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Homeostatic functions of tissue-resident macrophages and their role in tissue maintenance

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Abstract

Tissue-resident macrophages are best known for their indispensable role in immunological reactions, where they contribute to both the immune defense and tissue remodeling during the resolution phase of inflammation. However, recent studies have also revealed that these cells provide crucial tissue-specific functions that support organ homeostasis and maintenance. Defects in macrophage function or development can disrupt the delicate balance of organ homeostasis, leading to pathological manifestations. Thus, unraveling the functions and development of macrophages within a tissue is critical for understanding the interplay between immune and stromal cells, which together maintain organ physiology. This knowledge can have clinical implications, such as during organ transplantation or irradiation when monocyte-derived cells that perform different functions may replace the original macrophage population. In this chapter, we aim to provide an overview of the tissue-specific homeostatic functions of various macrophage populations, highlighting that macrophages are essential components of each organ and play a vital role in ensuring the survival of the organism, irrespective of their role in immunity.

Keywords

Innate immunity, macrophages, differentiation, tissue imprinting, homeostatic function

Introduction

Phagocytosis is a cellular process by which cells engulf and digest foreign particles such as pathogens or cellular debris to remove them from the body. The process of phagocytosis was first observed by Alexander Ecker in 1847 when he described the presence of numerous red blood cells within vacuole-rich splenic cells (Ecker 1847). That a specialized cell type, the so-called phagocytes and especially macrophages ('large eaters', from Greek word *μακρός* (*makrós*) = large, *φαγεῖν* (*phagein*) = to eat), are mainly involved in the cellular uptake and immune response was discovered by Ilya Mechnikoff's ground-breaking research (Metchnikoff 1905). Mechnikoff conducted his first experiments on starfish larvae and observed, after inserting a rose thorn into the transparent larval body, the accumulation of cells that were involved in phagocytosis at the site of the wound, suggesting their critical function in the innate immune response. From today's point of view, two things are remarkable about this observation: of course, the identification of phagocytes as immune sentinels by Mechnikoff and the existence of a functioning innate immune system in starfish larvae, a conclusion that could be easily overlooked. In fact, all eukaryotic organisms, starting from the single-cell amoeba *Dictyostelium discoideum*, rely on phagocytes as the main innate immune component. Under unfavorable conditions, *D. discoideum* transforms into a social organism and creates multicellular aggregates, known as the slug state, which can travel greater distances in search of better conditions. In this state, the newly assembled multicellular "organism" initiates the differentiation of a specialized cell type called sentinel cells (Chen et al. 2007), which exhibit phagocytic, extracellular trap-forming, and reactive oxygen species-producing functions (Zhang et al. 2016). Sentinel cells share these functions with macrophages and granulocytes in humans. Similar effector cell types can be found in other eukaryotes such as sponges (amebocytes in their mesoglea), cnidarians (interstitial cells), invertebrates (hemolymph-borne hemocytes), and coelomate animals (coelomocytes), showing that they and their functions have been conserved throughout the entire phylogeny of eukaryotes up to macrophages and granulocytes in more highly developed species such as humans. Consequently, the innate immune system, with phagocytes as the primary effector cell population, is present in all eukaryotes. In contrast, an advanced immune system with an additional adaptive arm is only found in vertebrates, beginning with early-jawed fish (emergence of the RAG gene) or jawless fish (the variable lymphocyte receptor (VLR) family). To put it another way, more than 95% of eukaryotes (all invertebrates) rely exclusively on innate immunity, while the remaining 5% of eukaryotes (vertebrates) additionally possess an adaptive immune system.

It is possible that the primary function and evolutionary force behind phagocytes is pathogen elimination and defense, as exemplified by the above-mentioned case of *D. discoideum,* but phagocytes and especially macrophages also play key homeostatic roles during development, aging, and wound healing. As a consequence, macrophages are beside red blood cells and megakaryocytes, one of the first hematopoietic cell types that appear during embryogenesis and that seed every organ at these early developmental stages. Their main function during development is the removal of apoptotic cells, which accumulate during organ expansion and organization. The importance of macrophages for proper embryonic development can be observed in hemocyte-deficient *Drosophila melanogaster*. When hemocytes are depleted during embryogenesis by the genetic induction of apoptosis specifically in this cell lineage, the animals show embryonic lethality with strong developmental defects in the central nervous system (CNS) (Defaye et al. 2009). However, no morphological changes were detected when hemocyte depletion was induced during

the later larval stage, highlighting the important role of phagocytes during embryogenesis. The coordinated and complex functions of macrophages in development can also be observed during epimorphic regeneration. The amphibian model organism axolotl, an aquatic salamander, utilizes macrophages to promote limb regeneration after amputation. The depletion of macrophages in this model leads to excessive fibrosis and collagen accumulation, which permanently hinders limb regeneration (Godwin et al. 2013). This demonstrates that macrophages are crucially involved in tissue organization, remodeling, and outgrowth.
From these examples, it is easy to infer that all macrophage populations are similar and that phagocytosis is a uniform process, but this is not actually the case. Therefore, an important question is: what determines the versatile identities and functions of macrophages in tissues? Within the past decade a growing number of studies have contributed to providing an answer to this question. Within their tissue of residence, macrophages are exposed to local environmental factors and signals that vary substantially depending on the specific tissue and its cellular composition. These tissue-specific signals and cell contacts/interactions act on macrophages inducing signaling pathways, influencing the chromatin accessibility of genes and gene regulatory sequences and driving tissue-specific gene programs in macrophages that ultimately determine their cellular functions. For instance, the CNS is composed of specialized glial cells, which clearly differ from the cells found in the lungs or spleen and form a unique environment. Microglia, the macrophages of the CNS, receive factors from neurons that imprint microglial functions that reciprocally allow them to participate in neuronal maintenance and synaptic pruning. Alveolar macrophages in the lungs are *per se* not able to perform the same functions as microglia. Instead, they are exposed to high concentrations of surfactant lipids that need to be degraded. Accordingly, lung epithelial cells provide specific factors that enable alveolar macrophages to catabolize and degrade these lipids. Red pulp macrophages in the spleen on the other hand are exposed to senescent red blood cells. Phagocytosis and degradation of these iron-containing cells requires a specific gene program that is induced by red blood cell-derived heme in macrophages. These examples indicate that the surrounding tissue dictates the identity and function of tissue-resident macrophages. It further shows that macrophages and tissue-specific cells perform a bidirectional communication and mutually influence each other to secure the functionality of an organ.
Given the emerging evidence that macrophages form an integral part of every tissue by fulfilling organ-specific functions and developmental cues, this chapter concentrates on the homeostatic role of various tissue-resident macrophages, including alveolar macrophages in the lungs, splenic macrophages, Kupffer cells in the liver, cardiac macrophages, microglia in the CNS, macrophages in the kidneys, and adipose tissue macrophages. This chapter summarizes how disruption of macrophage homeostasis in these selected organs can affect organ development and function and consequently lead to pathological manifestations. For a detailed description of macrophage origin or their role in pathology, the reader is referred to other recent, excellent reviews (Hoeffel and Ginhoux 2018; Park et al. 2022; Lazarov et al. 2023; Mass et al. 2023).

Precursors of tissue-resident macrophages

To better comprehend and contextualize the functions of tissue-resident macrophages, it is important to understand their ontogeny and developmental processes. For decades, tissue-resident macrophages in humans and mice were

believed to derive from circulating monocytes. Even though monocytes are able to differentiate into tissue-resident macrophages, the necessity of macrophages to perform apoptotic cell clearance during organ development precedes the appearance of monocytes during embryogenesis, at least in the case of the CNS (Alliot et al. 1999; Hoeffel et al. 2015). This indicates that another origin of macrophages must exist and indeed it was shown that tissue-resident macrophages can derive from two sources: from precursors in the yolk sac (YS) and from monocytes, either fetal or adult. Currently, two scenarios are being discussed:

The hematopoietic potential of the YS was first observed in mammals during two distinct phases, also referred to as waves. The earliest hematopoietic precursor cells in the mouse embryo derive from the posterior plate mesoderm of the extra-embryonic YS around embryonic days E7-E7.5. These “early” or primitive erythro-myeloid progenitors (EMPs) can differentiate into megakaryocytes, (nucleated) red blood cells, and primitive macrophages (**Figure 1**; Scenario 1). Using a fate-mapping model based on the expression of *Runx1*, Ginhoux and colleagues observed that labeling YS hematopoietic cells on day E7 led to labeled tissue-resident macrophages on days E10-E13, which in some tissues such as the brain persisted into adulthood (Ginhoux et al. 2010). This indicates that “early” EMPs are the precursors of tissue-resident macrophages, at least of microglia in the CNS. Starting from around E8.0, hematopoiesis expands to the YS hemogenic endothelium, initiating the second transient definitive wave of “late” EMPs. “Late” EMPs can also give rise to the erythroid and megakaryocytic lineages, but in addition they can also produce macrophages, mast cells, and granulocytes (Palis et al. 1999; Bertrand et al. 2005). These *Myb*-dependent EMPs can seed other tissues, especially the fetal liver, where they differentiate into fetal monocytes that subsequently colonize other tissues and further differentiate into tissue-resident macrophages (Hoeffel et al. 2015) (**Figure 1**). When “late” EMPs are targeted using the *Runx1*-dependent fate mapping approach after E8, the number of labeled tissue-resident macrophages in embryos increases significantly, whereas labeled microglia are nearly absent in the adult brain (Ginhoux et al. 2010). This suggests that most tissue-resident macrophages originate from “late” EMPs, while microglia derive from “early” EMPs, a hypothesis supported by subsequent studies (Hoeffel et al. 2015). However, the classification of EMPs into “early” and “late” stages has also been met with skepticism. *Runx1* is not specific for “early” and “late” EMPs and so far, no marker or fate mapping system could be established that definitely distinguishes these functionally overlapping cell types. It was also questioned if *Runx1* is expressed by “early” EMPs (Gomez-Perdiguero and Geissmann 2013; Lazarov et al. 2023), and therefore the contribution of this subset to macrophage establishment is controversially discussed in the field (Schneider and Kopf 2015; Hoeffel and Ginhoux 2018; Lazarov et al. 2023). Instead, some laboratories argue that only *Myb*-independent but *Runx1*-dependent non-primitive EMPs give rise to “pre-macrophages” that seed tissues and are precursors of almost all tissue-resident macrophage subsets (Schulz et al. 2012; Gomez-Perdiguero et al. 2015; Mass et al. 2016; Lazarov et al. 2023) (**Figure 1**; Scenario 2). After the establishment of EMPs in the YS at approximately E9, these cells colonize the fetal liver through the circulatory system (McGrath et al. 2003; Rantakari et al. 2016), where they develop into and coexist with pre-macrophages at E9.5 (Mass et al. 2016). Pre-macrophages share a common gene signature that includes *Csf1r*, *Cx3cr1*, and the transcription factors *Maf*, *Batf3*, *Pparg*, *Irf8*, and *Zeb2* (Mass et al. 2016). Subsequently, pre-macrophages migrate to the organs in a CX3CR1-dependent manner and receive tissue-instructive signals, which initialize the tissue-

specific signature and form the basis of macrophage heterogeneity (Mass et al. 2016).

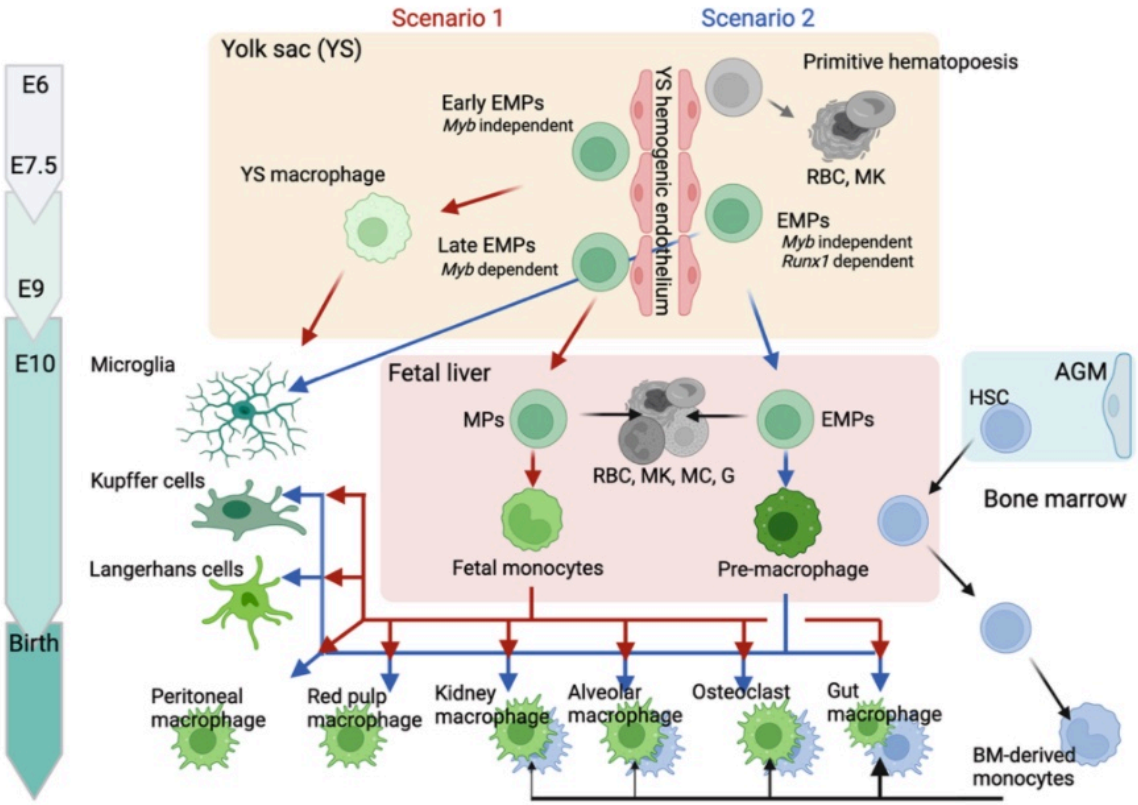

Fig. 1.1 Development of tissue-resident macrophages in mice. Two main differentiation routes are currently being discussed based on (Lazarov et al. 2023; Hoeffel and Ginhoux 2018). Scenario 1 (left; in red) postulates that early *Myb*-independent erythromyeloid precursors (EMPs) give rise to red blood cells (RBC), megakaryocytes (MK), and yolk sac (YS) macrophages, which then infiltrate the developing central nervous system and differentiate into microglia. Late *Myb*-dependent EMPs colonize the liver, where these myeloid precursors (MPs) can differentiate into RBC, MK, mast cells (MC), granulocytes (G), and fetal monocytes. Fetal monocytes are the main progenitors of tissue-resident macrophages. Some macrophage pools can then be progressively replaced or supplemented with bone marrow-derived monocytes during adulthood. In scenario 2 (right; in blue), primitive hematopoiesis results in the production of RBC and MK, but not EMPs. EMPs arise later and are *Myb*-independent and *Runx1*-dependent. These cells can expand to the fetal liver and can differentiate into RBC, MK, MC, G, and pre-macrophages. From there, pre-macrophages infiltrate in a CX3CR1-dependent manner all organs and their tissue-specific identity is regulated by specific sets of transcription factors. Definitive hematopoiesis begins in the aorta-gonad-mesonephros (AGM), from which hematopoietic stem cells (HSC) migrate to the liver and subsequently to the bone marrow. HSCs give rise to monocytes that infiltrate certain tissues and differentiate into tissue-resident macrophages. Created with BioRender.com

In most organs, embryonic macrophages are gradually replaced by bone marrow (BM) monocyte-derived macrophages in adulthood. The speed of the replacement process varies between organs. Tissue-resident macrophages of some organs such as the intestine or heart are replenished quickly by classical monocytes during postnatal phases, while other organs such as the skin or CNS are replenished slowly, if at all (Patel et al. 2021) (**Figure 1**). It is important to mention that all these results were obtained from experiments conducted in laboratory inbred mice that were housed under specific-pathogen-free conditions. It is essential to determine whether the repopulation of tissue-resident macrophages by monocytes occurs more

frequently in humans who are constantly exposed to changing environmental influences causing stress, infections, and sleep deprivation, as these factors may compromise macrophage competition. To achieve this, further research is needed to establish a correlation between these findings in mice and humans (Hume 2023).
One reason for the different replacement rates between organs observed in mice may be related to the inflammatory or stress state of a particular organ. For example, intestinal macrophages are exposed to microbial products that can cause immune activation and oppose their homeostatic self-renewal capacity. Moreover, shear stress, as observed in the heart due to muscle contraction, might contribute to a compromised macrophage niche that is replenished by monocytes. Under specific conditions, such as irradiation or transplantation (Ajami et al. 2007; Mildner et al. 2007), infection (Li et al. 2022), or macrophage niche liberation by depletion models, such as diphtheria toxin injections (Lund et al. 2018), monocytes can rapidly colonize all affected organs and differentiate into tissue-resident macrophage subsets. However, it remains a matter of debate whether monocyte-derived cells that infiltrate a compromised niche completely adapt to the new tissue environment and are equivalent to and transcriptionally indistinguishable from their embryonic counterparts in terms of self-renewal capacity and function (Lund et al. 2018; Shemer et al. 2018). Nevertheless, the experimental findings that monocytes are able to replenish macrophages under specific conditions have led to the “macrophage niche” concept (Guilliams et al. 2020), which considers and incorporates factors like tissue accessibility, availability, and macrophage precursor plasticity, thereby representing an excellent theoretical model for the understanding of macrophage replenishment by monocytes.

Development and homeostatic function of alveolar macrophages

The primary function of the lungs is to facilitate gas exchange, i.e., diffusion of oxygen from inhaled air diffuses into the bloodstream within the lung alveoli while carbon dioxide is expelled during exhalation. Since the lungs are constantly exposed to external factors that are part of the inhaled air, the lungs also play a vital role in immune defense. Alveolar macrophages (AMs) represent primary tissue-resident immune cells of the lungs and serve as the first line of defense against pathogens such as bacteria, viruses, dust, and man-made pollutants. Located in the alveolar lumen and therefore outside of the body, these cells play a crucial role in maintaining effective immune surveillance of the lung tissue. However, the alveolar space undergoes significant changes in oxygen pressure and microflora, and AMs must be able to adapt to these changes to maintain homeostatic tissue integrity. Therefore, the development and function of AMs is closely linked to the development and function of the lung tissue and is regulated by tissue-specific signals that induce a unique transcriptomic signature in AMs.
During mouse embryogenesis, the lungs are colonized by various macrophage lineages. Fetal lung macrophages derived from EMPs in the yolk sac are the first macrophages to inhabit the pulmonary tissue, preceding E12.0 (Guilliams et al. 2013). Subsequently, liver-derived fetal monocytes accumulate in the interstitial lung tissue starting at approximately E14.0. At the same time, the number of fetal lung macrophages begins to decrease before they almost completely disappear by the first postnatal week (Guilliams et al. 2013; Tan and Krasnow 2016). A small fraction of F4/80^{high} Mac2^{low} fetal-derived cells remained detectable in the lung interstitium along the mesothelium as well as perivascularly in adult *Runx1*$^{Cre/wt}$ *Rosa26*$^{TdTomato/TdTomato}$ reporter mice (Tan and Krasnow 2016). Although the precise

function of fetal lung macrophages is not yet clear, they are likely involved in the elimination of apoptotic pulmonary cells during organ development.
Once in the lung tissue, fetal monocytes are exposed to local environmental factors that induce their differentiation into immature alveolar macrophages (preAMs) (Guilliams et al. 2013). One important factor involved in this process is the granulocyte/macrophage colony-stimulating factor (GM-CSF, encoded by *Csf2*), which is expressed by type 2 alveolar epithelial cells and influences the development and maintenance of preAMs and mature AMs throughout their lifetime (Gschwend et al. 2021). In addition, TGF-β was shown to regulate early AM differentiation in an autocrine manner and reduce AM frequency when deleted (Yu et al. 2017).
Fate mapping and parabiosis experiments in mice have indicated that monocytes rarely infiltrate the alveolar niche and do not develop into AMs during adulthood (Hashimoto et al. 2013), although they can differentiate into AMs when transferred to an empty alveolar niche (van de Laar et al. 2016). Accordingly, 5-10% of the AM population undergoes homeostatic proliferation, as measured by Bromodeoxyuridine incorporation (Guilliams et al. 2013). However, experiments with newly developed mouse models that allow the permanent labeling of the entire monocyte pool indicated that monocytes do develop into AMs during aging and account for 50-80% of all tissue-resident macrophages in the aged lung (Liu et al. 2019b; Zhang et al. 2024). In addition, *Ccr2*-deficient humans are characterized by reduced numbers of AMs, suggesting that BM-derived monocytes might differentiate into AMs and are recruited to the lung in a CCR2-dependent manner (Neehus et al. 2024).
After the differentiation of preAMs, the external environment and high oxygen levels after birth appear to be crucial for the further maturation process of AMs. Mice lacking the L-plastin (encoded by *Lpl*) protein, which helps with accurate AM colonization in the alveolar lumina, show compromised development from preAMs to mature AMs (Todd et al. 2016). Additionally, a deficiency in the Von Hippel-Lindau tumor suppressor protein (encoded by *Vhl*) desensitizes AMs to oxygen after birth and inhibits the formation of mature AMs (Izquierdo et al. 2018).
Because the number of alveoli exceeds the number of AMs in the lungs (Westphalen et al. 2014), AMs need to be motile cells to survey all alveoli for pathogen colonization. Indeed, confocal intravital microscopy has indicated that AMs constantly patrol multiple alveoli through the pores of Kohn (Neupane et al. 2020). While this migration might secure immune surveillance, AMs are also involved in homeostatic functions, of which removal of pulmonary surfactant lipoproteins is the most important. Surfactant components are constantly secreted by type II alveolar epithelial cells and form a thin fluid film on the air-liquid interface that covers the entire alveolar wall (**Figure 2**).

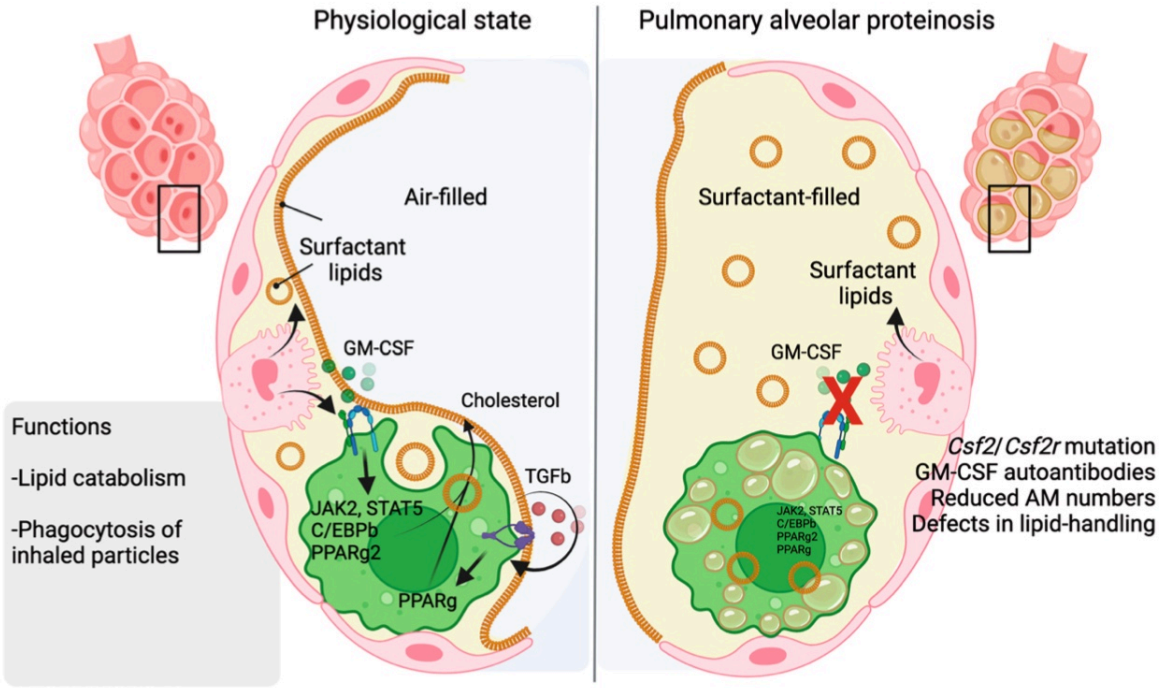

Fig. 1.2 Representation of alveolar macrophage function. Left: Under physiological conditions, alveolar macrophages are dependent on GM-CSF, which is secreted by lung type II epithelial cells. GM-CSF induces JAK2 activation in alveolar macrophages, which activates *Pparg* isoform 2 via C/EBPβ, and possibly STAT5. *Pparg* expression can also be induced by autocrine TGF-β signaling. PPARγ is crucial for the catabolism of surfactant lipids into cholesterol. Right: When alveolar macrophage functions are disturbed either because of autoantibodies against GM-CSF, mutations in the GM-CSF pathway, or genes relevant for alveolar macrophage maintenance and function such as lipid handling capacity, alveolar macrophages are not able to catabolize surfactant lipids, which subsequently accumulate, reduce air-filled space, and cause pulmonary alveolar proteinosis. Created with BioRender.com

Surfactants play an immunological role as opsonizing agents of pathogens but are mainly involved in reducing the surface tension of the alveoli, thereby preventing alveoli collapse during respiration. However, constant production requires constant degradation to prevent accumulation and to maintain physiological balance. Therefore, the turnover of these surfactant components is tightly regulated: while approximately half of the surfactant is recycled and catabolized by type II alveolar epithelial cells, the other half is cleared by AMs. The regular consumption of substantial quantities of phospholipids can result in lipotoxicity, necessitating a specialized metabolic machinery in AMs to facilitate the complete breakdown of lipids and the recycling of cholesterol. The induction of these lung-specific catabolic programs is ensured by GM-CSF, which is secreted by type II alveolar epithelial cells and acts as an instructive cytokine. Defects in surfactant degradation can cause accumulation of surfactant in the alveoli, a syndrome termed pulmonary alveolar proteinosis (PAP). Primary PAP is a condition characterized by impaired GM-CSF signaling, which can either be caused by hereditary factors, such as mutations in *Csf2* or its receptor *Csf2r*, or by autoimmunity resulting from autoantibodies against GM-CSF. Therefore, reduced numbers or dysfunctional AMs can only insufficiently clear surfactants, which accumulates, hinders proper gas exchange, and causes PAP. These findings highlight the essential constitutive role of GM-CSF in AM and surfactant homeostasis. Secondary PAP, in contrast, is defined by reduced numbers or impaired functions of AMs due to hematological disorders or other gene mutations.

This is for instance the case in *Ccr2*-deficient patients who suffer from secondary PAP due to recruitment defects of monocytes into the alveoli (Neehus et al. 2024). Reconstitution of AMs numbers and function, for instance, by subcutaneous or inhaled administration of recombinant GM-CSF, is an effective treatment option for patients with *Csf2* deficiency and restores the expression of lipid handling genes such as PPARγ and CD36 in AMs (Seymour and Presneill 2002). Deficiency of several genes involved in lipid handling have been identified to induce PAP-like symptoms in mice, including *Pparg* (Schneider et al. 2014), *Cebpb* (Cain et al. 2013; Dörr et al. 2022), *Abcg1* (de Aguiar Vallim et al. 2017), *Abca1* (Bates et al. 2005), *Stat5* (Eddy et al. 2017), *Tgfb1* (Yu et al. 2017), and *Bach2* (Ebina-Shibuya et al. 2017). Among these genes, the transcription factor PPARγ appears to be essential for lipid metabolism. Myeloid-restricted *Pparg* deficiency in mice leads to the accumulation of surfactants in the lung alveoli and neutral lipids and cholesterol within AMs (Schneider et al. 2014). AM cell numbers were also reduced in these mice, indicating that PPARγ is important for the survival of AMs, or that lipid-laden AMs undergo cell death, leading to a long-term compromised AM pool. Therefore, PPARγ might prevent lipotoxicity in macrophages, similar to the situation observed in adipocytes (Medina-Gomez et al. 2007). However, the exact molecular sequences and interplay of the aforementioned genes involved in AM maintenance remain to be completely resolved (**Figure 2**). Activation of CSF2R by GM-CSF results in phosphorylation of JAK2 and subsequent activation of either STAT3 or STAT5. Currently, there are no reports describing the homeostatic role of STAT3 in AM biology, while *Stat5*-deficient mice that lack both *Stat5* genes, *Stat5a* and *Stat5b*, are characterized by reduced AM numbers and develop PAP-like symptoms (Eddy et al. 2017). Of the two genes, *Stat5a* is the more likely candidate to influence AM development since it is involved in GM-CSF-dependent B cell transdifferentiation (Nguyen et al. 2024) and orchestrates the epigenetic remodeling of chromatin during early adipogenesis (Jung et al. 2012). The molecular orchestration of adipogenesis indeed shows high similarities to the GM-CSF-dependent lipid metabolism in AMs. STAT5a for instance participates with the transcription factor C/EBPβ in the formation of chromatin 'hotspots' and initiates chromatin remodeling at early stages of adipogenesis (Siersbæk et al. 2011). C/EBPβ binding is particularly important for priming chromatin regions, cause the open chromatin can be subsequently bound by late-acting adipogenic factors, including PPARγ (Siersbæk et al. 2011). Accordingly, a high binding overlap between PPARγ and C/EBPβ is evident during adipogenesis (Siersbæk et al. 2011). C/EBPβ is also involved in AM maintenance as its depletion reduces the number of AMs (Cain et al. 2013). Subsequent research showed that C/EBPβ specifically induces in a GM-CSF-dependent manner the expression of *Pparg* isoform 2 (PPARγ2) (Dörr et al. 2022), which is the main isoform involved in lipotoxicity prevention in adipocytes (Medina-Gomez et al. 2007). Under homeostatic conditions, *Pparg*2 is specifically expressed by AMs under homeostatic conditions, while kidney, spleen, peritoneal, and adipose tissue-resident macrophages only express *Pparg* isoform 1 (PPARγ1) (Dörr et al. 2022). Although PPARγ1 and PPARγ2 are identical except for an additional 30 amino acids at the N-terminus of PPARγ2, both isoforms seem to regulate distinct sets of target genes with genes involved in the PPAR signaling pathway mainly controlled by PPARγ2, at least in white adipose tissue (Hu et al. 2022). Further research is needed to dissect the distinct functions of PPARγ1 and PPARγ2 in macrophage biology, but the high molecular similarity between adipogenesis and AMs in terms of C/EBPβ and PPARγ2

usage might indicate a conserved gene regulatory program beyond these cell lineages.
Not only GM-CSF-induced signaling can drive the expression of *Pparg*. TGFβ was also shown to induce *Pparg*. Myeloid-restricted deficiency of either *Tgfbr2* or *Tgfb1* led to reduced AM development and blocked their differentiation during mouse embryogenesis (Yu et al. 2017). These results indicate that TGFβ functions in an autocrine manner.
The conserved natural killer cell receptor *Nkrp1b* also affects AM maintenance through the control of metabolic genes responsible for lipid turnover (Scur et al. 2022). However, this regulation appeared to be independent of GM-CSF and PPARγ. Instead, increased lipid uptake, storage, and processing pathways can be detected in *Nkrp1b*-deficient AMs, which is accompanied by downregulation of cholesterol efflux pumps (Scur et al. 2022). Therefore, increased uptake and reduced efflux account for the foamy phenotype of *Nkrp1b*$^{-/-}$ AMs.
In addition to tissue-specific gene expression that characterizes AMs, the metabolic state can influence the biology of macrophages. It was recently shown that a myeloid-restricted deficiency of mitochondrial transcription factor A (encoded by *Tfam*) disrupted oxidative phosphorylation (Oxphos) in macrophages and influenced the development of lipid-associated macrophages, particularly AMs (Wculek et al. 2023). *Tfam*-deficiency leads to increased cholesterol accumulation in AMs, cellular stress, cell-cycle arrest, and culminates in PAP-like syndrome in mice (Wculek et al. 2023). This emphasizes that the ability to perform Oxphos might be a deterministic requirement for proper lipid handling and potentially applicable to other diseases in which macrophages encounter high lipid concentrations (Wculek et al. 2022). However, further research is required to link the metabolic state of macrophage subsets with tissue-specific gene signatures. In particular, it needs to be shown how tissue-specific niche instruction influences the metabolism of macrophages, and *vice versa*.
Of note, the lung interstitium also contains two or three other macrophage subsets, which develop in a GM-CSF independent but macrophage colony-stimulating factor (M-CSF; encoded by *Csf1*) dependent manner. The two main subsets are phenotypically characterized as Lyve1^{low}MHCII^{hi} and Lyve1^{hi}MHCII^{low}, respectively (Chakarov et al. 2019). Lyve1^{low}MHCII^{hi} interstitial macrophages are associated with nerve bundles, whereas Lyve1^{hi}MHCII^{low} macrophages locate in proximity to blood vessels. Depletion of Lyve1^{hi}MHCII^{low} cells influences bleomycin-induced fibrosis, but the physiological role of both subsets in lung tissue maintenance is unknown.

In conclusion, AMs adapted to a lipid-rich environment and express a unique set of genes involved in lipid catabolism, which enables them to participate in surfactant clearance. Defects in this crucial function can cause pathological manifestations, which highlight the importance of macrophages for tissue homeostasis.

Development and homeostatic function of splenic macrophages

The spleen is a highly vascularized secondary lymphoid organ. The primary functions of the spleen include the elimination of senescent or damaged red blood cells, storage of platelets, T-cell priming, and B cell maturation. Furthermore, the spleen represents an important filtering system for blood components and detection of circulating pathogens. Macrophages play a crucial role in all of these functions.
Splenic macrophage heterogeneity was first described using conventional microscopy. Histological stainings of spleen sections with F4/80 and Ser-4 antibodies

(CD169) indicated that different macrophage subsets occupy discrete spatial areas of the spleen (Rabinowitz and Gordon 1991). Marginal metallophilic macrophages (MMMs) and marginal zone macrophages (MZMs) are located in the marginal zone, which separates the white and red pulp of the spleen. Macrophages in the white pulp (WPMs) are located in a lymphocyte-rich environment, whereas red pulp macrophages (RPMs) are in contact with the cords of Billroth and splenic sinusoids in the red pulp (**Figure 3**).

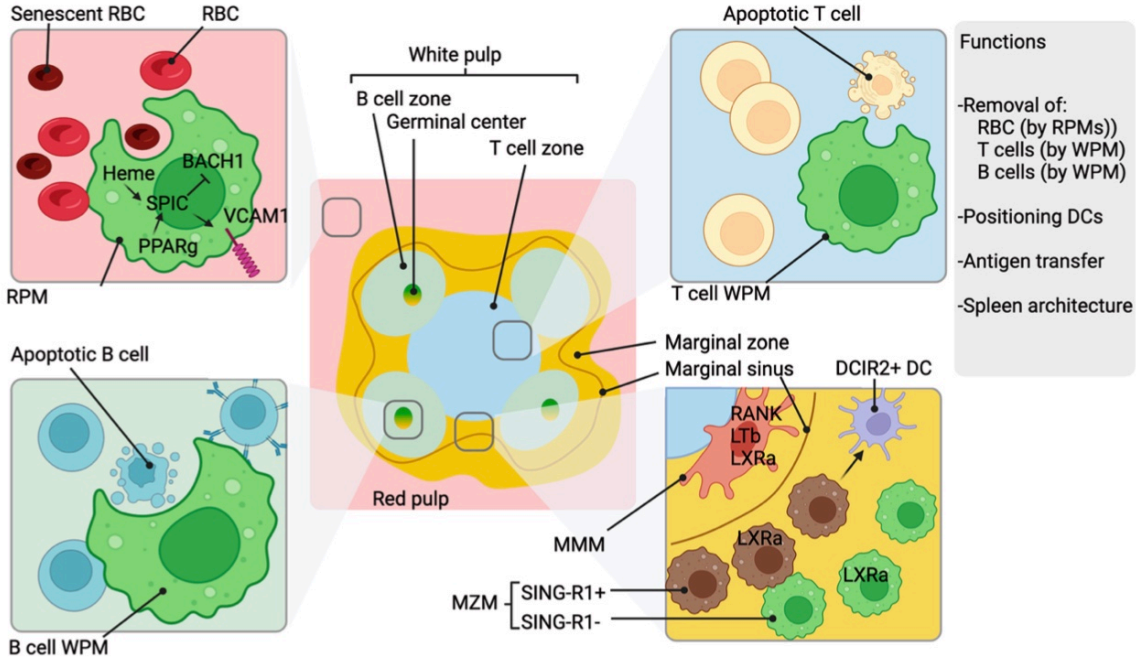

Fig. 1.3 Schematic representation of macrophage localization in the spleen. Five main tissue-resident macrophage subsets have been identified in different areas of the spleen. Red pulp macrophages (RPM) are involved in the clearance of senescent and damaged red blood cells (RBC) via heme, *SpiC*, and the degradation of BACH1. White pulp macrophages (WPM) participate in the phagocytosis of apoptotic T and B cells. Marginal zone macrophages (MZM) and marginal metallophilic macrophages (MMM) are involved in antigen sampling and marginal zone microarchitecture. MZMs influence the positioning of dendritic cells (DC) at the bridging channels. Created with BioRender.com

Nowadays, single-cell RNA-sequencing (scRNA-Seq) experiments have deeply expanded our knowledge regarding macrophage heterogeneity in some tissues, but scRNA-Seq datasets with a comprehensive representation of splenic macrophage subsets are still missing. This might be because the disaggregation of organs necessary for single-cell isolation often leads to macrophage fragmentation and thereby to the underrepresentation of this particular immune cell subset in scRNA-Seq datasets (Millard et al. 2021). It is also possible that certain enrichment strategies such as negative magnetic cell separation are less efficient in the spleen because of the intrinsic magnetism of splenic macrophages (Franken et al. 2015). More tissue-sensitive approaches, such as single-nucleus RNA-Seq experiments or spatial transcriptomics at the single-cell level, are needed to unravel the heterogeneity of splenic macrophages on a large scale.

Nevertheless, owing to the specific localization of macrophage subsets and the resulting unique cellular microenvironment, each splenic macrophage population is phenotypically distinct and equipped with specialized functions.

Marginal zone macrophages. MMMs and MZMs share a close functional and developmental relationship, which explains why some gene deletions or cell

depletion strategies affect both macrophage populations in a similar manner. Both subsets are involved in apoptotic cell clearance, antigen capture from the blood, and B-cell communication. Therefore, assigning a specific function to one subset, but not to the other, is problematic without mice that allow the specific targeting of the individual subsets. However, some specific functions for each subset have emerged during the past years, even though the influence of perturbations on other splenic macrophage subsets has not been investigated in all studies.

The majority of blood that flows into the spleen initially reaches the marginal zone surrounding the B-cell follicles. Accordingly, MZMs in this anatomical niche are suitable for filtering disseminated particles and apoptotic cells from the blood (McGaha et al. 2011), controlling *Listeria monocytogenes* infections (Aichele et al. 2003), and providing antigens to B cells (Prokopec et al. 2016). The role of MZMs was investigated in mice that received low-dose clodronate liposome injections in conjunction with chronic injections of apoptotic cells. Under these circumstances, apoptotic bodies are no longer entrapped in the marginal zone and instead accumulate in the white pulp, which alters adaptive immune responses during lupus erythematosus (McGaha et al. 2011). However, MZMs do not phenotypically form homogenous populations. The middle section of the marginal zone is composed of macrophages that express both SIGN-R1 (encoded by *Cd209a*) and MARCO, whereas the outer area is inhabited by MZMs that only express MARCO (Ito et al. 1999; Pirgova et al. 2020) (**Figure 3**). Interestingly, compared to $MARCO^+$ MZMs, $SIGN\text{-}R1^+$ $MARCO^+$ MZMs seem to be independent of M-CSF signaling (Takahashi et al. 1994; Ito et al. 1999) and only slowly reappear after clodronate liposome depletion (van Rooijen et al. 1989). $SIGN\text{-}R1^+$ $MARCO^+$ MZMs depend on the presence of B cells for differentiation (Nolte et al. 2004; You et al. 2009). This emphasizes that the maintenance of the marginal zone cellular architecture necessitates constant cell-to-cell communication. MZM localization and maintenance depend on *Mertk* (Soni et al. 2018), *Marco*, and *Msr1* (Chen et al. 2005). Furthermore, $SIGN\text{-}R1^+$ MZMs are important for the correct localization of $DCIR2^+$ cDC2 dendritic cells. Upon depletion of $SIGN\text{-}R1^+$ MZMs in $Cd209a^{Dtr\text{-}Cre}$ mice, $DCIR2^+$ dendritic cells lost their clustered positioning in the bridging channels, thereby impairing germinal center B cell responses (Pirgova et al. 2020).

Marginal metallophilic macrophages. MMMs reside at the border between the marginal zone and the white pulp, and their development is similarly dependent on B cells (Nolte et al. 2004) (**Figure 3**). These cells express CD169 and MOMA-1 and thereby show similarities to the subcapsular sinus macrophages of the lymph node. MMMs rely on receptor activator of NF-κB (RANK) and lymphotoxin beta signaling. Deletion of either factor in $Cd169^{Cre}$ mice leads to a strong reduction in MMMs, whereas MZMs remain unaffected (Camara et al. 2022). Marginal zone reticular cells were identified as the main producers of RANK ligand, thereby influencing MMM differentiation (Camara et al. 2022). In addition, the nuclear receptor LXRα (encoded by *Nr1h3*) and M-CSF are involved in MMM differentiation, even though they also affect MZM development (Takahashi et al. 1994; A-Gonzalez et al. 2013). MMMs can transfer antigens to splenic cDC1 dendritic cells for the cross-presentation and activation of cytotoxic T lymphocytes (Backer et al. 2010), which might represent an efficient vaccination strategy to induce cytotoxic T-cell immunity. It has also been discussed that MMMs participate in oxysterol biosynthesis and thereby affect the migration of activated B cells to the outer follicular niche (Liu et al. 2011), but cell-specific gene deletion approaches are necessary to formally prove this point.

Red pulp macrophages. Among all splenic tissue-resident macrophage populations, RPMs form the largest fraction in terms of cell numbers. In mice, RPMs

develop from early *Myb*-independent YS precursors (Schulz et al. 2012), but fetal monocyte-derived macrophages may contribute to the RPM pool (Epelman et al. 2014). However, detailed knowledge regarding RPM fate and replenishment during adulthood is still lacking.

RPMs are intrinsically magnetic (Franken et al. 2015), which emphasizes their role in iron recycling and red blood cell clearance. The causal link between red blood cell clearance and iron recycling was demonstrated earlier in different knockout mice. CD47 is normally expressed by viable red blood cells and interacts with the inhibitory receptor signal regulatory protein alpha (SIRPα) in RPMs, which prevents phagocytosis. The injection of CD47-deficient red blood cells led to erythrophagocytosis in RPMs, which was independent of red blood cell viability (Oldenborg et al. 2000). However, because *Cd47*- and *Sirpa*-deficient mice do not show an overt phenotype with respect to anemia and splenomegaly under physiological conditions (Bian et al. 2016), the exact *in vivo* contribution of CD47 / SIRPα interaction in RPMs awaits further research.

In contrast, *SpiC*-deficient mice show strongly reduced numbers of RPMs, whereas MZMs and MMMs are present in normal cell numbers (Kohyama et al. 2008). The SPIC-dependent absence of RPMs resulted in splenomegaly with increased iron accumulation in the spleen. Furthermore, proerythroblast numbers decreased in the steady state, supporting the idea that iron recycling is essential to sustain iron-sufficient erythropoiesis. It was postulated that SPIC induced the expression of *Vcam1* (Kohyama et al. 2008), and a direct cell-intrinsic role for VCAM1 in RPM maintenance was shown later (Ulyanova et al. 2016). However, the exact molecular role of *Vcam1* in RPM differentiation requires further investigation. Subsequent work revealed a critical role for heme in the induction of the SPIC-dependent pathway, which involves proteasomal degradation of the transcription factor BACH1 (Haldar et al. 2014) (**Figure 3**). The fact that heme plays an important role in RPM biology can also be observed in mice with heme oxygenase-1 (HO-1, encoded by *Hmox1*) deficiency. HO-1 is involved in heme metabolism, thereby releasing free iron, which can then return to circulation. Patients with HO-1 mutations suffer from tissue iron overload and anemia. Similarly, *Hmox1*-deficient mice accumulate cytotoxic heme, which leads to elevated oxidative stress and subsequent cell death in RPMs (Kovtunovych et al. 2010).

However, heme-*SpiC* induction seems to be important for the function of RPMs, but not directly for their development, since young *SpiC*-deficient mice show normal or only slightly reduced cell numbers in RPMs (Ulyanova et al. 2016; Okreglicka et al. 2021). Instead, *Pparg* is a critical transcription factor for the establishment of RPMs (Okreglicka et al. 2021). Lack of PPARγ caused the upregulation of genes involved in the immune response, while pathways related to erythrocyte development and heme biosynthesis were decreased in the remaining spleen macrophages (Okreglicka et al. 2021). Functionally, PPARγ regulates the adhesion and migration of early RPM precursors, which is in line with the PPARγ independence of adult RPMs (Okreglicka et al. 2021). Since RPMs do not express *Pparg2* (Dörr et al. 2022), this phenotype is likely based on *Pparg1* expression, which explains the independence of RPMs from GM-CSF and C/EBPβ (Cain et al. 2013). In addition, the combined deletion of the transcription factors *Irf4* and *Irf8* prevents RPM development, whereas deficiency of each factor alone does not affect RPM cell numbers (Yamamoto et al. 2011). However, the molecular basis of this phenotype remains unknown.

Vcam1 expression by RPMs is also involved in the regulation of extramedullary hematopoiesis. This was investigated in *Cd169*^{Dtr} mice, in which diphtheria toxin

injection not only depleted MMMs, but also strongly reduced RPMs. This led to a *Vcam1*-dependent reduction in splenic hematopoietic precursor cells, while simultaneously evaluating their frequency in the circulation (Dutta et al. 2015). These data suggested that VCAM1^{+} macrophages interact with hematopoietic precursors via the VCAM1 ligand VLA-4, which is important for the formation of an extramedullary hematopoietic niche (Williams et al. 1991). However, as the mouse *Cd169*^{Dtr} depletion system is not specific to RPMs, the contribution of MMMs to this phenotype should be investigated in the future.

White pulp macrophages. WPMs are located in the B- and T-cell areas of the spleen and do not express F4/80 (Rabinowitz and Gordon 1991) but are immunoreactive to CD68, MFG-E8, and TIM-4 (Rabinowitz and Gordon 1991; Hanayama et al. 2004; Wong et al. 2010). The B cell area can also contain germinal centers, in which WPMs can accumulate 'tingible bodies' that consist of condensed apoptotic nuclei (Rabinowitz and Gordon 1991). The intracellular accumulation of apoptotic cells suggests the involvement of WPMs in the clearance of apoptotic cells. In germinal centers, B cells undergo clonal selection, hyperproliferation, and somatic hypermutation. The randomness of receptor editing increases the risk of errors, which are associated with high rates of apoptosis. Efficient removal of cellular corpses is crucial because intracellular components can induce otherwise unwanted immune cell activation and the formation of self-reactive antibodies. This is evident in *Mfge8*-deficient mice, which suffer from autoimmune symptoms, such as glomerulonephritis, as a result of autoantibody production, splenomegaly, and hyperactive germinal centers (Hanayama et al. 2004). A similar phenotype was detected in mice lacking the proto-oncogene tyrosine-protein kinase MER, which exhibited increased non-phagocytized apoptotic cells on the surface of WPMs (Rahman et al. 2010). However, whether these phenotypes are the consequence of *Mfge8*- or *Mertk*-deficiency in WPMs specifically and not related to other splenic macrophage populations require subset-specific gene deletion approaches. Macrophages in the germinal center are stationary cells that do not migrate in search of apoptotic cells, at least in lymph nodes. Instead, they use long protrusions to scan the environment dynamically (Grootveld et al. 2023; Gurwicz et al. 2023). Interestingly, germinal center macrophages in the lymph node develop via a CD169-lineage independent pathway and do not require M-CSF for differentiation, which emphasizes the existence of a follicle resident precursor that can be locally activated by the presence of dead cells. This stimulus may be sufficient to induce differentiation into tingible body macrophages (Grootveld et al. 2023; Gurwicz et al. 2023). However, the exact precursor and molecular pathways require further research. It also needs to be shown whether splenic germinal center WPMs follow a similar developmental route.

However, macrophages in the white pulp are not confined to B-cell follicles but are also present in the periarteriolar sheath of the T-cell zone. Currently, detailed knowledge of the biology of T-cell zone macrophages in the spleen is lacking. Notably, a CX3CR1^{hi}CD64^{+}MERTK^{+} macrophage subset was recently identified in the T-cell area of the lymph node (Baratin et al. 2017). These macrophages are derived from BM monocytes after birth and differentiate into long-lived T-cell zone macrophages with a slow replacement during adulthood (Baratin et al. 2017). In the lymph node T-cell zone macrophages are involved in CX3CR1-dependent apoptotic cell clearance, but do not contribute to naïve CD4^{+} T-cell priming or tolerance. If their splenic counterpart fulfils a similar function and follows the same differentiation route, remains to be shown.

The primary functions of splenic macrophages are closely tied to the role of the spleen as an immunological organ. Tissue-resident macrophages in the spleen are uniquely equipped to degrade and recycle senescent red blood cells and apoptotic lymphocytes. Additionally, they are involved in the anatomical microarchitecture of the spleen, sample antigens from circulation, and transfer them to dendritic cells and B cells. However, it has not been shown yet whether these activities promote peripheral tolerance under physiological conditions.

Development and homeostatic function of Kupffer cells

The liver, being the largest solid internal organ in the body, is of crucial importance when it comes to maintaining metabolism, digestion, detoxification, and immune regulation. Hepatocytes, the primary cell type in the liver, perform essential functions, such as detoxifying harmful substances like drugs and alcohol, synthesizing proteins like albumin and clotting factors necessary for blood coagulation, storing glycogen as energy source, and metabolizing fats, carbohydrates, and proteins. Furthermore, the liver produces bile, which aids in the digestion and absorption of fat in the small intestine. The liver also plays a critical role in metabolizing hormones, cholesterol, and waste products such as bilirubin.
Kupffer cells (KCs), the tissue-resident macrophages of the liver, are among the first described macrophages. Karl Wilhelm von Kupffer observed in 1876 “Sternzellen” that could be stained with a gold chloride solution (Kupffer 1876), years before Ilya Metchnikoff identified macrophages (Metchnikoff 1892). Pathologist Tadeusz Browicz further described KCs (Browicz 1899) and because of his seminal contribution, some authors refer to liver macrophages as Kupffer-Browicz cells.
Evidence that macrophages first appear in the liver during embryogenesis may be attributed to Sorokin et al. (Sorokin et al. 1992). The authors used peroxidase-coupled isolectin B4 to identify macrophages in the rat brain (E12), liver (E12-13), and lungs (E13-14) but not in the spleen, BM, and thymus. Although KCs do not express CX3CR1 in adulthood, they are derived from a CX3CR1^{+} precursor, as shown in *Cx3cr1*^{Cre} reporter mice (Yona et al. 2013). These precursors are YS-derived EMPs and fetal monocytes (Schulz et al. 2012; Hoeffel et al. 2015). Because the liver undergoes significant expansion and reorganization in the first postnatal weeks in mice, BM-derived monocytes can integrate into the KC niche and acquire a KC-specific signature when transferred during this developmental window (Scott et al. 2016). The established KC pool is subsequently able to maintain itself throughout life. Together with microglia in the CNS and Langerhans cells in the skin, KCs are one of the investigated macrophage populations that don’t seem to be complemented by monocytes during adulthood as observed in *Ms4a3*^{Cre} reporter mice (Liu et al. 2019b). However, monocytes can adopt a KC signature when the original embryonic-derived macrophage pool is compromised, particularly after diphtheria toxin-mediated cell depletion in *Clec4f*^{Dtr} mice (Scott et al. 2016; Bonnardel et al. 2019; Sakai et al. 2019). This method was also used to unravel niche-specific signals that are important for instructing KC precursors and contributing to the tissue-specific identity of KCs (Bonnardel et al. 2019; Sakai et al. 2019). KCs line the sinusoidal endothelium of the liver and are in contact with hepatocytes, liver sinusoidal endothelial cells (LSECs), and hepatic stellate cells (**Figure 4**). Hepatic stellate cells provide M-CSF and bone morphogenic proteins (BMPs) to induce monocyte differentiation into KCs. LSECs further produce BMPs, as well as the Notch pathway ligands DLL1 and DLL4, which induce through RBPJ the transcription factors LXRα and SPIC in KCs (Bonnardel et al. 2019; Sakai et al. 2019). Deletion of *Nr1h3* in KCs is accompanied by significant

transcriptional and phenotypic changes, including the loss of KC signature genes (Sakai et al. 2019) and reduced TIM4 expression (Scott et al. 2018). Loss of TIM4^{+} cells in *Nr1h3*-deficient mice indicates that the survival or differentiation of embryonic-derived KCs is LXRα dependent. This is supported by the fact that *Nr1h3*, together with *Id3*, was identified as a possible pre-deterministic candidate for pre-macrophage-to-KC differentiation (Mass et al. 2016). However, the empty hepatic niche in *Nr1h3*-deficient animals is subsequently filled by BM-derived monocytes, which still show significant gene changes compared to wildtype controls (Scott et al. 2018).

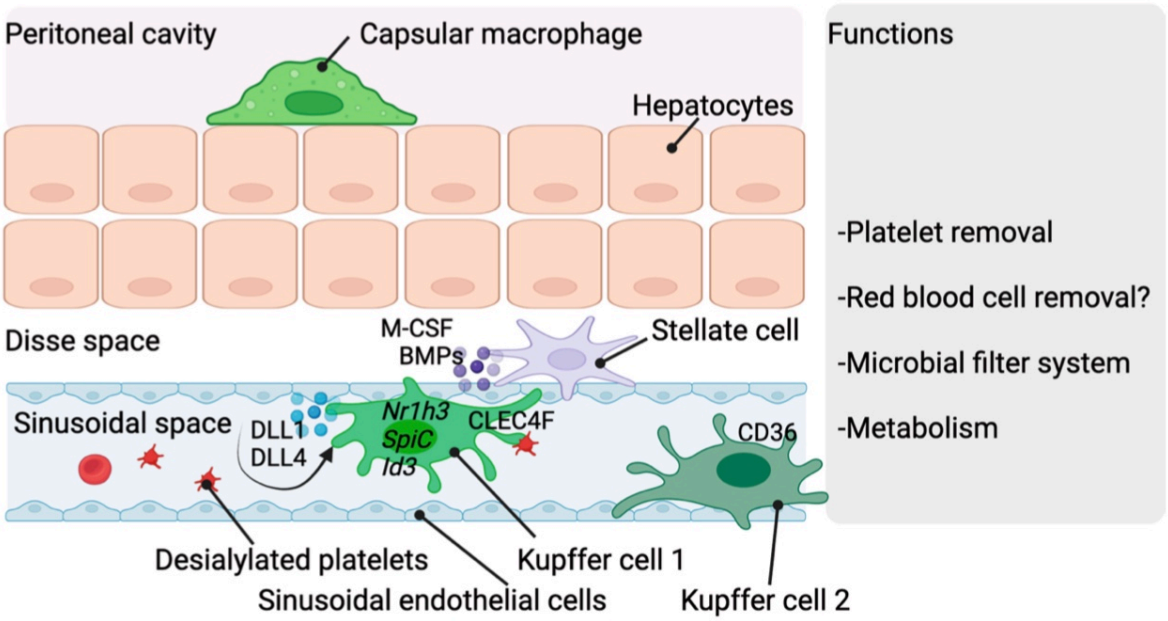

Fig. 1.4 Macrophage localization in the liver. Kupffer cell subsets 1 and 2 are located in the sinusoidal space, where they receive instructive signals from stellate cells (M-CSF, BMPs) and sinusoidal endothelial cells (Notch ligands DLL1 and 4). Kupffer cell subset 1 can remove damaged red blood cells and desialylated platelets via CLEC4F, whereas subset 2 participates in metabolic diseases via CD36. Created with BioRender.com

SpiC expression suggests that KCs are involved in iron recycling, similar to RPMs in the spleen and BM macrophages (Haldar et al. 2014). Indeed, the intravenous injection of ^{51}Cr-labeled oxidatively damaged red blood cells into mice showed that the main uptake of these cells occurred in the liver (Terpstra and van Berkel 2000). However, whether KCs are also involved in red blood cell clearance under homeostatic conditions remains to be shown. Compared to RPMs, KCs only show partial labeling in *SpiC*^{Gfp} reporter animals, and KC numbers seem to be unchanged in *SpiC*-deficient mice (Haldar et al. 2014). These data suggest that KCs might not use a SPIC-dependent pathway for the removal of senescent red blood cells and may participate in iron homeostasis in a different way.

KCs also respond to erythropoietin (EPO), which is the main hormone that drives mammalian erythropoiesis. Upon repeated injections of EPO, KC numbers increased in mice, indicating a direct or indirect role of EPO in mediating KC proliferation (Gilboa et al. 2017). Furthermore, KCs have also been shown to scavenge hemoglobin released by erythrocytes in the form of red blood cell-derived vesicles (Willekens et al. 2005). Using radiolabeled vesicles, researchers found that 80% of all radiolabeling is cleared from the bloodstream by the liver in just 5 minutes after intravenous injection in rats (Willekens et al. 2005). Older observations also suggest

that KCs play a role in erythrophagocytosis, particularly in conditions leading to hemolysis (Kaye et al. 1967; Loebl et al. 1973). However, even if these data indicate that KCs can clear damaged red blood cells after injection or hemolysis, the role of KCs in red blood cell clearance under steady-state conditions remains unclear. Instead, KCs may be the main phagocytic cells for the removal of desialylated platelets. Megakaryocyte-derived platelets are the second most common cell type in the blood, and because of their short life span of only a few days, they require constant removal. Platelets express high levels of sialylated N- and O-linked glycans The level of sialylation can change under pathological conditions or during aging (Goswami and Koner 2002; Li et al. 2015), and desialylated glycans show high binding affinity to receptors such as CLEC4F (Jiang et al. 2021), a marker gene of KCs (**Figure 4**). Deletion of receptors such as *Clec4f* in KCs (Jiang et al. 2021), macrophage galactose lectin (Deppermann et al. 2020), or genes involved in the generation of O-linked glycans, which reduces the levels of sialic acids on platelets (Li et al. 2017), leads to the accumulation of platelets in the liver.

The functions of KCs may change when the liver becomes the primary organ of extramedullary erythropoiesis, for instance, in splenectomized mice. Under these conditions, KCs upregulate *Vcam1* expression and therefore create a niche for hematopoietic precursor cells in emergency situations (Otsuka et al. 2011), similar to the function of MMMs during extramedullary hematopoiesis (Dutta et al. 2015). It is also possible that KCs can adopt an RPM gene signature in splenectomized mice, thereby securing the alternative removal of red blood cells by KCs.

A recent study explored in deeper detail the functions of KCs in homeostasis, by comparing KC dynamics in steady state with fibrotic livers. Extensive remodeling of the hepatic sinusoids was observed using a model of hepatic fibrosis, leading to the redistribution of blood flow from the sinusoids to collateral vessels (Peiseler et al. 2023). These changes cause deficits in liver sinusoid surveillance by KCs, a decrease in their ability to capture phagocyte-injected bacteria, and partial loss of KC identity (Peiseler et al. 2023). Unexpectedly, despite these changes, the survival of mice after microbial infection is not severely affected (Peiseler et al. 2023). This protection could be attributed to the formation of multinucleated structures (syncytia) in collateral vessels, resulting from the CD36-dependent fusion of BM-derived monocytes that adopt a KC-like signature (Peiseler et al. 2023). These syncytia showed enhanced bacterial clearance and phagocytic capacity compared to KCs, and compensated for the loss of KCs due to rarefied sinusoids. These data emphasize that KCs are involved in blood scavenging and represent an important filter system for preventing the systemic distribution of potentially pathogenic materials.

New reporter and fate mapping mouse models, as well as single-cell analysis, further revealed some heterogeneity in the liver-macrophage pool. More than three decades ago, an MHCII-expressing cell type was detected in the human hepatic capsule, which was classified as dendritic cells, despite their immunoreactivity towards CD68 and CD163 (Prickett et al. 1988). These cells were later identified as liver capsular macrophages, which are constantly replenished by monocytes in parabiotic mice (Sierro et al. 2017) but only partially in the *Ms4a3*^{Cre} fate mapping model (Liu et al. 2019b). Capsular macrophages are involved in immunosurveillance and recruitment of neutrophils (Sierro et al. 2017), but whether they fulfil physiological and niche-specific functions is unknown. Another hepatic macrophage subset in close proximity to the bile ducts in humans (Guilliams et al. 2022) partially shares a gene signature with TREM2^{+} lipid-associated macrophages (LAMs) (Jaitin et al. 2019). The

enrichment of genes involved in lipid handling suggests that these cells may play a role in metabolic diseases. Indeed, during metabolic-associated fatty liver diseases, monocytes can differentiate into hepatic LAMs and show different abilities to metabolize lipids compared to KCs (Remmerie et al. 2020). Moreover, during steatosis, the hepatic LAM population increases and relocates to steatotic zones (Guilliams et al. 2022); however, their physiological functions are unclear. The liver further harbors central vein macrophages under steady-state conditions, and a $CD206^{hi}$ $ESAM^{+}$ subset termed KC2 (**Figure 4**). While KC2 are ontogenetically related to KCs, arise from embryonic precursors, occupy the same cellular niche as KCs, and are characterized by the expression of KC marker genes (such as *Clec4f*, *Lyz2*, and *Csf1r*), they also share gene expression patterns with LSECs (Bleriot et al. 2021; De Simone et al. 2021). KC2s are enriched in genes involved in carbohydrate and lipid metabolism, and depletion of KC2 cells or deficiency of the fatty acid transporter CD36 in a mouse model of obesity or liver steatosis revealed that KC2s contribute to the regulation of glucose homeostasis and to the liver oxidative stress response (Bleriot et al. 2021). KC2s further contribute to the cross-presentation of hepatocellular antigens to prime and induce the antiviral functions of $CD8^{+}$ T-cells during hepatitis B virus infection (De Simone et al. 2021). Notably, data suggesting the existence of a KC subset that shows LSEC-related gene expression could also be attributed to the formation of doublets or tissue fragmentation (Millard et al. 2021; Guilliams et al. 2022; Hume et al. 2022).

In summary, KCs act as an indispensable central microbial filter system and are involved in platelet clearance. They may contribute to senescent red blood cell removal under steady-state conditions and participate in metabolism.

Development and homeostatic function of cardiac macrophages

The heart is a muscular organ responsible for pumping oxygenated blood throughout the body and deoxygenated blood into the lungs for gas exchange. The rhythmic contractions are based on the electrical conduction system of the heart, which ensures an efficient blood flow throughout the body. Central to this are specialized muscle cells called cardiomyocytes, which are able to rhythmically contract and relax. Cardiomyocytes are long-lived cells and have limited regenerative capacity, with less than 50% being replaced during the normal life span in humans (Bergmann et al. 2009). This longevity combined with the extreme physical requirements (one cardiomyocyte contracts approx. $>10^{7}$ times per year) exposes cardiomyocytes to constant cellular stress, suggesting that cardiomyocytes require specific protection mechanisms that support their functionality.

Cardiac macrophages are integral components of the immune cell repertoire of the heart but also fulfil tissue homeostatic functions and are involved in developmental aspects of the heart. The origin of cardiac macrophages has been an area of extensive research, leading to the understanding that they are derived from different cellular sources during the development of an organism. In mice, the heart is colonized by fetal YS-derived myeloid precursors around E10 (characterized by an $F4/80^{hi}CD11b^{low}$ phenotype), which are supplemented by $F4/80^{low}CD11b^{hi}$ fetal monocyte-derived cells at E12 (Epelman et al. 2014). Self-maintaining embryo-derived macrophages can further diversify into $MHCII^{low}$ and $MHCII^{hi}$ subsets postnatally (Molawi et al. 2014). While the $MHCII^{low}$ subset is additionally characterized by TIMD4, LYVE1, and FOLR2 expression, the $MHCII^{hi}$ subset only shows low expression of these markers (Rizzo et al. 2023). During late embryogenesis and after birth, a small fraction of BM monocyte-derived

macrophages further contribute to the tissue-resident cardiac pool and coexist with embryonic-derived macrophages (Epelman et al. 2014; Heidt et al. 2014; Leid et al. 2016). These cells can be distinguished by CCR2 expression and slowly outcompete their embryonic counterparts during aging (Molawi et al. 2014). Similar observations were made in patients who received a sex-mismatched heart transplant (Bajpai et al. 2018).

In terms of cardiac development, fetal cardiac macrophages have been shown to play important roles in coronary remodeling during late embryonic stages. The hearts of *Csf1*$^{op/op}$ and *LysM*^{Cre} *Rosa26*^{Dta} mice, which show a general macrophage deficiency, exhibited higher coronary vessel density when compared to controls, and their vascular plexuses failed to remodel into blood vessels of different diameters (Leid et al. 2016). Furthermore, macrophage depletion resulted in diminished growth of perfused blood vessels, whereas an increase in the proliferation of non-perfused blood vessels was observed (Leid et al. 2016) (**Figure 5**). This implies that cardiac macrophages directly or indirectly influence the proliferation of coronary endothelial cells. Factors that could play a role in vascularization are insulin-like growth factors 1 and 2, which are preferentially expressed by embryonic cardiac macrophages (Leid et al. 2016). This is in agreement with a study that investigated the role of embryonic-derived macrophages versus monocyte-derived cells in neonatal and adult cardiac injury models. Genetic cardiomyocyte ablation in neonatal mice increased the population of embryonic-derived cardiac macrophages, whereas the same injury in adults led to an influx of monocyte-derived macrophages (Lavine et al. 2014). Monocyte-derived cells show an inflammatory signature and are devoid of reparative functions, while embryonic-derived macrophages show a minimal inflammatory response and promote cardiac recovery through cardiomyocyte proliferation and angiogenesis (Lavine et al. 2014). These results suggest that embryonic cardiac macrophages are superior in supporting angiogenesis, whereas monocyte-derived cells are involved in inflammatory reactions that can contribute to tissue damage.

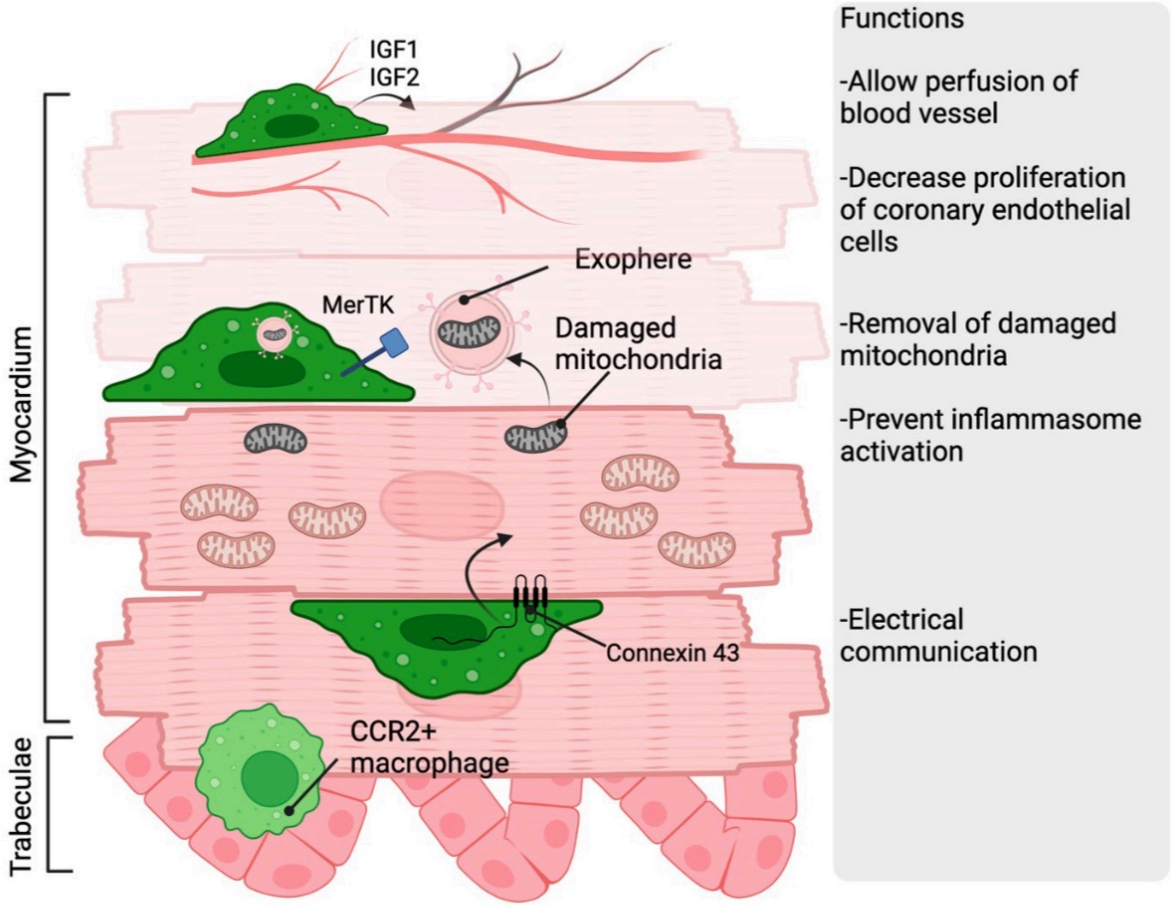

Fig. 1.5 Functions of cardiac macrophages. Embryonic-derived cardiac macrophages (dark green) are involved in perfused cardiac blood vessel formation during development. They are also involved in MerTK-dependent removal of damaged mitochondria, which are released from cardiomyocytes in the form of exophers. Cardiac macrophages also form connexin 43-dependent gap junctions, which positively depolarize the resting membrane potential in cardiomyocytes. $CCR2^{+}$ monocyte-derived macrophages were specifically identified in the trabecular projections. The function of this subset under homeostatic conditions is currently unknown. Created with BioRender.com

Interestingly, spatial analysis revealed that $CCR2^{-}$ embryonic-derived macrophages were almost exclusively found within the myocardium, whereas $CCR2^{+}$ macrophages were located within the trabecular projections of the endocardium (Leid et al. 2016). This indicates that embryonic-derived macrophages may support cardiomyocyte function. Contractile large cardiomyocytes have a high ATP demand to drive cardiac contraction/relaxation and ionic homeostasis, which is reflected by the high mitochondrial density in this cell population (Barth et al. 1992). Furthermore, cardiomyocytes have a low turnover rate (Bergmann et al. 2009), suggesting that mechanisms are needed to protect cardiomyocytes from malfunction. One layer of protection is represented by embryonic-derived $CX3CR1^{+}MHCII^{+}CD169^{+}$ cardiac macrophages. Depletion of macrophages in *Cd169*^{Dtr} mice increases the number of morphologically altered mitochondria in cardiomyocytes (Nicolás-Ávila et al. 2020). Further experiments have revealed that cardiac macrophages are able to uptake exophers that contain dysfunctional mitochondria from cardiomyocytes in an MER-dependent manner (Nicolás-Ávila et al. 2020). Depletion of macrophages increases mitochondria deposition in the extracellular space and causes inflammasome activation, leading to defects in ATP generation and diastolic and systolic

dysfunctions (Nicolás-Ávila et al. 2020). Therefore, embryonic-derived cardiac macrophages likely protect cardiomyocytes by removing the old and damaged mitochondria (**Figure 5**).
As cardiomyocytes are on average surrounded by five macrophages (Nicolás-Ávila et al. 2020), it is possible that macrophages also modulate electrical conduction. Disruptions in macrophage function can thus have direct implications for cardiac electrophysiology and arrhythmia development. Indeed, cardiac macrophage depletion in *Cd11b*^{Dtr} mice resulted in complete third-degree atrioventricular blockade (Hulsmans et al. 2017). This phenotype could be partially attributed to the physical interaction between atrioventricular node macrophages and cardiomyocytes. Macrophages directly couple with cardiomyocytes through gap junctions containing connexin 43 to facilitate electrical communication and exhibit rhythmic depolarization, resulting in a more positively depolarized resting membrane potential in cardiomyocytes (Hulsmans et al. 2017) (**Figure 5**). Therefore, they possibly allow for accelerated repolarization and a shorter action potential. Importantly, the cardiomyocyte support function of macrophages might change under pathological conditions when monocyte-derived cells rather than embryonic-derived macrophages dominate affected cardiac tissue areas. Both subsets execute different functions within the heart (Lavine et al. 2014; Hulsmans et al. 2023). This raises the possibility that an infarcted heart is functionally impaired due to the increased presence of monocyte-derived cells that do not form connexin 43-dependent contacts with cardiomyocytes and can cause atrioventricular blockade.

Collectively, these recent data demonstrate that macrophages are the guardians of cardiomyocytes and influence their function through mitochondrial removal and electrical conduction. Therefore, a detailed understanding of the origin and function of cardiac macrophages is pivotal to identify the causes of arrhythmias, a common feature of many cardiac diseases.

Development and homeostatic function of microglia

The CNS is responsible for processing sensory information obtained from the peripheral nervous system, thereby regulating vital processes, such as respiration and heartbeat, but also controls cognitive functions, including memory, learning, and behavior. The ability to perform these functions is a consequence of the interplay between four specialized cell subsets in the CNS: neurons, astrocytes, oligodendrocytes, and microglia.
Research on microglia, the primary immune cells and tissue-resident macrophages of the CNS and once considered the “almost forgotten third glial element” (Streit et al. 1988), attracted considerable attention in the early 2000s, and the number of microglia-related research articles is currently increasing nearly exponentially. Accordingly, microglia are now with 5.000 publications per year possibly the most studied tissue-resident macrophage population.
Microglia originate from “early” EMPs in the YS during embryogenesis, a process that depends on the transcription factors PU.1 (encoded by *Spi1*). Primitive macrophages colonize the developing brain around E9.5, which is before the blood-brain barrier (BBB) is fully established around E14.5 (Ginhoux et al. 2010; Gomez-Perdiguero et al. 2015; Hoeffel et al. 2015). Microglial development and function are finely tuned by a combination of intrinsic genetic programs that are linked to the expression of *Sall1* (Buttgereit et al. 2016), *Irf8* (Masuda et al. 2012; Kierdorf et al. 2013), *P2ry12* (Haynes et al. 2006), *Hexb* (Masuda et al. 2020), and extrinsic signals from the

surrounding glial environment, such as M-CSF (Kana et al., 2019), IL-34 (Wang et al. 2012; Greter et al. 2012), and TGFβ (Qin et al. 2018).
Microglia influx into the developing brain occurs before the peak of neurogenesis and neuronal migration. During this time, microglia help regulate the size of neural precursor cell populations in the cerebral cortex through selective engulfment of apoptotic and/or excess neural precursors (Cunningham et al. 2013), in the hippocampus by clearing dying neuroblasts that fail to transition into mature neurons (Sierra et al. 2010), and in the developing cerebellum by promoting the death and efferocytosis of Purkinje cells (Marín-Teva et al. 2004). It has also been shown that during postnatal development, the CX3CL1-CX3CR1 axis and supply of insulin growth factor 1 (IGF1) by microglia are important trophic factors supporting neuronal survival (Ueno et al. 2013) (**Figure 6**). During the formation of neuronal circuits, microglial processes further form contacts with developing neurons, reminiscent of purinergic junctions that are crucial for neuron-microglia communication. Constitutive deletion of P2RY12 leads to an aberrant cortical cytoarchitecture, spanning from development throughout adulthood (Cserép et al. 2022). Additionally, microglia modulate dopaminergic axon outgrowth and interneuron positioning in the neocortex as well as axon tract fasciculation during corpus callosum development through the adaptor protein DAP12 (Pont-Lezica et al., 2014; Squarzoni et al., 2014).

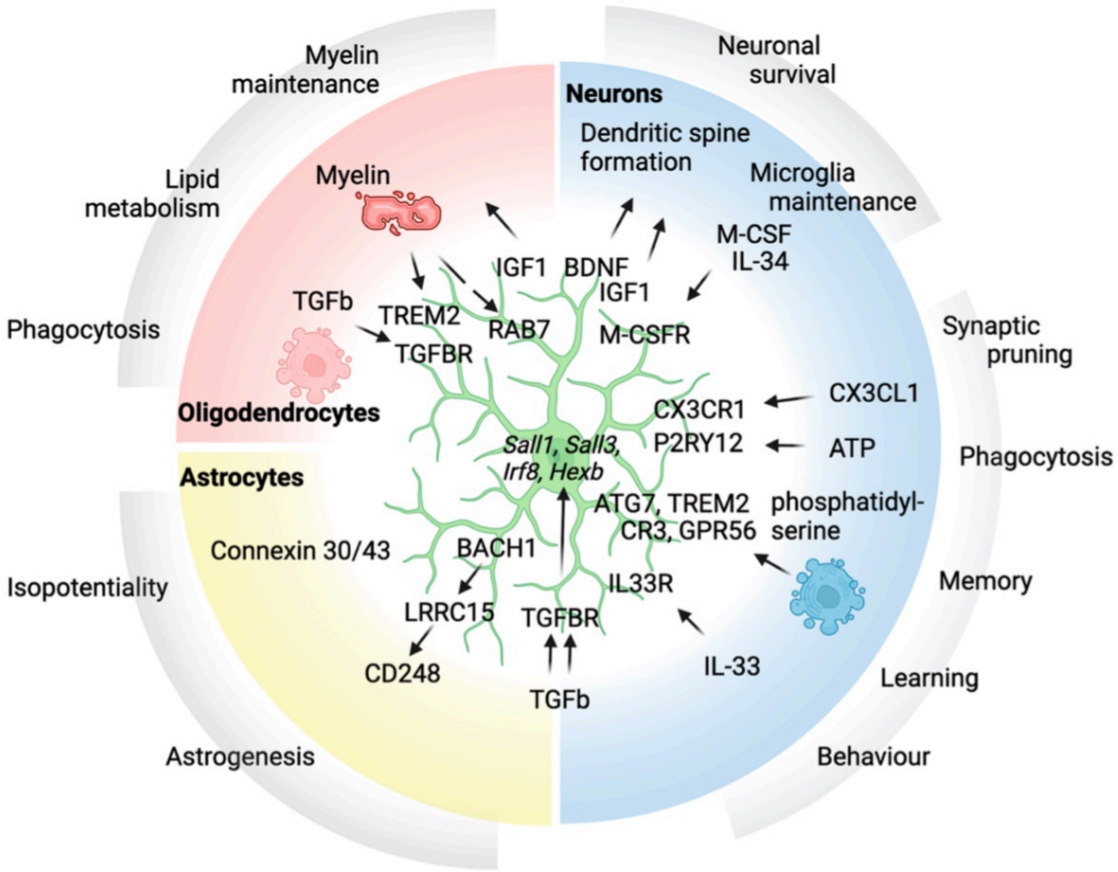

Fig. 1.6 Interaction of microglia with glial cells. Microglia are involved in intercellular communication with neurons, astrocytes, and oligodendrocytes, by contributing crucial factors and activities that are necessary for proper brain function. These functions include apoptotic processes (as synaptic pruning), provision of survival factors, and myelin maintenance. Microglial dysfunction can lead to deficits in learning, memory, and behavior. Created with BioRender.com

Upon formation of nerve tracts during the early postnatal period, a distinct amoeboid microglial population can be detected in the ventricular zone. Accordingly, this area was termed the “fountain of microglia” (Kershman 1939). This subset, also recently called axon-tract-associated microglia (ATM), has been demonstrated to aid in maintaining the structural integrity of the developing brain at vulnerable boundaries, such as the fetal cortico-striato-amygdalar and cortico-septal boundaries, where they prevent the formation of large cavitary lesions. This protective mechanism involves the pleiotropic ATM factor osteopontin (encoded by *Spp1*) (Lawrence et al. 2024). ATMs further interact with oligodendrocyte progenitor cells (OPCs) before myelination and contribute to oligodendrogenesis by controlling OPC numbers through phagocytosis (Hagemeyer et al. 2017; Nemes-Baran et al. 2020). This process of maintaining normal OPC-to-axon ratio as a homeostatic mechanism for proper myelination relies on the CX3CR1 pathway and IGF1 (Wlodarczyk et al. 2017; Nemes-Baran et al. 2020).

However, little is known about how microglia influence astrocyte development and function under steady-state conditions in vivo. Microglia regulate astrocyte-mediated control of neuronal structure and function, as evidenced by their role in modulating the astrocyte syncytium and synaptic transmission in the mouse hippocampus. Depletion of microglia disrupts astrocyte syncytial isopotentiality, decreases the expression of gap junction proteins such as connexins 30 and 43, and weakens synaptic transmission, whereas priming microglia enhances synaptic transmission without affecting astrocyte network function, suggesting context-dependent effects of microglia on astrocytes and neurons (Du et al. 2022) (**Figure 6**). Recent evidence shows that this microglia-astrocyte crosstalk is potentially mediated by the transcription factor BACH1, known for its involvement in microglial metabolic adaptations during early brain development (Wang et al. 2024).

As the CNS matures, microglia play a crucial role in maintaining myelin integrity throughout adulthood. The absence of microglia in *Csf1r*$^{\Delta FIRE/\Delta FIRE}$ mice leads to hyper- and/or demyelination, confirming their fundamental role in preserving myelin integrity (McNamara et al. 2022). The absence of microglia causes oligodendrocytes to accumulate cholesterol and impairs lipid export. This phenotype is driven by TGFβ1 (microglia)-and TGFβR1 (oligodendrocyte)-dependent pathways (McNamara et al. 2022). Myelin breakdown in aged microglia is partially mediated by RAB7, a GTPase involved in neuropathic and lipid metabolism diseases. The absence of *Rab7* in microglia causes the accumulation of endosomal and lysosomal inclusions that are enriched with myelin-derived lipids (Safaiyan et al. 2016) (**Figure 6**). At the same time, microglia showed an activated phenotype with high expression of MHCII molecules (Safaiyan et al. 2016). Microglial TREM2 has also been shown to play a crucial role in controlling neuronal bioenergetics during development (Tagliatti et al. 2024).

In surveying their microenvironment, adult microglia use highly motile processes, as documented by seminal in vivo two-photon microscopic imaging of the mouse neocortex (Nimmerjahn et al. 2005). With these processes, microglia can form purinergic junctions at the neuronal soma, which inhibit neuronal activity and protect neurons from excitotoxicity (Cserép et al. 2020). Neuronal synapses are characterized by the accumulation of externalized phosphatidylserine (Scott-Hewitt et al. 2020; Kurematsu et al. 2022), which can be recognized by different microglial receptors, such as the fractalkine receptor CX3CR1 (Paolicelli et al. 2011), complement receptor 3 (CR3 or CD11b) (Schafer et al. 2012), GPR56 (Li et al. 2020), or the innate immune receptor TREM2 (Filipello et al. 2018). This process

involves microglial P2Y12 receptors, which bring microglial processes near hyperactive ATP-releasing neurons (Haynes et al. 2006; Cserép et al. 2020). Microglia further express the surface ectoenzymes CD39 and CD73, which mediate the rapid breakdown of ATP into adenosine, which reduces neuronal firing through adenosine receptors (Badimon et al. 2020). The interaction between neurons and microglia involves brain-derived neurotrophic factor (BDNF) and tropomyosin receptor kinase B (TrkB) signaling. Deletion of microglial BDNF results in a significant decrease in learning-induced formation of postsynaptic dendritic spines in young and mature adult mice (Parkhurst et al. 2013), even though there is some controversy regarding the role of microglia-derived BDNF (Honey et al. 2022). Synapse reorganization in the adult stage also relies on neuronal cytokine IL-33. Disruption of the IL-33R axis in microglia negatively affects engulfment of the extracellular matrix, affecting the synaptic environment and memory formation (Nguyen et al. 2020). Notably, IL-33 levels decline in aged mice, which contributes to reduced plasticity and memory (Nguyen et al. 2020).

Due to the prominent role of microglia in shaping neuronal connectivity, defects in microglial function can also cause behavioral phenotypes. Deletion of *Csf1* from neuroectodermal derived cells causes Purkinje cell alterations and deficits in motor learning and social memory in adult mice, suggesting cerebellar dysfunction in these animals (Kana et al. 2019). Deficits in social behavior have also been observed in *Trem2*- (Filipello et al. 2018) and *Atg7*-deficient mice (Kim et al. 2017). Also increased elimination of inhibitory synapses in the ventral thalamus can cause hyperexcitability in the thalamocortical circuits and obsessive grooming behaviors as observed in progranulin-deficient mice (Lui et al. 2016). Other approaches, such as microglial ablation and conditional gene knockout, further emphasize the role of microglia in regulating learning and memory (Parkhurst et al. 2013), compulsive behaviors (Chen et al. 2010), and sleep (Liu et al. 2021; Hristovska et al. 2022). Whether dysregulation of these mechanisms is directly linked to autism and other neurodevelopmental and neuropsychiatric conditions is still a matter of debate (Mordelt and de Witte 2023).

In summary, microglia orchestrate various aspects of early brain development. In addition, microglia are involved in synaptic remodeling, perform intercellular communication with other glial elements and thereby represent an indispensable integral element of the CNS.

Development and homeostatic function of kidney macrophages

The kidneys serve to maintain fluid and electrolyte balance, regulate blood pressure, and eliminate metabolic waste products from the body by producing urine. Nephrons, which are the functional units of the kidney (**Figure 7**), filter blood to eliminate waste products such as urea and creatinine, as well as excess substances such as electrolytes and water, while simultaneously reabsorbing important nutrients and maintaining the acid-base balance. Additionally, the kidneys play a critical role in hormone production, including erythropoietin and renin production.

Kidney macrophages (KMs) develop from YS-derived precursors during early embryogenesis (Schulz et al. 2012; Epelman et al. 2014; Hoeffel et al. 2015). However, the proportion of YS-derived macrophages decreases with the arrival of fetal monocyte-derived cells around E14-16 (Epelman et al. 2014). Cell proliferation analysis of KMs indicates that a large proportion of the cells undergo cell division during the first three postnatal weeks, which subsequently declines (Liu et al. 2020). Using the *Ms4a3*^{Cre} lineage tracing mouse model, it was shown that 40-50% of KMs

at the age of three months showed reporter labeling, indicating that a substantial part of the KM pool is derived from monocytes during adulthood (Liu et al. 2019a). Similar results were obtained using the *Cx3cr1*^{CreERT2} tamoxifen-inducible fate mapping system. Labeled embryonic-derived KMs declined during the first two months of age, which was accompanied by an increase in unlabeled adult monocyte-derived KMs (Liu et al. 2020). Accordingly, two populations of tissue-resident macrophages of different origins might coexist in the kidney: embryo-derived renal macrophages (E-KMs) and BM-derived renal macrophages (BM-KMs). E-KMs are characterized by higher expression of F4/80 and CX3CR1, while BM-KMs show increased expression of CCR2 and CD11c (Liu et al. 2020). Unbiased single-cell sequencing also suggested the presence of two distinct KM subsets, of which one expressed high levels of *C1qa*, *Cd81*, *Cd63*, and *Pf4* (Cd63^{+} KMs), while the other subset was expressed in addition to the KM markers *C1qa* and *Cd81* and the genes *Ccr2*, *Mmp12*, and *Clec12a* (Ccr2^{+} KMs) (Yashchenko et al. 2023). However, both subsets were found to be equally labeled in *Ms4a3*-Cre reporter animals, suggesting that both subsets were at least partially derived from monocytes (Yashchenko et al. 2023).

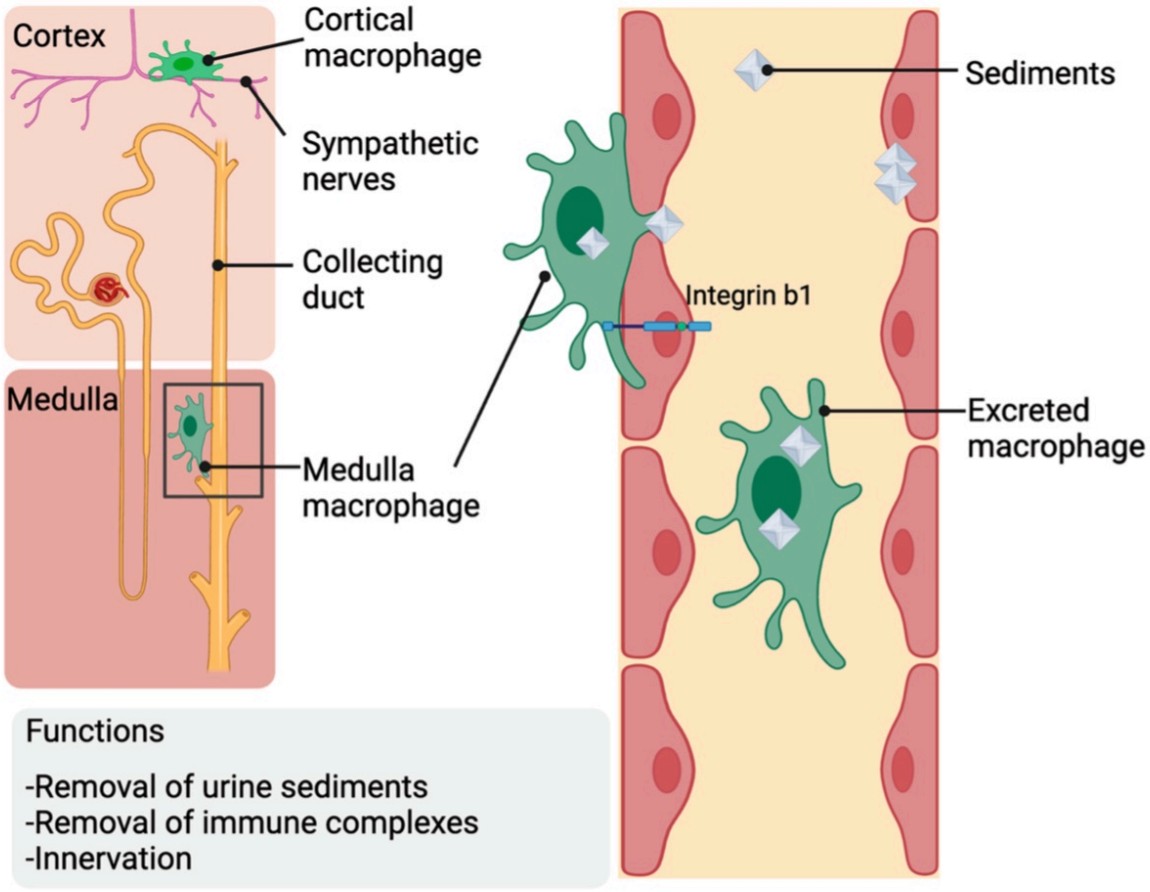

Fig. 1.7 Localization and function of renal macrophages. The nephron spans the cortical and medullary areas of the kidney (left). The culminating collecting ducts harbor macrophages that form transepithelial protrusions to scavenge larger particles in the urine, which can otherwise potentially induce kidney stone formation and obstruction (right). These macrophages can also be excreted in urine. Cortical renal macrophages are associated with sympathetic nerves, innervation control, and nerve activity. Created with BioRender.com

E-KMs were found in higher densities in the medulla compared to the cortex and papilla of the kidney (Liu et al. 2020), where they locate in spatial proximity to renal tubules. These juxtatubular macrophages extend long integrin β1-dependent

transepithelial protrusions through the tubular epithelial monolayer, which is less frequently observed in the cortex (He et al. 2024) (**Figure 7**). These protrusions have been described to penetrate the laminin^{+} basement membrane and epithelial cells, suggesting that they are exposed in the tubular lumen (He et al. 2024). Indeed, transepithelial protrusions were found to show extension-and-retraction movements, with signs of late endosome/lysosome accumulation. The sampling ability of KMs was demonstrated using fluorescent latex bead injection experiments into the kidney pelvis. Beads associated with macrophage transepithelial protrusions were readily visible, and depletion of macrophages led to longer retention of beads in the lumen of the collecting ducts, suggesting that urine flushing alone was insufficient for the removal of larger particles (He et al. 2024). However, KMs are not only involved in transepithelial removal of large particles. They are also able to transmigrate into the lumen, associate with particles that otherwise potentially lead to tubule blockade, and be ultimately excreted in the urine (He et al. 2024) (**Figure 7**). Compared to embryonic-derived macrophages in the brain, heart, lung or liver, E-KMs in the kidney medulla have higher proliferative rates (He et al. 2024). One possible explanation for this observation might be that the proportion of transmigrated macrophages lost in the urine must be continuously replaced.

KMs localized on the abluminal side of the endothelium of peritubular capillaries are also involved in immune surveillance. By sampling the collection ducts, renal macrophages can instantly detect opsonized particles. These particles can be small immune complexes that can otherwise cause type III hypersensitivity reactions. Renal macrophages are located close to the endothelial cells around the glomeruli and tubules, where they can pick up particles through trans-endothelial transport. The uptake of these foreign particles is dependent on the scavenging receptor FcγRIV, and triggers a local immune response characterized by the release of cytokines, which leads to the recruitment of neutrophils and monocytes to the target tissue (Stamatiades et al. 2016).

KMs express high levels of CX3CR1. Genetic deletion of this chemokine receptor significantly reduces medullary KM numbers by almost 50% in adult mice (Mass et al. 2016; He et al. 2024), and leads to intratubular sediments containing calcium and lipids (He et al. 2024). Since the kidney niche is not replenished by monocytes in *Cx3cr1*-deficient mice, it can be speculated that CX3CR1 is indispensable for KM maintenance, independent of cellular origin. Indeed, *Cx3cr1*-deficiency strongly affected the gene expression of Cd63^{+} KMs with a shift towards immune activation (Yashchenko et al. 2023). Of note, not medullary Cd63^{+} KMs but cortical *Ccr2*$^{+}$ KMs (identified in *Ccr2*$^{Rfp/wt}$ *Cx3cr1*$^{Gfp/wt}$ animals) were numerically reduced in *Cx3cr1*-deficient mice (Yashchenko et al. 2023). However, if *Ccr2*$^{+}$ KMs differ in function from medullary E-KM, needs to be shown in the future. Cortical E-KMs are also associated with sympathetic nerves (**Figure 7**). Deletion of KMs in *Cx3cr1*^{CreER} *Rosa26*^{Dtr} mice causes a pronounced reduction in renal innervation and reduced renal sympathetic nerve activity (Zhu et al. 2023). Consequently, a decline in plasma renin concentration, decreased kidney noradrenaline levels, and an increase in glomerular filtration rate could be detected, culminating in excessive Na^{+}, K^{+}, and Cl^{-} excretion and water loss in KM-deficient mice (Zhu et al. 2023).

Collectively, these results indicate that KMs play an important role in immune surveillance and urine monitoring by facilitating the removal of immune complexes and urine particles, suggesting that KMs may critically participate in kidney stone formation. KMs further contribute to the sympathetic innervation and ion homeostasis in the kidneys.

Development and homeostatic function of adipose tissue macrophages

Adipose tissue is a specialized connective tissue primarily composed of adipocytes, which are fat cells that store energy in the form of triglycerides. However, it also has other important functions such as insulation to maintain body temperature, thermoregulation, and providing energy during periods of fasting or increased energy demands. Additionally, adipose tissue secretes adipokines, hormones, and cytokines, which regulate metabolism, inflammation, and appetite. Imbalances in these functions can contribute to metabolic disorders such as type 2 diabetes, cardiovascular diseases, and inflammatory conditions.

The earliest observation of adipose tissue macrophages (ATMs) in fat tissue can possibly be attributed to Gilchrist and Ketron in 1916. They observed macrophages that phagocytosed adipocytes in subcutaneous fat samples. Decades later, studies reported chronic inflammation in multiple tissues and organs of obese and/or diabetic patients (Hartroft 1960; Braillon et al. 1985). It was also observed that macrophage-secreted cytokines could induce insulin resistance in preadipocytes in vitro (Pekala et al. 1983) and that *Tnf* expression in the adipocyte fraction correlated with obesity (Hotamisligil et al. 1993; Hotamisligil et al. 1994). Accordingly, neutralization of TNF-signaling partially restored the uptake of glucose in response to insulin, which emphasizes that chronic inflammation of the white adipose tissue (WAT) might contribute to obesity (Uysal et al. 1997). The subsequent discovery that BM-derived macrophages in irradiated chimeras accumulate in the expanding adipose tissue during obesity further supports the idea that inflammation contributes to pathology (Weisberg et al. 2003; Xu et al. 2003). These studies also demonstrated that macrophages, but not adipocytes, are the major source of TNFα in WAT. Lumeng and colleagues described phenotypic changes in ATMs as consequence of obesity, and suggested that BM-derived macrophages are different cells compared to ATMs in homeostasis (Lumeng et al. 2007a; Lumeng et al. 2007b). Around the same time, $LYVE1^{+}$ macrophages can be detected in early postnatal epididymal WAT (Cho et al. 2007; Han et al. 2011), suggesting the presence of a tissue-resident macrophage population under homeostatic conditions. Nevertheless, it was only in 2017 that the embryonic, BM-independent origin of tissue-resident ATMs was demonstrated in *Cx3cr1*^{CreERT2} fate mapping mice (Hassnain Waqas et al. 2017). However, WAT contains not only one homogenous subset of resident macrophages, as shown by scRNA-Seq of cell suspensions derived from multiple tissues (Chakarov et al. 2019). Similar to the situation in the lung, two general macrophage populations could be identified across different tissues: a population characterized by low expression of LYVE1, but high expression of MHCII and CX3CR1 (named $Lyve1^{low}MHCII^{hi}$), and a population with high LYVE1 expression, but low MHCII and CX3CR1 expression ($Lyve1^{hi}MHCII^{low}$) (Chakarov et al. 2019). Both subsets were derived from embryonic precursors, but were progressively replenished by BM-derived monocytes in WAT.

White adipose tissue macrophages: $LYVE1^{+}$ macrophages can be identified at a high density in the hypoxic tip region of epididymal WAT, which harbors a dense vascular network (DVN) (Cho et al. 2007). Systemic depletion of macrophages by clodronate liposome injection reduced vascular sprouting and DVN size in adult mice (Cho et al. 2007). These macrophages expressed CXCR4 and various matrix metalloproteinases (MMPs). Proteolytic inactivation of MMPs not only reduced DVN formation and vascular sprouting but also prevented macrophage infiltration. A similar function was observed when VEGF-VEGFR1 and SDF1-CXCR4 were blocked (Cho et al. 2007). ATMs are also important for angiogenesis during the early postnatal epididymal adipose tissue development. Systemic depletion of

macrophages causes avascular regions with reduced adipocyte coverage (Han et al. 2011). These data support the notion that Lyve1^{hi}MHCII^{low} macrophages participate in angiogenesis and WAT formation. Systemic depletion of myeloid cells in *LysM*^{Cre} *Rosa26*^{Dtr} mice has also suggested that macrophages play a role in energy homeostasis, contributing to the hypothalamic regulation of appetite (Lee et al. 2014). After depletion, mice lost weight owing to decreased food intake, which was linked to lower STAT3 signaling in the hypothalamus. However, the *LysM*^{Cre} *Rosa26*^{Dtr} approach is not specific to WAT macrophages and results in cell death of multiple macrophage subsets. Therefore, it is difficult to assign complex behavioral observations to a single macrophage population.

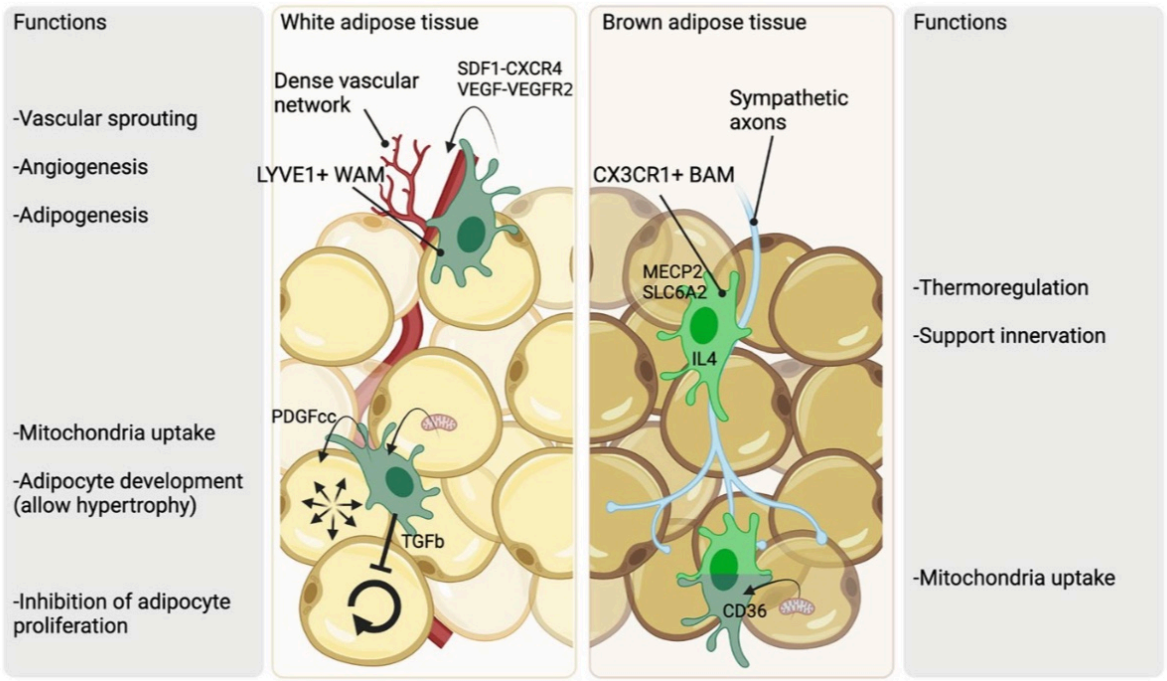

Fig. 1.8 Adipose tissue macrophages. Two main subsets of adipose tissue macrophages can be identified in white (left) and brown adipose tissue (right): Lyve1^{hi}MHCII^{low} (dark green) and CX3CR1^{+}Lyve1^{low}MHCII^{hi} (light green) macrophages. Lyve1^{hi}MHCII^{low} macrophages are involved in angiogenesis and blood vessel sprouting in white adipose tissue. They further contribute to adipocyte maintenance by inhibiting proliferation and mediating hypertrophy. CX3CR1^{+} macrophages in brown adipose tissue are involved in thermoregulation, which is mediated by IL-4, MECP2, and SLC4A1. Adipose macrophages can take up damaged mitochondria from adipocytes. WAM - White Adipose tissue-resident Macrophage; BAM - Brown Adipose tissue-resident Macrophage. Created with BioRender.com

Other depletion methods, such as *Csf1r*^{Cre} *Csf1r*$^{fl/fl}$ or *Tnfrsf11a*^{Cre} *Spi1*$^{fl/fl}$ mice, have also revealed that embryonic-derived macrophages are important modulators of adipocyte hypertrophy during the first postnatal week. The absence of macrophages in ATMs decreases fat pad size, which harbors smaller adipocytes with low lipid content (Cox et al. 2021). Importantly, the introduction of *Csf1r* deletion to the hematopoietic lineage using *Flt3*^{Cre} mice did not affect adipocyte development and hypertrophy, thereby excluding the role of monocyte-derived cells in neonatal adipose tissue expansion (Cox et al. 2021).

Further evidence came from experiments in which CD206^{+} WAT macrophages, a subset that likely corresponds to *Cd206*-expressing Lyve1^{hi}MHCII^{low} macrophages (Chakarov et al. 2019), were depleted in adult *Cd206*^{Dtr} mice. Under these circumstances, increased proliferation of adipocyte precursor cells was evident,

which was TGFβ-dependent (Nawaz et al. 2017). This resulted in improvements in glucose tolerance and insulin sensitivity under physiological conditions, suggesting that WAT macrophages may regulate the balance between quiescence and proliferation of adipocyte precursor cells in response to nutritional changes. Genetic deletion of tribbles homolog 1 (*Trib1*) also causes a significant reduction in different tissue-resident macrophage subsets, including epididymal WAT macrophages (Satoh et al. 2013). Furthermore, epididymal WAT mass was significantly reduced in *Trib1*-deficient mice, indicating a lipodystrophic phenotype, which could be rescued when *Trib1*-deficient mice were reconstituted with *Trib1*-proficient BM cells (Satoh et al. 2013). Hematopoietic *Trib1*-deficiency also caused increased levels of non-esterified fatty acids and glycerol in the serum, indicative of enhanced lipolysis (Satoh et al. 2013). Another factor contributing to the function of WAT macrophages is platelet-derived growth factor C (*Pdgfc*). Similar to the defects observed in macrophage-deficient mice, macrophage-restricted deletion of *Pdgfc* decreased white adipose tissue size, and adipocytes were smaller and contained fewer lipids (Cox et al. 2021). These data suggest that macrophage-derived PDGFcc promotes lipid storage via lipid synthesis in white adipocytes within the fat pads.

Similar to the situation in the heart, WAT macrophages are able to uptake mitochondria from adipocytes, which influences their transcriptome and causes the upregulation of the hypoxia-inducible factor (HIF)-1 α-related pathway and downregulation of genes associated with electron transport (Brestoff et al. 2021). Genes involved in heparan sulfate biosynthesis, including *Ext1*, are crucial for this mitochondrial uptake. The deletion of *Ext1* in macrophages increases body weight and absolute epididymal WAT mass, resulting in impaired glucose and insulin tolerance under steady-state conditions (Brestoff et al. 2021). Accordingly, macrophages remain in immunometabolic crosstalk with adipocytes and are able to adapt to microenvironmental changes through mitochondrial transfer.

Brown adipose tissue macrophages: Brown adipose tissue (BAT) in homeotherms is crucial for maintaining a constant body temperature even in colder environments. Previously, it was thought that this mechanism relies exclusively on the hypothalamus, which triggers sympathetic discharge upon cold temperature exposure, resulting in the release of catecholamines into WAT and BAT. Norepinephrine then binds to the adipocyte β3 adrenergic receptor and activates thermogenesis via the cyclic AMP/protein kinase A (cAMP/PKA) pathway (Chouchani et al. 2019). However, a seminal study showed that ATMs can also participate in the regulation of norepinephrine levels and thereby contribute to thermoregulation (Nguyen et al. 2011). It was first observed that cold exposure increased CD206, CD301, and arginase 1 protein levels in BAT and WAT macrophages, indicative of an alternatively activated macrophage phenotype (Nguyen et al. 2011). Mice with a defective IL-4 signaling pathway, the main cytokine involved in alternative activation, were then shown to be susceptible to cold-induced hypothermia, which was accompanied by decreased expression of cold-inducible thermogenic genes, reduced noradrenaline levels, and low levels of circulating free fatty acids (Nguyen et al. 2011). Injection of IL-4 reversed these effects in wild-type mice. This indicates that alternative M2 activated macrophages contribute to the adaptive and facultative aspects of thermogenesis. Notably, IL-4 is not only required for alternative activation, but also regulates the proliferation and cellular aging of some tissue-resident macrophages, such as peritoneal macrophages and splenic RPMs (Jenkins et al. 2011; Jenkins et al. 2013; Okreglicka et al. 2021; Zhou et al. 2024). Therefore, it is possible that IL-4 may be similarly involved in ATM maintenance.

The role of BAT macrophages in thermogenesis was further studied in mice with a *Cx3cr1*^{CreERT2}-restricted deletion of *Mecp2*, which is related to neurodegenerative Rett syndrome (Wolf et al. 2017). Interestingly, the *Mecp2*-deficiency in CX3CR1^{+} cells (which include microglia) did not cause neurodevelopmental symptoms in mice but resulted in spontaneous obesity. Mice with *Mecp2*-deficient macrophages produced less heat than controls, suggesting impairment of BAT thermogenesis. Indeed, microscopy revealed fewer sympathetic axons innervating BAT upon deletion of *Mecp2* and reduced local titers of norepinephrine, which resulted in lower expression of thermogenic factors by adipocytes (Wolf et al. 2017). The molecular mechanism involves the clearance of norepinephrine by BAT macrophages via the solute carrier family 6 member 2 (SLC6A2) transporter, and monoamine oxidase A, a degradation enzyme (Pirzgalska et al. 2017). Deletion of *Slc6a2* increases BAT content, causes browning of white fat, increases thermogenesis, and leads to weight loss in obese mice. These results indicate that CX3CR1^{+} macrophages play a crucial role in BAT innervation and thermogenesis. Since both studies used a CX3CR1 dependent system for the identification and targeting of BAT macrophages, the role of Lyve1^{hi}MHCII^{low} macrophages in BAT remains unclear.

BAT macrophages are also able to take up oxidatively damaged mitochondria from adipocytes in a CD36-dependent manner (Rosina et al. 2022). Accordingly, the *in vivo* depletion of BAT macrophages causes the accumulation of extracellular vesicles containing mitochondrial proteins, accumulation of lipids, and failure to initiate the thermogenic response to cold exposure (Rosina et al. 2022).

In summary, adipose macrophages are important for adipose tissue development, angiogenesis, innervation, thermogenesis, and the regulation of lipid metabolism. However, despite significant advances in adipose tissue macrophage research, the exact subset-specific functions of Lyve1^{hi}MHCII^{low} and CX3CR1^{+} Lyve1^{low}MHCII^{hi} macrophages remain to be elucidated.

Macrophages in other tissues and their homeostatic functions

The previous paragraphs represent a selection of some of the most studied tissue-resident macrophage populations to date. However, macrophages inhabit all vertebrate organs and there are accordingly numerous other populations beyond those discussed in this chapter. Given the crucial role of macrophages for organ and tissue integrity, research of these cells is rapidly increasing and extending to additional, less studied tissue populations. A detailed discussion of all of these populations is beyond the scope of this chapter, but to further illustrate the diversity and tissue specificity of macrophages, a few additional populations are (briefly) mentioned below.

Within the *peritoneal cavity*, large peritoneal macrophages (PMs) express the lineage determining transcription factor *Gata6*, which is induced by retinoic acid, a metabolite of vitamin A secreted by stromal cells in the omentum and visceral adipose tissues and essential for PM identity (Okabe and Medzhitov 2014; Buechler et al. 2019). Embryonic-derived PMs are long-lived cells and are gradually outcompeted by monocyte-derived cells with age (Bain et al. 2016). Primed by the peritoneal microenvironment and regulated by the transcription factors KLF2 and KLF4, PMs monitor visceral organs and related mesothelium and efficiently clear apoptotic cells. This mitigates inflammation caused by self-derived nucleic acids, while retaining responsiveness to infections (Roberts et al. 2017). PMs can also be recruited to inflammatory reactions that occur in peritoneal cavity-associated organs,

such as the intestine (Honda et al. 2021) or the heart (Deniset et al. 2019). As a result, they may act as a local source of immune cells in these locations.

Lymph nodes are home to two main types of macrophages: subcapsular sinus macrophages (SSMs) and medullary sinus macrophages (MSMs). The subcapsular sinus is a significant entry point for pathogens where the afferent lymph flows into the lymph nodes. At this location, $CD169^{+}F4/80^{-}SIGNR1^{-}$ SSMs act as a physical barrier that prevents the entry of large molecules into the conduit system and the migration of cells into the parenchyma. Consequently, SSMs are well positioned to filter any material that reaches the lymph nodes via the lymph from the periphery, including viruses, bacteria, pathogen-derived antigens, and immune complexes. They are able to translocate antigens across the subcapsular sinus and to activate B cells (Phan et al. 2007; Phan et al. 2009). Similar to MMMs in the spleen, differentiation of SSMs relies on lymphotoxin signaling (Phan et al. 2009; Moseman et al. 2012). $CD169^{+}F4/80^{+}SIGNR1^{+}$ MSMs, on the other hand, are lymphotoxin-independent and can be found in the cortical interfollicular region between the B cell follicles in the cortex and medullary sinuses. MSMs appear to be highly phagocytic cells with increased endocytic and degradative capabilities compared to SSMs (Phan et al. 2009), but it remains unclear to what extent the two macrophage subsets participate in antigen presentation and contribute to lymph node architecture under steady-state conditions.

Similar to the spleen and lymph nodes, the *thymus* also contains macrophages, which play an essential role in efferocytosis during negative and positive selection of thymocytes. This metabolic process is driven by the pentose-phosphate pathway (Esashi et al. 2003; Tsai et al. 2022). Two distinct subsets of thymic macrophages have been identified: embryonic-derived $TIM\text{-}4^{+}$ cells in the cortex, and monocyte-derived $CX3CR1^{+}$ macrophages at the cortico-medullary junction. Embryonic thymic macrophages bear similarities to splenic red pulp macrophages and Kupffer cells because of their *SpiC* expression (Zhou et al. 2022). However, the precise function of each thymic subset during T-cell development and tolerance formation remains unclear.

The *bone* contains at least three types of macrophages: erythroblastic island macrophages (EIMs), osteoclasts, and osteal macrophages (sometimes also referred to as osteomacs) (Chang et al. 2008). EIMs are similar to red pulp macrophages in the spleen and are likewise involved in red blood cell clearance. Accordingly, *Pparg* and *SpiC* crucially orchestrate their development and function (Okreglicka et al. 2021). Osteoclasts are unique multinucleated giant cells in the BM that are involved in bone resorption. This specialized cell type develops from EMP-derived myeloid cells (Jacome-Galarza et al. 2019) that fuse in a M-CSF and RANK ligand dependent manner (Lacey et al. 1998). Downstream of the RANK ligand pathway, the activation of NF-kB and MAPK signaling results in metabolic reprogramming of osteoclasts, which involves NFATc1, a crucial regulator of osteoclastogenesis (Takayanagi et al. 2002). In addition, monocytes can contribute to multinucleated giant cell formation during a defined postnatal time window (Jacome-Galarza et al. 2019). The functional ability of osteoclasts to remodel the bone can have consequences for the hematopoietic niche, which affects HSC mobilization, proliferation, and retention (Adams et al. 2006; Kollet et al. 2006; Lymperi et al. 2011). Defective osteoclast activity further contributes to osteopetrosis and BM failure, and significantly impairs erythropoiesis (Chow et al. 2013). Non-multinucleate osteal macrophages can, in conjunction with megakaryocytes, also influence the hematopoietic niche (Mohamad et al. 2017), but are mainly involved in bone homeostasis by promoting bone

deposition instead of bone resorption as osteoclasts. Accordingly, osteal macrophages regulate osteoblast function, as observed in *Cd169*^{Dtr} mice. Deletion of osteal macrophages in this model system causes a significant reduction in osteoblasts (Batoon et al. 2019), thereby reducing bone mass (Cho et al. 2014) and demonstrating the role of osteal macrophages in bone anabolism.

Langerhans cells are located in all *epithelial barriers*. While skin Langerhans cells are of embryonic origin and only minimally replenished by monocyte-derived cells (Hoeffel et al. 2012), other Langerhans cell subsets such as in the oral cavity can be of mixed origin, including monocyte and pre-dendritic cell precursors (Capucha et al. 2015). The development of epidermal Langerhans cells is regulated by the three cytokines TGF-β (Zahner et al. 2011), IL-34 (Greter et al. 2012), RANKL (Barbaroux et al. 2008), the mTOR signaling pathway (Kellersch and Brocker 2013; Sparber et al. 2014), and the transcription factors *Rara* (Hashimoto-Hill et al. 2018), *Runx3* (Chopin et al. 2013), and *Id2* (Hacker et al. 2003), of which the latter two are direct targets of TGF-β. It was shown that Langerhans cells selectively and specifically induce the activation and proliferation of skin-resident regulatory T-cells and thereby maintain tolerance in normal skin (Seneschal et al. 2012). In addition to their role as immunological gatekeepers of tissue barriers, Langerhans cells are involved in apoptotic cell clearance and intercellular communication. Langerhans cells directly communicate with keratinocytes, thereby maintaining a constant epithelial cell-to-immune cell ratio across the basal layer of the epidermis (Park et al. 2021). Accordingly, immune cell density increases when the number of keratinocytes increases (Park et al. 2021). However, the depletion of skin-resident immune cells does not affect epidermal thickness or epithelial basal density, and the role of Langerhans cells in homeostatic skin requires further investigation. Langerhans cells are also involved in skin histiocytosis, a condition characterized by the accumulation of mononuclear phagocytes in granulomatous lesions (Allen et al. 2018). It was shown that activating somatic mutations in genes involved in the mitogen-activated protein kinase pathway are sufficient to drive the development of histiocytosis in a Langerhans cell-dependent manner (Bigenwald et al. 2021).

The *intestine* contains short-lived monocyte-derived *Cx3cr1*-expressing macrophages in the mucosal lamina propria below the intestinal epithelium, which serve as a frontline defense against invading pathogens. These cells require constant IL-10-dependent immune silencing, which otherwise results in epithelial hyperplasia and erosion and causes severe spontaneous colitis (Zigmond et al. 2014). Moreover, intestinal macrophages are involved in the efferocytosis of apoptotic material, which contributes to the repression of pro-inflammatory pathways, thereby securing epithelial integrity and mitigating colitis (Cummings et al. 2016). Additional embryonic-derived gut macrophage populations include blood vessel-associated macrophages, which are crucial for vascular integrity (Honda et al. 2020), and neuron-associated macrophages within the muscularis region essential for enteric neuron survival (Gabanyi et al. 2016; De Schepper et al. 2018).

Two macrophage subsets with distinct phenotypes (F4/80^{hi} and F4/80^{lo} subsets) are evident within *mammary glands*, where they are involved in various developmental processes, including ductal development and epithelial remodeling (Jäppinen et al. 2019; Dawson et al. 2020). Additionally, a distinct CX3CR1^{+} macrophage subset arises during lactation and may play a role in immune surveillance, and possibly milk production (Cansever et al. 2023).

Similar to the situation observed in adipose tissue and the lungs, Lyve1^{hi}MHCII^{low} and Lyve1^{low}MHCII^{hi} macrophages can be found in various organs,

including the bladder, testis, ganglia, trachea, tongue, and others (Chakarov et al. 2019; Dick et al. 2022). It seems that both subsets are derived from fetal precursors during embryogenesis, but that the *Cx3cr1*-expressing Lyve1^{low}MHCII^{hi} population is replenished faster by BM-derived monocytes during the postnatal phase (Lyras et al. 2022). Lyve1^{low}MHCII^{hi} macrophages have been suggested to be more frequently associated with nerve bundles, whereas Lyve1^{hi}MHCII^{low} macrophages are located in proximity of blood vessels and are transcriptionally enriched in pathways related to angiogenesis (Chakarov et al. 2019). It is possible that the anatomy of an organ with distinct areas of high vessel density and nerve bundles, respectively, creates distinguished macrophage niches. *Cx3cr1*-expressing Lyve1^{low}MHCII^{hi} macrophages in the tongue, for instance, are preferentially located in the highly innervated lamina propria beneath the tongue epidermis, whereas FOLR2^{+} (Lyve1^{hi}MHCII^{low}) macrophages reside in deeper muscular tissues (Lyras et al. 2022). As described above, an association of *Cx3cr1*-expressing Lyve1^{low}MHCII^{hi} macrophages with nerves and of Lyve1^{hi}MHCII^{low} cells with vessels could also be detected in adipose tissue (**Figure 8**) and skin (Kolter et al. 2019). These data suggest that a general functional division of labor may exist between the two macrophage subsets, which can be found in interstitial or connective tissues. Therefore, it is possible that Lyve1^{low}MHCII^{hi} macrophages are functionally connected to neurons in many tissues and that Lyve1^{low}MHCII^{hi} participates more preferentially in angiogenesis. However, if this holds true for all interstitial tissues must be individually proven.

Conclusion

For a long time, macrophages have been solely recognized as immune cells that primarily perform functions in immune defense and surveillance. This prevailing view is currently changing, and macrophages are slowly starting to be appreciated for their role in tissue homeostasis and maintenance. Therefore, the fact that functional or developmental defects of macrophages can lead to organ dysfunctions and pathological manifestations is a relevant finding that also needs to be considered in the clinic. Furthermore, it is possible that self-renewing macrophages that arise during embryogenesis cannot be functionally replaced, at least not entirely, by monocytes during adulthood. However, these observations are currently based on experimental inbred mice, and await confirmation in humans. Nevertheless, macrophages occupy discrete niches, which provide crucial support to essential cell populations such as cardiomyocytes, neurons, and blood vessels. Breaking intercellular communication during inflammation, drug treatment such as chemotherapy, or organ transplantation can cause, at least temporarily, fluctuations in these delicate functions, which may delay healing and contribute to disease complications.

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