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Physiology and diseases of tissue-resident macrophages

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Abstract

Embryo-derived tissue-resident macrophages are the first representatives of the haematopoietic lineage to emerge in metazoans. In mammals, resident macrophages originate from early yolk sac progenitors and are specified into tissue-specific subsets during organogenesis—establishing stable spatial and functional relationships with specialized tissue cells—and persist in adults. Resident macrophages are an integral part of tissues together with specialized cells: for instance, microglia reside with neurons in brain, osteoclasts reside with osteoblasts in bone, and fat-associated macrophages reside with white adipocytes in adipose tissue. This ancillary cell type, which is developmentally and functionally distinct from haematopoietic stem cell and monocyte-derived macrophages, senses and integrates local and systemic information to provide specialized tissue cells with the growth factors, nutrient recycling and waste removal that are critical for tissue growth, homeostasis and repair. Resident macrophages contribute to organogenesis, promote tissue regeneration following damage and contribute to tissue metabolism and defence against infectious disease. A correlate is that genetic or environment-driven resident macrophage dysfunction is a cause of degenerative, metabolic and possibly inflammatory and tumoural diseases. In this Review, we aim to provide a conceptual outline of our current understanding of macrophage physiology and its importance in human diseases, which may inform and serve the design of future studies

Macrophages were initially described in invertebrates in the influential works of Elie Metchnikoff as serving as cellular sensors and healers of infection and tissue damage[1]. More recent work has confirmed and extended these findings and characterized an embryo-derived macrophage lineage that is conserved in metazoans from *Drosophila* to human[2–9]. These macrophages are characterized by their persistence in adults and their stable and close association with specialized tissue cells and are thus termed ‘resident macrophages’[10]. This resident macrophage lineage is genetically, developmentally and functionally distinct from macrophages derived from haematopoietic stem cells (HSCs) and circulating monocytes[5,6,9,11–16] (Fig. 1).

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Contributions

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Tissue-resident macrophages are present in most tissues and are endowed with general as well as tissue-specific functions that can be described as ancillary. Resident macrophages integrate signals from a wide range of environmental sensors[17–32] to orchestrate adaptative cellular responses that are critical for the growth, remodelling and homeostasis of specialized tissue cells[30,33–58] (Fig. 2). In support of this wide spectrum of functions, embryos lacking macrophages are frequently not viable, and neonates are severely diminished in size or exhibit early-onset developmental and degenerative diseases, depending on their genetic background[33,38,55,59–62]. In mice and humans, inherited genetic defects in macrophages result in severe and often fatal developmental disorders that include neurodevelopmental delay and dementia[59,60,63–66], bone deformities[38,40,59–62,64,67,68], tissue repair and remodelling defects[42,69–71], liver[72], spleen[72], reproductive[73], lung[74,75] and cardiac[43,74] dysfunction, and chronic inflammation and autoimmunity[33].

Over the past decade, key conceptual advances arising from the analysis of fate-mapping models and transcriptional and epigenetic studies[14,15,76–78] have enabled investigators to begin to decipher the functions of macrophages on the basis of their origin and anatomical location[55,79–81]. More importantly, these tools have started to enable the identification of precise molecular determinants of macrophage function in health, as well as developmental, degenerative, tumoural and inflammatory diseases. In this Review, we outline our current understanding of the biology of resident macrophages and their roles in health and disease.

Developmental origins underly functions

The origin and the differentiation process of resident macrophages from their early embryonic haematopoietic progenitors are expected to shape their functional properties and roles in homeostasis and disease. Therefore, understanding macrophage ontogeny and specification is fundamental for understanding their function. Early haematopoiesis remains incompletely understood, but at least three types of temporally and genetically distinct haematopoietic progenitor have been described in vertebrates[16] (Fig. 1).

Primitive haematopoiesis

The first blood cell progenitor arises from posterior plate mesoderm in the extra-embryonic yolk sac in a RUNX1-independent manner in zebrafish and mice. This progenitor gives rise to primitive red blood cells and megakaryocytes in both species and to early granulocytes and macrophages in zebrafish[3,82–85]. It has been suggested that microglia in the mouse brain originate from this primitive haematopoietic progenitor[9]. However, macrophages cannot develop in the absence of RUNX1 in mice, and human macrophages do not develop in vitro in the absence of RUNX1[33,83]; thus, in the absence of further experimental evidence, a primitive haematopoietic origin of macrophages is uncertain.

Erythro–myeloid progenitors

A second ‘wave’ of haematopoietic progenitors, EMPs, has been shown to give rise to erythroid cells, megakaryocytes, mast cells, granulocytes and resident macrophages[4,14,15,85–89]. EMPs arise from the yolk sac haemogenic endothelium in mice[14,86,87,90,91] in a RUNX1-dependent and MYB- and NOTCH1-independent manner,

and colonize the fetal liver[4,14,92]. EMP-derived macrophage precursors (PreMacs) colonize the entire embryo and differentiate into tissue-resident macrophages concurrently with the onset of organogenesis, before the emergence of HSCs[14,15]. EMPs disappear during fetal life, but resident macrophages and some mast cells persist and self-renew in adults[2,4–6,14,15,86–89,93].

Haematopoietic stem cells

Finally, HSCs represent the third well-characterized wave of haematopoietic progenitors, and emerge from the intra-embryonic haemogenic endothelium in mice[90,94–96]. HSCs migrate first to the fetal liver, where they expand and then colonize the bone marrow before birth[16]. Unlike EMPs, HSCs are endowed with self-renewing capacity and give rise to new erythroid, lymphoid and myeloid cells—including macrophages—throughout the life of the animal[6,33,97]. All fate-mapping studies but one suggest that fetal HSCs do not give rise to resident macrophages[14,78,90,98]. Thus the available data strongly support the EMP origin of resident macrophages. Further work using new technical approaches including DNA barcoding[99] should help to elucidate this model further.

On the basis of experimental evidence[5,6,10,13,16,80,100–102], it can be proposed that resident macrophages are long-lived cells that self-renew locally in tissues, whereas HSC-derived macrophages are short-lived, rely on circulating monocytes for their renewal, and can massively expand upon challenge. Exceptions are noted[97,103], as the gut lamina propria is populated by HSCs and bone marrow monocyte-derived macrophages (BMDMs) which may self-maintain[6,97], whereas resident macrophages are present in submucosal and myenteric plexus and Peyer's patches[103]. The dichotomy between early self-renewing resident macrophages and short-lived myeloid cells is conserved in *Drosophila*. Resident-like macrophages originate from the procephalic mesoderm as the first haematopoietic lineage and colonize the larva[104]. They survive metamorphosis to be present in adult flies[105], together with a second haematopoietic lineage coming from the lymph gland in the larval stages[106]. Reminiscent of HSCs, lymph gland progenitors in *Drosophila* can give rise to large numbers of macrophages in response to infectious challenge[107,108]. The hypothesis that adult mouse tissues contain EMP- and HSC- derived myeloid cells with distinct functions has implications for the design of studies investigating the molecular determinants of their specialized functions[10,109].

Models of macrophage specification

Resident macrophages in different tissues exhibit distinct chromatin landscapes, transcriptional profiles and functional characteristics, which are built up in a process of macrophage specification that takes place during embryonic development and is maintained after birth[8,15,17,21,76,77,110].

Cell-intrinsic lineage-determining factors

At the onset of organogenesis, preMacs express a core macrophage transcriptional programme that includes the transcription factors PU.1, cMAF and IRF8. PreMacs rapidly diversify, simultaneously with colonization. Preferential expression of lineage-determining

factors (LDFs) is detectable in mice as early as embryonic day 10.25—for example, SALL1 is detected in macrophages from the cephalic area and ID3 is found in macrophages from the liver anlage[15]. The LDFs that specifically identify postnatal tissue-resident macrophages in the brain, liver, kidney, skin and lung are all upregulated around the time of colonization[15]. Genetic deletion of these LDFs leads to deficiencies in specific tissue-resident macrophage subsets—for example, deletion of ID3 in Kupffer cells[15], SALL1 in microglia[111], PPARγ in alveolar macrophages[75], SPI-C in red pulp macrophages[72] and GATA6 in large peritoneal macrophages[21,112] (Fig. 1). Therefore, it is possible that stochastic expression of tissue-specific LDFs by preMacs may drive the development of macrophage niches in developing tissues.

Signalling from macrophage niches

Conversely, developing macrophages may be instructed by local environmental signals within tissue-specific niches. In adult tissues, the expression of tissue-specific transcriptional regulators by tissue-resident macrophages is dependent on the niche. Such signals include cytokines such as TGFβ produced by epithelial cells and neurons, which controls the expression of PPARγ by lung alveolar macrophages, SALL1 by brain microglia, and ID3 by liver Kupffer cells[17,113,114]. CSF2 (also known as GM-CSF) also controls PPARγ expression in alveolar macrophages[75] and IL-34 is important for microglia and Langerhans cell identity[115]. Nuclear receptor ligands are also involved in macrophage specification—for instance, retinoic acid regulates GATA6 expression in peritoneal macrophages[21], and desmosterol signalling via LXRα controls Kupffer cell identity in the liver[17] (Fig. 1). Of note, *Drosophila* peripheral neurons produce TGFβ-like peptides that contribute to the maintenance of the resident-like macrophage niche known as sessile pockets[116]. Therefore, an instructive model in which specification of macrophages is driven by their location within specific niches is probably responsible for their maintenance in adults and may also account for their differentiation in the embryo.

Stochastic and instructive processes

The instructive model of macrophage specification requires tissue- specific growth factors to instruct PU.1-expressing core macrophages, in which the chromatin landscape is primed to be accessible by the tissue-specific LDFs when they receive a tissue-specific signal[110]. Because ‘tissue-specific’ signals such as TGFβ and retinoic acid are in fact broadly expressed, it is unlikely that single factors are sufficient to determine macrophage identity during development, but it is possible that a unique combination of cytokines and growth factors may encode the identity of each macrophage subset. However, we favour a stochastic model, in which differentiating preMacs that stochastically express certain sets of LDFs before they reach a given tissue preferentially survive and differentiate or settle—in other words, are selected—in microenvironments that provide suitable cues, which are not limited to growth factors. This process would result in tissue-specific macrophages and contribute to the formation of niches[15] that subsequently maintain macrophage identities. This model implies that the engineering of tissue-specific macrophage subsets will require the expression of specific LDFs rather than simply exposure to one or more external signals. A further analysis of the mechanisms and molecular determinants that control macrophage

specification would be useful for future efforts to leverage macrophages for therapeutic purposes.

Self-renewal and proliferation

Whether BMDMs can replace resident macrophages or replenish the macrophage niches, and in which circumstances, is of high interest for macrophage biology and has been the subject of many studies.

Under experimental homeostatic conditions, lineage tracing and parabiosis studies have demonstrated that resident macrophages self-renew locally and persist across the majority of tissues in adult mice independently of major contributions from BMDMs[4–7,11,12,14]. For example, microglia in the brain, liver Kupffer cells and epidermal Langerhans cells persist by local self-renewal, independently of monocytes, in one-year-old mice[11,12,14]. Resident macrophages in the peritoneum[112], arteries[117], heart[118], pancreatic stroma[119] and adipose tissue[7,55] also self-maintain, with negligible replacement by BMDMs, although they can decrease in number with age. Bone osteoclasts are multinucleated cells (syncytia), and represent a special case. Embryo-derived osteoclasts carry EMP-derived nuclei and self-maintain in adult bones, but they integrate new HSC-derived nuclei by fusion, resulting in individual adult mouse osteoclasts being chimeric, containing nuclei from both EMPs and HSCs[38] (Fig. 1).

Under experimental stress conditions, inflammatory processes lead to a massive recruitment of inflammatory monocytes into tissues[100,101,120,121]. This does not automatically lead to the replacement of resident macrophages by the newcomers. In several analyses of experimental brain inflammation (autoimmune encephalitis (EAE) and stroke), recruited monocytes and BMDMs in the brain mediate inflammation, whereas microglia do not; the recruited monocytes and BMDMs gradually disappear during remission, while the resident microglia persist[13,78]. Even after prolonged residence in the central nervous system (CNS), monocytes exhibit distinct epigenetic features and do not acquire expression of microglia key transcription factors such as SALL1[122]. Similar observations have been made for epidermal Langerhans cells[93], fat tissue macrophages[55] and Kupffer cells[17]. Nevertheless, in some experimental models such as whole-body irradiation followed by bone marrow graft, and in some infectious models, resident macrophages can disappear as BMDMs take their place[16], although tissue macrophage reconstitution by local proliferation of the surviving resident macrophages has also been observed[5,93,123]. Organ transplantation studies in human have demonstrated that resident (donor) macrophages can self-maintain for years in the skin[124], heart[125], lung[126] and liver[127], although host-derived myeloid cells are also present in these organs. Overall, it seems likely that resident macrophages are long-lived within their organs of residence in homeostatic and stress conditions. Whether self-renewal is achieved by mitosis of differentiated macrophages or by the presence of a yet unidentified macrophage progenitor compartment within tissues remains unknown.

The development, proliferation and survival of resident macrophages is under local and systemic control of three main factors derived from stromal cells (CSF1, IL-34 and CSF2) acting via two receptors on macrophages (CSF1R and CSF2R). CSF1 is

expressed ubiquitously as three isoforms, two of which are found in blood[128]. Injections of recombinant CSF1 into mice induces proliferation of most tissue-resident macrophages, followed by a gradual return of macrophage density to homeostatic levels[129]. Studies of individual CSF1 isoforms in mice show that the membrane-bound isoforms can fully restore macrophage densities in CSF1-deficient mice throughout many tissues but not in the liver, adrenal gland, spleen or peritoneal cavity[128]. IL-34 is produced mainly by neurons and keratinocytes[115]. Mice lacking CSF1 are deficient in most tissue-resident macrophages, but not in Langerhans cells or microglia[62], whereas IL-34-deficient mice exhibit selective reductions in Langerhans cells and microglia[115]. CSF1 and IL-34 signal to macrophages via the class III receptor tyrosine kinase CSF1R[130]. Finally, CSF2 produced by lung epithelial cells is required for the survival of lung alveolar macrophages; this signal is mediated via CSF2R[131].

Sensors and effectors for tissue homeostasis

Macrophages express a range of receptors that sense physiological parameters such as pH[31], temperature[28,29], osmolarity[29,30] hypoxia[32] and pressure[27], a wide range of metabolites including fatty acids[17–21], extracellular matrix (ECM) components[23–26], molecular signals associated with apoptotic, damaged or unfit cells[132–137], and pathogen-associated molecular motifs[22,133] (Fig. 2). Increasing evidence suggests that resident macrophages integrate these environmental signals to provide effector responses in support of the specialized cellular component of the tissue in which they reside. These responses include removal of cellular waste, regulation of inflammation, secretion of proteases and remodelling of ECM, and the production of growth factors. This enables macrophages to support cell growth and cellular functions—such as lipid storage—to assist in the maintenance of tissue homeostasis[19,138]. Macrophages thus appear to function as recycling factories and biochemical transducers that integrate environmental inputs to direct tissue growth, remodelling and proper tissue function.

Tissue-resident macrophages associate with different types of specialized cell in their respective tissues, mediating specific support functions; defects in these functions are sometimes associated with organ-specific diseases (Fig. 3). For example, large peritoneal macrophages are resident cells that contribute to protecting the peritoneal cavity against microorganisms and inflammation and support the functions of B cells[21,112], whereas small peritoneal macrophages are bone marrow-derived and pro-inflammatory. Kupffer cells are resident macrophages in the liver that take up circulating senescent or damaged red blood cells. They have an important role in iron metabolism, as they recycle iron from haemoglobin via the ferroportin transporter[72,139,140]. Of note, BMDMs have also been shown to contribute to this process when there is a massive demand for iron recycling—under these conditions, monocytes accumulate in the liver and differentiate into ferroportin-expressing BMDMs that ingest stressed and senescent erythrocytes and deliver iron to hepatocytes[141]. Unlike Kupffer cells, this population of BMDMs is transient and disappears when the increased demand for erythrophagocytosis subsides[141]. Lung alveolar macrophages reside on the epithelial surface of alveoli and are in direct contact with inhaled particulates, invading pathogens and surfactant produced by pneumocytes. They preserve airway integrity by phagocytosing bacteria and particulates, and by scavenging

and degrading lung surfactant. Their failure to do so, as a result of the production of autoantibodies against CSF2, CSF2R deficiency or PPARγ deficiency in human and mice, causes alveolar macrophages to be unable to degrade lung surfactant, resulting in alveolar proteinosis and increased pulmonary infections[75,131,142]. Kidney-resident macrophages[143] are located at the abluminal side of peritubular capillaries, where they continuously monitor endothelial transport and detect and scavenge circulating immune complexes and particles that enter the renal interstitium. This process is not intrinsically inflammatory, however when the immune complexes contain immunostimulatory viral-like nucleic acids, the kidney-resident macrophages can mount a strong MYD88-dependent inflammatory response that leads to the recruitment of leukocytes and the instigation of renal type III hypersensitivity[143].

Tissue remodelling and regeneration

A critical function of macrophages is the digestion and recycling of ECM. This is illustrated by the role of osteoclasts, which resorb bone and control its growth, shape and remodelling.

The case of bone osteoclasts

Osteoclasts are a highly specialized form of multinucleated marophage that reside in the bone endosteum[38] (see ‘Self-renewal and proliferation’). Osteoclasts secrete acid and specialized lytic enzymes such as cathepsin K that dissolve bone mineral and enable the continuous remodelling of the bone matrix[38–40]. Osteoclasts secrete IGF1[144], and resorption by osteoclasts of the bone matrix releases TGFβ and IGF1, which stimulate osteoblast activity and bone formation[144,145] (Fig. 3). Several genetic skeletal disorders result from an imbalance between bone resorption by osteoclasts and bone formation by osteoblasts. In cases with severe disruption of osteoclasts, impaired bone remodelling capacity leads to osteopetrosis, with increased bone density and skeletal deformities that may encroach internal haematopoietic bone cavities and neuronal tissue, resulting in neurological symptoms and haematological deficiencies[38,40,67,68]. Mono- or bi-allelic loss-of-function mutations in genes that affect osteoclast survival and function, such as *TCIRG1*, *CLCN7*, *TNFRSF11A*, *TREM2* and *CSF1R*, have been shown to cause osteopetrosis in humans and mice[38,40,67,68]. The severity of symptoms in osteopetrosis varies depending on the extent to which osteoclast function is impaired. Osteoclasts have also been implicated in the pathogenesis of disorders associated with an excess of bone resorption, such as osteoporosis, Paget’s disease and rheumatoid arthritis[39,146,147].

Cell growth and repair

Remodelling is not limited to osteoclasts. Macrophages produce a wide range of proteases, growth factors and WNT ligands, which drive tissue organization and cellular composition[30,37,46,138,148]. Macrophages support vascular anastomosis[34] and produce proangiogenic factors, such as VEGF-A in a HIF1- and HIF2-dependent manner, which induce the formation of vascular endothelial cells in response to hypoxia[32,138]. By contrast, retina macrophages also produce WNT ligands to suppress vascular overgrowth and fine-tune the retinal vascular plexus[35]. Tissue-resident macrophages sense osmolarity to regulate the growth of lymphatic vessels via VEGF-C[30,36] and the proliferation and

differentiation of various local parenchymal, stromal and progenitor cells[37]. Macrophages have the capacity to sense and respond to ECM components by producing proteases that aid in ECM degradation[23,42,138] (Fig. 2). In response to tissue damage sensed via signals such as extracellular ATP, low pH or ECM fragments[70], macrophages clear cellular debris and damaged cells, and secrete immunoregulatory molecules that mediate the recruitment of immune cells such as monocytes and neutrophils to disinfect the damaged tissue at the initial stages of injury[70,143]. They also secrete soluble mediators that stimulate the proliferation of local stromal and progenitor cells that participate in the repair process[70]. In response to tissue damage in the skin, dermal macrophages produce IGF1 and PDGF-CC to support myofibroblast proliferation and differentiation, a process that facilitates wound contraction and closure[148]. Depletion of macrophages at the early stages of wound repair in mouse skin prevents normal re-epithelialization and vascularization[69]. Depletion of macrophages in the late stages of tissue repair results in fibrosis in skin and liver[42]. Macrophages associated with muscle tissue secrete paracrine molecules such as IGF1[149], the metalloprotease ADAMTS1[150] and glutamine in response to injury, which stimulate proliferation and differentiation of muscle-resident stem cells (satellite cells) and induce myogenesis[45]. Kupffer cells promote liver regeneration upon injury by secreting WNT ligands and hepatocyte growth factors that stimulate the differentiation of hepatic progenitor cells into mature hepatocytes[41]. Accordingly, the absence of Kupffer cells results in a marked delay of liver repair and regeneration in a mouse model of acute liver injury[41,42]. Macrophages have also been shown to be important for mouse neonatal heart regeneration[43], kidney repair[44], digit tip regeneration[151] and bone fracture healing[152,153], as well as the regeneration of peripheral nerves and adult sensory neurons[154,155]. The regenerative properties of macrophages are conserved across species—macrophages are required for full limb regeneration in salamanders[156] and tail fin regeneration in zebrafish[157], as well as repair after injury in zebrafish[158] and *Drosophila*[8,159]. However, the experimental approaches used in several of the above studies do not allow the observed phenotype to be formally attributed to resident macrophages.

In contrast to the restorative functions attributed to resident macrophages, evidence suggests that BMDMs increase inflammation at the site of injury and exacerbate fibrosis. Specifically, *Ccr2*-deficient mice—which lack monocytes—exhibit substantially milder fibrosis in various injury models in the liver[160], heart[161], kidneys[162] and lungs[163]. These findings suggest that targeting of monocytes may be a viable therapeutic approach to stifle inflammation and potentially improve tissue repair processes in certain scenarios.

Microglia in neuron homeostasis and diseases

Microglia, the main macrophage subset of the CNS[81], provide trophic support to developing CNS cells, mediate synaptic pruning, and may even regulate neuronal activity[46–54] (Fig. 3). Microglia produce effector molecules including IGF1, NGF, BDNF, neurotropin-3 and TGFβ, which have been proposed to be important for the development and survival of neurons[47] and glial cells[46] and for normal oligodendrocyte development and myelination. Microglia modulate the positioning of neocortical interneurons and the outgrowth of dopaminergic neurons in the developing forebrain[48]. Microglia participate in synaptic patterning by engulfing synaptic material[49–52] and by the production of growth factors

such as BDNF[53,54]. Microglial BDNF is important for motor learning-dependent synapse formation and neuropathic pain[53,54].

CSF1R is important in mice and humans for brain and bone architecture[38,59–62,64,65]. Humans with CSF1R mutations present with a spectrum of neurological and skeletal phenotypes, the gravity of which appears to be dependent on the residual activity of mutant CSF1R[59,60]. For example, hypomorphic or heterozygous loss-of-function alleles cause a late-onset progressive neurodegenerative disorder known as adult-onset leukoencephalopathy with axonal spheroids and pigmented glia[64,65]. By contrast, bi-allelic *CSF1R* loss of function results in paediatric-onset leukoencephalopathy, with a near complete absence of microglia, and death in the first year of life[60]. Inherited bi-allelic mutations in *TREM2* or the gene encoding its adapter molecule DAP12 (*TYROBP*) causes a neurodegenerative and bone disease known as polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy[164,165] (also known as Nasu–Hakola disease). In addition, genome-wide association studies have identified *TREM2* polymorphisms associated with risk of dementia and Alzheimer's disease in particular[166,167]. Other variants in macrophage-expressed genes are statistically associated with dementia in genome-wide association studies and are preferentially located in microglia enhancers[66]. Conversely, it is well documented that HSC-derived monocytes that infiltrate the brain in conditions of CNS damage[13,78] differentiate into a transient population of highly phagocytic and inflammatory macrophages but do not contribute to the pool of resident microglia.

Adipose macrophages in energy metabolism

Tissue macrophages support lipid storage in white adipocytes. Adipocytes from CSF1R-deficient rodents do not store lipids[168], and TRIB1-deficient mice, which lack subsets of fat tissue macrophages, exhibit loss of fat tissues and lipodystrophy[169]. In addition, white adipose tissue-resident macrophages act as nutrient sensors that adapt energy storage in adipocytes to nutrient availability in *Drosophila* and mice[55] (Fig. 3). Fat tissue-resident macrophages promote lipid storage, in part via production of the growth factor PDGF-CC in response to increased dietary fat intake in mice, and a similar mechanism operates in *Drosophila*[55]. PDGF-CC production by fat-resident macrophages is regulated by diet, and acts in a paracrine manner on fat-storing white adipocytes to control lipid storage in fat tissues. Loss of energy storage in white adipose tissues of mice due to a lack of resident macrophages or PDGF-CC results in surplus unstored energy that is dissipated in the brown adipose tissue as heat[55]. Fat-resident macrophages can also control energy expenditure by promoting browning of white adipose tissue[57,58] or via production of IL-10[57], and possibly through production or scavenging of catecholamines[56,170], although there have been reports of fat tissue macrophages suppressing browning and thermogenesis[171]. Scavenging of sympathetic noradrenaline by macrophages expressing the transporter SLC6A2 controls lipid storage in adult mice[170]. Of note, in contrast to BMDMs, fat-associated resident tissue macrophages do not produce inflammatory mediators in obese mice[55]. CCR2-dependent BMDMs recruited into tissues of obese animals produce TNF and promote systemic inflammation, insulin resistance and ectopic lipid deposition[172,173]. Notably, TREM2 deficiency worsens obesity and metabolic syndrome, possibly through global control of a lipid-associated BMDM programme by TREM2[174].

Fat tissue macrophages from the brown adipose tissue may also be important for the control of energy expenditure[56]. For example, these cells are necessary to increase heat dissipation from brown adipose tissue in response to cold exposure[56]. MECP2 may have a role as its deficiency in tissue macrophages reduces energy expenditure and thermogenesis, possibly via decreased sympathetic innervation in the brown adipose tissue[175]. Overall, fat tissue macrophages from the white and brown adipose tissues are likely to have important roles in lipid and energy metabolism that deserve further investigation, as they may represent therapeutic targets.

Phagocytosis and nutrient recycling

One of the best studied aspects of macrophage biology is their role in phagocytosis and host immunity. Phagocytes in all metazoans as well as hydras and echinoderms exhibit nearly complete sets of homologues for key molecular components with macrophage functions, such as pathogen recognition and uptake, including Toll-like receptors, NOD-like receptors and scavenger receptors, indicating their importance in maintaining organismal homeostasis[176]. Germline-encoded pattern recognition receptors (PRRs), which mediate the initial sensing of invading pathogens, can be classified into five families on the basis of their protein domain homology: Toll-like receptors, RIG-I-like receptors, NOD-like receptors, AIM2-like receptors and C-type lectin receptors (Fig. 2). Collectively, PRRs can sense a wide variety of host- and pathogen-associated molecular patterns, including various classes of lipopeptides, glycoproteins and nucleic acids[133]. Binding of ligands to PRRs on macrophages initiates tightly controlled signalling cascades that induce the production of inflammatory mediators (TNF, IL-1, IL-6 and interferon) and in some cases phagocytosis, which together coordinate the elimination of pathogens and infected cells[133]. The role of monocyte-derived macrophages in the inflammatory responses is well described[101,120,121,177] and resident macrophages appear to be preferentially involved in repair and homeostasis[30,33–58,78], although there are some exceptions[143].

Also well known are the essential roles of macrophages in the clearance and recycling of billions of cells that undergo apoptosis daily in mammalian tissues[178]. Macrophages sense phosphatidylserine exposed on the surface of apoptotic cells directly with TIM4 or through TAM receptors (TYRO3, AXL and MERTK), which work in conjunction with soluble bridging molecules such as MFGE8, GAS6, protein S and complement receptors (reviewed in ref. 178). This initiates the engulfment and degradation of target cells, secretion of anti-inflammatory molecules (TGFβ, IL-10 and PGE2) and inhibition of pro-inflammatory cytokine production[178] (TNF, IL-1β and IL-6). This process, called efferocytosis, may prevent chronic inflammation and autoimmune disorders following tissue damage. Engulfed cells and debris are then degraded to their basic components by the lysosomal apparatus[178,179] (Fig. 2). The respective roles of monocytes and resident macrophages in this process remain unclear—although, as outlined below, Kupffer cells, for example, are involved primarily in recycling of senescent red blood cells and iron metabolism, whereas monocytes can contribute in conditions of stress[141].

Lysosomal storage diseases

Defective lysosomal functions can also lead to severe homeostatic defects and lysosomal storage diseases (LSDs). LSDs are a heterogenous group of more than 70 genetic disorders that are predominantly diagnosed in children[63]. LSDs are caused by mutations in lysosomal proteins, including hydrolases and transporters, and have a vast genetic diversity and heterogenous clinical presentations[63]. Gaucher disease (also known as lysosomal glucocerebrosidase deficiency) is one of the most frequent LSDs[180], with a birth prevalence of 2 per 100,000. Macrophages from individuals with Gaucher disease are foamy, and because they are critical for the clearance of apoptotic cells and debris, they are thought to be the key cell type affected in LSD. Most patients with LSD exhibit developmental problems and neurological phenotypes including ataxia, seizures and intellectual disability, suggesting microglial dysfunction. Patients with LSD also occasionally present with involvements of other tissues, such as bone, liver and lung. Bone marrow transplantation is an alternative to lysosomal enzyme replacement therapy for some, but not all, patients with LSD, suggesting that monocytes can be at least a source of the missing enzyme for therapeutic purposes. This suggests that an important function of resident macrophages may be to recycle the substantial amounts of nucleotides, proteins, lipids and sugars that result from cellular digestion to fuel cell proliferation, tissue growth, remodelling and regeneration. Lysosomal dysfunction in resident macrophages interferes with nutrient recycling, leading to cellular stress or damage and ultimately organ disfunction.

Autoimmune diseases

Impaired efferocytosis can also lead to prolonged inflammation, collateral tissue damage and induction of autoimmunity in mice, and is implicated in the pathogenesis of autoimmune and chronic inflammatory diseases[179]. Mice that are deficient in molecular components involved in the recognition and uptake of apoptotic or necrotic cells by macrophages such as MFGE8, MERTK, TIM4 or complement (such as C1Q) develop phenotypes associated with autoimmunity[178,181–186]. Patients with C1Q deficiency are highly susceptible to developing systemic lupus erythematosus and chronic glomerulonephritis[186,187]. Impairment of efferocytosis due to lack of these molecules causes accumulation of necrotic cells, nucleic acids and other immunogenic cellular components that activate surrounding macrophages and may contribute to inflammation in systemic lupus erythematosus and glomerulonephritis, chronic inflammatory lung diseases, atherosclerosis and systemic sclerosis[178,179,188,189] (Fig. 3).

Cancer

In certain circumstances, macrophages phagocytose live non-apoptotic cells. This process enables the elimination of infected, unfit or tumoural cells. Notably, Kupffer cells have an important role in limiting the growth of various tumour cell lines in the liver[134,190]. Converesely, BMDMs in the tumoural niches, broadly defined as tumour-associated macrophages, can promote tumour growth[191]. Thus, it is possible that distinct subsets of resident macrophages and monocyte-derived macrophages may have different roles in tumour growth[192]. The phagocytosis of live cells by macrophages is controlled by activating receptors, including dectins and the calreticulin receptor[134,136], and inhibitory

receptors, such as the immunoreceptor tyrosine-based inhibitory motif-containing receptor SIRPα[193,194] (also known as SHPS-1) and sialic acid-binding immunoglobulin-like lectins such as SIGLEC10[195]. SIRPα may protect normal tissue against excessive damage caused by macrophage activation in response to microbial products[180], but it also protects tumoural cells, which express the SIRPα ligand CD47. Targeting the SIRPα–CD47 axis is a promising strategy for cancer treatment[193,194]. In sum, the roles of macrophages in tumour growth may depend on the transcriptomes of macrophage subsets and the balance between engagement of activating and inhibitory receptors in distinct microenvironments or tumoural niches. Investigation of mechanisms such as those that underly the antitumour activity of Kupffer cells in the liver may help identify molecular targets for harnessing macrophages for therapeutic purposes.

Conclusion

Tissue-resident macrophages represent a conserved lineage in metazoans, with important ancillary functions that contribute to development and tissue and organismal homeostasis. Tissue-resident macrophages establish stable relationships with specialized cell types, from the onset of organogenesis and throughout adult life, and are distinct from monocytes and monocyte-derived macrophages. The word ‘macrophage’ is an umbrella term for many cell types with distinct and sometimes opposing roles in health and disease—future studies should therefore consider the contributions of individual macrophage subsets to given phenotypes. Such a model provides a conceptual framework for the exploration of the physiology of complex tissues that may also contribute to investigations into the pathophysiology and genetic basis of sporadic diseases such as dementia, obesity, autoimmunity and cancer, and will be useful to identify novel therapeutic strategies.

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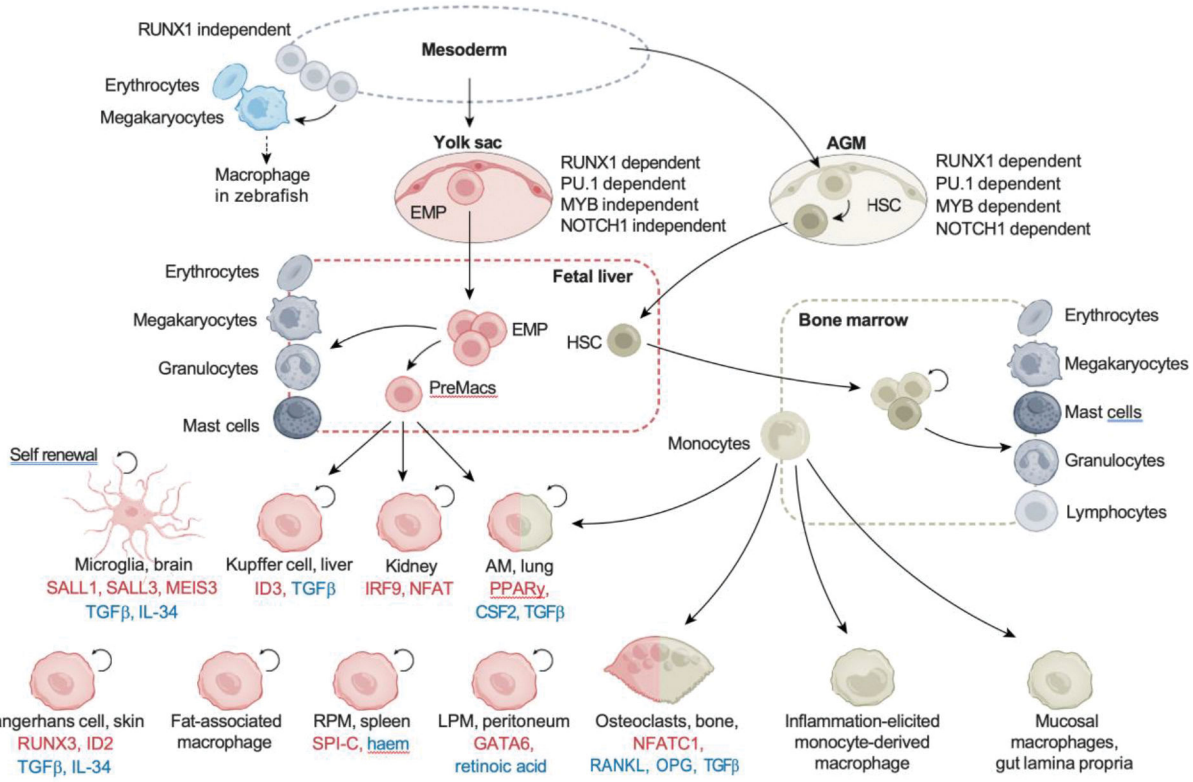

Fig. 1 |. Macrophage ontogeny and specification.

Cells ofthe primitive haematopoietic lineage are shown in blue, cells of the erythromyeloid progenitor (EMP) lineage are in red and cells of the HSC lineage are in taupe. Arrows indicate the developmental relationships between cells. Macrophage-specific transcription factors and tissue signals are labelled in red and blue, respectively. AGM, aorta– gonad–mesonephros; AM, alveolar macrophage; LPM, large peritoneal macrophage; preMacs, EMP-derived macrophage precursors; RPM, red pulp macrophage.

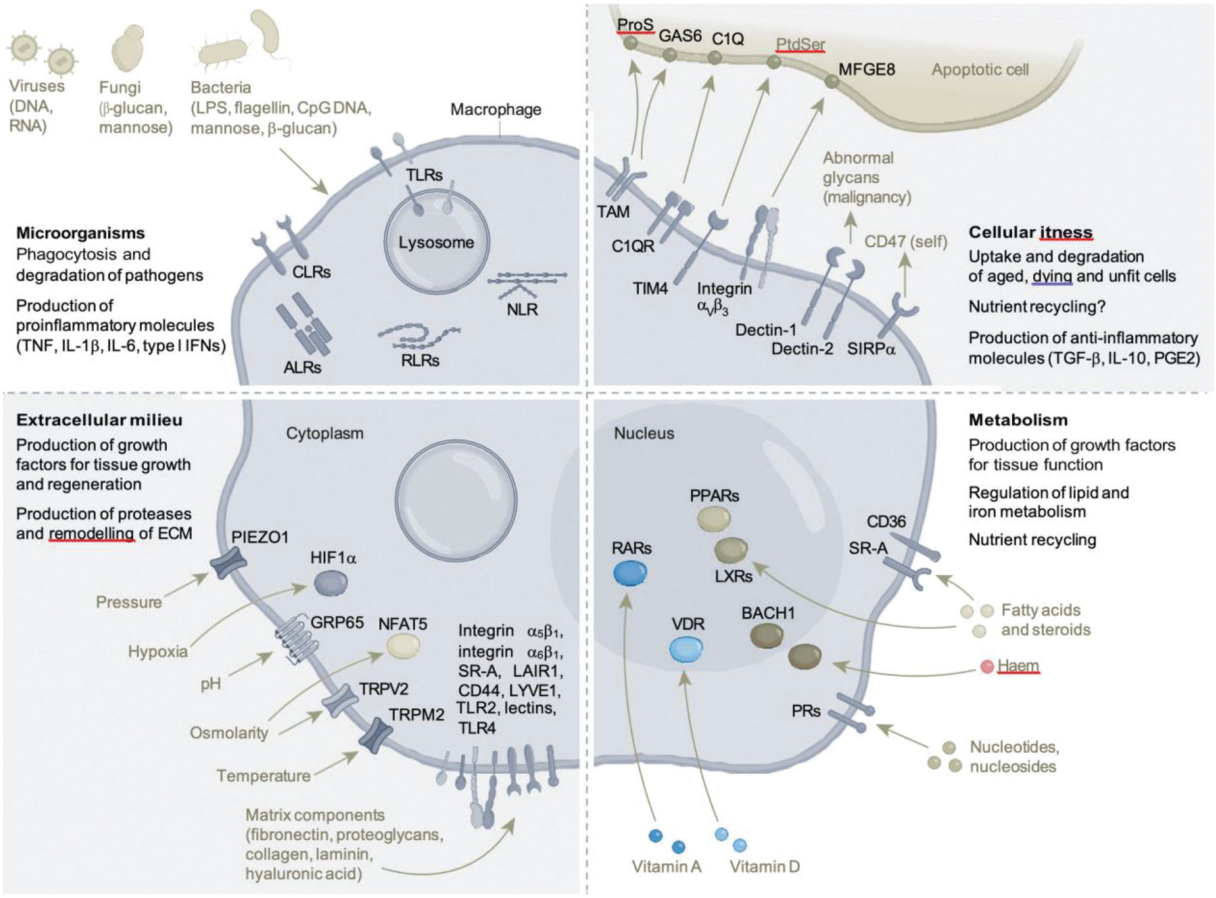

Fig. 2 |. Sensing repertoire of tissue-resident macrophages and related effector functions.

Macrophages display a wide range of sensors that detect and respond to pathogens, unfit cells, tissue microenvironmental signals and metabolites. Taupe arrows indicate an interaction between a specified signal (taupe text) and a macrophage sensor (black text). ALRs, AIM2-like receptors; CLRs, C-type lectin receptors; NLRs, NOD-like receptors; PPARs, peroxisome proliferator-activated receptors; PR, purinergic receptors; ProS, protein S; PtdSer, phosphatidylserine; RARs, retinoic acid receptors; RLRs, RIG-I-like receptors; TAM, TYRO3, AXL and MERTK; TLRs, Toll-like receptors; VDR, vitamin D receptor.

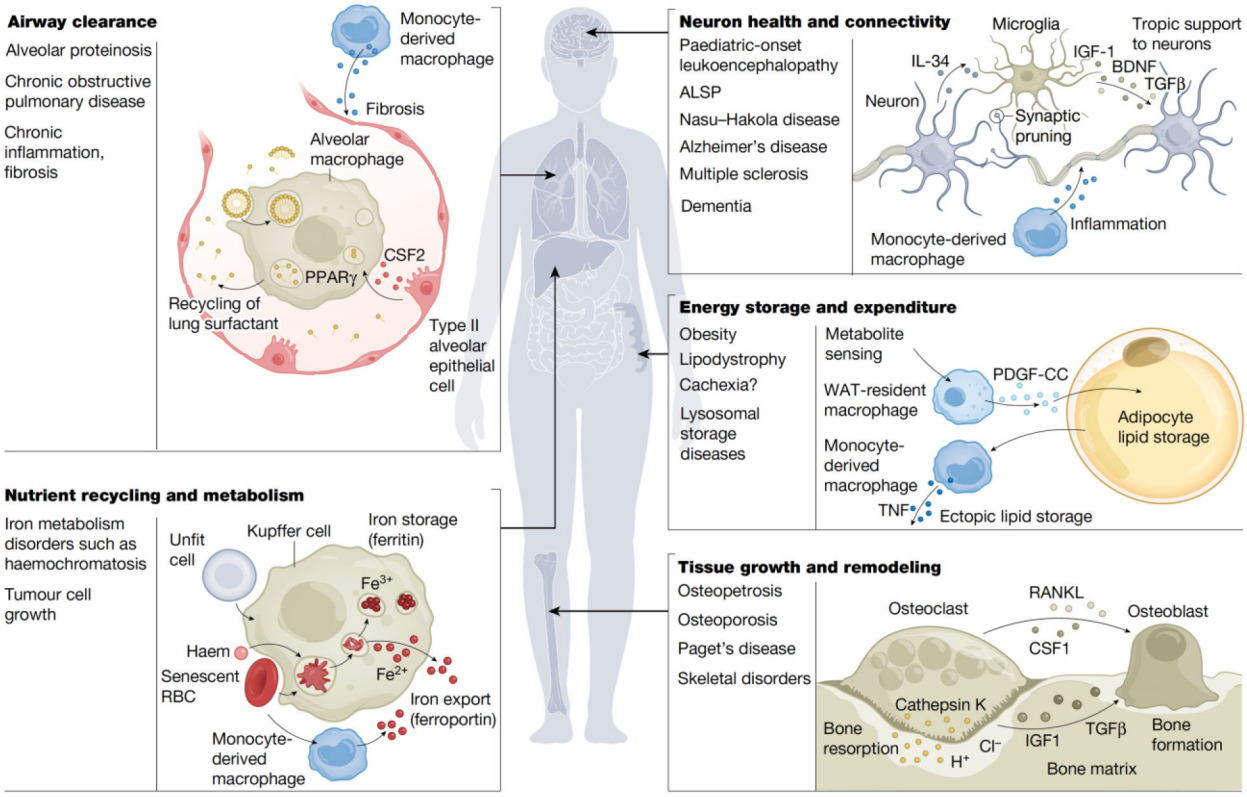

Fig. 3 |. Physiological roles of macrophages and associated disease processes.

Schematic depicting selected functions of macrophages and diseases associated with impairment of these functions. Black arrows indicate directional flow of molecules from the source to the acceptor cell or the sequence of events in a homeostatic process. ALSP, adult-onset leukoencephalopathy with axonal spheroids and pigmented glia; WAT, white adipose tissue; RBC, red blood cell; COPD, chronic obstructive pulmonary dysfunction