characterized the heterogeneity of each group of ILCs in different tissues by scRNA-seq analysis.⁸ They found a putative precursor subset in each group of ILCs (pre-ILCs) by high expression of cell cycle genes and low expression of ILC lineage genes, which could correspond to a committed precursor stage of each group of ILCs. Pre-ILC3s are likely to be LTi precursors since LTi cells are the major subset of group 3 ILCs at fetal stage in mouse.⁹

Liu et al. compared expression levels of transcription factors related with ILC development in ILCPs and pre-ILCs.⁸ The transcription factor TCF-1 is highly expressed in ILC progenitors in mouse and required for mouse ILCP development.⁴ Interestingly, the authors found that TCF-1 was poorly expressed in human fetal ILCPs, suggesting that TCF-1 may not be critical for human fetal ILCP development. The authors constructed regulatory transcription factor networks for ILCs based on their data, generating useful hypotheses to guide future work.

In summary, Liu et al. provide a large amount of information on human ILCs in multiple fetal tissues. Their results inform the distribution, heterogeneity and development of ILCs at early fetal

¹T Cell Biology and Development Unit, Laboratory of Genome Integrity, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA.

✉email: dingy3@nih.gov; avinash.bhandoola@nih.gov

Fig. 1 Early development of ILCs in mouse and human. **a** Early steps of ILC development in mouse. CLP, common lymphoid progenitor; EILP, early innate lymphoid progenitor; ILCP, ILC precursor; LTi cell, lymphoid tissue inducer cell; NK cell, natural killer cell; ILC1, 2, 3, innate lymphoid cell type 1, 2, or 3 that are considered helper ILCs; dashed arrow, lineage uncertain. EILPs express transcription factor TCF-1 and are the earliest identified progenitors that are specified towards ILC lineages. ILCPs express transcription factor PLZF, but mature ILCs do not. Results in mice indicate that LTi cells are specified first, followed by branching of NK cells from precursors such as EILPs^{2–4,10}. However, ILCPs retain NK cell lineage potential, and multiple pathways for development of NK cells likely exist. **b** Putative early steps of ILC development in human. HSPC, hematopoietic stem progenitor cell; LP, lymphoid progenitor. ILCPs have been identified in human; they give rise to both helper ILCs and NK cells. However, upstream progenitors of ILCPs remain undefined. Liu et al. identified Lin[−]CD34⁺CD127⁺IL3RA⁺ progenitors as ILC lineage-specified progenitors.

stages, and are highly complementary to other efforts such as the Human Cell Atlas. Further work should investigate lineage potential of these progenitors using models such as humanized mice and clonal assays in vitro, coupled with appropriate genetic perturbations to assess the roles of specific factors in ILC development. The results of the present study indicate that important differences may exist between human and mouse ILC development. The mechanism of ILC lineage specification in human is almost completely unknown, and the identification of ILC-specified progenitors in the present study is an important step towards realizing this goal.

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ACKNOWLEDGEMENTS

We thank Christelle Harly, Arundhoti Das and Marieke Lavaert for comments and criticism.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Y.D. or A.B.

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