Immunity

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Spotlight

Are we there yet? An immune field trip through human embryonic development

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Technical, analytical, and ethical challenges have obscured our understanding of immune cell subset ontogeny during human fetal development. Recently published in *Science*, Suo et al. (2022) apply multiple single-cell and spatial tools to provide a comprehensive roadmap during human gestation.

With our growing understanding of the impact that maternal cues have on the development of the fetal immune system, it is increasingly important to understand where and when immune cells in our tissues receive their education. Given the challenges, ethical considerations, and tremendous care and respect that must accompany human studies of fetal tissue, applying comprehensive and holistic profiling methods that can provide a detailed roadmap of the developing human immune system is paramount. Studies employing single-cell methods are geared toward learning as much as we can from these precious opportunities by surveying multiple cell types simultaneously. A recent study by Suo et al. (2022) in *Science* is part of the ongoing effort of the Human Developmental Cell Atlas to map human embryonic development using technologies including single-cell profiling of gene expression and immune repertoires (T and B cell receptors) and, more recently, spatial localization of transcripts within tissues (Park et al., 2020). In addition to a comprehensive list of myeloid and lymphocyte cell types present in developing tissues, the atlas brings insights into inflammatory activation state of myeloid cells throughout development, the biology of B1 cells, and the selection of unconventional T cells (Figure 1). Strikingly, while T cell education remains localized to the thymus, B cells appear to take a broader "field trip" outside of their bone marrow niche: surveying various organs during the refinement of their B cell receptor (BCR).

The earliest immune cells to arise during prenatal development, which include macrophages and innate lymphoid progenitors, are reported as early as 4 post-conception weeks (pcw), followed by lymphocytes appearing at 6–7 pcw (Park et al., 2020; Cao et al., 2020). While most of the development takes place in canonical locations such as the yolk sac, the fetal liver, and, later, the bone marrow and thymus, the contribution of development of immune cells in non-lymphoid tissues remains unresolved. Why do time and location matter for cells that grow up to be such migrants later in life? It has recently been shown that inflammatory events happening during prenatal development can entrain lifelong marks that will dictate the immune responses in adulthood within select barrier and non-barrier tissues (Lim et al., 2021). Moreover, the questions of how, when, and where adaptive immune cells establish tolerance to both fetal and maternal antigens have been challenging to ask in human prenatal development. To help in answering these questions, Suo et al. compiled newly generated and previously published single-cell RNA-sequencing (scRNA-seq) and immune repertoire data from various tissues of human fetuses covering the period from 4 to 17 pcw. The atlas contained close to one million cells of 127 different cell types that were integrated using scVI (Gayoso et al., 2022) and followed in time and across organs using Milo, a statistical tool developed by the authors that computes differential abundance using cellular neighborhoods (Dann et al., 2022). Importantly, the authors supplemented the cell type annotations with mapping onto human adult cell type references and spatial transcriptomics at 16 and 18 pcw, which enabled projection of the identified cell subsets and states during their travels to the far future.

Suo et al. begin their journey with monocyte, macrophage, and dendritic cell (DC) subsets across prenatal human development. Historically, macrophages in tissues are thought to be replenished by a pool of circulating monocytes but are now recognized as a cell type with various origins. Yolk-sac-derived LYVE1^{hi} macrophages were found in the earliest timepoints and show the highest self-renewal capacity, which makes them a likely candidate for long-lived precursors of tissue-resident macrophages. Iron-recycling macrophages arose in later timepoints and show notable similarities to adult erythrophagocytic macrophages. The majority of monocytes and dendritic cell subsets mapped cleanly to adult counterparts, with the notable exception of chemokine receptor CXCR4^{+} monocytes, which likely relate to recent

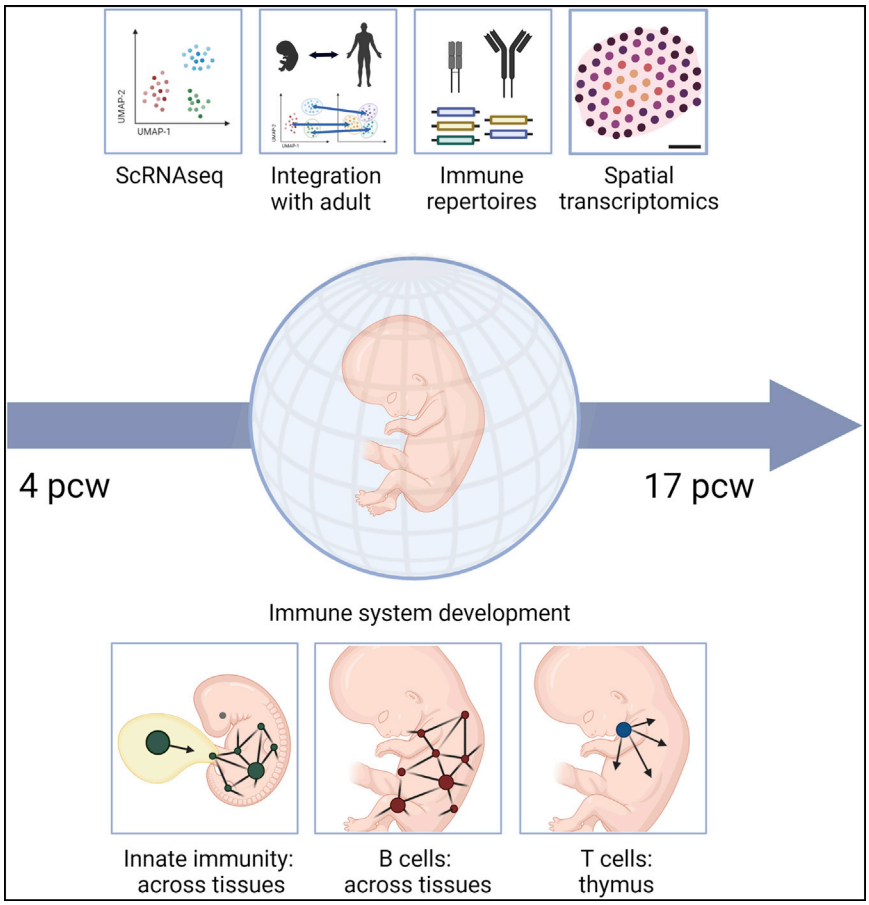

Figure 1. A cross-tissue atlas of human embryonic development reveals the spatial organization of immune system development

Suo et al. compiled newly generated and previously published scRNA-seq data to generate an atlas of human prenatal immune system development. They profiled samples from 4 to 17 post-conception weeks (pcw) using scRNA-seq, single-cell immune repertoire profiling, and spatial transcriptomics. The integrated data revealed that innate immune cells (myeloid lineage and innate lymphoid lineages) appear early in the development and the progenitors are found across tissues. Strikingly, developing B cells also appear in multiple tissues, including non-lymphoid tissues, while T cells develop exclusively in the thymus. Characterization of innate-like B1 and unconventional T cells revealed adaptive immune subsets for further study. Figure 1 was created with BioRender.com.

lineage-tracing experiments in mice that link these monocytes with maintenance of tissue-resident macrophages (Werner et al., 2020). Intriguingly, different macrophage subsets converge on very similar states depending on the time point sampled. During the first trimester of human embryogenesis, they expressed proinflammatory cytokines and chemokines such as *IL1A*, *IL1B*, *CXCL8*, *TNF*, *CCL3*, and *CCL4*. The abundance of these genes gradually decreased until the second trimester, when macrophages got ready for antigen presentation. The role of this pronounced early macrophage inflammation in the skin and yolk sac merits further exploration, especially as it pertains to how specific types of inflammation may influence canonical developmental pathways responsible for tissue growth and patterning.

Focusing on lymphoid cells, the authors revealed a concerted shift from innate to adaptive immune responses. From 4 to 10 pcw, the lymphoid compartment was dominated by natural killer (NK) cells and innate lymphoid cells (ILCs) that were supplemented by developing B and T cells after 12 pcw. We note that inherent limitations of scRNA-seq analysis prevent a thorough understanding of whether NK cells and ILCs were diminished later in development or whether their numbers were dwarfed by adaptive immune cells. Strikingly, B cell progenitors undergoing active BCR rearrangement (determined by *RAG1*, *VPREB1*, and *DNTT* expression) were found in most of the sampled tissues, expanding the current view of fetal liver and bone marrow as exclusive sites of B cell lymphopoiesis. The fact that, in contrast to thymo-centric T cell development, B cell development is not as spatially restricted reflects the importance of thymic T cell selection and raises the intriguing model that primary receptor recombination or light-chain editing in developing B cells occurs at mucosal tissues like the gut even prior to postnatal microbial colonization as demonstrated in murine models (Wesemann et al., 2013).

While conventional lymphocyte subsets are better defined in adult organisms, their unconventional counterparts are poorly understood. B1 cells provide rapid protection against pathogens by constitutive production of natural IgM antibodies. Consistent with earlier reports (Griffin et al., 2011), Suo et al. identified $CD5^+CD27^+CD43^+$ B cells that were able to secrete high levels of IgM and IgD and had self-renewal capacity, shorter CDR3 junctions, and a restricted BCR repertoire. A $CCR10^+$ B1 cell subset showed the highest capacity to produce IgM *ex vivo*. As antigen exposure is tightly controlled during development, the functional role of prenatal B1 cells remains elusive.

Exploration of T cell heterogeneity revealed naive and regulatory $CD4^+$ and $CD8^+$ populations of conventional T cells mapping to adult counterparts. T effector and memory subsets found after birth were absent in prenatal samples. Additionally, Suo et al. observed large numbers of unconventional T cells expressing *ZBTB16* (PLZF), which separated on the basis of transcription factor expression into type 3 innate, type 1 innate, and $CD8\alpha\alpha^+$ T cells. While the $CD8\alpha\alpha^+$ T cells can also be found in adolescence, the innate subsets had no discernable adult counterparts and shrank in relative proportion after 7–9 pcw, suggesting important roles in early development. Through T cell receptor (TCR) sequencing, the authors demonstrated that these unconventional T cells lacked clonal expansion and exhibited TRAV-TRAJ usage more similar to double-positive (DP) cells than conventional T cells. Positing that DP cells give rise to these unconventional subsets via

thymocyte-thymocyte interactions (Georgiev et al., 2021), the authors utilized an *in vitro* differentiation model lacking thymic epithelial cells and generated *ZBTB16* cells with similar transcriptional profiles to the unconventional type 1 innate subset discovered *in vivo*.

Synthesizing this large scRNA-seq dataset of prenatal immune cells, we can begin to stitch together a concerted view of immunological development. Myeloid cell development was characterized by an early inflammatory burst of gene expression. A few weeks after, T cells began undergoing development in the thymus, which shifted output toward more mature populations in distal tissues as gestation progresses. Precursors for most cells, but particularly for B cells, could be found everywhere in the body, supporting a model of centralized expansion with increasing layers of peripheral education for some cell types. Assessing the function of B1 and unconventional T cells outside the bone marrow, spleen, and thymus may help elucidate their transience, roles, and fate.

One of the major challenges for the field will be contextualizing studies of inflammatory or infectious perturbation performed in murine models with these types of human data. Whether specific findings are shared with human development—and which novel adaptations may take place in humans relative to mice and model organisms—presents an exciting area for future work. The ability to use computational methods to integrate across studies also presents opportunities for tool development to maximally uncover core biology while minimizing nuisance batch effects. Understanding the temporal periods and specific cues that regulate immune cell development during fleeting windows in early life may lead to opportunities to harness this knowledge to restore missing or altered cell subsets in young children. Whether cells of the same type that arrive at their adult destination via different places and times of education are completely interchangeable or whether there is functional specialization presents an opportunity for the application of lineage-tracing techniques. While it may appear that the immune system of a newborn is in a naive and tranquil state, the multiple lenses used in this study have allowed us to take a Magic School Bus-style "field trip" to see just how active and inflammatory early development really is.

DECLARATION OF INTERESTS

J.O.-M. reports compensation for consulting services with Cellarity and Hovione.

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