RESEARCH HIGHLIGHT

Time and place: mapping human prenatal macrophages across tissues

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In a recent *Cell* paper, Wang et al. characterized human immune cells at the single-cell level across 19 tissues and throughout prenatal developmental phases. This work revealed two new macrophage populations: microglia-like macrophages outside the central nervous system, notably in the skin where they interact with neural crest cells, and perivascular proangiogenic macrophages throughout embryonic tissues.

A colossal effort is at play to better understand the prenatal immune system development in humans, with each study bringing new information toward completing this atlas.[1–4] Wang et al.[5] combined human single-cell data across 19 tissues and across developmental phases to create a Human Atlas of the prenatal immune system. These samples were collected from embryonic stages (Carnegie stage 11, CS11), across postconceptional weeks (PCW) 4–26 until the early third trimester at 26 PCW. Sorted $CD45^+$ immune cells were processed with MARS-seq and the meta-analysis focused on 15 spatially and timely resolved macrophage populations. Wang et al. focus on the development of macrophages and discover two prenatal macrophage subsets, microglia-like macrophages and proangiogenic macrophages, and study their developmental trajectory, spatial distribution and functional specialization.

The first unexpected finding from this novel single-cell analysis was the detection of a macrophage population with a microglia-like signature outside the central nervous system. These microglia-like macrophages were found in the skin, heart and testicles. Importantly, these microglia-like cells express *SALL1*, a core transcription factor that was shown to be specific to yolk sac-derived microglia in mice.[6] These microglia-like macrophages could be identified as $MRC1^-P2RY12^+$ cells and were found in multiple tissues in successive waves (Fig. 1). These cells first emerge in the skin around CS12 and disappear by 20 PCW. Appearing in the heart at CS13, they initially expand in the atrium and then the aorta and are detected until 26 PCW, but not in adulthood. Finally, they are detected in the testicles at CS14 close to the mesonephric tubules and later at 26 PCW in the epididymis. In the skin, they are located in the epidermis and more abundant in the back and head skin. There, they are in close contact with $SOX10^+$ neural crest cells located along the dorsoventral axis, on their way to differentiate into melanocytes. By culturing the fetal back epidermis ex vivo and depleting the macrophages with clodronate liposomes or a CSF1R inhibitor, the authors demonstrated that these microglia-like macrophages promote the development of neural crest cells. The molecular mechanism underlying this process remains to be identified, but it will also be interesting to study the role of these cells in the other tissues such as the developing heart and testicles. During embryonic development, neural crest cells migrate to the various peripheral tissues where they can differentiate into melanocytes, Schwann cells or peripheral nerves. It is tempting to speculate that the embryonic microglia-like macrophages could play a crucial role in the nurturing of neural crest cells and in the subsequent neuronal remodeling of the developing peripheral nervous system during this period. We learned from murine and human studies that *SALL1* expression and its downstream gene module, as well as the epigenetic landscape conferring the microglia identity, are strictly dependent on signals from the central nervous system microenvironment.[6] One could suppose that embryonic epidermis, testicle and heart microenvironments share common factors that will temporarily promote the maintenance of microglia-like macrophages. As these cells have distinct kinetics in each of these tissues, these niche signals could also be expressed in waves. The disappearance of the microglia-like cells could be due to the loss of the microglia maintenance signals in these peripheral tissues, or the competition for survival factors such as CSF1 with the novel tissue-resident macrophages better adapted to each of the developing tissues. Thus, neural crest cells could also be producing factors, in reciprocal crosstalk, to sustain microglia-like macrophage niche before their terminal differentiation.

The Wang et al. study also revealed the presence of proangiogenic macrophages (PraMs) and their precursors (pre-PraMs) across organs, such as the ovary, testicle, lung, heart, kidney, skin, adrenal gland, and brain (Fig. 1). PraMs can be identified as $MRC1^+P2RY12^-$ macrophages. The distinction between precursors and mature PraMs is based on the higher expression of proangiogenic genes, such as *IL1B*, *VEGFA*, or *CXCL8*. Mature PraMs display a higher expression of CD83 and are better at promoting angiogenesis when cultured with human umbilical vein endothelial cells. Even though endothelial cells are probably a crucial actor in the pre-PraM/PraM niche, we could consider a multicellular niche comprising both endothelial cells and pericytes, as the main source of perivascular niche factors.

Finally, this novel scRNA-seq atlas of the developing human prenatal system proposes that yolk sac-derived progenitors differentiate into two distinct subsets of macrophages: PraMs and microglia-like macrophages. These developmental trajectories were predicted using an in silico pseudotime analysis. Lately, whole-genome sequencing has been used to trace cell lineage in human

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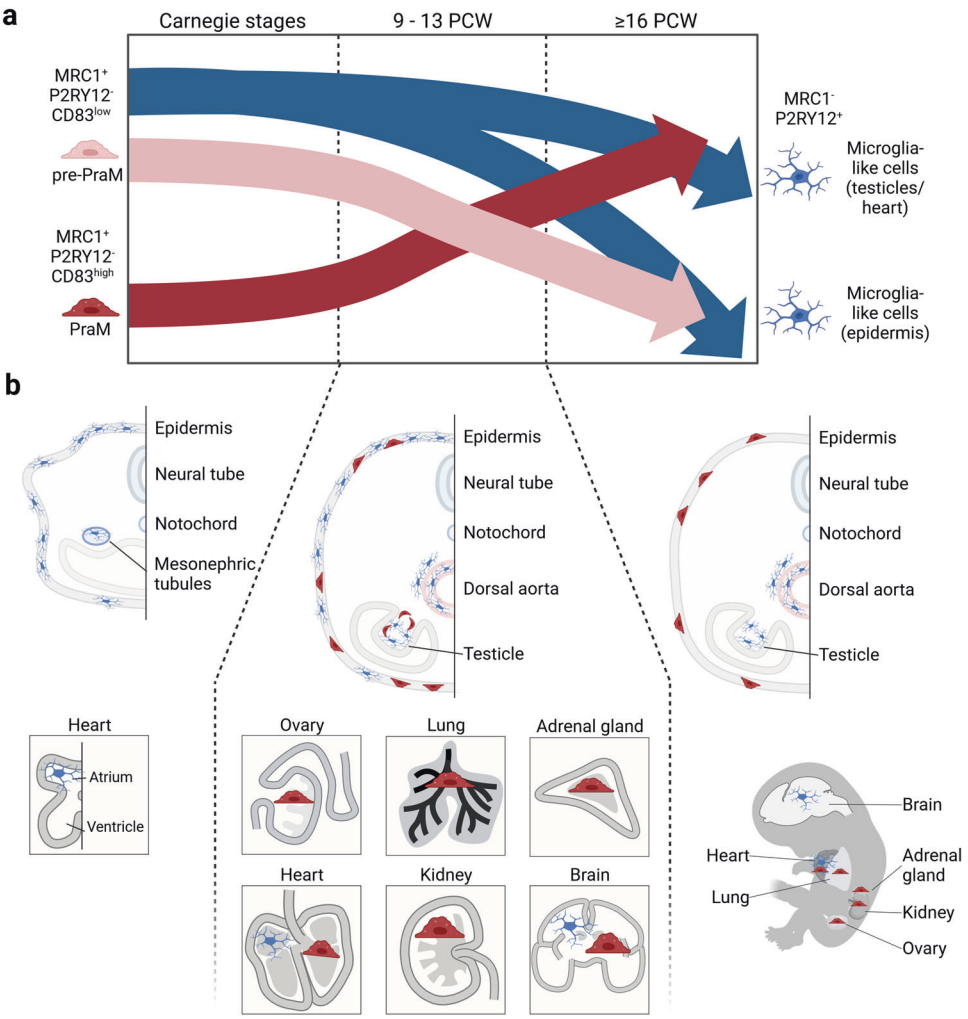

Fig. 1 Human Prenatal Macrophage Development. a Kinetics of expansion and contraction of microglia-like macrophages and proangiogenic macrophages (PraMs) during different embryonic stages. **b** Location of microglia-like macrophages and PraMs in embryonic tissues across the different developmental periods. PCW: postconceptional week. Created with BioRender.com.

cells based on the transmission of discrete somatic mutations,[7–9] generating natural in vivo barcoding. This method could represent a complementary approach to unravel the developmental trajectory of cells during human development. PraMs differentiate in the perivascular space and regulate angiogenesis, while microglia-like macrophages are distributed outside the central nervous system and interact with neural crest cells. This study therefore challenges the current dogma that microglia are restricted to the central nervous system and poses the fascinating question of the role of these cells in peripheral tissues during prenatal development. It will in the future be interesting to profile the non-immune compartment of each of these tissues to unravel the cellular composition of each of these macrophage niches and identify the local signals that drive the development and the functional specialization of these macrophages during prenatal development. Lastly, it should be noted that some macrophage subsets, including liver-resident Kupffer cells, splenic macrophages and osteoclasts, are underrepresented in the current study. By combining all the datasets from the massive efforts from the Human Cell Atlas community, we are slowly but surely unraveling the developmental trajectories of cells during human prenatal development. This could reveal successive waves of myeloid precursors, as has been found in the mouse.[10] Understanding how cells develop within the human embryo should help develop better human tissue organoids. Moreover, unraveling the molecular signals involved in the functional specialization of these cells during embryogenesis could lead to the production of functional human cells in vitro that could one day be used as cellular therapies in regenerative medicine.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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