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DEVELOPMENT AT A GLANCE

Biology of resident tissue macrophages

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ABSTRACT

Although best known for their phagocytic and immunological functions, macrophages have increasingly been recognised as key players in the development, homeostasis and regeneration of their host tissues. Early during development, macrophages infiltrate and colonise all tissues within the body, developing symbiotically with their host tissues and acquiring unique functional adaptations based on the tissue microenvironment. These embryonic resident tissue macrophages (RTMs) are ontogenically distinct from the later adult bone marrow-derived monocytes, and in some tissues are self-maintained independently of general circulation at a steady state. In this article, we briefly discuss the ontogeny, maintenance and unique tissue adaptions of RTMs focusing on microglia, Kupffer cells, Langerhans cells, intestinal macrophages, cardiac macrophages and tumour-associated macrophages, and highlight their role in development, homeostasis and dysfunction.

KEY WORDS: Development, Kupffer cell, Macrophage, Microglia, Monocyte, Tissue resident macrophage

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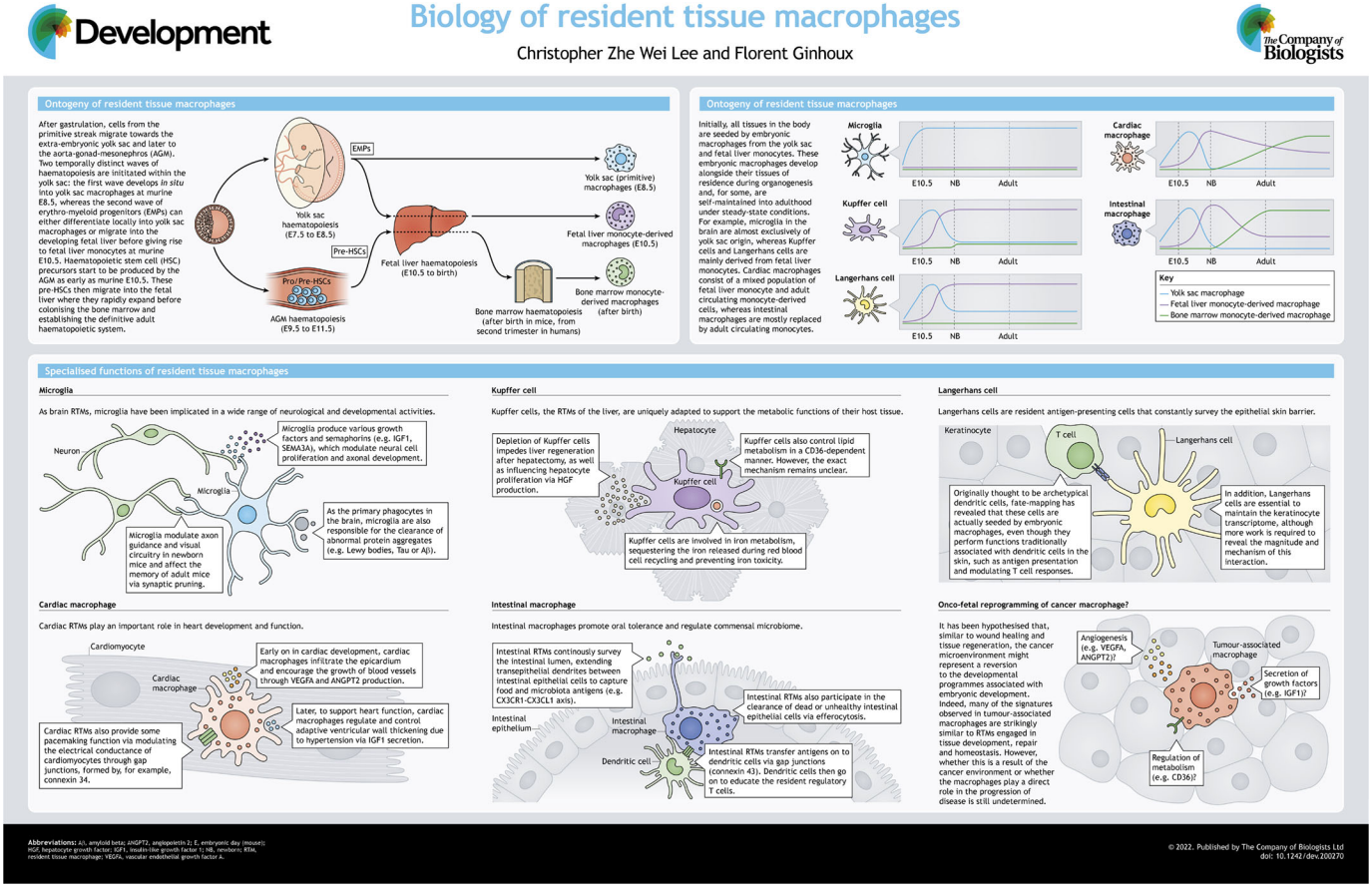
Introduction

Originally thought to be uniformly maintained from monocytic precursors in the blood, recent advances in fate-mapping and single-cell technologies have confirmed that there are at least three separate and distinct lineages of progenitors that can give rise to resident tissue macrophages (RTMs). Based on their ontogeny and tissue niches, these RTMs can exhibit vastly different transcriptomic and epigenetic profiles (Gautier et al., 2012; Gosselin et al., 2014).



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Here, we aim to provide a simplified overview of the ontogeny of RTMs and their tissue-dependent replenishment/renewal kinetics, and to highlight examples of their specialised functions in tissue development, regeneration and dysfunction.

Ontogeny of resident tissue macrophages

Mammalian embryonic haematopoiesis is a complex process, involving multiple temporally overlapping programmes (reviewed by Palis and Yoder, 2001) that are difficult to pry apart and study in isolation. Moreover, given the ethical and technical limitations of studying early embryonic development in humans, most of what we understand about embryonic haematopoiesis comes from the use of animal models, especially mouse or zebrafish. However, significant conservation of this process is seen across the animal models used, and recent studies employing cutting edge single-cell sequencing technologies and analyses of human embryos have, thus far, corroborated these conclusions (Popescu et al., 2019; Bian et al., 2020).

Soon after gastrulation, mesodermal progenitors from the posterior primitive streak migrate first towards the extra-embryonic yolk sac, forming the yolk sac blood islands, then later towards the region that becomes the aorta-gonad-mesonephros (AGM) (Shalaby et al., 1997). It is from these two tissues (the yolk sac and the AGM) that all subsequent haematopoietic lineages develop, although the exact relationship between these progenitors remains unclear. Within the yolk sac, two temporally distinct waves of haematopoietic progenitors are formed at murine embryonic day (E)7.5 and E8.5 (Hoeffel et al., 2015). The first wave, which matures rapidly into yolk sac macrophages by E8.5, overlaps temporally with the emergence of primitive nucleated erythrocytes, which is why they are sometimes referred to as ‘primitive macrophages’, that will give rise to the first embryonic macrophages. The second wave of progenitors at E8.5, initially termed ‘erythro-myeloid progenitors’ for their ability to produce both erythrocytic and myeloid colonies *in vitro* (reviewed by Frame et al., 2013), although some natural killer cell potential has also been observed recently (Dege et al., 2020), either differentiate locally into non-monocytic yolk sac macrophages (Mass et al., 2016) or migrate at E10.5 to the fetal liver where they give rise later on to fetal liver monocytes and definitive enucleated erythrocytes. These fetal liver monocytes will migrate to fetal tissues and differentiate into fetal macrophages. Whether the first early ‘primitive’ wave, which is phenotypically similar to the second late one (Hoeffel et al., 2015), exhibits the same erythro-myeloid potential as the second remains to be formally established. Concurrently, the precursors of haematopoietic stem cells (HSCs), with lymphoid and engraftment potential, start to emerge from the AGM between E9.5 and E11.5. These pre-HSCs also migrate towards the fetal liver, where they mature into short-term and long-term HSCs before colonising the bone marrow and giving rise to monocytes and other definitive haematopoietic cells (Ema and Nakauchi, 2000).

Kinetics of macrophage tissue residency at steady state

As the production of embryonic (yolk sac-derived and fetal liver monocyte-derived) macrophages is superseded by the development of adult (bone marrow monocyte-derived) macrophages, it has long been assumed that adult RTM populations consist entirely of adult macrophages that replace their original embryonic counterparts. This paradigm was challenged by the observation that Langerhans cells (skin RTMs) in parabiotic mice do not appear to be naturally replaced by host-derived circulating cells (Merad et al., 2002), and later shattered when it was discovered that microglia (brain RTMs) consist almost entirely of embryonic yolk sac-derived macrophages, even well into adulthood (Ginhoux et al., 2010). Since then, the RTM populations in many other tissues have been found to harbour embryonic macrophages that self-renew under steady-state conditions (Hashimoto et al., 2013, reviewed by Ginhoux and Guilliams, 2016). For example, Kupffer cells (the resident macrophage of the liver) are replaced by fetal liver monocyte-derived macrophages, but not bone marrow monocyte-derived ones (Yona et al., 2013), whereas cardiac macrophages are derived from cells from all three lineages (Epelman et al., 2014). Conversely, most intestinal macrophages are of adult monocytic origin (Bain et al., 2014), although there is a minor population associated with the enteric ganglions that appear to be of embryonic origin and persists long into adulthood (De Schepper et al., 2018). This begs the interesting question of what makes the niche ‘open’ or ‘closed’ to replacement by adult macrophages. In some tissues, such as the brain, skin (Hoeffel et al., 2012) or lung alveolar (Guilliams et al., 2013), the presence of a physical barrier could explain, at least in part, the difficulty of replacement during steady state. However, in other tissues such as the liver or mammary glands (Jäppinen et al., 2019) it is not as obvious. Adding to the confusion is the fact that, in some cases, such as during hepatectomy, liver RTMs (also named Kupffer cells) repopulate preferentially from the existing compartment (Ait Ahmed et al., 2021), whereas in others, such as chemical depletion (Scott et al., 2016) or pathogen infection (Blériot et al., 2015), Kupffer cell repopulation is mainly monocyte driven. This suggests that the different modes of RTM repopulation may be driven in part by cellular cues arising from RTM death, which is supported by some recent evidence using monocyte fate-mapping (Liu et al., 2019).

Understanding the dynamics of macrophage tissue residency beyond the steady state might be the next step towards understanding the development of age-related pathology and dysfunction, as embryonic and adult macrophages have vastly differing transcriptomic and epigenetic landscapes (Lavin et al., 2014; Gosselin et al., 2014), and could influence their tissues differently in subtle but nevertheless important ways.

Specialised functions of resident tissue macrophages

Macrophages are incredibly plastic cells, capable of taking on exquisitely different functions based on host tissue cues. Given the early emergence and infiltration of embryonic macrophages, it has long been suggested that they play an important role in aiding the proper development and function of their host tissues. However, only recently have we had a clearer understanding of the complex cellular crosstalk between macrophages and their host tissues, thanks in no small part to the increased ease and affordability of single-cell sequencing technologies and high-resolution microscopy. Listed below are a few tissue-specialised functions of key RTM populations.

Microglia

As the resident macrophages of the brain, microglia interact constantly with neurons and other glial tissues. The presence of synaptic fragments within the phagosomes of microglia have sparked the idea that they might actively prune synapses (reviewed by Thion et al., 2018; Mehl et al., 2022), whereas a physical examination of retinogeniculate synapses has indicated that a subset of microglia can suppress the formation of dendritic spines in their immediate surroundings (Cheadle et al., 2020). Behavioural studies also suggest that microglia are deeply involved in modulating neural circuitry, because mice depleted of microglia are unable to forget fear conditioning (Wang et al., 2020). In addition to direct interactions

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with synapses, microglia also secrete various growth factors, such as insulin-like growth factor 1 (IGF1) and transforming growth factor β (TGFβ) (Ueno et al., 2013), and semaphorins (reviewed by Carulli et al., 2021), supporting neuronal proliferation as well as guiding axon development. Finally, microglia are also involved in the clearance of abnormal protein aggregates, such as those seen in Tau pathologies or Parkinson's disease (reviewed by Li and Haney, 2020).

Kupffer cell

In line with the role of the liver as the key site of metabolism and detoxification, Kupffer cells are also highly involved in metabolic processes. As the liver is the primary site of red blood cell removal and iron recycling, Kupffer cells participate in iron metabolism and sequestration (reviewed by Scott and Guilliams, 2018), not only helping to store excess iron but also controlling the release of iron from hepatocytes by hepcidin production. In cases of injury or toxicity, Kupffer cells can produce growth factors, such as hepatocyte growth factor (HGF), to encourage the regeneration and proliferation of hepatocytes (reviewed by Wen et al., 2020). Also, a subset of Kupffer cells directly controls lipid metabolism in a CD36-dependent manner, because their depletion in mice results in significantly lower weight gain and liver steatosis, even under high-fat diet conditions (Blériot et al., 2021). These data suggest functional specialisation among RTM subsets.

Cardiac macrophages

Cardiac macrophages are also involved in the development, regeneration (Simões and Riley, 2022) and function of their host tissues. Early in embryonic development, embryonic macrophages infiltrate into the epicardial layer, where they promote angiogenesis by the secretion of growth factors, such as vascular endothelial growth factor A (VEGFA) and angiopoietin 2 (ANGPT2) (Gula et al., 2021). They encourage the growth of cardiomyocytes by IGF1 production, most notably when under hypertensive stress (Zaman et al., 2021), ensuring proper cardiac function and health. Most interestingly, there is increasing evidence that cardiac macrophages might have electrical conductance activity as a pacemaker via modulating cardiomyocytes through gap junctions formed by, for example, connexin 34 (Hulsmans et al., 2017), and that absence of these macrophages might have severe consequences in the case of acute right ventricular stress (Sugita et al., 2021).

Intestinal macrophages

The gut is the largest mucosal membrane in the body and is continuously in contact with food and pathogenic antigens. As the RTMs of the gut, intestinal macrophages play an important role in promoting antigenic tolerance by first sampling food and bacterial antigens from the intestinal lumen via the extension of CX3CR1-CX3CL1-dependent transepithelial dendrites, then passing the antigens to intestinal dendritic cells via gap junctions (connexin 43; reviewed by Bain and Schridde, 2018). In the colon, intestinal macrophages can use another mechanism, involving balloon-like protrusions that do not extend into the lumen, to sample fungal metabolites dissolved in the reabsorbed liquid (Chikina et al., 2020). In addition, intestinal macrophages uniquely express the efferocytosis receptor αvβ5, which helps maintain the health of intestinal epithelial cells by the removal of apoptotic or unhealthy cells.

Langerhans cells

Langerhans cells were initially thought of as a classical example of a dendritic cell; however, fate-mapping models have recently revealed that they are derived from embryonic macrophages, rather than bone marrow-derived dendritic-cell progenitors (Hoeffel et al., 2012). Due to this misidentification, much of the functional characterisation of these cells had been restricted to migration, antigen presentation and immune tolerance (reviewed by Doebel et al., 2017). However, it is hypothesised that they could also play important roles in maintaining tissue homeostasis, much like their other RTM counterparts. Constitutive depletion of Langerhans cells results in an altered keratinocyte transcriptome (Su et al., 2020b), although much more work needs to be done to determine the exact role of Langerhans cells in skin homeostasis, especially regarding their role in the maintenance of keratinocyte biology.

Onco-fetal reprogramming of tumour-associated cancer macrophages

The role of macrophages in the development and progression of cancer remains a controversial one, with studies attributing both pro- and anti-tumourigenic functions to these cells. This discrepancy is perhaps due to the ambiguous nature of bona fide RTMs versus infiltrating monocyte-derived macrophages, especially in an environment as immunologically complex as a tumour. However, it is being increasingly recognised that, similar to wound healing and regeneration, the cancer microenvironment might represent a reversion to embryonic developmental programmes, albeit to an extreme, a phenomenon termed 'onco-fetal reprogramming'. Under such a hypothesis, it is conceivable that the original embryonic-derived RTMs might be 'hijacked' into supporting the growth and metastasis of the cancer, similar to how they would support the growth and maturation of their host tissues during embryogenesis (Sharma et al., 2020). Although this remains a hypothesis at present, some evidence supports this idea; tumour-associated macrophages (TAMs) express many of the same genes observed in other tissues during tissue development and remodelling. For example, CD36 expression is upregulated in TAMs (Su et al., 2020a), similar to a subpopulation of Kupffer cells associated with metabolism. Furthermore, the role of TAMs in promoting angiogenesis and secreting growth factors is well-recognised (reviewed by DeNardo and Ruffell, 2019). It would be interesting to take a closer look at these overlaps between normal RTM and TAM functions, especially regarding TAM subsets, perhaps allowing us to use cancer to study the embryonic interactions between RTMs and their host tissues, and vice versa.

Conclusion and perspectives

The past decade has greatly enhanced our understanding of RTM biology and brought about an increased appreciation for the role of RTMs in the development and maintenance of their host tissues. However, many questions remain unanswered, especially in the context of human RTMs and whether the animal models are sufficiently representative of real-world conditions. For example, the mouse models used to study the long-term residency of RTMs are raised in controlled, specific pathogen-free environments, and only live for a maximum of 2 years, as opposed to the average human lifespan of 73 years. Although we now have some evidence that embryonic RTMs develop and seed the organs in a similar way in humans (Bian et al., 2020), whether these populations are maintained into adulthood, and for how long, remains unknown. Limited evidence from human allograft transplants appears to indicate that, at least in the case of Langerhans cells (Kanitakis et al., 2004) and microglia (Unger et al., 1993), RTMs are similarly long-lived in humans. Hence, the need for human models of yolk sac-derived and fetal liver monocyte-derived macrophages has never

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been stronger, as most of the work currently focusing on human RTMs uses adult bone marrow monocyte-derived macrophages, which might not be able to fully recapitulate the phenotype and function of embryonic macrophages. Thankfully, there exists some evidence that induced pluripotent stem cells (iPSC)-derived macrophages (iMacs) might be able to bridge this gap, although much more work needs to be done to assess the ontogenic identity of these cells (reviewed by Lee et al., 2018). Indeed, using iMacs with CRISPR-CAS9 and other genome editing techniques will aid in the understanding of human macrophage-related genetic diseases that may otherwise be difficult or impossible to test with traditional models. Finally, iPSC-derived organoid systems have also become increasingly popular as a tool to study human development and organogenesis, but many of them are lacking in the developmental cues provided by RTMs and, as such, might not be fully representative. It would be interesting to see whether the addition of iMacs improves tissue maturation and function, providing a more accurate model of *in utero* development.

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Competing interests
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