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Regulation of macrophage development and function in peripheral tissues

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Abstract

Macrophages are immune cells of haematopoietic origin that provide crucial innate immune defence and have tissue-specific functions in the regulation and maintenance of organ homeostasis. Recent studies of macrophage ontogeny, as well as transcriptional and epigenetic identity, have started to reveal the decisive role of the tissue stroma in the regulation of macrophage function. These findings suggest that most macrophages seed the tissues during embryonic development and functionally specialize in response to cytokines and metabolites that are released by the stroma and drive the expression of unique transcription factors. In this Review, we discuss how recent insights into macrophage ontogeny and macrophage–stroma interactions contribute to our understanding of the crosstalk that shapes macrophage function and the maintenance of organ integrity.

Macrophages are key components of the innate immune system that reside in tissues, where they function as immune sentinels. They are uniquely equipped to sense and respond to tissue invasion by infectious microorganisms and tissue injury through various scavenger, pattern recognition and phagocytic receptors[1–4]. Macrophages also have homeostatic functions, such as the clearance of lipoproteins, debris and dead cells using sophisticated phagocytic mechanisms[5,6]. Accordingly, macrophages are crucial for maintaining a balanced response to homeostatic or tissue-damaging signals and, when this delicate balance is disturbed, inflammatory disease can occur.

Recent studies have revealed the ontogeny and functional diversity of tissue-resident macrophages. These studies have established that tissue-resident macrophages are maintained by distinct precursor populations that can be recruited from either embryonic haematopoietic precursors during fetal development or bone marrow-derived myeloid precursors during adult life[7]. In addition to developmental diversity, macrophages have unique functions in maintaining homeostasis and exhibit extensive plasticity during disease progression.

Macrophages have classically been defined by their dependence on colony-stimulating factor 1 (CSF1; also known as M-CSF). However, in some tissues, macrophages also

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depend on other cytokines and meta bolites for their differentiation and maintenance. Recent data acquired by high-throughput sequencing have characterized the transcriptional and epigenetic programmes of tissue-resident macrophages and revealed the extent of diversity in these populations[1,8]. In addition to differences in ontogeny, locally derived tissue signals can explain some of this diversity, as they drive the expression of unique transcription factors in tissue-resident macrophages, leading to distinct epigenetic profiles, transcriptional programmes and, ultimately, different functions.

In this Review, we discuss the unique ontogeny of tissue-resident macrophages, the interactions of macrophages with their tissue environment and how these interactions shape macrophage function in the steady state and during inflammation.

The mononuclear phagocyte system

A central dogma in immunology posits that monocytes and macrophages are part of a continuum that forms the mononuclear phagocyte system (MPS). According to this system, macrophages are fully differentiated cells that have lost proliferative potential and are constantly repopulated by circulating monocytes produced by bone marrow-derived myeloid progenitors[9]. The definition of this cellular system stems mostly from studies tracing the differentiation of radiolabelled monocytes in mice with inflammation and thus describes the contri bution of monocytes to inflammatory macrophages that accumulate in injured tissues.

Reinvestigating macrophage ontogeny using congenic parabiotic mice that share the same circulation provided insight into the physiological contribution of circulating monocytes to macrophages residing in healthy tissues. Congenic parabionts have mixed haematopoietic cell precursors in the bone marrow, mixed lymphocytes and monocytes in the blood, and mixed dendritic cells (DCs) in the lymphoid organs[10]. Therefore, if tissue-resident macrophages were derived from monocytes, they should harbour the same level of chimerism as circulating monocytes. However, the mononuclear phagocytes of the epidermis (known as Langerhans cells)[10] and the brain-resident macrophages (known as microglia)[11,12] were found not to mix in tissues even after a year of parabiosis, which suggested that they could be maintained independently of circulating precursors in adult mice. More recently, several other tissue-resident macrophages, including alveolar macrophages, spleen red pulp macrophages and Kupffer cells[13–17], were also shown to be maintained independently of circulating precursors either through longevity or self-renewal. Several studies in humans were consistent with a circulation-independent maintenance of tissue-resident macrophages: patients with severe monocytopenia have normal numbers of Langerhans cells in the epidermis[18,19]; donor Langerhans cells can be detected for years in a recipient of a human limb graft[20]; and host Langerhans cells remained in patients that received sex-mismatched allogeneic bone marrow transplants[21,22]. Similarly to Langerhans cells, numerous tissue-resident macrophages are present in patients with severe monocytopenia[18,19] and in patients who received a sex-mismatched allogeneic bone marrow transplant[22].

Together, these studies have challenged the linearity of the MPS, reigniting debate on the contribution of early haematopoiesis to tissue-resident macrophages[23], as discussed below.

Origins of tissue-resident macrophages

Embryonic haematopoietic precursors

Early studies reported the presence of a primitive macrophage lineage that has self-renewal capacity and arises before the development of definitive haematopoiesis, without a monocyte intermediate[24–26]. Primitive macrophages were first identified in the blood islands of the extra-embryonic yolk sac (YS) around embryonic day 8 (E8) and were shown to migrate to various tissues from E8.5 to E10 to give rise to proliferative fetal macrophages independently of monocytes[24–26]. Similar data were also obtained in zebrafish[27], in which the emergence of macrophages was shown to precede the onset of blood circulation. However, whether these primitive macrophages contributed to adult tissue-resident macrophages has never been directly addressed.

In mice, the first haematopoietic cells appear in the YS blood islands around E7.5 and produce primitive erythrocytes and macrophages but not lymphocytes[28,29]. In humans, haematopoiesis is also initiated in the extra-embryonic YS during the third week of development and is limited to erythromyeloid cells[30]. YS-derived progenitors migrate and seed the fetal liver through the bloodstream after E8.5, once the circulation is established, to rapidly initiate the first wave of intra-embryonic haematopoiesis[31,32]. A second wave of haematopoiesis beginning at E10.5 arises in the mouse embryo from major arterial vessels and gives rise to definitive haematopoietic stem cells (HSCs) with multi-lineage potential[33]. HSC activity subsequently expands in the fetal liver and peaks at E16.5 before transitioning to the bone marrow, which becomes the main site of haematopoiesis in adult life[34,35]. Although the primitive and definitive haematopoiesis waves have myeloid potential, the contribution of each embryonic wave of haematopoiesis to the adult tissue-resident macrophage pool has not been directly tested.

Experimental models

The first fate mapping model that was used to probe the contribution of embryonic precursors to adult tissue-resident macrophages traced the progeny of YS runt-related transcription factor 1 (RUNX1)$^+$ haematopoietic cells. RUNX1$^+$ haematopoietic precursors are restricted to YS-derived cells between E6.5 and E8, and RUNX1 starts to be expressed by definitive haematopoietic precursors around E8.5 (REFS 11,36). Conditional labelling of RUNX1$^+$ cells in E7–E7.5 embryos allows the contribution of YS haematopoietic cells to fetal and adult macrophages to be traced. RUNX1$^+$ YS-derived macrophages started to infiltrate the whole embryo following the formation of blood circulation around E8.5–E9.5, and a high number of macrophages were labelled in E10 and E13 embryos[11,37,38]. Strikingly, labelled microglia were retained in adult brains, whereas most tissue macrophages lost their labelling in adult tissues, which suggests that they were being replaced by non-labelled precursors before birth[11,37,38]. These results led to the hypothesis that microglia that populate adult mouse brains uniquely derive from E7–E7.5 YS-derived cells.

Several studies have since confirmed the embryonic origin of tissue-resident macrophages[15,16,38–41]. Despite agreeing on a paradigm shift, a controversy arose regarding

the exact nature of the embryonic precursor that gives rise to adult tissue-resident macrophages, with some researchers suggesting that YS-derived precursors mainly give rise to microglia, whereas most tissue-resident macrophages derive from fetal liver monocytes[15,16,37,39], and other researchers being in favour of a universal YS origin of most adult tissue macrophages including microglia[40,41].

The suggestion of a universal YS origin of adult macrophages was based on results showing that mice that lacked the transcription factor MYB, which is required for definitive but not primitive haematopoiesis, retained F4/80^{hi} macrophages in E16.5 embryos[40] and that fate mapping of YS macrophages that express the macrophage marker CSF1 receptor (CSF1R) gave rise to these fetal F4/80^{hi} macrophages. However, although fate mapping of E8.5 CSF1R^{+} YS cells marked a large population of fetal F4/80^{hi} macrophages, a very small population of labelled macrophages remained in adult tissues[38,40], with the exception of microglia, which retained maximal labelling after birth[38]; these results are consistent with those obtained with the RUNX1 fate mapping model. The contribution of YS progenitors to adult tissue macrophages was further substantiated by a novel fate mapping model showing that the angiopoietin 1 receptor TIE2^{+} YS-derived progenitors with restricted erythroid and myeloid potential — known as erythromyeloid precursors (EMPs) — seed and expand in the fetal liver and give rise to both fetal and adult macrophages[41], a result that was not observed with either the RUNX1 or CSF1R fate mapping model described earlier. Pulse labelling of E7.5 TIE2^{+} cells also labelled definitive HSCs, although at a lower level than YS macrophages[41], which raises the possibility that labelled adult macrophages in this model may also derive from definitive HSCs.

A recent study[38] might help to resolve the apparent discrepancy between these studies. Consistent with previous studies[42,43], it was shown that YS-derived EMPs are heterogeneous and arise in two waves that differentially contribute to adult microglia and other macrophages[38]. An early wave of YS-derived EMPs appears around E7.5 and colonizes the brain and other fetal tissues around E9 to give rise to F4/80^{hi} macrophages, as previously described[11,40]. A later wave of YS-derived EMPs colonizes and expands in the fetal liver to differentiate into fetal liver monocytes, which subsequently replace early F4/80^{hi} macrophages around E14.5 (with the exception of the brain microglia) and maintain macrophages in adult tissues (FIG. 1). Interestingly, whereas the early YS wave lacks the transcription factor MYB[40], the second wave of YS-derived haematopoietic cells do express MYB. Fate mapping of MYB-deficient and MYB-sufficient progenitors should help to compare their contribution to tissue-resident macrophages in adult mice.

Although the primitive YS-derived versus definitive HSC-derived haematopoiesis model influences many interpretations of haematopoietic cell emergence, it is likely to be an oversimplification. In fact, embryonic haematopoiesis probably occurs in overlapping waves. We currently still know very little about the molecular and cellular steps that control the development of embryonic haematopoietic waves and still lack the tools to genetically trace discrete precursor populations in the embryos. In addition, the use of tamoxifen-dependent Cre recombinase-dependent fate mapping models has caveats that may affect the interpretation of the fate mapping results (BOX 1). Thus, we need to be careful not to over-interpret fate mapping studies, especially when performed in embryos.

Origin of macrophages in inflamed adult tissues

In contrast to most healthy tissues, in which macrophages are maintained with minimal contribution of adult circulating monocytes, a large influx of monocytes that are produced by adult myeloid progenitors enter injured tissues and differentiate into macrophage-like and DC-like cells. Accumulating evidence suggests that monocyte differentiation into macrophage-like cells in inflamed tissues occurs in parallel with the expansion of tissue-resident macrophages in many tissues[13,15,44]. In particular, upon nematode infection, the production of interleukin-4 (IL-4) was shown to drive *in vivo* replication of tissue-resident pleural macrophages as well as resident macrophage populations in the peritoneum, liver and intestine[44,46].

The extent and duration of adult monocyte-derived cell engraftment in tissues remains unclear. In the lungs and brain, for example, monocyte-derived macrophages do not seem to substantially contribute to the resident macrophage pool after the infection or injury resolves[13,47]. However, in some healthy tissues, macrophages are constantly replenished by circulating monocytes, such as mouse intestinal macrophages[48,49] and some dermal macrophages[50]. In other tissues, such as the heart, epidermis and peritoneal cavity, a smaller subset of monocyte-derived macrophages can be found in healthy tissues and may derive from the physiological recruitment of a small monocyte population[15,45,51], or they may be remnant monocyte-derived macrophages that have been recruited during a tissue injury[52,53].

The contribution of monocyte-derived macrophages to the resident macrophage population may depend both on the organ and the nature of the injury. In the liver, for example, infection with *Listeria monocytogenes* induces Kupffer cell necrosis and, in this case, monocyte-derived macrophages contribute to repopulating the liver macrophage population[54]. However, after paracetamol-induced injury, resident Kupffer cells proliferate and expand, and monocytes do not notably contribute to the resident macrophage pool[55]. It is still unclear to what extent macrophage ontogeny — that is, embryonic versus monocyte-derived — determines macrophage function. Using bone marrow transplant experiments, adult bone marrow-derived macrophages acquire the enhancer profile of the embryonic-derived macrophages that they replace[8]. However, whether embryonic-derived or adult bone marrow-derived macrophages are functionally identical remains an open question. For example, embryonic and monocyte-derived cardiac macrophage subsets that coexist in the healthy heart have different propensities for promoting tissue repair after cardiomyocyte injury[56]. Thus, the balance of these different macrophage ontogeny programmes in tissue immunity and homeostasis needs to be addressed in each tissue.

Tissue factors control macrophage identity

In contrast to tissue-resident DCs, which have the same transcriptional programme regardless of the tissue in which they reside[57], tissue-resident macrophages share expression of only a few unique transcripts, with most of the transcriptional programme being specific to the tissue of residence[1,8]. These results suggest that, in addition to potential ontogeny cues, environmental signals contribute to shaping macrophage transcriptional regulation[58,59].

Ontogeny and environmental signals can shape cell identity through epigenetic modifications (such as chromatin accessibility) and open chromatin-containing regulatory elements (such as promoters and enhancers)[60]. During development, pioneer transcription factors open large swaths of chromatin, which allows the binding of other transcription factors that confer cell-type specificity[61]. In myeloid cells, PU.1 acts as a pioneer transcription factor, binding throughout the genome to both promoter and enhancer regions[62,63]. The binding of other macrophage-specific transcription factors, together with PU.1, further remodels the chromatin in a cell-specific manner[63,64]. CCAAT/enhancer-binding protein (CEBP) transcription factors, for example, also have a role in myeloid cell differentiation and act together with PU.1 by binding throughout the genome[61,63]. In tissue macrophages, CEBPβ may be more important in lung and peritoneal cavity macrophages as these cells are substantially reduced in its absence[65]. Interestingly, the binding motif of the MAF transcription factor family is enriched in most tissue-resident macrophage enhancers compared with monocytes and neutrophils[8]. MAF and MAFB are important for macrophage terminal differentiation, and macrophages that lack these transcription factors are immortalized and proliferate indefinitely in the presence of CSF1 (REFS 66,67). Therefore, PU.1, CEBP, MAF and MAFB transcription factors probably work together to shape common tissue-resident macrophage function.

In addition, macrophages receive specific signals from the tissue to drive tissue-specific transcription factor expression. Transcription factors, such as GATA-binding protein 6 (GATA6) in peritoneal cavity macrophages[68], peroxisome proliferator-activated receptor-γ (PPARγ) in alveolar macrophages[39] and SPIC in spleen red pulp macrophages[69], work together with PU.1 to bind enhancers and remodel the chromatin in a tissue-specific manner. Accordingly, whereas promoters are mostly shared between distinct tissue-resident macrophages, open enhancer regions differ between different tissue macrophages[8,64]. Thus, macrophages are shaped both by common developmental factors and tissue-specific transcription factors triggered by tissue-specific signals — that is, cytokines and metabolites — and together they drive macrophage transcriptional programmes and functional specialization, as discussed below.

The tissue secretome shapes tissue macrophages

Local cytokine and metabolite production in the tissue is important for the regulation, maintenance and functional specialization of macrophages (TABLE 1). Cytokines, such as CSF1, promote macrophage survival and proliferation in many tissues. Other cytokines, such as IL-34 (which also signals through CSF1R) and CSF2 (previously known as GM-CSF), are produced in specific tissues and maintain macrophages locally. Many of these cytokines have been widely explored for their functions *in vitro*; however, recent work has begun to elucidate their role in the regulation of macrophages *in vivo.* Together with cytokines, tissue metabolites such as fatty acids and oxysterols have been known to regulate macrophage function[70], but recently other metabolites such as haem have also been shown to contribute to shaping tissue-specific macrophage functional identity, as discussed here (FIG. 2).

Local production of CSF1 controls macrophage homeostasis in tissues

Most macrophages express high levels of CSF1R. The production of CSF1 in peripheral tissues is required for macrophage maintenance as mice that have a spontaneous null mutation in *Csf1* (*Csf1*$^{op/op}$ mice) have a broad reduction of tissue-resident macrophage populations. Transgenic local expression of CSF1 but not intravascular injection rescues macrophage development in *Csf1*$^{op/op}$ mice[71,72], and transgenic expression of cell surface CSF1 is sufficient to restore many tissue-resident macrophage populations[73], which indicates the importance of local CSF1 production in macrophage homeostasis. In *Csf1*$^{op/op}$ mice, as well as upon antibody-mediated blockade of CSF1R, numbers of LY6C^{hi} inflammatory monocytes are only slightly reduced[14,74–76], whereas LY6C^{low} monocytes are almost undetectable owing to defective differentiation of LY6C^{hi} monocytes into LY6C^{low} monocytes[14,77]. These results further indicate the distinct regulation and maintenance mechanisms of LY6C^{hi} monocytes and resident macrophages, and support the importance of local CSF1 production for macrophage maintenance *in vivo*. Studies using *Csf1* reporter mice identified CSF1 production in macrophage-enriched sites, such as the marginal zone and red pulp of the spleen, the bone marrow, the base of the crypts in the intestine, and the cortex and medulla of the lymph node[72]. However, the exact mechanisms and sources that produce CSF1 in most tissues remain unclear. In the muscularis layer of the gut, microbial signals drive enteric neurons to produce CSF1, which is required to maintain muscularis macrophage homeostasis[78]. Inflammatory signals can also drive high levels of CSF1 production, even in tissues that do not produce it in the steady state, such as the epidermis, indicating that CSF1 may have distinct roles in healthy and injured tissues[79].

IL-34 production by neurons and keratinocytes shapes microglia and Langerhans cells, respectively

IL-34 is an alternative ligand for CSF1R; it has a very restricted tissue expression pattern (mainly in the brain and epidermis)[79–83]. IL-34 has very little homology with CSF1 and binds to CSF1R with higher affinity than CSF1 (REF. 81). In the central nervous system, IL-34 and CSF1 are produced in non-overlapping regions of the brain[79,82,83]: IL-34 is produced mainly by neurons in the cortex, striatum, olfactory bulb and hippocampus, whereas CSF1 is highly expressed in the cerebellum[79–83]. IL-34-deficient mice have fewer microglia in the brain regions in which IL-34 is normally produced[79,83]. In *Csf1*$^{op/op}$ mice, microglia numbers are only slightly reduced[11], suggesting that IL-34 may compensate for the loss of CSF1, although regional microglial loss has not been precisely quantified in these mice (FIG. 3a).

Langerhans cells are the only mononuclear phagocytes that populate the epidermis in mice and humans. CSF1 is not produced in the healthy epidermis and, accordingly, Langerhans cells are maintained independently of CSF1 (REF. 74). By contrast, IL-34 is constitutively produced by keratinocytes, and IL-34-deficient animals lack Langerhans cells whereas dermal DCs and other macrophages remain unaffected in these mice[79,83] (FIG. 3b).

Langerhans cells and microglia also depend on transforming growth factor-β (TGFβ), as both of these populations are reduced in the absence of TGFβ[84,85]. The receptor for TGFβ, TGFβR1, is highly expressed by microglial cells compared with other myeloid cells[8]. In the

brain, TGFβ production by astrocytes drives synaptic pruning by microglia through C1q production by neurons[86]. TGFβ signals through the phosphorylation of SMAD transcription factors downstream of TGFβR1 (REF. 87), and a SMAD-binding motif is enriched in PU.1-bound enhancer sites in microglial cells[64]. TGFβR1 is also highly expressed by Langerhans cells, and TGFβ is required to retain Langerhans cells in the epidermis[85,88]. Interestingly, TGFβ works both in a paracrine and autocrine loop to retain Langerhans cells in the skin and prevent them from upregulating maturation markers that mediate trafficking to the lymph nodes[88,89]. TGFβ induces the expression of inhibitor of DNA binding 2 (ID2) and RUNX3, which are required for Langerhans cell maintenance in the skin[90,91] (FIG. 3b). By contrast, inflammatory monocytes that are recruited to the epidermis upon exposure to ultraviolet light are independent of ID2 (REFS 90,91) and infiltrate the skin independently of IL-34 (REF. 79).

These studies established that local production of both IL-34 and TGFβ is important for the maintenance and regulation of microglia and Langerhans cells. *In vitro*, monocytes cultured with CSF1 and TGFβ express microglia-specific genes[84], whereas they exhibit Langerhans cell-like characteristics when cultured with CSF2 and TGFβ[90]. Signalling through CSF1R promotes the expression of TGFβR1 as peritoneal macrophages cultured with either CSF1 or IL-34 start to upregulate this receptor[64]. However, how IL-34 and TGFβ cooperate to shape microglia and Langerhans cell function *in vivo* remains to be explored.

CSF2 produced by local radio-resistant cells shapes mucosal macrophages

CSF2 is an important myeloid growth factor with a key role in the maintenance and homeostasis of lung and intestinal tissue macrophages. In the absence of functional CSF2 or its receptor, the homeostasis of lung and intestinal macrophages is specifically altered, which results in an increased susceptibility to infection and impaired tissue repair[13,94–96]. CSF2 is produced by radio-resistant cell types that require IL-1-mediated activation[93,97,98]; it is produced mostly by epithelial cells in the lungs and by retinoic acid-related orphan receptor-γt (RORγt)-expressing innate lymphocytes in the intestine[93,97]. Interestingly, CSF2 promotes the survival of macrophages through signal transducer and activator of transcription 5 (STAT5)-mediated anti-apoptotic gene regulation[99]. Similar findings are documented for DCs, in which CSF2-mediated STAT5 phosphorylation leads to the expression of anti-apoptotic genes through the exchange of STAT3 at gene regulatory elements[100]. Thus, the balance of phosphorylated STATs is likely to drive gene expression in macrophages and thus allows for a distinction between steady state and activated state.

In addition, CSF2 promotes the differentiation of alveolar macrophage precursors from the fetal liver into mature alveolar macrophages through the induction of PPARγ expression[16,39,101]. PPARγ is highly expressed by lung alveolar macrophages and regulates their development and function, partly through the induction of a specific transcriptional programme that involves the expression of genes related to lipid degradation and fatty acid oxidation required for surfactant catabolism[39,101,102]. Besides acting as sentinels for bacterial infiltration, alveolar macrophages are crucial for maintaining lung homeostasis through the clearance of excess phospholipid surfactant. Mice that lack CSF2 or CSF2R, and patients with defective CSF2R signalling, develop severe lung inflammatory disease known

as pulmonary alveolar proteinosis owing to accumulation of surfactant in the lung tissues[103,104]. The transcriptional repressor BACH2 has also been implicated as a regulator of alveolar macrophages and in effective clearance of surfactant[105], suggesting that additional factors may be involved in the regulation of alveolar macrophages (FIG. 3c).

Similarly, CSF2 has an important role in maintaining homeostasis in intestinal macrophages. Macrophages in the lamina propria of the small and large bowel are permanently exposed to a very large number of commensal microorganisms and to ingested antigens and potential pathogens. Therefore, the mechanisms that help to distinguish between harmful or innocuous antigens are vital for macrophages of the gut mucosa. Macrophages actively sample the intestinal lumen and undergo functional changes in response to signals induced by intestinal microorganisms or their metabolites[106,107]. Recognition of luminal microorganisms by macrophages promotes CSF2 release by group 3 innate lymphoid cells. In turn, this helps to maintain DC and macrophage numbers and imprints their regulatory function that supports the induction and local expansion of regulatory T cells[93] (FIG. 3d). Regulatory T cells are the dominant producers of IL-10, which acts through a feedback loop on macrophages to dampen their inflammatory phenotype and prevent excessive immune activation and tissue damage[108,109]. Indeed, deletion of IL-10 receptor, but not the cytokine IL-10, from intestinal macrophages leads to the development of spontaneous colitis in mice[108]. Thus, microbial stimuli trigger CSF2- and IL-10-dependent dampening of macrophage stimulation and drive a tolerogenic programme required for tissue homeostasis. PPARγ expression also helps to dampen tissue damage and modulate the expression of inflammatory cytokines, ultimately contributing to the maintenance of intestinal homeostasis[110,111]. Thus, in both the lungs and intestine, CSF2 is required to maintain essential macrophage functions and ensure appropriate organ homeostasis (FIG. 3c,d).

The homeostatic role of cytokines in the maintenance of macrophage survival and function may depend on the location, the dose and the context in which the cytokine is produced. For example, CSF2 may promote macrophage survival and/or tolerogenic functions when expressed at low doses in healthy intestinal tissues[93], but at higher doses, concomitantly with injury signals, CSF2 may contribute to inflammatory disease[112].

It is unclear why different cytokines are required to maintain macrophage homeostasis. In particular, it is surprising that both CSF1 and IL-34, two cytokines that signal through the same receptor, have been evolutionary conserved in two restricted sites — the epidermis and specific regions of the brain, in mice, birds and humans[79,83,113]. It is also intriguing that both CSF1 and CSF2 are required to maintain two distinct macrophage populations in the lungs[16,114]. Macrophage dependency on distinct cytokines may reflect, in addition to viability requirements, additional genetic imprinting that confers functional specificity. CSF2 production by lung epithelial cells specifically confers alveolar macrophages with the ability to clear lung surfactant[115], whereas lung interstitial macrophages, which require CSF1 but not CSF2 for their homeostasis, are unable to clear surfactant but instead modulate overt inflammatory responses to innocuous airborne antigens[116].

Splenic red pulp macrophages are shaped by CSF1 and haem

In the red pulp of the spleen, macrophages phagocytose senescent erythrocytes and specialize in the recycling of iron stores[117]. CSF1 is expressed in the splenic red pulp[71] and is required for the maintenance of red pulp macrophages. Red pulp macrophages are constantly exposed to senescent red blood cells that contain high levels of haemoglobin and its iron- containing moiety haem. Haem, together with CSF1, acts on red pulp macrophages to induce the expression of SPIC[69], a transcription factor that drives the expression of key red pulp macrophage genes, such as vascular cell adhesion molecule 1 (*VCAM1*), and is required for macrophage maintenance[6,69].

Interestingly, high levels of intracellular haem are toxic to macrophages[118], and thus SPIC-driven signals may be crucial for expanding the macrophage pool to respond to increased levels of senescent red blood cells that may occur under conditions such as haemolysis. SPIC is also expressed by other macrophages that interact with erythrocytes, such as $VCAM1^+$ bone marrow macrophages and a subset of liver macrophages, implicating a role for haem in regulating macrophages that are exposed to erythrocytes[69,119].

Retinoic acid controls the function of peritoneal cavity macrophages

Macrophages and B-1 cells are the main immune cell populations that reside in the peritoneum. Peritoneal cavity macrophages express CSF1R and depend on CSF1 for their maintenance[73], whereas retinoic acid signalling is required for the specialization of peritoneal cavity macrophages[68]. In the omentum, high levels of retinoic acid-converting enzymes allow for macrophage exposure to retinoic acid, which stimulates the expression of *GATA6*, probably through retinoic acid response elements in the *GATA6* promoter[68]. The GATA motif is enriched in open enhancers of peritoneal cavity macrophage-specific genes, such as *TGFB2*, suggesting that retinoic acid-induced GATA6 contributes to shaping the chromatin state of peritoneal cavity macrophages[8]. Retinoic acid-induced GATA6 expression promotes macrophage accumulation in the peritoneal cavity, macrophage self-renewal potential and the expression of many peritoneal cavity macrophage-specific genes, including *TGFB2* and *ASPA*[68,120,121]. TGFβ2 production by peritoneal macrophages drives peritoneal B-1 cell class switching and IgA production[68], and GATA6-deficient mice, which have reduced numbers of peritoneal cavity macrophages and can no longer make TGFβ2, have lower levels of IgA in the intestine[68].

Macrophages control organ homeostasis

Shaped by their environment, macrophages are key sensors of tissue signals and are crucial for the maintenance of organ functionality and immune homeostasis. Here, we discuss the crucial role of macrophage–tissue interactions in tissue homeostasis (FIG. 4).

Bone marrow-resident macrophages

Bone marrow-resident macrophages contribute to the maintenance of bone marrow HSCs by regulating the expression of key HSC retention factors, such as CXC-chemokine ligand 12 (CXCL12) and VCAM1, by $nestin^+$ stromal cells[122]. Following the depletion of macrophages, $nestin^+$ stromal cells lose expression of these retention factors, and HSCs are

released into the bloodstream at significantly higher rates than in macrophage-sufficient controls[122]. Bone marrow macrophages also regulate the circadian release of haematopoietic progenitor cells into the bloodstream by downregulating CXCL12 levels through phagocytosis of aged neutrophils[123]. Stimulation of liver X receptor-α (LXRα) controls macro phage ability to retain haematopoietic progenitors in the bone marrow[94]. Accordingly, absence of LXRα and LXRβ impairs the phagocytic potential of macrophages through the dysregulation of phagocytic receptors, such as tyrosine-protein kinase MER (MERTK)[92,93], and disrupts normal circadian fluctuations in the HSC niche upon engulfment of aged neutrophils[95]. In turn, nestin$^+$ niche cells express high levels of CSF1, although *CSF1* deletion in these cellsdoes not significantly reduce bone marrow macrophage numbers[122,124], indicating that there are probably other regulators of macrophage homeostasis in the bone marrow such as agonists of LXRs[123] (FIG. 4a).

Bone marrow macrophages are also crucial for the production of erythroblasts in the bone marrow, and depletion of macrophages can help to control malignant proliferation of erythroblasts *in vivo*[119]. Together, these results suggest a crucial role for macrophages in the regulation of HSC release into the bloodstream.

Intestinal muscularis macrophages

Macrophages in the intestinal muscularis layer also engage in crosstalk with their surrounding cells. The surrounding muscle cells, enteric neurons and tissue-resident macrophages all contribute to ensuring normal intestinal peristalsis. Intestinal muscularis macrophages produce bone morphogenetic protein 2 (BMP2), which signals through the BMP receptor type 2 expressed by enteric neurons, promoting SMAD translocation to the nucleus and neuronal control of peristaltic activity of the gut muscularis layer[78]. In turn, enteric neurons in response to microbial signals produce CSF1, which is required to maintain muscularis macrophage homeostasis *in vivo*. Alterations in the gut flora, the intestinal macrophages or BMP2 production affect peristaltic activity and subsequently alter colonic transit time[78] (FIG. 4b).

Lymph node subcapsular sinus macrophages

Subcapsular sinus (SCS) macrophages are located in the floor of the SCS and are directly exposed to the afferent lymphatic vessels, allowing them to trap particulate tissue antigens from the lymph and limit systemic dissemination of virus. SCS macrophages present lymph-derived antigens to follicular B cells, probably through immune complexes, to promote the induction of antiviral humoral immunity[125–127]. These macrophages interact closely with B cells and depend on B cell production of lymphotoxin α1β2 (LTα1β2) for their maintenance, differentiation and function[128,129]. Upon viral infection, B cell production of LTα1β2 drives permissive viral replication within SCS macrophages, which in turn promotes macrophage production of type I interferons required for viral clearance[129,130]. Conversely, the positioning of macrophages in the SCS is required for effective B cell responses, and disruption of SCS macrophage positioning alters B cell responses[131]. The close interaction of B cells and SCS macrophages is therefore crucial for the induction of innate and adaptive immunity to tissue antigens that travel via lymphatic vessels (FIG. 4c).

Splenic marginal zone macrophages

Marginal zone (MZ) macrophages are important for the capture of blood-borne antigens and for the proper positioning of MZ B cells[132,133]. Absence of MZ macrophages in LXRα-deficient mice impairs the capture of blood-borne antigens[134] and affects B cell positioning in the MZ, reducing antibody responses to thymus-independent antigens[134]. However, the exact source and contribution of CSF1 and LXRα agonists to macrophage maintenance remain to be established.

Kupffer cells

Embedded in the hepatic sinusoids of the liver, Kupffer cells are well positioned to capture blood antigens. They express the transcription factor LXRα, and the LXR motif is enriched in the enhancers of Kupffer cell-specific genes[8,135]. Kupffer cells are also involved in recycling erythrocytes and therefore, similar to red pulp macrophages, they express haem-related genes such as haem oxygenase 1 (*HMOX1*) and may be partially dependent on the transcription factor SPIC[8,69,136]. The direct role of hepatocytes in Kupffer cell maintenance is unclear but, during infection, hepatocyte-derived IL-33 leads to the release by basophils of IL-4 and promotes the proliferation of monocyte-derived macrophages[54]. Local CSF1 production also contributes to Kupffer cell maintenance[71], but the exact source of CSF1 in the liver is unknown.

Cardiac macrophages

Cardiac macrophages are in close contact with cardiomyocytes throughout the myocardium where they have important homeostatic roles through the phagocytosis of dying cardiomyocytes and debris, and the production of pro-angiogenic and tissue-protective molecules[137]. CSF1 is highly expressed in the heart tissue in comparison to other organs, probably contributing to the maintenance of macrophages[80].

Cardiac monocytes are heterogeneous in origin and derive from embryonic and adult haematopoiesis (as discussed earlier), with adult monocyte-derived macrophages increasing with age as embryonic macrophages lose their capacity for self-renewal[15,51]. Embryonic and adult monocyte-derived macrophages have different propensities for phagocytosis and inflammasome activation[15]. Monocyte-derived macrophages have pro-inflammatory potential and lack reparative activities, and inhibition of monocyte recruitment to the adult heart preserves embryonic-derived macrophage subsets, reduces inflammation and enhances tissue repair[56]. The mechanisms that regulate the maintenance and proliferation of these distinct populations remain to be analysed.

Macrophages and tissue cells thus engage in crosstalk to modulate both immune and tissue homeostasis. Distinguishing between monocytes and resident macrophages has provided further clarity in establishing the role of local tissue signals that regulate the maintenance and proliferation of tissue-resident macrophages. These local communication networks are being elucidated in both the steady state and inflammation and provide important insights into the mechanisms by which macrophages contribute to tissue maintenance.

Tissue control of macrophage plasticity

The plasticity of macrophages in inflammatory settings has been well described and extensively studied in the setting of tumours (BOX 2), in which macrophages can alternatively express pro-inflammatory or immunemodulatory cytokines and other molecules[138,139]. More recently, it has been appreciated that tissue-specific regulation provides an additional layer that contributes to macrophage plasticity.

Bone marrow chimeric mice have been used to explore the contribution of tissue factors to reprogramming macrophage identity. Exposure to a lethal dose of ionizing radiation (approximately 12 Gy in C57BL/6 mice) eliminates most embryonic-derived tissue-resident macrophages — with the exception of microglia[11,140], Langerhans cells[10] and potentially Kupffer cells[141]— and promotes their replacement by donor-derived adult bone marrow progenitors. These studies revealed that macrophages that derive from donor bone marrow cells almost entirely recapitulate the enhancer profile and transcriptional programme of the embryonic-derived macrophages that they replace[8,142]. Moreover, in mice with impaired development of lung tissue-resident macrophages, bone marrow-derived cells can restore the function of alveolar macrophages, clear surfactant and protect from lung inflammatory diseases[142–144].

Recent results have also revealed that even terminally differentiated peritoneal macrophages can retain some levels of plasticity and adapt to new environmental cues. For example, peritoneal cavity macrophages, when adoptively transferred into the lung microenvironment, down-regulate *GATA6* and upregulate *PPAR*G expression, along with other lung macrophage-specific gene transcripts, although some transcripts remained fixed and retained the signature of the tissue of origin[8]. Accordingly, *in vitro* exposure to TGFβ alters the enhancer profile of differentiated peritoneal macrophages[64], and a vitamin A-deficient diet dramatically alters peritoneal macrophage expression of *GATA6*, which in turn compromises macrophage homing and function in the peritoneum[68]. Therefore, although tissue-derived signals have a key role in shaping macrophage functional identity, tissue-resident macrophages retain the ability to adapt to new environments. Determining the genes that can be remodelled and those that are fixed may help to identify new physiological cues and novel potential targets that modulate macrophage cell fate and differentiation in tissues.

Conclusion

Macrophages are an essential part of the tissue immune compartment. They are in close contact with the surrounding stroma where they constantly sense environmental cues. Tissue-specific cytokines and metabolites contribute to shaping the local steady-state programme of tissue-resident macrophages. In turn, macrophages are important regulators of tissue homeostasis during the steady state and inflammatory settings. The crucial nature of macrophage interactions with the local microenvironment adds another layer of complexity to macro phage functional plasticity. More work is needed to better define the tissue-specific metabolites and regulators of macrophage function in different tissues and how they are affected during inflammation and disease. It is also important to further probe whether the

tissue regulators and macrophage transcription factors that have been defined in mice have equally important roles in tissue-resident macrophages in humans.

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Box 1 | Novel tools for macrophage research

Inducible gene fate mapping models. These models rely on mice expressing an inducible Cre recombinase-encoding gene under the control of endogenous promoters that are expressed by the cell population of choice crossed with mice expressing floxed reporter transgenes that are driven by a ubiquitous reporter such as ROSA26. Upon induction of Cre recombinase expression, the stop-flox cassette is removed, and a reporter transgene is introduced in discrete cell populations at a specific time point. Introduction of the reporter transgene helps to trace the fate of specific populations that originated at a distinct time point during development or adulthood. In these models, the genetic marker that is introduced is irreversible and, therefore, all the progeny of the marked cells express the same genetic marker. However, there are caveats to the floxed-Cre approaches. Expression of the gene expressing Cre recombinase depends on a specific promoter or enhancer, which may be expressed in multiple cell types or expressed at levels that are insufficient to drive the level of Cre recombinase expression necessary for the removal of the *loxP* site and subsequent cell labelling. Thus, too little Cre expression can result in incomplete labelling of the target cell populations, whereas 'leaky' expression can result in undesired cells being labelled.

Mass cytometry (also known as CyTOF). This approach combines mass spectrometry with the principles of flow cytometry and allows samples to be tagged with approximately 40 different antibodies specific for surface markers or intracellular targets[145]. Compared with standard flow cytometry approaches, it provides a much more detailed analysis of the relative proportions of all immune cells within one sample. It has been used to identify new subpopulations of cells and has also stimulated the development of new tools to analyse the data. Tools such as viSNE and phenograph attempt to cluster populations based on unbiased approaches[146–148]. Using these improved tools, we now have a better ability to finely distinguish between tissue-resident macrophages and monocytes that accumulate at sites of inflammation and to understand how they change their phenotype in these contexts.

Molecular epigenetics. With improved techniques for high-throughput chromatin immunoprecipitation and sequencing[149], cells sorted from mice or humans can now be analysed at the chromatin level[8,59]. This will continue to expand our understanding of the DNA remodelling that occurs in phagocytes in the steady state and during inflammation. The past and future potential of cells are imprinted on the chromatin, making this dynamic layer of great interest[58]. Moreover, the role of chromatin modifiers in the context of inflammation has been of increasing interest, as such modifiers have been implicated in different diseases and cell types[150]. As fewer cells are needed, our understanding of how individual cells respond to a stimulus will continue to improve.

Single-cell genomics. Single-cell RNA sequencing (RNA-seq) is now a well-established technique that provides a snapshot of RNA presence and quantities at a given time[151,152]. This technique could soon be used to finely map how individual cells respond to a stimulus and to explore to what degree these responses are due to their lineage, epigenetics, cellular interplay or location.

Next-generation histology. With the identification of new cell subpopulations by mass spectrometry and transcriptomics, it is becoming increasingly important to now define their spatial distribution within the tissue. Multiplex immunohistochemistry techniques that allow for high dimensional analysis of tissues while preserving tissue architecture have recently been developed and should help to identify complex cell populations and cellular interactions in tissues. Similarly, *in situ* RNA-seq that assesses the transcriptome of cells within a structure has an important role in dissecting local interactions[153,154], and mass spectrometry-based immunohistochemistry can be used to detect many proteins in a single histological sample using large panels of metal-based antibodies[155,156]. Determining the effect of localization and local interactions on the range of macrophage phenotypes present in tissues will provide important insight into their function and response to stimuli.

Box 2 | Macrophages in tumours

Similarly to chronically injured tissues, tumour lesions are heavily populated by monocytes and macrophages. The quantification and prognostic value of macrophage accumulation in human tumours have mainly been assessed by histological staining for CD68 (also known as macrosialin)$^{+}$ cells. However, staining for CD68 does not distinguish between tissue-resident macrophages, monocytes and monocyte-derived cells and does not assess the functional state of the macrophages, which has led to considerable confusion regarding the prognostic value of tumour-associated macrophages.

Tumour-associated macrophages are highly heterogeneous, with a mixture of pro-inflammatory and anti-inflammatory gene expression signatures[157,158] that may reflect a range of macrophage activation pathways and/or responses by different macrophage populations. Indeed, macrophages at different stages in tumour development may function differently; the resident macrophages and monocyte-derived inflammatory macrophages may have distinct functions, and different macrophage effector functions are likely to be induced depending on the nature of the tumour tissue. This may explain why, in some tumours, the accumulation of CD68^{+} cells correlates with tumour growth and decreased survival[159,160], whereas in other tumours (such as non-small cell lung carcinoma and colorectal cancer) the presence of CD68^{+} cells correlates with prolonged survival[161,162]. Better characterization of macrophages in tumours through the use of multiple surface markers that define ontogeny and functional diversity should help to clarify the roles of macrophages in cancer.

Similar to healthy tissues, crosstalk between the tumour tissue and macrophages can benefit both tumour and macrophage homeostasis. One of the best examples of this crosstalk has been identified in mammary tumour lesions, in which mammary carcinoma cells produce colony-stimulating factor 1 (CSF1) to promote the recruitment and survival of macrophages, as well as the induction of epidermal growth factor (EGF) production by macrophages. Macrophage production of EGF promotes invasion of the tumour by blood cells and induces further CSF1 production by carcinoma cells, thereby generating a positive feedback loop that enhances both tumour spread and macrophage survival[163,164].

Our improved understanding of the distinction between tissue-resident macrophages and monocyte-derived cells has raised the question of whether they may have distinct roles in the context of tumours. Moreover, as the role of the environment and tissue–macrophage crosstalk has been shown to shape steady state macrophages, understanding the contribution of tumour-specific environmental signals to macrophage phenotype and function will become more important. Exploring how distinct tumour environments shape the function of resident macrophages and monocytes will provide important insights into the role of phagocytes in cancer and how they might be best modulated by immunotherapies.

Mononuclear phagocyte system

(MPS). A group of bone marrow-derived cells (monocytes, macrophages and dendritic cells) with different morphologies. These cells are mainly responsible for phagocytosis, cytokine secretion and antigen presentation.

Parabiotic mice

Mice in which the blood circulation has been joined surgically. Parabiotic mice share the same blood circulation and exchange blood precursor cells, thereby providing a model to trace the physiological contribution of circulating precursors to tissue-resident cells.

B-1 cells

An innate-like population of B cells found mainly in the peritoneal and pleural cavities. B-1 cell precursors develop in the fetal liver and omentum. B-1 cells recognize self-antigens as well as common bacterial antigens and secrete antibodies of low affinity and broad specificity.

Omentum

A fatty tissue in the peritoneum that connects the spleen, stomach, pancreas and colon.

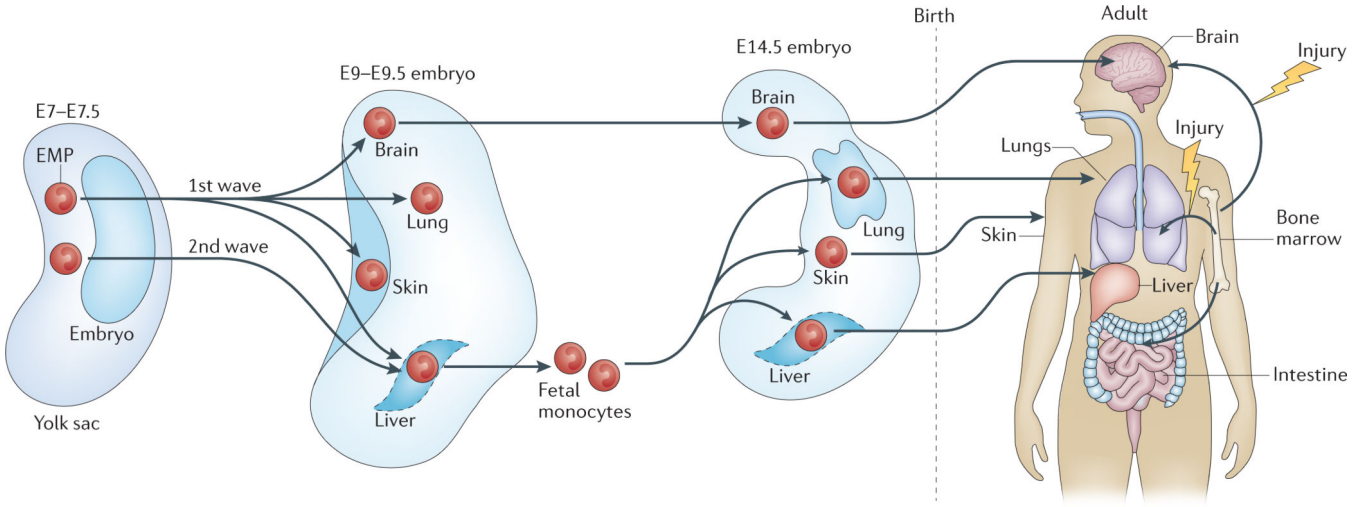

Figure 1. Origin of tissue-resident macrophages

Macrophages are maintained in most healthy tissues in mice by embryonic precursors (embryonic macrophages) independently of monocytes, with the exception of intestinal macrophages, dermal macrophages and a subset of cardiac macrophages. Two hypotheses dominate our thinking about the origin of embryonic macrophages. The first hypothesis suggests that all embryonic macrophages derive from yolk sac-derived erythromyeloid precursors (EMPs) that develop around embryonic day 7.5 (E7.5). The second hypothesis suggests that yolk sac-derived EMPs arise in two waves that differentially contribute to adult microglia and other macrophages: an early wave of yolk sac-derived EMPs that appear around E7.5 in the yolk sac colonize the brain and other fetal tissues around E9 to give rise to all tissue macrophages, and a later wave of yolk sac-derived EMPs that colonize and expand in the fetal liver to differentiate into fetal liver monocytes, which subsequently replace fetal macrophages, with the exception of microglia, and maintain them in adulthood.

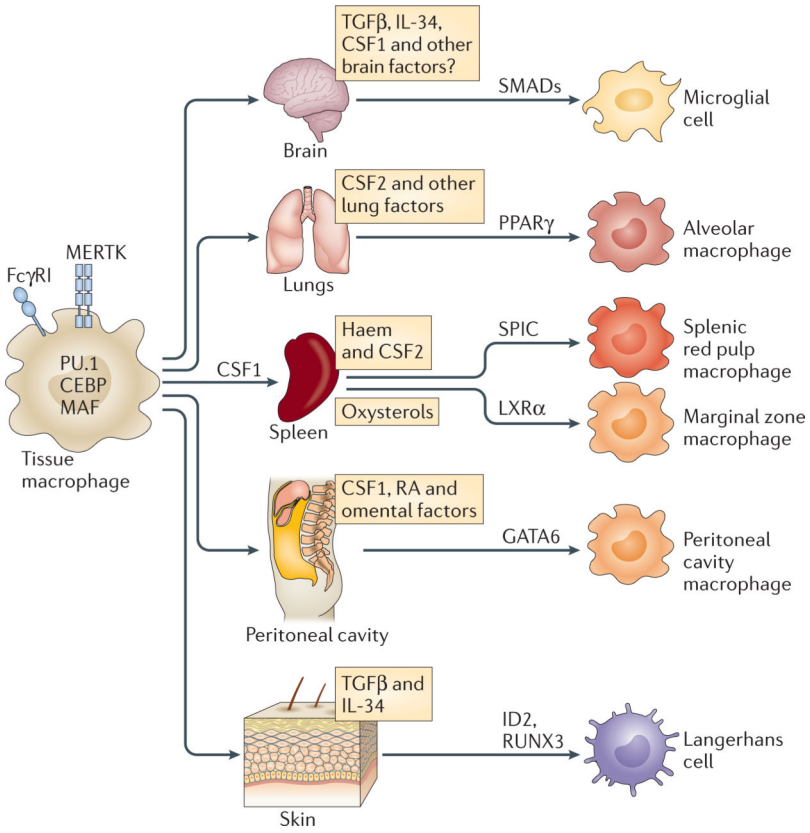

Figure 2. The tissue microenvironment determines macrophage differentiation cues

During embryonic development, macrophages enter the tissues where they self-renew and proliferate. Macrophages in all tissues are characterized by expression of the cell surface marker FcγRI (also known as CD64), tyrosine-protein kinase MER (MERTK) and the transcription factors PU.1, CCAAT/enhancer-binding protein (CEBP) family members, MAF and MAFB. In the tissues, macrophage identity and functions are shaped by cytokines and metabolites that are produced in the local environment and drive specific transcription factor expression. In the brain, incoming yolk sac-derived cells are exposed to locally expressed transforming growth factor-β (TGFβ), which drives SMAD phosphorylation and the expression of genes that are unique to microglia. In the lungs, fetal monocytes that are exposed to colony-stimulating factor 2 (CSF2) express peroxisome proliferator-activated receptor-γ (PPARγ), which drives their differentiation into alveolar macrophages. In the spleen, haem drives *SPIC* expression, which controls the differentiation and maintenance of red pulp macrophages and the expression of key splenic red pulp macrophage-specific molecules, including vascular cell adhesion molecule 1 (VCAM1). In the marginal zone of the spleen, macrophage maintenance depends on liver X receptor-α (LXRα)-mediated signals. Retinoic acid (RA) and omental factors induce the expression of GATA-binding protein 6 (GATA6), which promotes the differentiation of peritoneal cavity macrophages. ID2, inhibitor of DNA binding 2; IL-34, interleukin-34; RUNX3, runt-related transcription factor 3.

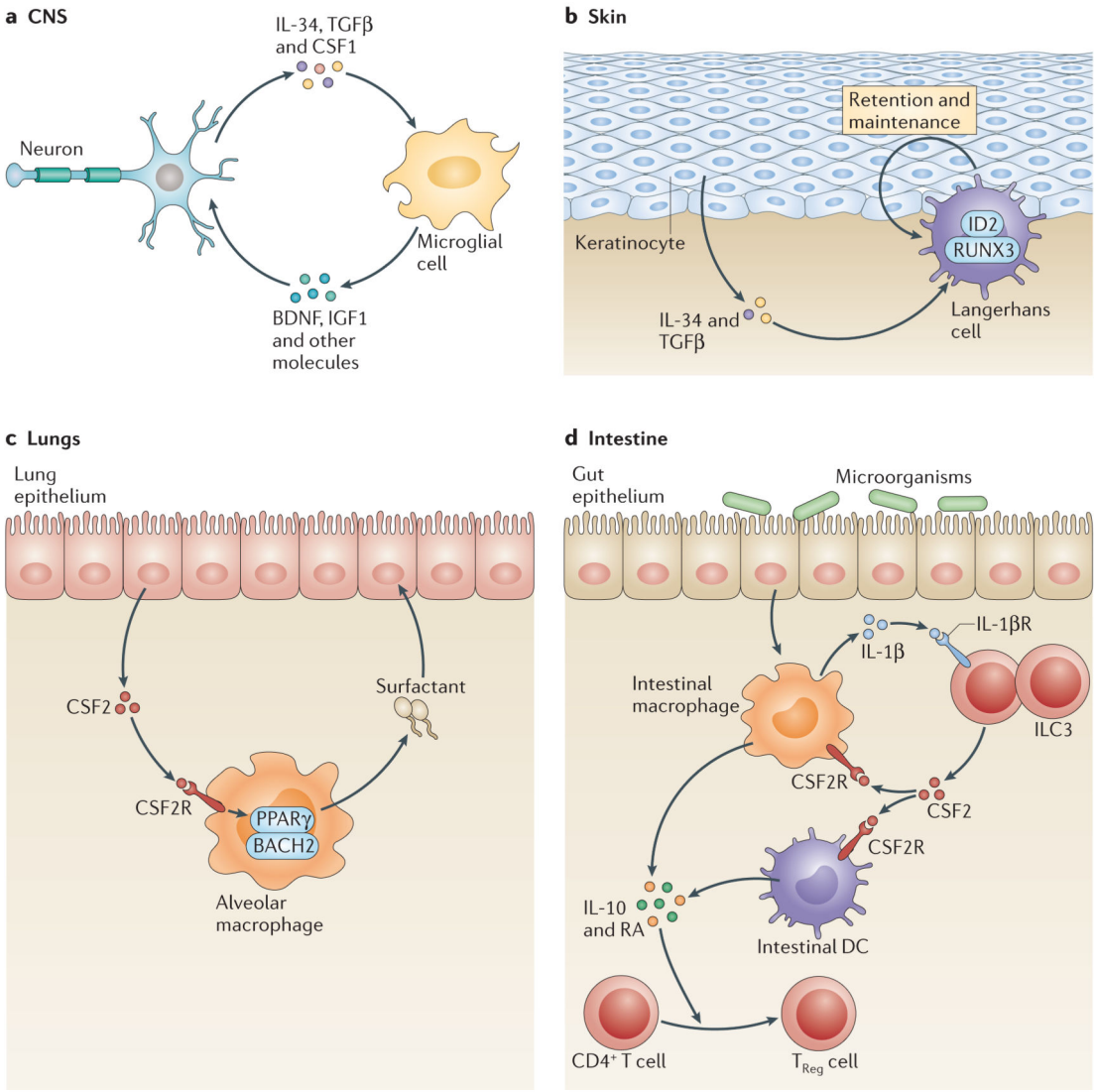

Figure 3. IL-34 and CSF2 regulate specific tissue macrophage maintenance

a | In the brain, interleukin-34 (IL-34) is produced by neurons in the cortex, hippocampus and striatum and is necessary for microglial cell maintenance. Transforming growth factor-β (TGFβ) may be important for microglia maintenance, but it is still unclear which cells produce it in the brain. Conversely, microglia produce brain-derived neurotrophic factor (BDNF), which is important for learning-dependent synapse formation, and insulin-like growth factor 1 (IGF1), which is crucial for survival of layer V cortical neurons. **b** | In the skin, keratinocytes produce IL-34 and TGFβ, which are required for Langerhans cell homeostasis in the epithelium. TGFβ drives the expression of the transcription factors inhibitor of DNA binding 2 (ID2) and runt-related transcription factor 3 (RUNX3) in Langerhans cells and promotes their retention in the epidermis. The exact mechanism by which IL-34 drives Langerhans cell maintenance in the epidermis is unclear. **c** | In the lungs, epithelial cell-derived colony-stimulating factor 2 (CSF2) promotes peroxisome proliferator-activated receptor-γ (PPARγ) expression, and final maturation of alveolar macrophages is required for surfactant catabolism, which clears excess surfactant and maintains lung

homeostasis. **d** | In the intestine, microbial products drive macrophage production of IL-1β that stimulates group 3 innate lymphoid cells (ILC3s) to produce CSF2. ILC3-mediated CSF2 production promotes the survival of intestinal macrophages and dendritic cells (DCs) and their production of IL-10 and retinoic acid (RA), which are required for the maintenance of regulatory T(T_{Reg}) cell homeostasis in the intestine. CSF2R, CSF2 receptor.

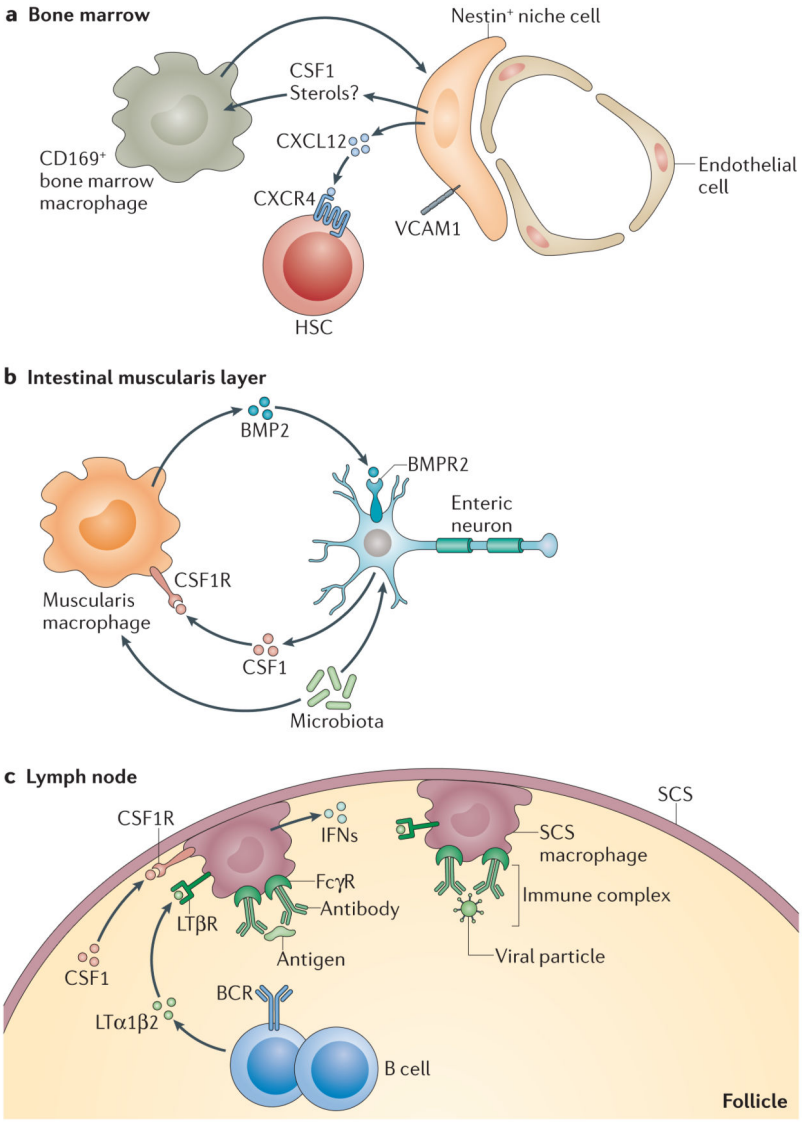

Figure 4. Macrophage–tissue crosstalk

a | Bone marrow macrophages are important for stimulating nestin^{+} niche cells to produce haematopoietic stem cell (HSC) retention factors, such as CXC-chemokine ligand 12 (CXCL12) and vascular cell adhesion molecule 1 (VCAM1). In turn, niche cells may produce colony-stimulating factor 1 (CSF1) to help to maintain macrophages in the niche. **b** | Intestinal muscularis macrophages produce bone morphogenetic protein 2 (BMP2), which signals through BMP receptor type 2 (BMPR2) expressed on enteric neurons and promotes neuronal control of peristaltic activity of the gut muscularis layer. In turn, enteric neurons, in response to microbial signals, produce CSF1, which is required to maintain muscularis macrophage homeostasis *in vivo*. **c** | In the lymph nodes, macrophages are situated directly beneath the subcapsular sinus (SCS) surrounding the B cell zone. They present large particulate antigen to B cells in the form of immune complexes and are important for trapping viral particles. In turn, B cells help to retain the SCS macrophage layer through the secretion of lymphotoxin α1β2 (LTα1β2). SCS macrophages also depend on local

production of CSF1, although its source is unknown. BCR, B cell receptor; CSF1R, CSF1 receptor; FcγR, Fc receptor for IgG; IFNs, interferons; LTβR, lymphotoxin-β receptor.

Table 1

Tissue-resident macrophage phenotypes in healthy tissues

Macrophage subset	Tissue	Homeostatic function	Specific surface markers	Specific transcription factors	Cytokine and metabolite regulation
Alveolar macrophages	Lung, alveoli	Surfactant clearance	F4/80, Siglec-F, CD11c^{hi}, CD169	PPARγ, BACH2 (REF. 105)	CSF2 (REFS 16,115)
Red pulp macrophages	Spleen red pulp	Erythrocyte clearance, iron recycling	F4/80, VCAM1	SPIC[6,69], PPARγ?	Haem[69], CSF2?, CSF1 (REF. 71)
Marginal zone macrophages	Spleen marginal zone	Trap circulating particulates, marginal zone B cell maintenance	SIGN-R1, MARCO	LXRα[134]	CSF1 (REF. 72)? sterols[134]?
Metallophilic macrophages	Spleen, adjacent to white pulp and marginal sinus	Sample the circulation	CD169, MOMA1	LXRα[134]	CSF1 (REF. 71), sterols[134]?
Kupffer cells	Liver, sinusoids	Erythrocyte clearance, bilirubin metabolism, particulate antigen clearance from portal circulation	F4/80, CD169, CLEC4F	LXRα[135]? SPIC[69]?	CSF1 (REF. 71), haem?
Microglial cells	Brain, CNS	Synaptic pruning, learning-dependent synapse formation	CX_3CR1, CD45^{mid}, FCRLS, Siglec-H	SMADs[64,87]? SALL1 (REF. 84)? MEF2C[8]? IRF8 (REF. 165)	IL-34 (REFS 79,83), TGFβ[84,87]
Peritoneal cavity macrophages	Peritoneum	Maintain B-1 cell-derived IgA production	ICAM2, F4/80^{hi}	GATA6 (REFS 68, 120,121)	CSF1 (REF. 73), retinoic acid, omental factors[68]
Langerhans cells	Epidermis	Skin tolerance and immunity	CD11c^{hi}, MHC class II^{hi}, EPCAM	ID2, RUNX3 (REFS 90,91)	IL-34 (REF. 79,83), TGFβ[85,89]
Lamina propria intestinal macrophages	Large and small intestinal lamina propria	Gut tolerance and immunity	F4/80, CX_3CR1	RUNX3 (REF. 8)?	CSF1 (REF. 71), CSF2 (REF. 93), IL-10 (REF. 108), microbial products[166]
Intestinal muscularis macrophages	Muscularis layer of intestine	Maintain normal peristalsis	CX_3CR1, MHC class II^{hi}	ND	CSF1, microbial products[78]
Bone marrow macrophages	Bone marrow	Regulate retention factors on nestin^{+} HSC niche cells, erythroblast development and release, remove aged neutrophils	VCAM1, CD169	SPIC[69], LXRα[123]	CSF1 (REF 122), sterols[123]?
Subcapsular sinus macrophages	Lymph node	Trap particulate antigens	CD169	ND	CSF1 (REF. 71), LTα1β2 (REF. 128)
Cardiac macrophages	Heart	Phagocytose dying cardiomyocytes	Subpopulations distinguished by: CX_3CR1, MHC class II, CCR2	ND	CSF1 (REF. 80)

CCR2, CC-chemokine receptor 2; CLEC4F, C-type lectin domain family 4 member F; CNS, central nervous system; CSF, colony-stimulating factor; CX_3CR1, CX_3C-chemokine receptor 1; EPCAM, epithelial cell adhesion molecule; FCRLS, Fc receptor-like S; GATA6, GATA-binding protein 6; HSC, haematopoietic stem cell; ICAM2, intercellular adhesion molecule 2; ID2, inhibitor of DNA binding 2; IL, interleukin; IRF8, interferon-regulatory factor 8; $LT\alpha1\beta2$, lymphotoxin $\alpha1\beta2$; LXRα, liver X receptor-α; ND, not determined; MEF2C, monocyte enhancer factor 2C; PPARγ, peroxisome proliferator-activated receptor-γ; RUNX3, runt-related transcription factor 3; SALL1, Sal-like protein 1; Siglec, sialic acid-binding immunoglobulin-like lectin; TGFβ, transforming growth factor-β; VCAM1, vascular cell adhesion molecule 1.