

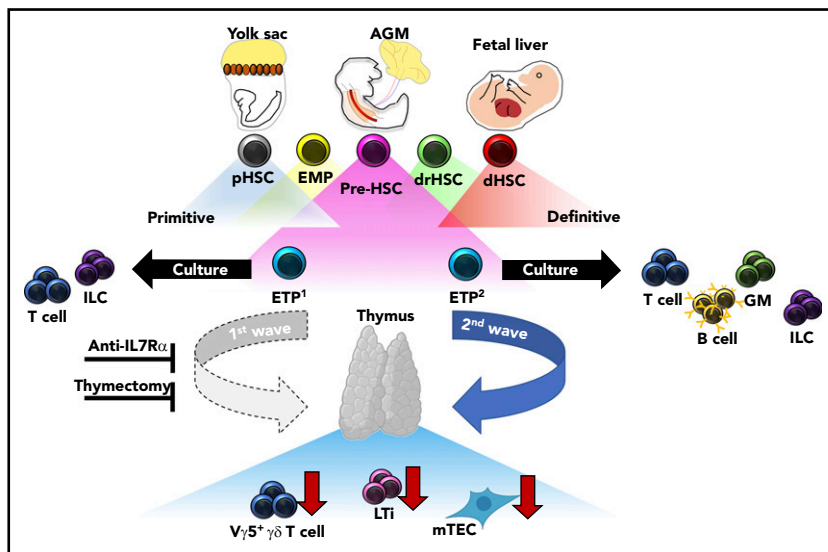
**HEMATOPOIESIS AND STEM CELLS**

Comment on Elsaid et al, page 1024

## Layered immunity of the developing thymus

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In this issue of *Blood*, Elsaid et al reinforce the temporal and spatial complexity of immune layering during embryogenesis by intricately dissecting 2 distinct waves of early thymic progenitors (ETPs) that differentially contribute to normal thymic organogenesis and homeostasis.<sup>1</sup> The concept of “layered immunity,” in the form of waves of different immune cells derived from developmentally distinct progenitor populations, was initially described for innate-like lymphocytes, including B1-B cells and  $\gamma\delta$  T cells.<sup>2,3</sup> Cellular compartments generated by layered immunity now extend beyond subsets of innate-like B and T cells to include tissue-resident macrophages,<sup>4</sup> innate lymphoid cells (ILC),<sup>5</sup> and mast cells.<sup>6</sup>



During fetal development, transient waves of progenitors arise and generate distinct immune cells that persist and contribute to the mature immune system. Elsaid et al elegantly demonstrate that 2 distinct waves of ETPs arise from HSC-derived progenitors and differentially contribute to thymic innate-immune cell establishment and mTEC maturation. Blockade of the first, but not the second, wave of ETPs by anti-IL7R $\alpha$  injection results in significantly less thymic LTi cells,  $V\gamma 5^+ \gamma\delta$  T cells, and mature mTECs. These results were recapitulated in neonates after complete thymectomy. Altogether, these data suggest that the initial wave of ETPs specifically contributes to the establishment of innate-immune cell compartments in the developing thymus and is required for the maturation of *AIRE*-expressing mTECs. AGM, aorta-gonad-mesonephros; dHSC, definitive HSC; drHSC, developmentally-restricted HSC; EMP, erythro-myeloid progenitor; GM, granulocyte/monocyte; pHSC, primitive HSC.

One of the outstanding questions regarding layered immunity is defining the unique interactions between waves of developmentally regulated immune cells and the developing tissues they seed. Importantly, these interactions serve to define both tissue-resident immune cell identity and function, and overall tissue homeostasis. When these interactions are dysregulated, they can lead to immune and tissue dysfunction. The authors contribute to this topic by defining differences in differentiation potential and innate-immune cell establishment between the first (embryonic day 13 [E13]) and second (E18) wave of ETPs in the developing thymus. Single-cell culture assays demonstrated restricted differentiation potential between E13 ETPs as compared to E18 ETPs, the former generating only lymphoid tissue inducer (LTi) cells and T cells. Furthermore, *in vitro* fetal thymic organ culture experiments, recapitulated *in vivo*, revealed LTi and  $V\gamma 5^+ \gamma\delta$  T-cell generation was specifically attributed to E13 ETPs and not E18 ETPs, highlighting that thymic innate-immune cell generation is temporally regulated. In agreement with their limited differentiation potential, single-cell transcriptional analysis revealed that E13 ETPs appear to be transcriptionally primed toward LTi and invariant T-cell profiles. Importantly, these data suggest a previously unappreciated source of LTi cell generation outside of the fetal liver (FL), which is currently understood to be the primary source of LTi cells during fetal development. Furthermore, dependency of thymic tissue-resident  $V\gamma 5^+ \gamma\delta$  T cells,  $V\gamma 6^+ \gamma\delta$  T cells in the lymph nodes, and medullary thymic epithelial cell (mTEC) maturation within the thymus was shown to be primarily attributed to E13 ETPs through experiments in which the first wave of thymic development was blocked with either a monoclonal anti-IL7R $\alpha$  antibody or neonatal thymectomy. Altogether, these data strongly suggest that a functional thymus depends on temporally regulated layering from ETPs with unique differentiation potentials (see figure).

It is noteworthy that in their attempt to dissect the earliest origins of ETP, the authors used multiple lineage-tracing approaches to test the origin of different lineages. Using an established *Il7ra<sup>Cre</sup>Rosa26<sup>YFP</sup>* lineage tracing model to detect the earliest progenitor of IL7R<sup>+</sup> ETPs, the authors demonstrated that although YFP<sup>+</sup> expressing cells marked by IL7R expression can be detected in the yolk sac (YS) as early as E9.5, as previously reported,<sup>7</sup> these IL7R-marked progenitors lacked lymphoid potential in ex vivo culture. This is in stark contrast to the same (IL7R<sup>+</sup>) YS Kit<sup>+</sup> progenitors at E10.5, which readily generated T-cell subsets under the same conditions. Interestingly, transcriptional profiling of E9.5 YFP<sup>+</sup> and YFP<sup>-</sup> cells indicated that these 2 populations did not significantly differ in transcript expression, and only 6 of 47 YFP<sup>+</sup> cells coexpressed *IL7Rα* transcripts. Transient expression of *IL7ra* message and surface expression was recently described in tissue-resident macrophages,<sup>8</sup> suggesting a decoupling of conventional lymphoid-associated lineage commitment and differentiation potential during fetal hematopoiesis. Importantly, the author's finding of transient expression of a lymphoid-associated lineage marker without accompanying lymphoid potential challenges our understanding of differentiation potential and lineage commitment, emphasizing the importance not only of transcriptional profiling but also of in vivo testing of lineage contribution.

The authors appropriately acknowledged that the constitutive *Il7ra<sup>Cre</sup>Rosa26<sup>YFP</sup>* lineage tracing model lacks the ability to faithfully trace the origin of embryonic-derived lymphoid cells due to YFP expression in downstream progenitor and mature cells. To address this point, they also employed a *Csf1<sup>r<sup>Meri</sup>CreMer</sup>Rosa26<sup>YFP</sup>* lineage tracing model to better define the origin of the first wave of ETPs during embryogenesis. As ETPs were only labeled upon 4-hydroxytamoxifen pulse at E10.5 coincident with the emergence of the hematopoietic stem cells (HSCs) and colabeling of FL stem and progenitor populations, but not pulse at E8.5, the authors convincingly propose that ETPs are exclusively HSC<sup>-</sup>, and not YS<sup>-</sup>, derived. It is important to note the caveat, however, that while fate-mapping is a very powerful approach, many lineage-tracing models are both imperfect and overlapping. Deployment of pulse labeling within different developmental

windows will and can suggest disparate origins depending on precisely when the label is induced and how the data are interpreted. There is still a need to identify more specific molecular markers of distinct embryonic progenitors so as to more precisely distinguish between the various waves of immune development during embryogenesis. Nonetheless, the work presented by Elsaid et al reflects a substantial contribution to understanding the mechanisms underlying developmental immune layering and how layered immunity contributes to the generation of a functional immune system.

**Conflict-of-interest disclosure:** The authors declare no competing financial interests. ■

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## IMMUNOBIOLOGY AND IMMUNOTHERAPY

Comment on Willier et al, page 1037

# CAR-T treatment of pediatric AML: a long and winding road

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**In this issue of *Blood*, Willier et al explored the differences in immunotarget expression in pediatric (ped) acute myeloid leukemia (AML) compared with adult AML. They identified CD33 and CLEC12A (CLL1) as the best combination for immunotherapy in ped AML. Their findings underscore the need to delineate immunotarget expression levels prior to (pre-)clinical application of chimeric antigen receptor (CAR)-T-cell treatment.<sup>1</sup>**

Ped AML is much less common than acute lymphoblastic leukemia (ALL) in children. Whereas the overall survival for ped AML currently reaches 75%, outcomes remain poor in patients with relapsed or refractory disease despite salvage therapy with intensive reinduction chemotherapy and allogeneic hematopoietic stem cell transplantation (HSCT).<sup>2</sup> Following the

clinically successful trials with CD19-directed CAR-T-cell treatment in childhood and young adult ALL,<sup>3</sup> substantial efforts were made to duplicate this success in AML. Unlike B-cell malignancies, which exclusively express B-cell lineage antigens, cellular therapy in AML has been hampered by the lack of AML-specific antigens and by coexpression of myeloid