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Macrophage-fibroblast circuits in the spleen

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Running title:

Splenic fibroblast-macrophage circuits

Summary:

Macrophages are an integral part of all organs in the body, where they contribute to immune surveillance, protection, and tissue-specific homeostatic functions. This is facilitated by so-called niches composed of macrophages and their surrounding stroma. These niches structurally anchor macrophages and provide them with survival factors and tissue-specific signals that imprint their functional identity. In turn, macrophages ensure appropriate functioning of the niches they reside in. Macrophages thus form reciprocal, mutually beneficial circuits with their cellular niches.

In this review, we explore how this concept applies to the spleen, a large secondary lymphoid organ whose primary functions are to filter the blood and regulate immunity. We first outline the splenic micro-anatomy, the different populations of splenic fibroblasts and macrophages and their respective contribution to protection of and key physiological processes occurring in the spleen. We then discuss firmly established and potential cellular circuits formed by splenic macrophages and fibroblasts, with an emphasis on the molecular cues underlying their cross-talk and their relevance to splenic functionality. Lastly, we conclude by considering how these macrophage-fibroblast circuits might be impaired by aging, and how understanding these changes might help identify novel therapeutic avenues with the potential of restoring splenic functions in the elderly.

Keywords:

Macrophage, Spleen, Stromal cell, Niche

1.1 Functions of the spleen

Located in the abdominal cavity between the diaphragm and the fundus of the stomach, the spleen is the largest secondary lymphoid organ in the body. It acts mainly as a blood filter that selectively removes immune complexes, circulating pathogens and senescent, dysfunctional, or infected red blood cells.¹ As a result, splenectomy is associated with an increased susceptibility to severe bacterial infections (*Streptococcus pneumoniae*, *Haemophilus influenzae*, *Neisseria meningitidis*...) and a major risk of overwhelming sepsis.^{2,3}

Besides its immune function, the spleen is involved in other important physiological processes such as iron homeostasis. Senescent or ruptured red blood cells are trapped in the spleen and their hemoglobin content is metabolized into free iron, which supplies most of the iron needed for erythropoiesis.^{4,5} The adult spleen is also a prominent site for extramedullary hematopoiesis. In conditions of infection, anaemia and genetic blood disorders or during pregnancy, hematopoietic stem cells (HSC) mobilized from the bone marrow (BM) migrate into newly formed splenic niches, where they differentiate into blood cells.⁶⁻⁸ The spleen also acts as a cell reservoir for monocytes, thrombocytes, plasmablasts, long-lived memory B cells and red blood cells.⁹⁻¹³ During hemorrhage, acute physiological stress ("fight or flight" reaction) or apnoeic diving in aquatic mammals, contraction of the spleen increases the number of systemic red blood cells and platelets.¹³⁻¹⁵ Finally, rapid deployment of splenic monocytes participates in healing of the affected cardiac tissue in response to ischemic myocardial injury.¹⁶

Appropriate functioning of the spleen depends on various cells acting in concert. Recent evidence suggest that fibroblasts and macrophages engage in bi-directional, virtuous dialogues crucial for tissue homeostasis. In this review, we will discuss how cooperation of these two cell types underlies the pleiotropic functions of the spleen.

1.2 Overview of the splenic anatomy

The spleen is surrounded by a contractile capsule and compartmentalized into distinct microanatomical zones, each fulfilling different purposes (Figure 1). The lymphoid part of the spleen, the **white pulp (WP)**, sheathes the smaller branches of the splenic arterial supply and is organized in a way reminiscent of lymph nodes, with peripheral B cell follicles surrounding inner T cell zones. As there are no afferent lymphatic vessels in the spleen, cells and antigens reach the WP via the blood. The WP is surrounded by the **marginal zone (MZ)**, which separates the lymphoid and non-lymphoid parts of the spleen (Figure 1). Most of the arterial blood entering the spleen flows through the MZ, pouring into a marginal sinus (Figure 1) that connects to the surrounding fibroblastic tissue of the MZ.¹⁷ Therefore, the MZ plays an important part in blood screening and trafficking of cells into the organ. The rest of the arterial blood is discharged by open-ended capillaries into the cords of the **red pulp (RP)** (Figure 1), a complex meshwork of fibroblasts and reticular fibres forming a structural frame of an open blood circulation. The bloodstream subsequently runs into peculiar structures called venous sinuses (Figure 1). The specific architecture of the cords' reticular meshwork and sinuses enables the selective removal of senescent red blood cells in the RP.^{1,18} Each of these splenic compartments is populated by distinct sets of fibroblasts, which assemble in complex 3D cellular meshworks that we will now briefly introduce.

2. Splenic fibroblasts

The spleen is divided into various areas delineated by cellular fibroblastic networks that underpin its structural integrity (Figure 2 and table 1). As these cells have been extensively described by others,^{19–21} we will here only summarize some of their key attributes as they pertain

to their potential interactions with splenic macrophages. Developmentally, all subsets of splenic stromal cells derive from early mesenchymal progenitors expressing the transcription factors *Islet-1* and *Nkx2.5*.²² These progenitors are found in the splenic primordium as early as embryonic day (E) 10.5 and ultimately differentiate into mature stromal subsets of both, the RP and WP.²² In addition to their structural functions, these stromal cells also directly impact on immune responses by controlling recruitment and distribution of immune cells to the different areas of the spleen.²³ Finally, in the spleen as well as other lymphoid and non-lymphoid tissues, stromal cells are also emerging as cellular partners within macrophage niches.

2.1 White Pulp and Marginal Zone fibroblasts

Splenic WP reticular cells broadly resemble those of the LN in their respective localization and functions. The center of the WP is populated by **T-cell zone reticular cells (TRCs)** (Figure 2 and table 1) characterized by expression of podoplanin (PDPN),²⁴ PDGFR α , PDGFR β and Vascular Cell Adhesion protein 1 (VCAM1). Much like in lymph nodes,^{25,23,26} TRCs attract and nurture CCR7⁺ T cells and dendritic cells (DC) to and in the splenic T cell zone through the production of the homeostatic chemokines CCL19, CCL21²⁷ and the T cell survival factor IL-7.²⁸ On the other hand, **follicular dendritic cells (FDCs)** (Figure 2), the reticular subsets of the B cell follicle, attract B and T follicular helper cells and direct them to the follicles via CXCL13.²⁹ Mature FDCs retain antigen on their surface via complement receptors and thereby also participate in the maturation of the immune response (for review,³⁰). CXCL12-producing cells in follicles might regulate B-cell trafficking and germinal center reactions.³¹

Marginal reticular cells (MRC) (Figure 2) populate the border of the MZ that lines the sinus, and they express MAdCAM-1 and CXCL13. These fibroblasts delineate the WP region³² and enhance the capture and trafficking of antigens into B cell follicles.^{33,34} MRCs attract CXCR5⁺ B

cells via the production of CXCL13 (for review,³⁵⁻³⁷). They also promote B cell-ILC interactions during the generation of antibody responses³⁸ and initiate antiviral immune responses.³⁹ They represent the niche for MZ B cells and probably the one of CD169⁺ metallophilic macrophages, as discussed in more detail below. Indeed, MRC express RANK and the Notch ligand Delta-like 1, which are important for the maintenance of the MZ myeloid cell populations⁴⁰ and the differentiation of MZ B cells and Esam⁺ DC, respectively.⁴¹ Finally, **perivascular reticular cells (PRCs)** (Figure 2 and table 1) are characterized by expression of PDGFR α , PDGFR β , and Sca-1. Intriguingly, this phenotype is reminiscent of CCL19⁺ fetal lymphoid tissue organizer cells, and PRCs indeed appear to retain stemness in adulthood and can differentiate into the different WP reticular cells in a LT β R-dependent manner.⁴² Inference of differentiation trajectories from single cell transcriptomic data suggests that PRCs can also generate splenic **mural cells**, a subset of perivascular cells surrounding splenic blood vessels that expresses pericyte markers (e.g. NG2, CD146).⁴²

2.2 Red Pulp fibroblasts

The RP cords are populated by RP fibroblasts (Figure 2 and table 1). In mice, they express PDGFR β and CXCL12, through which they can retain CXCR4⁺ differentiating plasma B cells.^{11,43} In agreement with this, human CD54⁺ RP stromal cells expressing CXCL12 produce IL-6 and are suspected to stimulate antibody production by CD11a⁺ plasma cells.¹¹ RP fibroblasts also produce stem cell factor (SCF), a critical regulator of HSC, potentially implicating them in splenic extra-medullary hematopoiesis.⁶ The most abundant population of VCAM1⁺ RP fibroblasts specifically expresses the transcription factor WT1.⁴⁴ Their transcriptome is enriched for genes associated with mesenchymal differentiation and endothelial cell function.^{42,44} These fibroblasts are key regulators of macrophage homeostasis in the RP,⁴⁴ as we will discuss below.

2.3 Capsule fibroblasts

The spleen is surrounded by a contractile capsule formed by a dense connective layer containing smooth muscle fibers, which enter the splenic parenchyma and form the trabeculae. With the exception of the hilum, the surface of the capsule is covered by a single layer of mesothelial cells. This capsule contains different stromal subsets⁴⁵ that can be found from neonates to adult mice. Interestingly, the neonatal capsule can fully regenerate all splenic tissues with functional capacity upon grafting. This potential has been attributed to the presence of putative stromal MAdCAM-1⁺LT β R⁺ lymphoid tissue organizer cells, which rely on LT α 1 β 2 signals for regeneration.⁴⁵ Contrary to WP FRCs, the fibroblasts of the mesothelium do not express Ccl19, as revealed recently by single cell transcriptomic analyses of splenic fibroblasts from Ccl19-*ieYFP* mice. This work also demonstrated that the outer layer of the splenic capsule is made up of specific fibroblasts expressing mesothelin, while a subcapsular layer is formed by fibroblasts expressing Lumican.⁴²

3. Splenic macrophages

The spleen comprises several subsets of macrophages (Figure 3 and table 2) that can be distinguished by their locations, developmental origins, phenotypic profiles and functional specializations. The major subsets of splenic macrophages will be reviewed in the following sections.

3.1 Red Pulp Macrophages (RPM)

RPM reside in the splenic cords, where they “bath” in the open blood circulation in close association with RP fibroblasts (Figure 3 and table 2).^{46,47,44} These macrophages control iron homeostasis via the recycling of iron contained in aged erythrocytes. RPM express high levels of F4/80, VCAM1, CD68, as well as scavenger receptors (CD163, CD91, signal regulatory protein alpha (SIRP α), CD36, complement receptor 3, Fc receptors), which allow them to clear free hemoglobin, free heme and senescent erythrocytes retained in the reticular network of the RP.^{4,5,48,49} The mechanisms by which senescent erythrocytes are recognized and degraded by RPMs are still ill-defined, since erythro-phagocytic events are rarely observed *in vivo* in RPM.^{50,48} *In vivo* imaging and transfusion experiments in mice showed that senescent erythrocytes interact with the extracellular matrix of the splenic architecture and ultimately release their cytoplasmic content through rupture of their cell membrane, a process known as hemolysis.⁴⁸ It is thought that RPM recognize and phagocytose the remnant erythrocyte “ghost shells”, now devoid of hemoglobin, along with scavenger-associated heme and hemoglobin. Once they have captured senescent red blood cells, RPM must degrade their toxic cargo. To this end, RPM are equipped with a molecular machinery capable of neutralizing the toxic effects of heme and metabolizing iron.⁵¹⁻⁵⁴ Following hemoglobin degradation in the phagolysosome, heme is transported into the cytosol by the heme-transporter HRG1, where it is catabolized into iron by heme oxygenase-1 (*HO1*). Cytosolic iron is either stored in ferritin complexes or exported into plasma through ferroportin (FPN).⁵ Intracellular heme is also a strong inducer of the transcription factor SPI-C, which regulates RPM homeostasis.^{51,52} Indeed, mice deficient in *SpiC* or *HO1* are largely devoid of RPM, underscoring that these genes are critical for RPM development and survival after erythrocyte clearance.^{51,53} Specifically, SPI-C was shown to increase transcription of *Fpn* in LPS-activated tissue macrophages, and *SpiC* KO pre-RPM display reduced expression of this transporter.⁵⁴ These observations indicate that

SpiC deficiency could cause lethal excess of intracellular iron in RPM due to insufficient FPN-mediated export.⁵⁴

Aside from their role in iron homeostasis, RPM are also strategically located to detect and defend the organism against blood-borne infections, such as malaria. Early microscopy studies in monkeys and mice have established that RPM phagocytose free or internalized parasites and infected erythrocytes.^{55,56} In mice, RPM also take part in the control of parasitemia by promoting immune responses during blood stage malaria. Infection of *Ifnb*^{-Yfp} reporter mice with the rodent malaria parasite *P. chabaudi* has demonstrated that RPM are a primary source of type I interferon, which promotes the inflammatory response needed to fight this parasite.⁵⁷ Yet, *SpiC* KO mice are still able to control the infection, supporting the notion that RPM are dispensable for the clearing of malaria.⁵⁷ However, *SpiC* KO mice do display increased numbers of monocytes in blood and spleen compared to WT controls, suggesting that the lack of RPM is compensated by recruitment of monocytes to the spleen.⁵⁷⁻⁵⁹ RPM are also involved in the recognition and elimination of fungal and bacterial infections. Histological examination of spleens from rodents injected with capsular polysaccharide from *Cryptococcus neoformans* showed selective capture of this microbial compound by RPM.⁶⁰ In mice lacking MZ macrophages as a result of chronic *Leishmania donovani* infection, resistance to co-infection with *Streptococcus pneumoniae* depends on RPM. Consequently, depletion of RPM with clodronate liposomes abrogates this protective effect and results in pneumococcal sepsis in these mice.⁶¹ Furthermore, co-culture experiments using isolated RPM and naive CD4⁺ T cells implicated these macrophages in the process of differentiation towards functional regulatory T cells.⁶²

Macrophages are known regulators of hematopoiesis in adult and fetal tissues.⁶³ Using CyTOF, flow cytometric, transcriptomic analyses, and vital time-lapse imaging in E10-E10.5 *Csf1rGFP* (“MacGreen”) embryos, Mariani et al. found that CD206⁺ macrophages seeding the aorta-gonad mesonephros (AGM) interact with nascent intra-aortic hematopoietic cells. Depletion of macrophages in AGM explants with clodronate-liposomes or the CSF1R inhibitor BLZ945 impairs the generation of hematopoietic cells both from *in vitro* cultures and following *in vivo* transplantation of explant-derived cells into adult irradiated mice, thus demonstrating that these macrophages are crucial components of the niche of emerging intra-aortic hematopoietic progenitors.⁶⁴ Similarly, several studies have established a prominent role for RPM in the support of splenic extramedullary hematopoiesis (EMH). In LPS-induced EMH, nanoparticle-mediated silencing of VCAM-1 specifically in RPM impairs splenic anchoring of HSC, which normally is mediated via Very Late Antigen-4, a VCAM-1 ligand. This highlights an essential contribution of RPM to the retention of HSC in the splenic niche.⁶⁵ In line with this, depletion of CD169⁺ macrophages in diphtheria toxin-treated *Cd169^{DTR}* mice or depletion of phagocytes with clodronate-loaded liposomes reduces splenic erythropoiesis during experimental anaemia induced by myeloablation, polycythemia vera and β -thalassemia.^{66–68} Additional evidence was provided by Paulson and colleagues, who demonstrated that RPM are required to maintain erythroid homeostasis during anemia following inflammation, myeloablation or phenylhydrazine treatment.^{69–71} Monocyte lineage tracing in tamoxifen-treated *Cx3cr1^{YFP}CreER;tdTomato* mice revealed that labelled monocytes massively infiltrate the spleen of these mice following phenylhydrazine treatment.⁶⁹ Upon transplantation of BM from the same model, labelled monocytes also extensively seed the spleen of recipient WT mice recovering from myeloablation. Transfer of purified labelled monocytes into irradiated recipients further confirmed that recruited monocytes differentiate into RPM during the

recovery phase.⁶⁹ These monocyte-derived RPM associate with erythroid progenitors, thereby becoming erythroblastic island macrophages that expand the stress erythropoietic niche. Transfer of dye-labelled erythrocytes prior to myeloablation revealed increased erythrophagocytotic behaviour in RPM, which causes them to produce CCL2, a potent monocyte chemoattractant. CCL2-producing RPM thus might regulate the monocytic influx into the growing splenic erythroid niche.⁶⁹ Erythro-phagocytosis by RPM is also increased in response to stimulation of Toll-like receptors (TLR) during sterile inflammation. Subsequent activation of SPI-C promotes stress erythropoiesis through expression of *Gdf15* in RPM, a signal initiating the expansion of stress erythroid progenitors. In the same conditions, *SpiC* KO mice fail to activate *Gdf15* transcription, which severely impairs expansion of these progenitors in the RP.⁷⁰ RPM are also a source of canonical Wnt ligands and prostaglandin E2, which respectively promote proliferation and differentiation of stress erythroid progenitors.⁷¹ In conclusion, RPM regulate key functions of the spleen, including iron recycling and stress hematopoiesis, whilst also protecting from infections.

3.2 Marginal Zone Macrophages (MZM)

The MZ contains several layers of macrophages (Figure 3 and table 2). Although typically referred to as a single population, these can be distinguished based on specific surface markers. The inner rim of the MZ comprises macrophages that co-express SIGN-R1 and macrophage receptor with collagenous structure (MARCO), while the outer rim is populated by MZM that express MARCO but lack SIGN-R1.^{72,73} MZM are embedded in a network of reticular fibroblasts and are constantly exposed to the blood.^{17,2} Owing to their unique location and set of immune receptors (i.e. SIGN-R1, MARCO, but also Scavenger receptor A), MZM represent the first line

of defence against circulating pathogens, a feature shared with marginal metallophilic macrophages (MMM, see below). *In vivo* depletion of MZM and MMM through the uptake of clodronate liposomes demonstrated that these cells are crucial for the early control of *Listeria monocytogenes* and lymphocytic choriomeningitis virus infections.^{74,75} Histological studies have shown that MZM and MMM phagocytose pathogens like *Leishmania donovani*, *L. monocytogenes*, adenovirus, murine cytomegalovirus (MCMV) and cowpox virus.^{76–79} Studies performed in SIGN-R1^{-/-} mice showed that MZM exert protection against lethal *S. pneumoniae* infection.⁸⁰ MZMs are also involved in central tolerance through ingestion of circulating apoptotic cells.⁸¹ Indeed, in clodronate liposome-treated mice depleted of MZM, chronically injected apoptotic cells are no longer trapped in the MZ and instead, accumulate in the WP. This dysregulated efferocytosis drives adaptive inflammatory responses and eventually fatal autoimmunity.⁸² The immunological activities of MZM also include their ability to efficiently trap and present antibody-coated blood-borne antigens to MZ B cells.^{72,83} MZ B cells further migrate inside the B follicles, and deposit their antigenic cargo onto FDC, eventually generating germinal center reactions in a MARCO and SIGN-R1-dependent manner.⁸⁴ In line with this, engagement of MARCO with specific antibodies reduces the transport of CD21-dependent antigens to the follicles by MZ B cells.⁸³ MARCO also mediates interactions between MZM and MZ B cells, and binding of its known ligand *S. aureus* induces relocation of MZ B cells to B follicles.⁸⁵ Selective ablation of SIGN-R1 macrophages using short-term clodronate liposome treatment or SIGN-R1^{Cre/DTR} mice impairs the development of germinal center B cells and the establishment of a mature germinal center reaction.⁷³ The absence of SIGN-R1⁺ macrophages further hinders normal clustering of CD4⁺ DC expressing dendritic cell inhibitory receptor 2 (DCIR2) to the interfollicular regions of the spleen, where they normally play a key role in priming Tfh responses.⁷³ Thus, SIGN-R1⁺ MZM specifically contribute to the regulation of

splenic humoral immunity. Collectively, a rich body of work has shown that MZM have a key role in antimicrobial immunity through screening of circulating antigens and regulation of MZ and GC B cell responses.

3.3 Marginal Metallophilic Macrophages (MMM)

MMM populate the inner side of the marginal sinus that surrounds B follicles (Figure 3 and table 2). MMM are closely associated with MRC and are defined by high expression of CD169 (Sialoadhesin), thus resembling subcapsular sinus macrophages (SSM) of the lymph node.^{33,86} Like MZM, MMM are important for scavenging of blood-borne pathogens and apoptotic bodies.² Phagocytosis assays using BM-derived macrophages from wild type and CD169-deficient mice revealed that CD169 promotes the uptake of meningococci and *Campylobacter jejuni*.^{87,88} CD169 was also found to recognize porcine reproductive and respiratory syndrome virus in porcine alveolar macrophages, highlighting a more general and evolutionarily conserved role of CD169⁺ macrophages in the clearance of viruses.⁸⁹ Interestingly, MMM can indirectly activate T cell responses through the transfer of CD169-bound antigens to cross-presenting splenic DC. Following *in vivo* antigen targeting to MMM by Ovalbumin (OVA)-conjugated anti-CD169 antibodies, isolated splenic CD8⁺ DC are able to stimulate OVA-specific OT-I T cells *in vitro*. This confirms antigen transfer from MMM to DC. Depletion of MMM with clodronate liposomes before intravenous infection with adenovirus abolishes activation of cytotoxic T cell by splenic DC.⁹⁰ SSM have been characterized as important antigen-presenting cells controlling B cell immunity in lymph nodes. Imaging studies have shown that they capture opsonized antigens and viral particles from lymph and present them to B cells for subsequent follicular delivery.^{39,91–93} Interestingly, upon stimulation with LPS or chimeric Fc protein tar-

getting CD169, MMM migrate from the marginal sinus into B follicles, providing further evidence that they also contribute to the regulation of B cell immunity.^{94–97} While this remains to be formally demonstrated, MMM might even recruit follicular B cells. Activated splenic follicular B cells express Epstein-Barr virus-induced G protein-coupled receptor 2, and migrate towards the MZ in response to its endogenous ligand oxysterol (such as 7 α ,25-dihydroxycholesterol).^{98,99} Splenic macrophages express transcripts for enzymes involved in oxysterol biosynthesis such as *Cyp7b1* and *Cyp27a1*, and their levels are increased following LPS activation.⁹⁹ Given the location of MMM, this could implicate them as the potential producers of chemotactic factors guiding activated B cells to the outer follicular niche.^{99,100} In return, B cells might regulate MMM through poorly known feedback mechanisms. Transgenic mice in which all B cells are gradually depleted due to overexpression of the TNF family member CD70 concomitantly lose MZM and MMM, while other splenic macrophages are unaltered. These data suggest that B cells are essential for the maintenance of these macrophages, presumably through expression of Lymphotoxin $\alpha 1\beta 2$.^{101–103} Besides their crucial role in scavenging antigens from the circulation, MMM might thus present antigens to B cells and attract them during infection through production of chemotactic cues, thereby contributing to efficient B cell responses in the spleen.

3.4 Tingible Body Macrophages (TBM)

TBM are predominantly found in active germinal centers (Figure 3 and table 2). They contain efferocytic vacuoles filled with apoptotic B cells in various states of degradation, which are referred to as tingible bodies.¹⁰⁴ These macrophages are equipped with efferocytotic receptors and soluble molecules such as CD68, Mer receptor tyrosine kinase (MerTK), Tim4, CD36, milk fat globule epidermal growth factor 8 (MFG-E8), Gas6 and C1q.² Impaired efferocytosis

in MerTK-deficient mice induces accumulation of apoptotic B cells and hyperactivated germinal centers upon exposure to the conjugated hapten NP16-CGG.¹⁰⁵ Moreover, electron transmission microscopy identifies intact apoptotic B cells outside TBM in MFG-E8-deficient mice immunized with keyhole limpet hemocyanin, hence further implicating this efferocytic receptor in the clearance of apoptotic B cells by TBM.¹⁰⁶ In these mice, impaired efferocytosis eventually leads to autoimmune symptoms,^{105,106} suggesting that TBM are crucial for the regulation of germinal center reactions and the maintenance of peripheral B tolerance.

3.5 T Zone Macrophages (TZM)

T Zone macrophages are equipped with phagocytic receptors (CD68, MerTK, TIM4, CD36) and are found in the T cell zone of the spleen, but also other secondary lymphoid organs such as lymph nodes and Peyer's patches (Figure 3 and table 2).^{107,108,44} Lymph node TZM have recently been characterised in detail.¹⁰⁷ These macrophages readily remove apoptotic irradiated T cells and OT-II CD4 T cells injected respectively into naive and OVA-immunized mice, suggesting that they represent the counterparts of TBM in the B cell zone. Lymph node TZM are also intimately associated with the TRC meshwork.^{86,107} Moreover, chimeric reconstitution with a mixture of *Cx3cr1*-sufficient and -deficient BM cells revealed that *Cx3cr1*-sufficient lymph node TZM captured more apoptotic T cells than *Cx3cr1*-deficient TZM. As apoptotic lymphocytes have been shown to release CX3CL1,¹⁰⁹ the ligand of CX3CR1, it is likely that the CX3CL1-CX3CR1 axis regulates TZM mediated efferocytosis.¹⁰⁷ Whilst splenic TZM remain to be studied with state-of-the-art approaches, the striking similarity in structure and macrophage colonisation between the spleen and lymph nodes suggests that their TZM exert analogous functions and exploit similar pathways.

4 Establishment and maintenance of splenic macrophage populations

All macrophages derive from precursors sharing the same transcriptome and epigenetic landscape. The core macrophage program is driven by major lineage-determining transcription factors that support macrophage identity and common functions like phagocytosis and immune defence. These include PU.1, CEBP, MAF and MAFB.^{110–114} Upon seeding their target organ, these precursors receive tissue-specific cues, which trigger the expression of additional transcription factors and ultimately endow macrophages with tissue-specific functions.^{114–116} The mechanisms regulating the transcriptional program of other tissue-resident macrophages have been reviewed elsewhere, and we will now focus on the imprinting of splenic macrophage identity.^{113,117}

During embryonic and postnatal development, the spleen is seeded by different waves of macrophage precursors. The embryonic spleen contains a single population of F4/80^{bright} macrophages that predominates in the RP until the second postnatal week.^{118–120} Lineage tracing experiments demonstrated that these F4/80^{bright} RPM originate from erythro-myeloid progenitors produced in the extra-embryonic yolk sac.¹²¹ Studies in parabiotic mice and models in which BM-derived monocytes are lineage traced further established that adult RPM self-maintain at steady state with low contribution from circulating monocytes.^{122,123} However, during blood stage malaria infection, increased erythrophagocytosis, drug-induced hemolysis or following experimental depletion, the damaged RPM network is rapidly replenished by monocytes^{59,124,52,44} and monocyte-derived RPM ultimately acquire the characteristics of their embryonic counterparts.⁵⁹

The segregation of RP and WP is initiated a few days after birth, and this coincides with the emergence of macrophages in the WP and MZ.¹²⁵ In the latter, MARCO⁺ MZM and CD169⁺ MMM are seeded at postnatal days 7-14, probably from monocytes, and acquire their final locations at four weeks of age.¹²⁰ Indeed, monocytes transferred into LXR α -deficient mice that lack MZ macrophages partially reconstitute MMM and MZM, supporting the notion that circulating monocytes are the precursors of these macrophages.¹²⁰ However, the repopulation kinetics differ for the distinct macrophages of the MZ, as shown in depletion experiments using clodronate liposomes: while MMM and MARCO⁺ MZM are replenished within two weeks, complete reappearance of SIGN-R1⁺ MARCO⁺ MZM takes up to six weeks.^{73,126} Data describing the ontogeny and homeostatic renewal mechanisms of splenic TZM are still missing. However, as the T zone develops when adult-type definitive hematopoiesis is already operational, they likely originate from monocytes.^{127,128} Renewal of lymph node TZM has also been studied under more physiological conditions in partial chimeras, in which lymph nodes were protected from irradiation. This demonstrated that adult TZM are replaced by monocytes, albeit at a slow pace.¹⁰⁷ Considering their overall similarity to their lymph node counterparts, these data strongly suggest that splenic TZM also renew from monocytes. Initial seeding and maintenance of WP macrophages is less well characterised. However, the appearance of TBM coincides with the generation of germinal center reactions after weaning.¹²⁵ This relatively late seeding with TBM suggests that they probably also originate from monocytes.

5. The concept of the macrophage niche

The identity of any tissue-resident macrophage population is thought to be imprinted and maintained by its immediate microenvironment, also referred to as niche. Macrophage niches are thus composed of a group of cells and their surrounding extracellular matrix. According to

this holistic concept, the macrophage niche meets several criteria: It constitutes a physical anchor to its macrophage, while also providing survival factors and tissue-specific cues that imprint its functional identity.^{129,130} Based on the fact that mice deficient in macrophages suffer from pleiotropic defects, the niche concept postulates that macrophages engage in mutually beneficial interactions with their niche. In the absence of macrophages, their niche would not function properly or even survive, hence explaining the multi-organ failure observed in mice deficient in CSF1 or its receptor CSFR1, key regulators of macrophage development and survival.^{131–134} In sum, macrophage niches appear to nurture and imprint their macrophages for their own benefit, establishing interdependent cell circuits that are essential for tissue homeostasis.¹³⁰ In the following sections, we will discuss our current understanding of splenic macrophage niches and their underlying molecular mechanisms (Figure 4). We will also draw from studies in other tissues, in particular lymph nodes, with which the spleen shares structural features and similar populations of stromal cells and macrophages, as outlined above.

5.1 Scaffolding and anchoring

Sessility of tissue-resident macrophages is thought to be facilitated by their constitutive expression of cellular adhesion proteins like integrins of the β 1, β 2, and β 5 families.^{110,135,136} Inhibition of integrin-mediated adhesion using PI3K inhibitors causes BM-derived macrophages to undergo anoikis, a specific type of apoptosis resulting from their inability to form cell–matrix interactions.¹³⁷ Matrix-derived intracellular signals have also been associated with the functionality of macrophages. For example, β 3-deficient mice develop osteosclerosis due to dysfunctional osteoclasts.¹³⁸ RPM express high levels of α 9 integrin, a known ligand of various extracellular matrix proteins such as fibronectin and tenascin-C, which are abundant

in the spleen.^{62,139–141} Transcriptional analyses revealed that developing RPM upregulate α D integrin, possibly contributing to their retention within the RP.¹⁴² As introduced, RPM also express *SpiC*, which potently induces VCAM1 in these macrophages.⁵¹ As a result, the few remaining RPM observed in *SpiC* KO mice lack VCAM1.⁵¹ Since *Vcam1* KO mice also display a paucity of RPM, VCAM1 might control their anchoring in the RP.¹⁴³

Adhesion of macrophages to their niche can also be mediated by chemotactic factors such as CX3CL1 and CSF1 (see section 5.2). Many macrophage subsets express the chemokine receptor CX3CR1. Its ligand, CX3CL1, is expressed as a transmembrane adhesion protein that can further be cleaved into a soluble chemokine.¹⁴⁴ An early study revealed that CX3CR1 allows efficient adherence of Leukemic monocyte-like THP-1 cells to membrane-bound CX3CL1, whilst enhancing adhesion to fibronectin and ICAM-1 in response to soluble-CX3CL1.¹⁴⁵ Another study reported that CX3CR1 promotes the insertion of F4/80⁺ macrophages into the wall of micro-vessels during neovascularisation following carotid artery ligation. CX3CL1 was further shown to regulate the morphogenesis of CX3CR1⁺ macrophages *in vitro* by stimulating their tubulogenesis between two matrigel layers.¹⁴⁶ These data indicate that CX3CR1/CX3CL1 signalling probably controls the tight attachment of CX3CR1⁺ arterial macrophages to CX3CL1⁺ endothelium.^{146,147} Similar mechanisms might regulate the association of CX3CR1⁺ TzM with CX3CL1⁺ found on cellular ramifications of TRC in the splenic T zone.⁸⁶ Interestingly, crosstalk between the cytokine CSF1 and integrin signalling might have an important role in macrophage biology. In support of this notion, high doses of CSF1 rescue the differentiation defect of β 3 integrin-deficient osteoclasts *in vitro*, and *in vivo* administration of an engineered bispecific antagonist that simultaneously targets CSF1R and α β 3 integrin inhibits osteoclast activity in a mouse model of ovariectomy-induced osteoporosis.^{148,149} It is therefore tempting to speculate that adhesion of splenic macrophages

(MZM and RPM) to their respective niches is crucial to maintain their homeostasis and functionality.

5.2 Nurturing

Like most tissue macrophages, RPM, MMM and MZM depend on CSF1 for their development and maintenance: These macrophages are drastically reduced or lacking in *Csf1-deficient* (*Csf1^{op/op}*) mice,^{62,85,150} and completely or partially restored upon administration of recombinant CSF1.^{134,150} The RPM population is quickly diminished upon blocking CSF1, and in turn, rapidly increases upon administration of recombinant CSF1.^{151–153} Histological examination of *Csf1-LacZ* reporter mice attested to the presence of a local source of CSF1 in the RP, and fibroblasts are known producers of this cytokine.^{154,155} Indeed, we confirmed the presence of CSF1 in RP fibroblasts by immunostaining, and further analysis of their transcriptome revealed that these cells express both membrane-bound and soluble isoforms of CSF1. Using genetic approaches differentially targeting distinct splenic stromal cells, we provided evidence that conditional deletion of *Csf1* in RP fibroblasts, but not WP fibroblasts or endothelial cells, drastically reduces RPM numbers without altering circulating levels of CSF1. This identified RP fibroblasts as the nurturing component of the RPM niche.⁴⁴

The transcription factor WT1 is expressed by embryonic mesenchymal spleen progenitors, and is critical for splenic organogenesis and the development of adult splenic stromal cells.¹⁵⁶ In *Wt1^{ΔCsf1}* mice, the deletion of *Csf1* in splenic fibroblastic cells also highly impacts the MMM and MARCO+ MZM populations.⁴⁴ Transcriptomic analysis of stromal and macrophage populations identified in scRNAseq datasets from murine spleen inferred that MRC and macrophages are equipped to communicate through CSF1/CSF1R. This suggests that MRC could provide CSF1 for MMM, an assumption that needs to be experimentally validated.⁴⁴

Similarly, the source of CSF1 for MZM remains to be identified. Our work strongly supports the idea that steady local supply of CSF1 is required for the development and maintenance of splenic macrophages.⁴⁴ Along these lines, re-establishing normal levels of membrane-bound *Csf1* in *Csf1^{op/op}* using a transgenic approach corrects the paucity of most macrophages normally observed in these mice, as well as their gross defects.¹⁵⁷ According to the nurturing scaffold model inspired by *in vitro* observations of Medzhitov and colleagues, membrane-bound CSF1 allows tight association between macrophages and nurturing cells, such as fibroblasts.¹⁵⁴ In line with this, experiments using the macrophage cell line BAC1.2F5 showed that exposure to CSF1 promotes the formation of focal complexes and cellular adherence to laminin or fibronectin substrates.¹⁵⁸ This might explain how phenotypically and functionally different macrophage populations co-exist in very close splenic anatomic locations like the RP and MZ.

Experimental data identifying the survival factors involved in the homeostasis of WP macrophages are still lacking. As TZM are not affected by the deletion of *Csf1* in splenic WP fibroblasts, it is conceivable that their maintenance depends on other survival factors. In lymph nodes, TZM are tightly associated with CX3CL1⁺ TRCs, suggesting that this axis might regulate their homeostasis.⁸⁶ Yet, the number and turnover of TZM seem normal in *Cx3cr1*-deficient mice, implicating an alternative mechanism such as IL-34 signalling through CSFR1.¹⁰⁷ Finally, systemic injection of CSF1R-blocking antibodies in MacGreen (*Csf1r*-EGFP) transgenic mice has no effect on lymph node TBM, while lymph node SSM are efficiently depleted, suggesting that TBM are also independent of CSF1/CSF1R signalling.¹⁵⁹

5.3 Imprinting

The local microenvironment has a profound influence on the functional identity of tissue-resident macrophages. Individual macrophages are exposed to a particular combination of niche cues that together induce signal-dependent transcription factors. Through activation of signature genes, these factors ultimately endow macrophages with specific functions that contribute to homeostasis within their tissue of residence.^{117,130} Such niche imprinting likely represents a multifactorial process that relies on cytokines, metabolites and cell-cell contact. This has recently been described for liver Kupffer cells (KC). Using conditional depletion of KC in the *Clec4f^{DTT}* transgenic model, Bonnardel et al. showed that stellate cells, endothelial cells, and hepatocytes collectively contribute to the imprinting of KC identity by providing CCL2, VCAM1, Bone Morphogenetic Proteins, Notch ligands, CSF1 and IL34 to recently recruited monocytes.¹⁶⁰

Phagocytosis, a defining functional property of all macrophages, also participates in this phenotypic imprinting within tissues, and hence, macrophage heterogeneity.¹⁶¹ Constant phagocytosis and degradation of apoptotic cells activates the transcription factors PPAR γ , RXR α and LXR through the accumulation of cellular lipids. Efferocytosis of apoptotic thymocytes by macrophages induces expression of critical phagocytic genes such as *Mer*, *Cd36*, *Axl*, *Fcgr1*, *C1q* or *Mfge8*.^{162,163} Hence, physiological cell clearance influences the transcriptional profile of macrophages and seems to engage them in a virtuous cycle perpetuating their phagocytic capacity.¹⁶² Experiments involving partners deficient in various phagocytosis-related genes further assured that the exact phagocytic molecules macrophages use to dispose of cellular debris differ between tissues.¹⁶¹ It is plausible that the diverse nature of cells and consequently the cargo phagocytosed by splenic macrophages in their respective niches, impact their transcriptional programs and thus, contribute to their phenotypic and functional heterogeneity. One such cue that macrophages are abundantly exposed to in the spleen is heme, a metabolite of

erythrocyte degradation. Indeed, heme was the first signal identified as a regulator of RPM differentiation. In conditions of intravascular hemolysis induced by phenylhydrazine, high levels of circulating heme induce SPI-C expression in splenic monocytes, which eventually become RPM.⁵² This observation was further confirmed by Lu et al., who showed that *in vivo* administration of heme increases CD11b^{high} F4/80^{low} SPI-C^{low} pre-RPM, though not mature RPM.¹⁶⁴ Indeed, this study also demonstrated a key role for erythrocyte-derived IL-33 signaling in the terminal differentiation of RPM. Longitudinal studies performed in WT and IL-33 receptor (IL1RL1)-deficient mice revealed that RPM numbers increase with age, and that IL-33 signalling is essential for the specific maintenance of RPM in adult mice. Erythrocyte transfer into RPM-deficient *Il33* or *Il1rl1* KO mice partially restores the RPM population.¹⁶⁴ Transcriptomic comparison between splenic monocytes, pre-RPM, and RPM from WT and IL1RL1-deficient mice identified the IL33-inducible transcription factor GATA2 as a master regulator of the pre-RPM to RPM transition. The extracellular matrix of the RP exposes laminin- α 5, which facilitates trapping and hemolysis of senescent erythrocytes specifically expressing activated Lu/BCAM (Lutheran blood group and basal cell adhesion molecule).⁴⁸ It is therefore tempting to speculate that imprinting of RPM by senescent erythrocytes is both direct, via the intake of IL33 and heme, as well as indirect, through stable positioning of RPM and erythrocyte “ghosts” within the cord. Since all macrophages are professional phagocytes, such indirect imprinting through stable positioning and access to unique cellular material likely explains how distinct populations of macrophages coexist in nearby sub-tissular niches of the spleen. Interestingly, other macrophages are known to perform erythrophagocytosis *in vivo*.^{52,124,165} Yet, they are distinct from RPM. This implies that additional local factors control the specification of RPM. Studies in *Vav1^{cre} Tgfbr2^{fl/fl}* and *LysM^{cre} PPAR γ ^{fl/fl}* mice, in which the genes encod-

ing Transforming Growth Factor Beta Receptor 2 or the transcription factor PPAR γ are respectively depleted in hematopoietic cells or macrophages, have reported reduced RPM numbers.^{166,167}

TGF β is a known inducer of the transcription factors IRF8 and PPAR γ , both of which are active in RPM.^{111,167–169} As IRF8-deficient mice also display a paucity of RPM,¹⁷⁰ TGF β might represent an additional major specifier of RPM identity. Interestingly, RP fibroblasts and RPM express *Tgfb* isoforms (*Tgfb1* for RPM, *Tgfb1/3* for RP Fibroblasts) which implies that paracrine and/or autocrine signalling could be involved, the latter mimicking mechanisms known to govern alveolar macrophage homeostasis.^{44,62,166} Additional insights into the complexity of RPM's imprinting has been provided by the comparison of PPAR γ - and *SpiC*-deficient mouse models. In their study, Okreglicka and colleagues have revised the pivotal roles played by both transcription factors in the seeding and homeostasis of RPM.¹⁴² While PPAR γ is required for the neonatal expansion of RPM, SPI-C appears essential to their presence in adult mice.¹⁴² The early *SpiC*-independent development of RPM could be explained by the possible absence of senescent erythrocytes in newborn pups.¹⁷¹ Hence, the survival of neonatal RPM would not rely on their ability to metabolize iron.

Different molecules and particles are also probed or phagocytosed by macrophage subsets in distinct splenic compartments. Conditional deletion of *LXR α* in hematopoietic cells induces selective loss of MZM and MMM.¹²⁰ *LXR α* and *LXR β* are key transcription factors transactivated by endogenous oxysterols and involved in the control of sterol homeostasis.¹⁷² Mice deficient in both, *LXR α* and β suffer from an overload of neutral lipids in the spleen, suggesting that sterol metabolites accumulate in the absence of MZ macrophages.^{173,120} It is therefore plausible that spleen-specific sterol metabolites activate *LXR α/β* during the development of

MZ macrophages. Furthermore, pharmacological hyperactivation of LXR α alone does not induce MZ macrophage markers in other splenic subsets, suggesting that additional MZ specific cues are required for the development of MZ macrophages.¹²⁰ The identity and cellular sources of such cues remain to be identified.

6. Feedback mechanisms

The trophic functions of macrophages are most evident from the defects observed in mice devoid of macrophages, such as the *Csf1*- and *Csf1r*-deficient models.^{131–133} Their pleiotropic alterations suggest that macrophages are crucial for the proper development and function of many cellular microenvironments. Indeed, it is now well-established that these cells are involved in processes as diverse as surfactant degradation and lung alveolar homeostasis,^{174,175} electrical conduction in the heart,¹⁷⁶ gastro-intestinal viability and motility,¹⁷⁷ and neuronal development, homeostasis and excitability.^{178–180}

Indeed, it has been proposed that macrophages engage in mutually beneficial interactions with their niches.^{130,154} According to this concept, macrophages control homeostasis of their niche through feedback mechanisms, akin to those described for macrophages and fibroblasts by Medzhitov and colleagues.¹⁵⁴ They reported that co-cultured macrophages and fibroblasts locally and reciprocally exchange growth factors (CSF1 and platelet-derived growth factor (PDGF)) and engage in cellular contacts.¹⁵⁴ Similar macrophage-niche circuits might exist in several organs. Testicular Leydig cells and interstitial macrophages interactions are thought to control male fertility.^{181–183} In the bone, osteoblasts and osteoclasts constantly interact to control bone homeostasis,^{184–189} while in the mammary gland, mammary epithelial stem cells and mammary gland macrophages collaborate to regulate the morphogenesis and remodelling of the mammary gland.^{190–193} We therefore consider it very likely that

macrophages also contribute to the homeostatic and functions of their niches in the spleen (Figure 5).

6.1 Trophic functions

Tissue-resident macrophages are a recognized source of trophic factors for their neighbouring cells. For example, interstitial testicular macrophages stimulate steroidogenesis by Leydig cells via the production of 25-OH cholesterol, while osteoclasts regulate the activity and differentiation of osteoblasts through the production of semaphorin 4D and complement component 3a.^{182–185}

It is thus highly conceivable that RPM also regulate the activity of RP fibroblasts, their niche. Transcriptomic analysis revealed that RPM express transcripts for TGF β , while RP fibroblasts express TGF β RIII (also called β glycan), a co-receptor for active TGF β .^{44,62,194} TGF β signalling facilitates the activation and production of extracellular matrix components by fibroblasts, in particular through epigenetic regulation.^{195–199} In a swine model of ureteral injury, TGF β 1-producing macrophages located at the injury site enhance collagen secretion and activation of fibroblasts.¹⁹⁸ In cardiac fibroblasts, inducible deletion of canonical TGF β signalling (*Smad2*, *Smad3*, *Tgfbr1*, *Tgfbr2*) results in reduced expression of genes involved in fibrosis, adhesion, and extracellular matrix production in response to pressure overload stimulation.¹⁹⁹ RPM-derived TGF β potentially affects the behaviour of RP fibroblasts in a similar manner. RPM and RP fibroblasts could also communicate through progranulin and its receptors TNFRSF1A/B, which are respectively expressed by RPM and RP fibroblasts.⁴⁴ The growth factor progranulin binds to TNFR and acts as a competitor for TNF α , hence inhibiting TNF α -induced apoptotic and inflammatory pathways.^{185,186} Progranulin also acts as a growth factor for fibroblasts, both un-

der homeostatic conditions and during wound repair. *In vivo*, progranulin promotes proliferation of isolated rat dermal fibroblasts.²⁰⁰ Similarly, progranulin locally increases the numbers of fibroblasts when applied to transcutaneous puncture wounds.²⁰⁰ In analogy with the mechanisms described by Medzhitov in the two-cell circuit model,¹⁵⁴ RPM could also regulate the homeostasis of PDGFR α / β ⁺ RP fibroblasts via the production of PDGF β , a growth factor essential for survival and proliferation of fibroblasts that is expressed by several tissue-resident macrophages, including RPM.^{111,154} As the identity of the cellular niches of other splenic macrophages is still unknown, it is currently difficult to envision the exact nature of the macrophage-niche circuits in the MZ and WP.

6.2. Maintenance of the splenic architecture

Macrophages contribute to extracellular matrix remodelling through production of proteases, modulation of fibroblastic activity²⁰¹ and in some instances, even direct collagen deposition.²⁰² Interestingly, the subcutaneous connective tissue of *Csf1^{op/op}* mice displays increased interstitial extracellular matrix with altered composition.²⁰³ Tissue-resident macrophages have also been implicated in the regulation of extracellular matrix homeostasis in the nulliparous mammary gland and arterial wall. Using transcriptomic profiling and CSFR1 inhibitor-mediated depletion approaches (pexidartinib), Wang et al., demonstrated that LYVE-1⁺ (hyaluronan receptor) F4/80⁺ CD206⁺ macrophages associated with hyaluronan-enriched stroma are key modulators of extracellular matrix turnover in the mammary gland.²⁰⁴ Similarly, LYVE-1⁺ aortic-resident macrophages contribute to arterial homeostasis by secreting MMP-9 that locally degrades collagen. Deletion of these macrophages in *Lyve1^{cre} Csf1^{fl/fl}* mice and the associated loss of their collagenolytic effect induces arterial stiffness and dysfunction.²⁰⁵ As discussed, RPM are in direct contact with RP fibroblasts and are thus ideally

positioned to regulate their homeostasis. Expression of collagen (*Col14a*) and matrix remodelling genes such as *Mmp13*, *Adam23*, *Ctsd* by RPM supports the idea that these cells could be involved in the remodelling of the RP extracellular matrix.^{110,206,207} Thus, in addition to performing erythrophagocytosis, RPM would also indirectly be involved in controlling the quality of blood filtration in the RP via maintaining its reticular structure. A striking feature of LXR-deficient mice is the drastic alteration of the MZ microenvironment concomitant to the selective lack of MMM and MZM. Chimeric mice reconstituted with LXR-deficient BM show a major disruption of the MZ 3 months after transplantation, while LXR-deficient mice reconstituted with WT BM recover both, MZ macrophages and MZ space.¹²⁰ These observations suggest that MZ macrophages not only help positioning MZ B cells, but also support maintenance of the MZ architecture.

6.3 Compromised splenic macrophage populations lead to stromal cell damage

By scavenging the hemoglobin content from senescent erythrocytes, RPM are crucial for protection from the cytotoxic effect of iron. Indeed, iron overload develops in the RP of *SpiC* KO and *Wt1^{ΔCsf1}* mice, which profoundly lack RPM.^{44,51} Iron deposition in the RP exposes stromal cells to oxidative toxicity. With time, this appears to alter splenic morphology and function. Indeed, histologic examination of *Hmox1* KO spleens revealed chronic collagen deposition in the RP, which is gradually replaced by fibrotic tissue. This severely impacts the blood filtering capacity of the spleen. Ultimately, aging *Hmox1* KO mice display functional hyposplenism, a condition characterized by the presence of numerous abnormal erythrocytes and increased platelets in peripheral blood.⁵³

Splenic macrophages also constitute an immune barrier that shields splenic fibroblasts from the deleterious consequences of intracellular infections. MCMV productively infects ER-TR7⁺

reticular fibroblasts in the MZ and RP, causing degeneration of these cells and subsequent destruction of these zones.⁷⁹ During acute MCMV infection, depletion of splenic macrophages with clodronate liposomes significantly increases viral replication in the spleen.^{208,209} *In vitro*, co-culture of MCMV-infected c-3T3 fibroblasts with isolated splenic macrophages reduces viral lysis of infected cells, thus suggesting that splenic macrophages protect virus-permissive fibroblasts from MCMV replication.²⁰⁸ Interestingly, in the lymph node, depletion of SSM with liposomal clodronate or diphtheria toxin (in the *Cd169^{DTR}* model) facilitates infection and lysis of ER-TR7⁺ fibroblasts by MCMV. These data demonstrate that SSM have the potential to prevent the deadly infection of fibroblasts by MCMV.²¹⁰ By analogy, splenic macrophages such as MMM, the counterparts of lymph node SSM, might protect MCMV-permissive splenic fibroblasts from MCMV infection and subsequent lytic cell death.

FRC and MZ fibroblastic cells are also targets of infection by the lymphocytic choriomeningitis virus CL-13 and hemorrhagic fever viruses like Ebola, Marburg and Lassa. Such infections lead to the disruption of the lymphoid structural integrity, altered conduit function, and sustained splenic atrophy, collectively causing immune dysregulation and compromised immunity.^{211–213} Infection with *Leishmania donovani* and *Plasmodium yoelli* parasites was shown to respectively kill TRC and FDC and cause concomitant loss of germinal centers as well as marked changes in metabolic activity and morphology of RP fibroblasts.^{214–217} Given the crucial role played by macrophages in the uptake and scavenging of diverse virus and parasites,^{2,3,125} it is more than likely that by virtue of these immune functions, macrophages shield splenic fibroblasts from infection. Macrophages thereby partake in maintaining local immunity in the spleen.²¹⁸ However, splenic stromal cells are central to the generation of systemic adaptive immune responses, as they feed and guide immune cells within the WP.^{218–220} These responses are severely hampered by pathogens targeting the splenic stroma. Through protecting splenic

fibroblasts, their macrophage partners are important players in facilitating immunity on the organismic level.

6.4 Extramedullary hematopoiesis: stress erythropoiesis

Stress erythropoiesis is a specific type of extramedullary haematopoiesis intended to rapidly produce mature erythrocytes in instances when medullary production is insufficient to correct anaemia. In mice, stress erythropoiesis frequently takes place in the splenic RP. During this process, the RP expands to accommodate emerging erythropoietic niches.²²¹ The creation of these niches involves the initial recruitment of short-term reconstituting HSC and monocytes, and the subsequent proliferation of RP fibroblasts and vascular endothelial cells.^{6,7,69} As previously mentioned, RPM are essential in supporting extramedullary haematopoiesis, as their absence impairs the recovery of normal red blood cell counts.^{66–68,70} RPM were identified as a source of GDF15, a critical regulator of the expansion of stress erythropoietic niches. In conditions of sterile inflammation, phenylhydrazine-induced anemia and bone marrow transplantation, GDF15 signalling maintains expression of BMP4 in the splenic erythroid niche, a critical step that conditions the expansion of stress erythroid progenitors.^{70,222} Adult stress erythropoiesis and fetal erythropoiesis share similar responsiveness to BMP4 signalling, which is evident in the murine *flexed-tail* model that carries a mutated *Smad5* gene, a transcription factor downstream of the BMP4 receptor. Due to insufficient BMP4 signalling, these mice exhibit fetal neonatal anemia and delayed recovery from acute anemia when challenged with phenylhydrazine.^{70,221,223,224} Interestingly, stromal cells in the fetal liver express BMP4 during embryogenesis,²²³ and RP fibroblasts appear to be a source of BMP4 during stress erythropoiesis. Earlier observations in the MSS31 splenic stromal cell line reported that under hypoxic conditions, these cells express *Bmp4*.²²⁴ Confocal imaging further suggested that RP

stromal cells produce BMP4 during phenylhydrazine-induced anaemia.²²⁵ It is thus possible that through GDF15, RPM induce BMP4 secretion by RP fibroblasts and thereby control the maintenance of a microenvironment suited for stress erythropoiesis.

7- Emerging questions: macrophage-fibroblast circuits in the aged spleen

Like all long-lived cells of the body, macrophages and fibroblastic cells are subject to age-related changes.^{226–228} As discussed in this review, splenic macrophages and stromal cells engage in molecular dialogues that reciprocally assure maintenance of both cellular populations through positive feedback mechanisms, creating virtuous cell circuits. This therefore poses important questions: Could the circuits established between splenic macrophages and their niches be impaired during aging, and could this contribute to the progressive loss of healthy physiology?

7.1 Ageing of the mouse spleen

It is widely acknowledged that the decline of cellular functions with time has a complex and systemic impact that disrupts physiological integrity and increases susceptibility to infections, cancers and autoimmunity in aged subjects. The spleen displays age-associated changes in its structural organisation and immune function, which might explain vulnerability of older patients to infectious diseases like streptococcal pneumonia.^{229–231} In aging mice, the MZ undergoes several structural changes, ultimately leading to a distorted anatomy.^{229,232} As the density of MZ macrophages (both, MMM and MZM) decreases, their distribution is more scattered, whilst the MRC lining becomes discontinuous and thicker.^{229,232,233} Aged MZ macrophages have a reduced ability to uptake dextran particles injected intravenously,²³² and splenic macrophages isolated from irradiation-induced senescent mice display a decrease in

phagocytic activity *in vitro*.²³⁴ These observations suggest that the scavenging efficiency is diminished in aged splenic macrophages. Furthermore, aging increases the production of cell debris, while their disposal by macrophages through phagocytosis and autophagy becomes less efficient.^{226,235,236} Imbalance causes cellular debris to accumulate, a process called “garb-aging”, which is believed to promote inflammatory responses in tissue macrophages.²³⁶ In addition, aged macrophages might also be metabolically distinct, producing increased levels of pro-inflammatory cytokines, which eventually would contribute to chronic low-grade inflammation (“inflammaging”).²²⁶ With splenic macrophages being highly involved in the clearance of systemic apoptotic cells, it is tempting to speculate that they are exposed to such alterations.

Similarly, aged splenic fibroblasts display morphological and secretory changes, and these could be amplified by local inflammation driven by aging splenic macrophages.^{233,237–239} Shuttling of B cells between the MZ and B cell follicles is significantly impaired in aged mice, as assessed by *in vivo* antibody pulse-labelling with anti-complement receptor 2 (CD21). Strikingly, this effect on shuttling is not rescued by reconstitution of aged recipients with young donor BM, implicating the aged splenic stroma, rather than radio-sensitive immune cells, as the main drivers behind the decrease in MZ B cell migration.^{229,233} The production of key cytokines (CCL19/21, CXCL13) is dysregulated in aged splenic TRC and FDC, causing lymphoid compartments to merge.^{237–239} Moreover, splenic stromal cells from aged mice respond to an *in vivo* LPS challenge with enhanced production of inflammatory cytokines such as IL-1 β , IL-6 and IL-17, suggesting that they develop a senescence-associated secretory phenotype that could further contribute to inflammaging.²⁴⁰

7.2 Ageing of the human spleen

Human and rodent spleens share many features, but there are also several distinctive anatomical characteristics specific to humans (for review,^{18,229,242}). The RP predominates in humans and the WP is mainly occupied by B cell follicles.¹⁸ The human MZ is split into an inner and an outer layer that are not observed in the mouse.²⁴³ The human spleen further contains a unique structure called perifollicular zone that encloses the WP and the MZ. As in rodents, the human WP is structured by FRC and FDC, while a peculiar type of MRC-like cells covers the surface of B cell follicles, the T cell zone and the perifollicular zone.²⁴⁴ Interestingly, the human RP contains fibroblasts and macrophages that closely resemble those described in mice.^{18,48,245–247} Notably, human RP fibroblasts express WT1 and CSF1, which strongly suggests that they might also nurture RPM like in the mouse.⁴⁴

Alterations in the microarchitecture of the aging human spleen have been reported, yet a comprehensive study of the changes occurring in its anatomy over time is still lacking. With age, the human splenic capsule becomes thinner, the volume of the RP increases and the B cell follicles gradually atrophy.^{241,248} Elderly and individuals with splenic dysfunctions or splenectomy are highly susceptible to infection with *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Neisseria meningitidis* or other encapsulated bacteria, and this susceptibility correlates with a deficit in circulating MZ B-cells.^{231,249–251} In line with this, the human spleen harbours less precursors of MZ B cells with time, as a comparison between children and adults showed.²⁵² Overall, these results are very similar to the ones observed in the aging mouse, suggesting that MZ remodeling in the elderly might be responsible for the defective immune protection mediated by MZ B cells.^{229,233}

The efficacy of vaccination is compromised with age.²⁵³ Meta-analysis of influenza vaccination trials revealed that vaccine effectiveness is greatly reduced in adults aged 65 years or older.²⁵⁴

Immunization of old mice with a polyvalent vaccine containing a mixture of pneumococcal capsular polysaccharides suggested that deficiencies in splenic DC and macrophages might explain the poor antibody response induced by the vaccine, a defect believed to contribute to unresponsiveness of older patients to vaccination.^{255,256} Interestingly, studies comparing human peripheral blood mononuclear cells from young and old adults showed that monocytes of older individuals display decreased levels of TLR, TLR-induced expression of cytokines and costimulatory markers.^{257,258} As with time, human splenic macrophages might be replaced by monocytes, it is tempting to speculate that the immune and scavenging functions of these macrophages are impeded in aging humans. Aged splenic macrophages would thereby fail to protect against circulating pathogens and support vaccination-induced prophylaxis in the elderly.

In addition to immune system alterations, heightened baseline inflammation, a phenomenon known as inflammaging, exacerbates multi-system functional decline and serious pathophysiologic processes in elderly (for review,^{259,260}). This chronic systemic inflammation is most notably driven by elevated pro-inflammatory cytokines in the circulation. IL-6, for example, is normally low or non-detectable in healthy young adults, but dramatically increased in the blood of individuals over 70 years.²⁶¹ Interestingly, splenic stroma obtained from aged mice produces more IL-6 *in vitro* compared to stroma from younger mice, as stated in the previous section.²⁴⁰ Although these results need to be confirmed in human, it is conceivable that also in humans, splenic fibroblasts might contribute to inflammaging through the secretion of inflammatory cytokines.

Finally, the increased susceptibility to infections observed in aged patients could be further exacerbated by a combination of age-driven changes affecting splenic T cell homeostasis. A single-cell transcriptomic atlas across the lifespan of mice reported that the proportion of

splenic T cells decreases with age, and this might also occur in humans.²³⁰ This reduction in splenic T cells can at least in part be attributed to the decline of naive T cell output from thymus, which is undergoing age-related atrophy.^{262,263} However, age-related disturbance of the splenic microenvironment could be involved as well.

Intriguingly, whilst the proliferation and survival of naive T cells is compromised in the spleens of aged mice, these defects are not due to a decrease in splenic IL-7, a key signal regulating the survival of naive T cell.²⁶⁴ Similarly, the concentration of circulating IL-7 is maintained in elderly humans.²⁶⁴ This suggests that rather than production of IL-7, its accessibility becomes a limiting factor for lymphocytes upon aging. In line with this, *in vivo* administration of exogenous IL-7 fails to restore homeostatic proliferation of transferred naive T cells in irradiated, aged donor mice. This is in contrast to administration of IL-7 bound to a neutralizing antibody (M25), a complex that presumably promotes IL-7 presentation and persistence in the lymphoid microenvironment.²⁶⁴ Hence, the dramatic loss of T cell homeostasis in aged individuals might stem from the inability of stromal cells to bind and present key survival factors to T cells.

In closing, the convergence of aging-related changes in the splenic microarchitecture, loss of functionality in senescent splenic lymphocytes, macrophages and fibroblasts paired with chronic low-grade inflammation collectively hamper the ability of elderly to efficiently respond to circulating pathogens and limit the efficacy of commonly used vaccination strategies, leaving aged individuals highly susceptible to infectious diseases. In the light of the intricate control of splenic functions by resident macrophages, and their intimate, reciprocal interactions with the splenic stroma, delineating if and by which mechanisms their crosstalk

is dysregulated over the course of life may unravel therapeutic strategies that hold the premise of restoring full functionality in the aged spleen.

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Declaration of interests

The authors declare no competing interests

Bibliography

1. Mebius RE, Kraal G. Structure and function of the spleen. *Nat Rev Immunol.* 2005;5:606-16.
2. den Haan JMM, Kraal G. Innate Immune Functions of Macrophage Subpopulations in the Spleen. *J Innate Immun.* 2012;4:437-45.
3. Borges da Silva H, Fonseca R, Pereira RM, et al. Splenic Macrophage Subsets and Their Function during Blood-Borne Infections. *Front Immunol.* 2015;6.
4. Klei TRL, Meinderts SM, van den Berg TK, van Bruggen R. From the Cradle to the Grave: The Role of Macrophages in Erythropoiesis and Erythrophagocytosis. *Front Immunol.* 2017;8.
5. Alam MZ, Devalaraja S, Haldar M. The Heme Connection: Linking Erythrocytes and Macrophage Biology. *Front Immunol.* 2017;8.
6. Inra CN, Zhou BO, Acar M, et al. A perisinusoidal niche for extramedullary haematopoiesis in the spleen. *Nature.* 2015;527:466-71.
7. Oda A, Tezuka T, Ueno Y, et al. Niche-induced extramedullary hematopoiesis in the spleen is regulated by the transcription factor Tlx1. *Sci Rep.* 2018;8:8308.
8. Yang X, Chen D, Long H, Zhu B. The mechanisms of pathological extramedullary hematopoiesis in diseases. *Cell Mol Life Sci.* 2020;77:2723-38.
9. van der Laan AM, ter Horst EN, Delewi R, et al. Monocyte subset accumulation in the human heart following acute myocardial infarction and the role of the spleen as monocyte reservoir. *Eur Heart J.* 2014;35:376-85.
10. Heyns A, Badenhorst P, Lotter M, Pieters H, Wessels P. Kinetics and mobilization from the spleen of indium-111-labeled platelets during platelet apheresis. *Transfusion (Paris).* 1985;25:215-8.
11. Ellyard J, Avery D, Mackay C, Tangye S. Contribution of stromal cells to the migration, function and retention of plasma cells in human spleen: potential roles of CXCL12, IL-6 and CD54. *Eur J Immunol.* 2005;35:699-708.
12. Mamani-Matsuda M, Cosma A, Weller S, et al. The human spleen is a major reservoir for long-lived vaccinia virus-specific memory B cells. *Blood.* 2008;111:4653-9.
13. Shephard RJ. Responses of the human spleen to exercise. *J Sports Sci.* 2016;34:929-36.
14. Stewart IB, McKenzie DC. The Human Spleen During Physiological Stress: *Sports Med.* 2002;32:361-9.

15. Cabanac A, Folkow LP, Blix AS. Volume capacity and contraction control of the seal spleen. *J Appl Physiol*. 1997;82:1989-94.
16. Swirski FK, Nahrendorf M, Etzrodt M, et al. Identification of Splenic Reservoir Monocytes and Their Deployment to Inflammatory Sites. *Science*. 2009;325:612-6.
17. Kraal G, Mebius R. New Insights into the Cell Biology of the Marginal Zone of the Spleen. In: *International Review of Cytology*. Elsevier; 2006. p. 175-215.
18. Steiniger BS. Human spleen microanatomy: why mice do not suffice. *Immunology*. 2015;145:334-46.
19. Perez-Shibayama C, Gil-Cruz C, Ludewig B. Fibroblastic reticular cells at the nexus of innate and adaptive immune responses. *Immunol Rev*. 2019;289:31-41.
20. Alexandre YO, Mueller SN. Stromal cell networks coordinate immune response generation and maintenance. *Immunol Rev*. 2018;283:77-85.
21. Malhotra D, Fletcher AL, Turley SJ. Stromal and hematopoietic cells in secondary lymphoid organs: partners in immunity. *Immunol Rev*. 2013;251:160-76.
22. Castagnaro L, Lenti E, Maruzzelli S, et al. Nkx2-5+Islet1+ Mesenchymal Precursors Generate Distinct Spleen Stromal Cell Subsets and Participate in Restoring Stromal Network Integrity. *Immunity*. 2013;38:782-91.
23. Nolte MA, Beliën JAM, Schadee-Eestermans I, et al. A Conduit System Distributes Chemokines and Small Blood-borne Molecules through the Splenic White Pulp. *J Exp Med*. 2003;198:505-12.
24. Onder L. A novel bacterial artificial chromosome-transgenic Podoplanin–Cre mouse targets lymphoid organ stromal cells in vivo. *Front Immunol*. 2011;2.
25. Bajénoff M, Glaichenhaus N, Germain RN. Fibroblastic Reticular Cells Guide T Lymphocyte Entry into and Migration within the Splenic T Cell Zone. *J Immunol*. 2008;181:3947-54.
26. Worbs T, Mempel TR, Bölter J, von Andrian UH, Förster R. CCR7 ligands stimulate the intranodal motility of T lymphocytes in vivo. *J Exp Med*. 2007;204:489-95.
27. Luther SA, Tang HL, Hyman PL, Farr AG, Cyster JG. Coexpression of the chemokines ELC and SLC by T zone stromal cells and deletion of the ELC gene in the plt/plt mouse. *Proc Natl Acad Sci*. 2000;97:12694-9.
28. Link A, Vogt TK, Favre S, et al. Fibroblastic reticular cells in lymph nodes regulate the homeostasis of naive T cells. *Nat Immunol*. 2007;8:1255-65.
29. Ansel KM, Ngo VN, Hyman PL, et al. A chemokine-driven positive feedback loop organizes lymphoid follicles. *Nature*. 2000;406:309-14.

30. Heesters BA, Myers RC, Carroll MC. Follicular dendritic cells: dynamic antigen libraries. *Nat Rev Immunol.* 2014;14:495-504.
31. Rodda LB, Bannard O, Ludewig B, Nagasawa T, Cyster JG. Phenotypic and Morphological Properties of Germinal Center Dark Zone *Cxcl12* -Expressing Reticular Cells. *J Immunol.* 2015;195:4781-91.
32. Katakai T, Suto H, Sugai M, et al. Organizer-Like Reticular Stromal Cell Layer Common to Adult Secondary Lymphoid Organs. *J Immunol.* 2008;181:6189-200.
33. Katakai T. Marginal reticular cells: a stromal subset directly descended from the lymphoid tissue organizer. *Front Immunol.* 2012;3.
34. Koning JJ, Mebius RE. Interdependence of stromal and immune cells for lymph node function. *Trends Immunol.* 2012;33:264-70.
35. Cyster JG. CHEMOKINES, SPHINGOSINE-1-PHOSPHATE, AND CELL MIGRATION IN SECONDARY LYMPHOID ORGANS. *Annu Rev Immunol.* 2005;23:127-59.
36. Randall TD, Carragher DM, Rangel-Moreno J. Development of Secondary Lymphoid Organs. *Annu Rev Immunol.* 2008;26:627-50.
37. Roozendaal R, Mebius RE. Stromal Cell–Immune Cell Interactions. *Annu Rev Immunol.* 2011;29:23-43.
38. Magri G, Miyajima M, Bascones S, et al. Innate lymphoid cells integrate stromal and immunological signals to enhance antibody production by splenic marginal zone B cells. *Nat Immunol.* 2014;15:354-64.
39. Junt T, Moseman EA, Iannacone M, et al. Subcapsular sinus macrophages in lymph nodes clear lymph-borne viruses and present them to antiviral B cells. *Nature.* 2007;450:110-4.
40. Habbeddine M, Verthuy C, Rastoin O, et al. Receptor Activator of NF- κ B Orchestrates Activation of Antiviral Memory CD8 T Cells in the Spleen Marginal Zone. *Cell Rep.* 2017;21:2515-27.
41. Fasnacht N, Huang H-Y, Koch U, et al. Specific fibroblastic niches in secondary lymphoid organs orchestrate distinct Notch-regulated immune responses. *J Exp Med.* 2014;211:2265-79.
42. Cheng H-W, Onder L, Novkovic M, et al. Origin and differentiation trajectories of fibroblastic reticular cells in the splenic white pulp. *Nat Commun.* 2019;10:1739.
43. Hargreaves DC, Hyman PL, Lu TT, et al. A Coordinated Change in Chemokine Responsiveness Guides Plasma Cell Movements. *J Exp Med.* 2001;194:45-56.
44. Bellomo A, Mondor I, Spinelli L, et al. Reticular Fibroblasts Expressing the Transcription Factor WT1 Define a Stromal Niche that Maintains and Replenishes Splenic Red Pulp Macrophages. *Immunity.* 2020;53:127-142.e7.

45. Tan JKH, Watanabe T. Murine Spleen Tissue Regeneration from Neonatal Spleen Capsule Requires Lymphotoxin Priming of Stromal Cells. *J Immunol*. 2014;193:1194-203.
46. Fujita T. A Scanning Electron Microscope Study of the Human Spleen. *Arch Histol Jpn*. 1974;37:187-216.
47. Hataba Y, Kirino Y, Suzuki T. Scanning electron microscopic study of the red pulp of mouse spleen. *J Electron Microsc (Tokyo)*. 1981;30:46-56.
48. Klei T robert leon, Dalimot JJ, Nota B, et al. Hemolysis in the spleen drives erythrocyte turnover. *Blood*. 2020;blood.2020005351.
49. Nairz M, Theurl I, Swirski FK, Weiss G. "Pumping iron"—how macrophages handle iron at the systemic, microenvironmental, and cellular levels. *Pflüg Arch - Eur J Physiol*. 2017;469:397-418.
50. Gottlieb Y, Topaz O, Cohen LA, et al. Physiologically aged red blood cells undergo erythrophagocytosis in vivo but not in vitro. *Haematologica*. 2012;97:994-1002.
51. Kohyama M, Ise W, Edelson BT, et al. Role for Spi-C in the development of red pulp macrophages and splenic iron homeostasis. *Nature*. 2009;457:318-21.
52. Haldar M, Kohyama M, So AY-L, et al. Heme-Mediated SPI-C Induction Promotes Monocyte Differentiation into Iron-Recycling Macrophages. *Cell*. 2014;156:1223-34.
53. Kovtunovych G, Eckhaus MA, Ghosh MC, Ollivierre-Wilson H, Rouault TA. Dysfunction of the heme recycling system in heme oxygenase 1-deficient mice: effects on macrophage viability and tissue iron distribution. *Blood*. 2010;116:6054-62.
54. Alam Z, Devalaraja S, Li M, et al. Counter Regulation of Spic by NF- κ B and STAT Signaling Controls Inflammation and Iron Metabolism in Macrophages. *Cell Rep*. 2020;31:107825.
55. Schnitzer B, Sodeman T, Mead ML, Contacos PG. Pitting Function of the Spleen in Malaria: Ultrastructural Observations. *Science*. 1972;177:175-7.
56. Tosta CE, Ruiz G, Wedderburn N. The role of spleen macrophages in malaria: an ultrastructural study. *Rev Soc Bras Med Trop*. 1984;17:31-6.
57. Kim CC, Nelson CS, Wilson EB, et al. Splenic Red Pulp Macrophages Produce Type I Interferons as Early Sentinels of Malaria Infection but Are Dispensable for Control. *Rénia L, éditeur. PLoS ONE*. 2012;7:e48126.
58. Sponaas A-M, Freitas do Rosario AP, Voisine C, et al. Migrating monocytes recruited to the spleen play an important role in control of blood stage malaria. *Blood*. 2009;114:5522-31.
59. Lai SM, Sheng J, Gupta P, et al. Organ-Specific Fate, Recruitment, and Refilling Dynamics of Tissue-Resident Macrophages during Blood-Stage Malaria. *Cell Rep*. 2018;25:3099-3109.e3.

60. De Jesus M, Gyu Park C, Su Y, et al. Spleen deposition of *Cryptococcus neoformans* capsular glucuronoxylomannan in rodents occurs in red pulp macrophages and not marginal zone macrophages expressing the C-type lectin SIGN-R1. *Med Mycol.* 2008;46:153-62.
61. Kirby AC, Beattie L, Maroof A, van Rooijen N, Kaye PM. SIGNR1-Negative Red Pulp Macrophages Protect against Acute Streptococcal Sepsis after *Leishmania donovani*-Induced Loss of Marginal Zone Macrophages. *Am J Pathol.* 2009;175:1107-15.
62. Kurotaki D, Kon S, Bae K, et al. CSF-1-Dependent Red Pulp Macrophages Regulate CD4 T Cell Responses. *J Immunol.* 2011;186:2229-37.
63. McCabe A, MacNamara KC. Macrophages: Key regulators of steady-state and demand-adapted hematopoiesis. *Exp Hematol.* 2016;44:213-22.
64. Mariani SA, Li Z, Rice S, et al. Pro-inflammatory Aorta-Associated Macrophages Are Involved in Embryonic Development of Hematopoietic Stem Cells. *Immunity.* 2019;50:1439-1452.e5.
65. Dutta P, Hoyer FF, Grigoryeva LS, et al. Macrophages retain hematopoietic stem cells in the spleen via VCAM-1. *J Exp Med.* 2015;212:497-512.
66. Chow A, Huggins M, Ahmed J, et al. CD169+ macrophages provide a niche promoting erythropoiesis under homeostasis and stress. *Nat Med.* 2013;19:429-36.
67. Ramos P, Casu C, Gardenghi S, et al. Macrophages support pathological erythropoiesis in polycythemia vera and β -thalassemia. *Nat Med.* 2013;19:437-45.
68. Jacobsen RN, Forristal CE, Raggatt LJ, et al. Mobilization with granulocyte colony-stimulating factor blocks medullar erythropoiesis by depleting F4/80+VCAM1+CD169+ER-HR3+Ly6G+ erythroid island macrophages in the mouse. *Exp Hematol.* 2014;42:547-561.e4.
69. Liao C, Prabhu KS, Paulson RF. Monocyte-derived macrophages expand the murine stress erythropoietic niche during the recovery from anemia. *Blood.* 2018;132:2580-93.
70. Bennett LF, Liao C, Quicquel MD, et al. Inflammation induces stress erythropoiesis through heme-dependent activation of SPI-C. *Sci Signal.* 2019;12:eaap7336.
71. Chen Y, Xiang J, Qian F, et al. Epo receptor signaling in macrophages alters the splenic niche to promote erythroid differentiation. *Blood.* 2020;136:235-46.
72. You Y, Myers RC, Freeberg L, et al. Marginal Zone B Cells Regulate Antigen Capture by Marginal Zone Macrophages. *J Immunol.* 2011;186:2172-81.
73. Pirgova G, Chauveau A, MacLean AJ, Cyster JG, Arnon TI. Marginal zone SIGN-R1 + macrophages are essential for the maturation of germinal center B cells in the spleen. *Proc Natl Acad Sci.* 2020;117:12295-305.

74. Aichele P, Zinke J, Grode L, et al. Macrophages of the Splenic Marginal Zone Are Essential for Trapping of Blood-Borne Particulate Antigen but Dispensable for Induction of Specific T Cell Responses. *J Immunol.* 2003;171:1148-55.
75. Seiler P, Aichele P, Odermatt B, et al. Crucial role of marginal zone macrophages and marginal zone metallophils in the clearance of lymphocytic choriomeningitis virus infection. *Eur J Immunol.* 1997;27:2626-33.
76. Gorak PM, Engwerda CR, Kaye PM. Dendritic cells, but not macrophages, produce IL-12 immediately following *Leishmania donovani* infection. *Eur J Immunol.* 1998;28:687-95.
77. Aoshi T, Carrero JA, Konjufca V, et al. The cellular niche of *Listeria monocytogenes* infection changes rapidly in the spleen. *Eur J Immunol.* 2009;39:417-25.
78. Di Paolo NC, Miao EA, Iwakura Y, et al. Virus Binding to a Plasma Membrane Receptor Triggers Interleukin-1 α -Mediated Proinflammatory Macrophage Response In Vivo. *Immunity.* 2009;31:110-21.
79. Hsu KM, Pratt JR, Akers WJ, Achilefu SI, Yokoyama WM. Murine cytomegalovirus displays selective infection of cells within hours after systemic administration. *J Gen Virol.* 2009;90:33-43.
80. Lanoue A, Clatworthy MR, Smith P, et al. SIGN-R1 Contributes to Protection against Lethal Pneumococcal Infection in Mice. *J Exp Med.* 2004;200:1383-93.
81. McGaha TL, Karlsson MCI. Apoptotic cell responses in the splenic marginal zone: a paradigm for immunologic reactions to apoptotic antigens with implications for autoimmunity. *Immunol Rev.* 2016;269:26-43.
82. McGaha TL, Chen Y, Ravishankar B, van Rooijen N, Karlsson MCI. Marginal zone macrophages suppress innate and adaptive immunity to apoptotic cells in the spleen. *Blood.* 2011;117:5403-12.
83. Prokopec KE, Georgoudaki A-M, Sohn S, et al. Cutting Edge: Marginal Zone Macrophages Regulate Antigen Transport by B Cells to the Follicle in the Spleen via CD21. *J Immunol.* 2016;197:2063-8.
84. Cinamon G, Zachariah MA, Lam OM, Foss FW, Cyster JG. Follicular shuttling of marginal zone B cells facilitates antigen transport. *Nat Immunol.* 2008;9:54-62.
85. Karlsson MCI, Guinamard R, Bolland S, et al. Macrophages Control the Retention and Trafficking of B Lymphocytes in the Splenic Marginal Zone. *J Exp Med.* 2003;198:333-40.
86. Bellomo A, Gentek R, Bajénoff M, Baratin M. Lymph node macrophages: Scavengers, immune sentinels and trophic effectors. *Cell Immunol.* 2018;330:168-74.
87. Jones C, Virji M, Crocker PR. Recognition of sialylated meningococcal lipopolysaccharide by siglecs expressed on myeloid cells leads to enhanced bacterial uptake: Siglec-mediated uptake of sialylated meningococci. *Mol Microbiol.* 2003;49:1213-25.

88. Heikema AP, Bergman MP, Richards H, et al. Characterization of the Specific Interaction between Sialoadhesin and Sialylated *Campylobacter jejuni* Lipooligosaccharides. *Infect Immun*. 2010;78:3237-46.
89. Vanderheijden N, Delputte PL, Favoreel HW, et al. Involvement of Sialoadhesin in Entry of Porcine Reproductive and Respiratory Syndrome Virus into Porcine Alveolar Macrophages. *J Virol*. 2003;77:8207-15.
90. Backer R, Schwandt T, Greuter M, et al. Effective collaboration between marginal metallophilic macrophages and CD8+ dendritic cells in the generation of cytotoxic T cells. *Proc Natl Acad Sci*. 2010;107:216-21.
91. Phan TG, Grigorova I, Okada T, Cyster JG. Subcapsular encounter and complement-dependent transport of immune complexes by lymph node B cells. *Nat Immunol*. 2007;8:992-1000.
92. Phan TG, Green JA, Gray EE, Xu Y, Cyster JG. Immune complex relay by subcapsular sinus macrophages and noncognate B cells drives antibody affinity maturation. *Nat Immunol*. 2009;65:786-93.
93. Carrasco YR, Batista FD. B Cells Acquire Particulate Antigen in a Macrophage-Rich Area at the Boundary between the Follicle and the Subcapsular Sinus of the Lymph Node. *Immunity*. 2007;27:160-71.
94. Groeneveld PHP, van Rooijen N, Eikelenboom P. In-vivo effects of lipopolysaccharide on lymphoid and non-lymphoid cells in the mouse spleen: Migration of marginal metallophils towards the follicle centres. *Cell Tissue Res*. 1983;234:201-8.
95. Martínez-Pomares L, Kosco-Vilbois M, Darley E, et al. Fc chimeric protein containing the cysteine-rich domain of the murine mannose receptor binds to macrophages from splenic marginal zone and lymph node subcapsular sinus and to germinal centers. *J Exp Med*. 1996;184:1927-37.
96. Milićević ĐN, Despotović SZ, Westermann J, Milićević NM. Tumour necrosis factor receptor-1 is dispensable for the migration of marginal metallophilic macrophages into the B-cell zone of the mouse spleen. *Anat Histol Embryol*. 2018;47:560-5.
97. Yu P, Wang Y, Chin RK, et al. B Cells Control the Migration of a Subset of Dendritic Cells into B Cell Follicles Via CXC Chemokine Ligand 13 in a Lymphotoxin-Dependent Fashion. *J Immunol*. 2002;168:5117-23.
98. Hannedouche S, Zhang J, Yi T, et al. Oxysterols direct immune cell migration via EBI2. *Nature*. 2011;475:524-7.
99. Liu C, Yang XV, Wu J, et al. Oxysterols direct B-cell migration through EBI2. *Nature*. 2011;475:519-23.
100. Pereira JP, Kelly LM, Cyster JG. Finding the right niche: B-cell migration in the early phases of T-dependent antibody responses. *Int Immunol*. 2010;22:413-9.

101. Nolte MA, Arens R, Kraus M, et al. B Cells Are Crucial for Both Development and Maintenance of the Splenic Marginal Zone. *J Immunol.* 2004;172:3620-7.
102. Mackay F, Majeau GR, Lawton P, Hochman PS, Browning JL. Lymphotoxin but not tumor necrosis factor functions to maintain splenic architecture and humoral responsiveness in adult mice. *Eur J Immunol.* 1997;27:2033-42.
103. Ngo VN, Cornall RJ, Cyster JG. Splenic T Zone Development Is B Cell Dependent. *J Exp Med.* 2001;194:1649-60.
104. Swartzendruber DC, Congdon CC. ELECTRON MICROSCOPE OBSERVATIONS ON TINGIBLE BODY MACROPHAGES IN MOUSE SPLEEN. *J Cell Biol.* 1963;19:641-6.
105. Rahman ZSM, Shao W-H, Khan TN, Zhen Y, Cohen PL. Impaired Apoptotic Cell Clearance in the Germinal Center by Mer-Deficient Tingible Body Macrophages Leads to Enhanced Antibody-Forming Cell and Germinal Center Responses. *J Immunol.* 2010;185:5859-68.
106. Hanayama R. Autoimmune Disease and Impaired Uptake of Apoptotic Cells in MFG-E8-Deficient Mice. *Science.* 2004;304:1147-50.
107. Baratin M, Simon L, Jorquera A, et al. T Cell Zone Resident Macrophages Silently Dispose of Apoptotic Cells in the Lymph Node. *Immunity.* 2017;47:349-362.e5.
108. Bonnardel J, Da Silva C, Henri S, et al. Innate and Adaptive Immune Functions of Peyer's Patch Monocyte-Derived Cells. *Cell Rep.* 2015;11:770-84.
109. Truman LA, Ford CA, Pasikowska M, et al. CX3CL1/fractalkine is released from apoptotic lymphocytes to stimulate macrophage chemotaxis. *Blood.* 2008;112:5026-36.
110. the Immunological Genome Consortium, Gautier EL, Shay T, et al. Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. *Nat Immunol.* 2012;13:1118-28.
111. Lavin Y, Winter D, Blecher-Gonen R, et al. Tissue-Resident Macrophage Enhancer Landscapes Are Shaped by the Local Microenvironment. *Cell.* 2014;159:1312-26.
112. Gosselin D, Link VM, Romanoski CE, et al. Environment Drives Selection and Function of Enhancers Controlling Tissue-Specific Macrophage Identities. *Cell.* 2014;159:1327-40.
113. Link VM, Gosselin D, Glass CK. Mechanisms Underlying the Selection and Function of Macrophage-Specific Enhancers. *Cold Spring Harb Symp Quant Biol.* 2015;80:213-21.
114. Mass E, Ballesteros I, Farlik M, et al. Specification of tissue-resident macrophages during organogenesis. *Science.* 2016;353:aaf4238-aaf4238.
115. van de Laar L, Saelens W, De Prijck S, et al. Yolk Sac Macrophages, Fetal Liver, and Adult Monocytes Can Colonize an Empty Niche and Develop into Functional Tissue-Resident Macrophages. *Immunity.* 2016;44:755-68.

116. Gordon S, Plüddemann A. Tissue macrophages: heterogeneity and functions. *BMC Biol.* 2017;15:53.
117. T'Jonck W, Guilliams M, Bonnardel J. Niche signals and transcription factors involved in tissue-resident macrophage development. *Cell Immunol.* 2018;330:43-53.
118. Morris L, Graham CF, Gordon S. Macrophages in haemopoietic and other tissues of the developing mouse detected by the monoclonal antibody F4/80. *Dev Camb Engl.* 1991;112:517-26.
119. Bertrand JY. Fetal spleen stroma drives macrophage commitment. *Development.* 2006;133:3619-28.
120. A-Gonzalez N, Guillen JA, Gallardo G, et al. The nuclear receptor LXR α controls the functional specialization of splenic macrophages. *Nat Immunol.* 2013;14:831-9.
121. Gomez Perdiguero E, Klapproth K, Schulz C, et al. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. *Nature.* 2015;518:547-51.
122. Hashimoto D, Chow A, Noizat C, et al. Tissue-Resident Macrophages Self-Maintain Locally throughout Adult Life with Minimal Contribution from Circulating Monocytes. *Immunity.* 2013;38:792-804.
123. Liu Z, Gu Y, Chakarov S, et al. Fate Mapping via Ms4a3-Expression History Traces Monocyte-Derived Cells. *Cell.* 2019;178:1509-1525.e19.
124. Youssef LA, Rebbaa A, Pampou S, et al. Increased erythrophagocytosis induces ferroptosis in red pulp macrophages in a mouse model of transfusion. *Blood.* 2018;131:2581-93.
125. A-Gonzalez N, Castrillo A. Origin and specialization of splenic macrophages. *Cell Immunol.* 2018;330:151-8.
126. van Rooijen N, Kors N, Kraal G. Macrophage Subset Repopulation in the Spleen: Differential Kinetics After Liposome-Mediated Elimination. *J Leukoc Biol.* 1989;45:97-104.
127. Desanti G, Bertrand JY, Golub R. Fetal spleen development, the ride toward multiple functions. In 2007.
128. Ginhoux F, Guilliams M. Tissue-Resident Macrophage Ontogeny and Homeostasis. *Immunity.* 2016;44:439-49.
129. Guilliams M, Scott CL. Does niche competition determine the origin of tissue-resident macrophages? *Nat Rev Immunol.* 2017;17:451-60.
130. Guilliams M, Thierry GR, Bonnardel J, Bajenoff M. Establishment and Maintenance of the Macrophage Niche. *Immunity.* 2020;52:434-51.
131. Dai X-M, Ryan GR, Hapel AJ, et al. Targeted disruption of the mouse colony-stimulating factor 1 receptor gene results in osteopetrosis, mononuclear phagocyte deficiency,

- increased primitive progenitor cell frequencies, and reproductive defects. *Blood*. 2002;99:111-20.
132. Pridans C, Raper A, Davis GM, et al. Pleiotropic Impacts of Macrophage and Microglial Deficiency on Development in Rats with Targeted Mutation of the *Csf1r* Locus. *J Immunol*. 2018;201:2683-99.
 133. Wiktor-Jedrzejczak W, Bartocci A, Ferrante AW, et al. Total absence of colony-stimulating factor 1 in the macrophage-deficient osteopetrotic (op/op) mouse. *Proc Natl Acad Sci*. 1990;87:4828-32.
 134. Cecchini MG, Dominguez MG, Mocci S, et al. Role of colony stimulating factor-1 in the establishment and regulation of tissue macrophages during postnatal development of the mouse. *Dev Camb Engl*. 1994;120:1357-72.
 135. Brown EJ. Integrins of Macrophages and Macrophage-Like Cells. In: Gordon S, éditeur. *The Macrophage as Therapeutic Target*. Berlin, Heidelberg: Springer Berlin Heidelberg; 2003. p. 111-30.
 136. Schittenhelm L, Hilkens CM, Morrison VL. β 2 Integrins As Regulators of Dendritic Cell, Monocyte, and Macrophage Function. *Front Immunol*. 2017;8:1866.
 137. Roach TIA, Slater SE, White LS, et al. The protein tyrosine phosphatase SHP-1 regulates integrin-mediated adhesion of macrophages. *Curr Biol*. 1998;8:1035-9.
 138. McHugh KP, Hoidalva-Dilke K, Zheng M-H, et al. Mice lacking β 3 integrins are osteosclerotic because of dysfunctional osteoclasts. *J Clin Invest*. 2000;105:433-40.
 139. Høye AM, Couchman JR, Wewer UM, Fukami K, Yoneda A. The newcomer in the integrin family: Integrin α 9 in biology and cancer. *Adv Biol Regul*. 2012;52:326-39.
 140. Lokmic Z, Lämmermann T, Sixt M, et al. The extracellular matrix of the spleen as a potential organizer of immune cell compartments. *Semin Immunol*. 2008;20:4-13.
 141. Lo CG, Lu TT, Cyster JG. Integrin-dependence of Lymphocyte Entry into the Splenic White Pulp. *J Exp Med*. 2003;197:353-61.
 142. Okreglicka K, Iten I, Pohlmeier L, et al. PPAR γ is essential for the development of bone marrow erythroblastic island macrophages and splenic red pulp macrophages. *J Exp Med*. 2021;218:e20191314.
 143. Ulyanova T, Phelps SR, Papayannopoulou T. The macrophage contribution to stress erythropoiesis: when less is enough. *Blood*. 2016;128:1756-65.
 144. Jones BA, Beamer M, Ahmed S. Fractalkine/CX3CL1: A Potential New Target for Inflammatory Diseases. *Mol Interv*. 2010;10:263-70.
 145. Goda S, Imai T, Yoshie O, et al. CX3C-Chemokine, Fractalkine-Enhanced Adhesion of THP-1 Cells to Endothelial Cells Through Integrin-Dependent and -Independent Mechanisms. *J Immunol*. 2000;164:4313-20.

146. Kumar AHS, Martin K, Turner EC, et al. Role of CX3CR1 Receptor in Monocyte/Macrophage Driven Neovascularization. Eisenberg L, éditeur. PLoS ONE. 2013;8:e57230.
147. Ensan S, Li A, Besla R, et al. Self-renewing resident arterial macrophages arise from embryonic CX3CR1+ precursors and circulating monocytes immediately after birth. Nat Immunol. 2016;17:159-68.
148. Faccio R, Takeshita S, Zallone A, Ross FP, Teitelbaum SL. c-Fms and the $\alpha\beta 3$ integrin collaborate during osteoclast differentiation. J Clin Invest. 2003;111:749-58.
149. Zur Y, Rosenfeld L, Keshelman CA, et al. A dual-specific macrophage colony-stimulating factor antagonist of c-FMS and $\alpha\beta 3$ integrin for osteoporosis therapy. McGonnell I, éditeur. PLOS Biol. 2018;16:e2002979.
150. Takahashi K, Umeda S, Shultz LD, Hayashi S-I, Nishikawa S-I. Effects of macrophage colony-stimulating factor (M-CSF) on the development, differentiation, and maturation of marginal metallophilic macrophages and marginal zone macrophages in the spleen of osteopetrosis (op) mutant mice lacking functional M-CSF activi. J Leukoc Biol. 1994;55:581-8.
151. Lin W, Xu D, Austin CD, et al. Function of CSF1 and IL34 in Macrophage Homeostasis, Inflammation, and Cancer. Front Immunol. 2019;10:2019.
152. Gow DJ, Sauter KA, Pridans C, et al. Characterisation of a Novel Fc Conjugate of Macrophage Colony-stimulating Factor. Mol Ther. 2014;22:1580-92.
153. Pridans C, Sauter KA, Irvine KM, et al. Macrophage colony-stimulating factor increases hepatic macrophage content, liver growth, and lipid accumulation in neonatal rats. Am J Physiol-Gastrointest Liver Physiol. 2018;314:G388-98.
154. Zhou X, Franklin RA, Adler M, et al. Circuit Design Features of a Stable Two-Cell System. Cell. 2018;172:744-757.e17.
155. Ryan GR, Dai X-M, Dominguez MG, et al. Rescue of the colony-stimulating factor 1 (CSF-1)-nullizygous mouse (Csf1op/Csf1op) phenotype with a CSF-1 transgene and identification of sites of local CSF-1 synthesis. Blood. 2001;98:74-84.
156. Herzer U, Crocoll A, Barton D, Howells N, Englert C. The Wilms tumor suppressor gene wt1 is required for development of the spleen. Curr Biol. 1999;9:837-51.
157. Dai X-M, Zong X-H, Sylvestre V, Stanley ER. Incomplete restoration of colony-stimulating factor 1 (CSF-1) function in CSF-1-deficient Csf1op/Csf1op mice by transgenic expression of cell surface CSF-1. Blood. 2004;103:1114-23.
158. Pixley FJ, Lee PSW, Condeelis JS, Stanley ER. Protein Tyrosine Phosphatase ϕ Regulates Paxillin Tyrosine Phosphorylation and Mediates Colony-Stimulating Factor 1-Induced Morphological Changes in Macrophages. Mol Cell Biol. 2001;21:1795-809.

159. MacDonald KPA, Palmer JS, Cronau S, et al. An antibody against the colony-stimulating factor 1 receptor depletes the resident subset of monocytes and tissue- and tumor-associated macrophages but does not inhibit inflammation. *Blood*. 2010;116:3955-63.
160. Bonnardel J, T'Jonck W, Gaublomme D, et al. Stellate Cells, Hepatocytes, and Endothelial Cells Imprint the Kupffer Cell Identity on Monocytes Colonizing the Liver Macrophage Niche. *Immunity*. 2019;51:638-654.e9.
161. A-Gonzalez N, Quintana JA, García-Silva S, et al. Phagocytosis imprints heterogeneity in tissue-resident macrophages. *J Exp Med*. 2017;214:1281-96.
162. A-Gonzalez N, Bensinger SJ, Hong C, et al. Apoptotic Cells Promote Their Own Clearance and Immune Tolerance through Activation of the Nuclear Receptor LXR. *Immunity*. 2009;31:245-58.
163. Rószter T, Menéndez-Gutiérrez MP, Lefterova MI, et al. Autoimmune Kidney Disease and Impaired Engulfment of Apoptotic Cells in Mice with Macrophage Peroxisome Proliferator-Activated Receptor γ or Retinoid X Receptor α Deficiency. *J Immunol*. 2011;186:621-31.
164. Lu Y, Basatemur G, Scott IC, et al. Interleukin-33 Signaling Controls the Development of Iron-Recycling Macrophages. *Immunity*. 2020;52:782-793.e5.
165. Theurl I, Hilgendorf I, Nairz M, et al. On-demand erythrocyte disposal and iron recycling requires transient macrophages in the liver. *Nat Med*. 2016;22:945-51.
166. Yu X, Buttgereit A, Lelios I, et al. The Cytokine TGF- β Promotes the Development and Homeostasis of Alveolar Macrophages. *Immunity*. 2017;47:903-912.e4.
167. Gautier EL, Chow A, Spanbroek R, et al. Systemic Analysis of PPAR γ in Mouse Macrophage Populations Reveals Marked Diversity in Expression with Critical Roles in Resolution of Inflammation and Airway Immunity. *J Immunol*. 2012;189:2614-24.
168. Humblin E, Thibaudin M, Chalmin F, et al. IRF8-dependent molecular complexes control the Th9 transcriptional program. *Nat Commun*. 2017;8:2085.
169. Kurotaki D, Uede T, Tamura T. Functions and development of red pulp macrophages: Biology of red pulp macrophages. *Microbiol Immunol*. 2015;59:55-62.
170. Yamamoto M, Kato T, Hotta C, et al. Shared and Distinct Functions of the Transcription Factors IRF4 and IRF8 in Myeloid Cell Development. Bunting KD, éditeur. *PLoS ONE*. 2011;6:e25812.
171. Baron MH, Isern J, Fraser ST. The embryonic origins of erythropoiesis in mammals. *Blood*. 2012;119:4828-37.
172. Repa JJ, Mangelsdorf DJ. The Role of Orphan Nuclear Receptors in the Regulation of Cholesterol Homeostasis. *Annu Rev Cell Dev Biol*. 2000;16:459-81.

173. Janowski BA, Willy PJ, Devi TR, Falck JR, Mangelsdorf DJ. An oxysterol signalling pathway mediated by the nuclear receptor LXR α . *Nature*. 1996;383:728-31.
174. Guillems M, De Kleer I, Henri S, et al. Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life via GM-CSF. *J Exp Med*. 2013;210:1977-92.
175. Yoshida M, Ikegami M, Reed JA, Chronos ZC, Whitsett JA. GM-CSF regulates protein and lipid catabolism by alveolar macrophages. *Am J Physiol-Lung Cell Mol Physiol*. 2001;280:L379-86.
176. Hulsmans M, Clauss S, Xiao L, et al. Macrophages Facilitate Electrical Conduction in the Heart. *Cell*. 2017;169:510-522.e20.
177. Muller PA, Koscsó B, Rajani GM, et al. Crosstalk between Muscularis Macrophages and Enteric Neurons Regulates Gastrointestinal Motility. *Cell*. 2014;158:300-13.
178. Kana V, Desland FA, Casanova-Acebes M, et al. CSF-1 controls cerebellar microglia and is required for motor function and social interaction. *J Exp Med*. 2019;216:2265-81.
179. Bessis A, Béchade C, Bernard D, Roumier A. Microglial control of neuronal death and synaptic properties. *Glia*. 2007;55:233-8.
180. Klawonn AM, Fritz M, Castany S, et al. Microglial activation elicits a negative affective state through prostaglandin-mediated modulation of striatal neurons. *Immunity*. 2021;54:225-234.e6.
181. Bergh A, Damber J-E, van Rooijen N. Liposome-mediated macrophage depletion: an experimental approach to study the role of testicular macrophages in the rat. *J Endocrinol*. 1993;136:407-NP.
182. Hutson JC, Garner CW, Doris PA. Purification and characterization of a lipophilic factor from testicular macrophages that stimulates testosterone production by Leydig cells. *J Androl*. 1996;17:502-8.
183. Lukyanenko YO, Chen J-J, Hutson JC. Production of 25-Hydroxycholesterol by Testicular Macrophages and Its Effects on Leydig Cells¹. *Biol Reprod*. 2001;64:790-6.
184. Matsuoka K, Park K, Ito M, Ikeda K, Takeshita S. Osteoclast-Derived Complement Component 3a Stimulates Osteoblast Differentiation: C3a AS A COUPLING FACTOR. *J Bone Miner Res*. 2014;29:1522-30.
185. Negishi-Koga T, Shinohara M, Komatsu N, et al. Suppression of bone formation by osteoclastic expression of semaphorin 4D. *Nat Med*. 2011;17:1473-80.
186. Amano H, Yamada S, Felix R. Colony-Stimulating Factor-1 Stimulates the Fusion Process in Osteoclasts. *J Bone Miner Res*. 1998;13:846-53.
187. Xiong J, Onal M, Jilka RL, et al. Matrix-embedded cells control osteoclast formation. *Nat Med*. 2011;17:1235-41.

188. McMichael BK, Meyer SM, Lee BS. c-Src-mediated Phosphorylation of Thyroid Hormone Receptor-interacting Protein 6 (TRIP6) Promotes Osteoclast Sealing Zone Formation. *J Biol Chem*. 2010;285:26641-51.
189. Chen X, Wang Z, Duan N, et al. Osteoblast–osteoclast interactions. *Connect Tissue Res*. 2018;59:99-107.
190. Stewart TA, Hughes K, Hume DA, Davis FM. Developmental Stage-Specific Distribution of Macrophages in Mouse Mammary Gland. *Front Cell Dev Biol*. 2019;7:250.
191. Van Nguyen A, Pollard JW. Colony Stimulating Factor-1 Is Required to Recruit Macrophages into the Mammary Gland to Facilitate Mammary Ductal Outgrowth. *Dev Biol*. 2002;247:11-25.
192. Chakrabarti R, Celià-Terrassa T, Kumar S, et al. Notch ligand Dll1 mediates cross-talk between mammary stem cells and the macrophageal niche. *Science*. 2018;360:eaan4153.
193. Dawson CA, Pal B, Vaillant F, et al. Tissue-resident ductal macrophages survey the mammary epithelium and facilitate tissue remodelling. *Nat Cell Biol*. 2020;22:546-58.
194. López-Casillas F, Cheifetz S, Doody J, et al. Structure and expression of the membrane proteoglycan betaglycan, a component of the TGF- β receptor system. *Cell*. 1991;67:785-95.
195. Thannickal VJ, Lee DY, White ES, et al. Myofibroblast Differentiation by Transforming Growth Factor- β 1 Is Dependent on Cell Adhesion and Integrin Signaling via Focal Adhesion Kinase. *J Biol Chem*. 2003;278:12384-9.
196. Ignatz RA, Massagué J. Transforming growth factor-beta stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix. *J Biol Chem*. 1986;261:4337-45.
197. Pan X, Chen Z, Huang R, Yao Y, Ma G. Transforming growth factor β 1 induces the expression of collagen type I by DNA methylation in cardiac fibroblasts. *PLoS One*. 2013;8:e60335.
198. Ueshima E, Fujimori M, Kodama H, et al. Macrophage-secreted TGF- β 1 contributes to fibroblast activation and ureteral stricture after ablation injury. *Am J Physiol-Ren Physiol*. 2019;317:F52-64.
199. Khalil H, Kanisicak O, Prasad V, et al. Fibroblast-specific TGF- β –Smad2/3 signaling underlies cardiac fibrosis. *J Clin Invest*. 2017;127:3770-83.
200. He Z, Ong CHP, Halper J, Bateman A. Progranulin is a mediator of the wound response. *Nat Med*. 2003;9:225-9.
201. Kim SY, Nair MG. Macrophages in wound healing: activation and plasticity. *Immunol Cell Biol*. 2019;97:258-67.

202. Simões FC, Cahill TJ, Kenyon A, et al. Macrophages directly contribute collagen to scar formation during zebrafish heart regeneration and mouse heart repair. *Nat Commun.* 2020;11:600.
203. Radi ZA, Guzman RE, Bell RR. Increased Connective Tissue Extracellular Matrix in the *op/op* Model of Osteopetrosis. *Pathobiology.* 2009;76:199-203.
204. Wang Y, Chaffee TS, LaRue RS, et al. Tissue-resident macrophages promote extracellular matrix homeostasis in the mammary gland stroma of nulliparous mice. *eLife.* 2020;9:e57438.
205. Lim HY, Lim SY, Tan CK, et al. Hyaluronan Receptor LYVE-1-Expressing Macrophages Maintain Arterial Tone through Hyaluronan-Mediated Regulation of Smooth Muscle Cell Collagen. *Immunity.* 2018;49:326-341.e7.
206. Scott CL, T'Jonck W, Martens L, et al. The Transcription Factor ZEB2 Is Required to Maintain the Tissue-Specific Identities of Macrophages. *Immunity.* 2018;49:312-325.e5.
207. Bonnans C, Chou J, Werb Z. Remodelling the extracellular matrix in development and disease. *Nat Rev Mol Cell Biol.* 2014;15:786-801.
208. Hamano S, Yoshida H, Takimoto H, et al. Role of Macrophages in Acute Murine Cytomegalovirus Infection. *Microbiol Immunol.* 1998;42:607-16.
209. Hanson LK, Slater JS, Karabekian Z, et al. Replication of Murine Cytomegalovirus in Differentiated Macrophages as a Determinant of Viral Pathogenesis. *J Virol.* 1999;73:5970-80.
210. Farrell HE, Davis-Poynter N, Bruce K, et al. Lymph Node Macrophages Restrict Murine Cytomegalovirus Dissemination. Frueh K, éditeur. *J Virol.* 2015;89:7147.
211. Steele KE, Anderson AO, Mohamadzadeh M. Fibroblastic reticular cell infection by hemorrhagic fever viruses. *Immunotherapy.* 2009;1:187-97.
212. Mueller SN, Matloubian M, Clemens DM, et al. Viral targeting of fibroblastic reticular cells contributes to immunosuppression and persistence during chronic infection. *Proc Natl Acad Sci.* 2007;104:15430-5.
213. Mbanwi AN, Wang C, Geddes K, Philpott DJ, Watts TH. Irreversible splenic atrophy following chronic LCMV infection is associated with compromised immunity in mice. *Eur J Immunol.* 2017;47:94-106.
214. Ato M, Stäger S, Engwerda CR, Kaye PM. Defective CCR7 expression on dendritic cells contributes to the development of visceral leishmaniasis. *Nat Immunol.* 2002;3:1185-91.
215. Smelt SC, Engwerda CR, McCrossen M, Kaye PM. Destruction of follicular dendritic cells during chronic visceral leishmaniasis. *J Immunol Baltim Md 1950.* 1997;158:3813-21.
216. Weiss L. Barrier cells in the spleen. *Immunol Today.* 1991;12:24-9.

217. Martin-Jaular L, Ferrer M, Calvo M, et al. Strain-specific spleen remodelling in *Plasmodium yoelii* infections in Balb/c mice facilitates adherence and spleen macrophage-clearance escape: Spleen adherence and macrophage escape in *Plasmodium yoelii* infections. *Cell Microbiol.* 2011;13:109-22.
218. Mueller SN, Germain RN. Stromal cell contributions to the homeostasis and functionality of the immune system. *Nat Rev Immunol.* 2009;9:618-29.
219. Golub R, Tan J, Watanabe T, Brendolan A. Origin and Immunological Functions of Spleen Stromal Cells. *Trends Immunol.* 2018;39:503-14.
220. Thierry GR, Gentek R, Bajenoff M. Remodeling of reactive lymph nodes: Dynamics of stromal cells and underlying chemokine signaling. *Immunol Rev.* 2019;289:42-61.
221. Paulson RF, Ruan B, Hao S, Chen Y. Stress Erythropoiesis is a Key Inflammatory Response. *Cells.* 2020;9:634.
222. Hao S, Xiang J, Wu D-C, et al. Gdf15 regulates murine stress erythroid progenitor proliferation and the development of the stress erythropoiesis niche. *Blood Adv.* 2019;3:2205-17.
223. Porayette P, Paulson RF. BMP4/Smad5 dependent stress erythropoiesis is required for the expansion of erythroid progenitors during fetal development. *Dev Biol.* 2008;317:24-35.
224. Lenox LE, Perry JM, Paulson RF. BMP4 and Madh5 regulate the erythroid response to acute anemia. *Blood.* 2005;105:2741-8.
225. Perry JM, Harandi OF, Porayette P, et al. Maintenance of the BMP4-dependent stress erythropoiesis pathway in the murine spleen requires hedgehog signaling. *Blood.* 2009;113:911-8.
226. van Beek AA, Van den Bossche J, Mastroberardino PG, de Winther MPJ, Leenen PJM. Metabolic Alterations in Aging Macrophages: Ingredients for Inflammaging? *Trends Immunol.* 2019;40:113-27.
227. Masters AR, Haynes L, Su D-M, Palmer DB. Immune senescence: significance of the stromal microenvironment: The aged microenvironment. *Clin Exp Immunol.* 2017;187:6-15.
228. Wang C, Jurk D, Maddick M, et al. DNA damage response and cellular senescence in tissues of aging mice: Senescent cells in aging mice. *Aging Cell.* 2009;8:311-23.
229. Turner VM, Mabbott NA. Influence of ageing on the microarchitecture of the spleen and lymph nodes. *Biogerontology.* 2017;18:723-38.
230. The Tabula Muris Consortium. A single-cell transcriptomic atlas characterizes ageing tissues in the mouse. *Nature.* 2020;583:590-5.

231. van der Poll T, Opal SM. Pathogenesis, treatment, and prevention of pneumococcal pneumonia. *The Lancet*. 2009;374:1543-56.
232. Birjandi SZ, Ippolito JA, Ramadorai AK, Witte PL. Alterations in Marginal Zone Macrophages and Marginal Zone B Cells in Old Mice. *J Immunol*. 2011;186:3441-51.
233. Turner VM, Mabbott NA. Ageing adversely affects the migration and function of marginal zone B cells. *Immunology*. 2017;151:349-62.
234. Palacio L, Goyer M, Maggiorani D, et al. Restored immune cell functions upon clearance of senescence in the irradiated splenic environment. *Aging Cell*. 2019;18.
235. Plowden J, Renshaw-Hoelscher M, Engleman C, Katz J, Sambhara S. Innate immunity in aging: impact on macrophage function: Innate immunity in aging, J. Plowden et al. *Aging Cell*. 2004;3:161-7.
236. Franceschi C, Garagnani P, Vitale G, Capri M, Salvioli S. Inflammaging and 'Garb-aging'. *Trends Endocrinol Metab*. 2017;28:199-212.
237. Lefebvre JS, Maue AC, Eaton SM, et al. The aged microenvironment contributes to the age-related functional defects of CD4 T cells in mice: The aged environment impairs CD4 T-cell functions. *Aging Cell*. 2012;11:732-40.
238. Minges Wols HA, Johnson KM, Ippolito JA, et al. Migration of immature and mature B cells in the aged microenvironment. *Immunology*. 2010;129:278-90.
239. Aw D, Hilliard L, Nishikawa Y, et al. Disorganization of the splenic microanatomy in ageing mice. *Immunology*. 2016;148:92-101.
240. Park J, Miyakawa T, Shiokawa A, et al. Splenic Stromal Cells from Aged Mice Produce Higher Levels of IL-6 Compared to Young Mice. *Mediators Inflamm*. 2014;2014:1-9.
241. Al'fonsova EV. [Functional morphology of conjunctive tissue stroma of spleen in the age aspect]. *Adv Gerontol Uspekhi Gerontol*. 2012;25:415-21.
242. Lewis SM, Williams A, Eisenbarth SC. Structure and function of the immune system in the spleen. *Sci Immunol*. 2019;4:eaau6085.
243. Steiniger B, Timphus EM, Barth PJ. The splenic marginal zone in humans and rodents: an enigmatic compartment and its inhabitants. *Histochem Cell Biol*. 2006;126:641-8.
244. Steiniger BS, Wilhelmi V, Seiler A, Lampp K, Stachniss V. Heterogeneity of stromal cells in the human splenic white pulp. Fibroblastic reticulum cells, follicular dendritic cells and a third superficial stromal cell type. *Immunology*. 2014;143:462-77.
245. Steiniger B, Barth P, Hellinger A. The Perifollicular and Marginal Zones of the Human Splenic White Pulp. *Am J Pathol*. 2001;159:501-12.
246. Steiniger B, Trabandt M, Barth PJ. The follicular dendritic cell network in secondary follicles of human palatine tonsils and spleens. *Histochem Cell Biol*. 2011;135:327-36.

247. Nagelkerke SQ, Bruggeman CW, den Haan JMM, et al. Red pulp macrophages in the human spleen are a distinct cell population with a unique expression of Fc- γ receptors. *Blood Adv.* 2018;2:941-53.
248. Alex L, Rajan M, Xavier B, et al. Microscopic study of human spleen in different age groups. *Int J Res Med Sci.* 2015;1701-6.
249. Quandelacy TM, Viboud C, Charu V, Lipsitch M, Goldstein E. Age- and Sex-related Risk Factors for Influenza-associated Mortality in the United States Between 1997–2007. *Am J Epidemiol.* 2014;179:156-67.
250. Cerutti A, Cols M, Puga I. Marginal zone B cells: virtues of innate-like antibody-producing lymphocytes. *Nat Rev Immunol.* 2013;13:118-32.
251. Leone G, Pizzigallo E. BACTERIAL INFECTIONS FOLLOWING SPLENECTOMY FOR MALIGNANT AND NONMALIGNANT HEMATOLOGIC DISEASES. *Mediterr J Hematol Infect Dis.* 2015;7:e2015057.
252. Descatoire M, Weller S, Irtan S, et al. Identification of a human splenic marginal zone B cell precursor with NOTCH2-dependent differentiation properties. *J Exp Med.* 2014;211:987-1000.
253. Ciabattini A, Nardini C, Santoro F, et al. Vaccination in the elderly: The challenge of immune changes with aging. *Semin Immunol.* 2018;40:83-94.
254. Osterholm MT, Kelley NS, Sommer A, Belongia EA. Efficacy and effectiveness of influenza vaccines: a systematic review and meta-analysis. *Lancet Infect Dis.* 2012;12:36-44.
255. Garg M, Luo W, Kaplan AM, Bondada S. Cellular basis of decreased immune responses to pneumococcal vaccines in aged mice. *Infect Immun.* 1996;64:4456-62.
256. Bondada S, Wu H-J, Robertson DA, Chelvarajan RL. Accessory cell defect in unresponsiveness of neonates and aged to polysaccharide vaccines. *Vaccine.* 2000;19:557-65.
257. van Duin D, Mohanty S, Thomas V, et al. Age-Associated Defect in Human TLR-1/2 Function. *J Immunol.* 2007;178:970-5.
258. Nyugen J, Agrawal S, Gollapudi S, Gupta S. Impaired Functions of Peripheral Blood Monocyte Subpopulations in Aged Humans. *J Clin Immunol.* 2010;30:806-13.
259. Yao X, Li H, Leng SX. Inflammation and Immune System Alterations in Frailty. *Clin Geriatr Med.* 2011;27:79-87.
260. Fülöp T, Larbi A, Witkowski JM. Human Inflammaging. *Gerontology.* 2019;65:495-504.
261. Alberro A, Iribarren-Lopez A, Sáenz-Cuesta M, et al. Inflammaging markers characteristic of advanced age show similar levels with frailty and dependency. *Sci Rep.* 2021;11:4358.

262. Steinmann GG, Klaus B, Muller-Hermelink H-K. The Involution of the Ageing Human Thymic Epithelium is Independent of Puberty.: A Morphometric Study. *Scand J Immunol.* 1985;22:563-75.
263. Murray JM, Kaufmann GR, Hodgkin PD, et al. Naive T cells are maintained by thymic output in early ages but by proliferation without phenotypic change after age twenty. *Immunol Cell Biol.* 2003;81:487-95.
264. Becklund BR, Purton JF, Ramsey C, et al. The aged lymphoid tissue environment fails to support naïve T cell homeostasis. *Sci Rep.* 2016;6:30842.

Figures legends

Figure 1. Anatomy of the rodent spleen

The spleen is compartmentalized into distinct zones organized according to their vascularization. The splenic artery branches into central arterioles sheathed by lymphoid tissue called the white pulp (WP). The central arterioles further divide into terminal arterioles which either end in the sinus of the marginal zone (MZ) that surrounds the WP or become open-ended capillaries in the cords of the red pulp (RP). In the RP, blood discharged by these capillaries runs into venous sinuses, which collect into the splenic vein. Large splenic arteries and veins are supported by trabeculae stemming from the contractile capsule of the spleen.

Figure 2. Splenic fibroblasts

The structural integrity of the splenic compartments relies on complex fibroblastic networks. In the WP (upper panels), the T zone, the central arteriole and B zone are supported respectively by T-cell zone reticular cells (TRC), perivascular reticular cells (PRC) and follicular dendritic cells (FDC). At the periphery of B follicles, marginal reticular cells (MRC) delineate the marginal sinus (bottom right). Around the WP, cohesion between fibroblasts in the MZ (bottom right) and the RP (bottom left) assure the rigidity of the venous part of the spleen.

Figure 3. Splenic macrophages

The spleen comprises several subsets of macrophages that contribute to its appropriate functioning by disposing of various apoptotic debris, senescent cells and circulating pathogens. In the T zone (upper left) and B zone (upper right), T zone macrophages (TZM) and tingible body macrophage (TBM) respectively phagocytose apoptotic T and B cells generated during antigenic responses. In the RP (bottom left), red pulp macrophages (RPM) clear senescent erythrocytes trapped in the splenic cords and recycle the iron contained in their hemoglobin, thereby controlling iron homeostasis. In the MZ (bottom right), marginal metallophilic macrophages (MMM) and marginal zone macrophages (MZM) screen the systemic blood for circulating antigens and pathogens. Through antigen capture and presentation, they contribute to efficient anti-microbial immunity.

Figure 4. Niches of splenic macrophages

According to the concept of macrophage niche, the diverse phenotypic profiles and functional specialization of splenic macrophages are regulated by specific cues present in their respective microenvironments. We propose that splenic fibroblasts are essential components of such niches. (Upper left) In the RP, RPM are imprinted by heme and IL33, acquired through phagocytosis of red blood cells. Such imprinting might also involve TGF β , which could be secreted by RP fibroblasts or RPM themselves. RP fibroblasts provide a steady supply of CSF1 and an anchoring scaffold, both critical for RPM homeostasis. CSF1 could further promote the establishment of contacts between the two cell types.

(Upper right) In the MZ, MMM and MZM are believed to be imprinted by cellular lipids derived from circulating apoptotic cells, oxysterols and yet to be identified MZ factors. MZ fibroblasts

might be the source of CSF1, the essential factor for survival of MMM and MZM. The maintenance of these macrophages probably depends on their anchoring to fibroblasts and associated extracellular matrix in the MZ. (Bottom right) in the T zone, TZM could be imprinted by cellular lipids derived from apoptotic T cells, and they might depend on IL34 for their survival, a factor potentially provided by FRC²⁴⁰. TZM homeostasis might be controlled by their adhesion to FRC and the extracellular matrix they secrete. (Bottom left) In the B zone, the maintenance of TBM could rely on imprinting by cellular lipids derived from apoptotic B cells and adhesion to FDC and their extracellular matrix.

ECM = extracellular matrix ; RBC = red blood cell

Figure 5. Feedback mechanisms

By engaging in mutually beneficial interactions with their niches, splenic macrophages likely contribute to their homeostasis, overall maintaining the functionality of the spleen.

(Upper left) By providing trophic factors such as progranulin, TGF β and PDGF β , RPM might support the survival, proliferation, and secretion of extracellular matrix by RP fibroblasts. (Upper right) Splenic macrophages might be involved in the maintenance of the architecture of the spleen. RPM could remodel the extracellular matrix of the RP through production of collagen and proteases. The presence of MZM/MMM might be critical for the maintenance of the MZ. (Bottom left) Splenic macrophages are specialized in the clearance of viruses, parasites (MMM/MZM/RPM) and iron (RPM), thereby protecting splenic fibroblasts from cellular damages arising from infection or oxidative toxicity. (Bottom right) During stress erythropoiesis, production of GDF15 by RPM might induce the secretion of BMP4 by RP fibroblasts. Both factors are critical for the expansion and maintenance of stress erythropoietic niches.

ECM = extracellular matrix ; MZF = MZ fibroblast ; SEP = stress erythroid progenitor

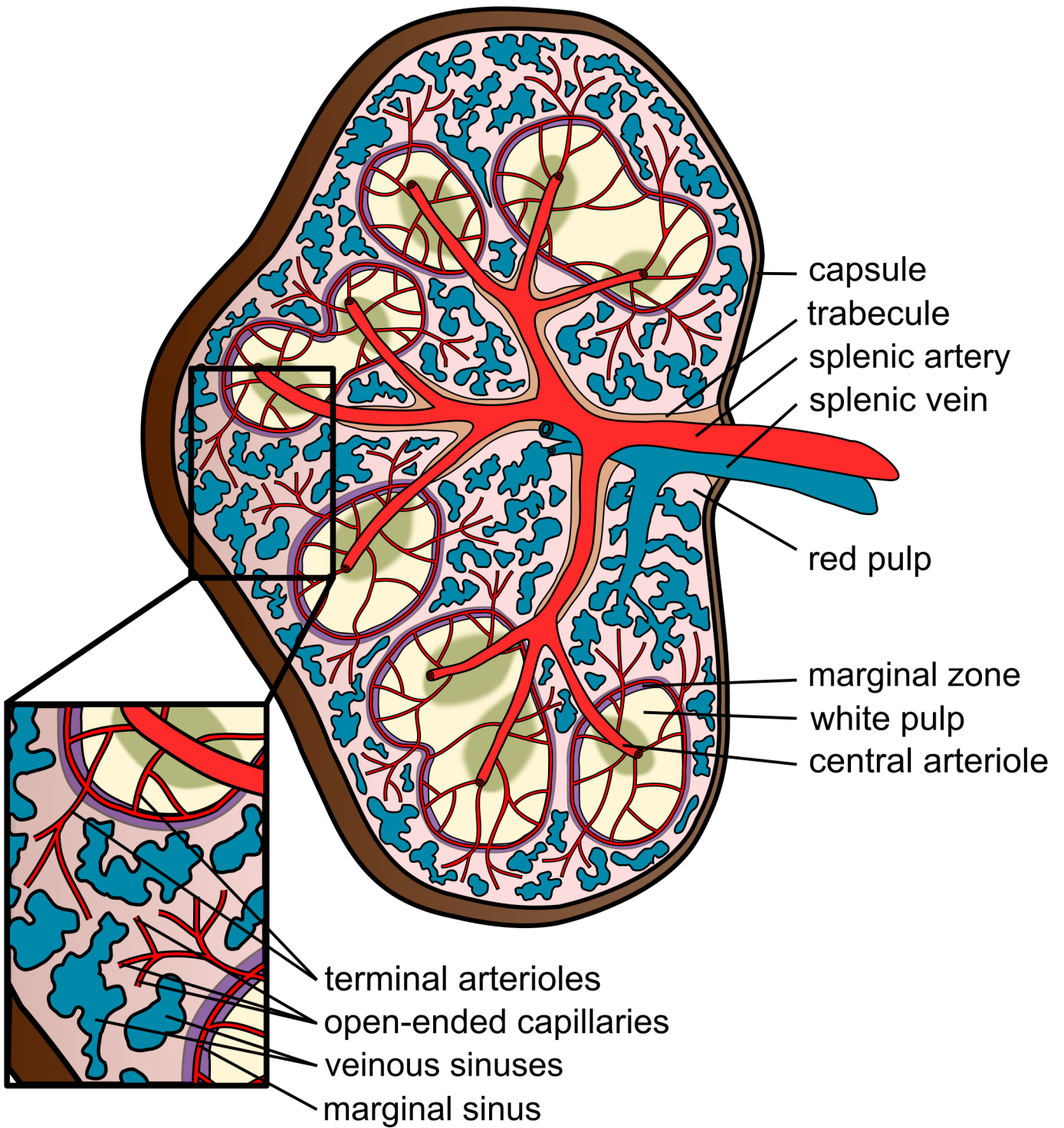


Figure 1

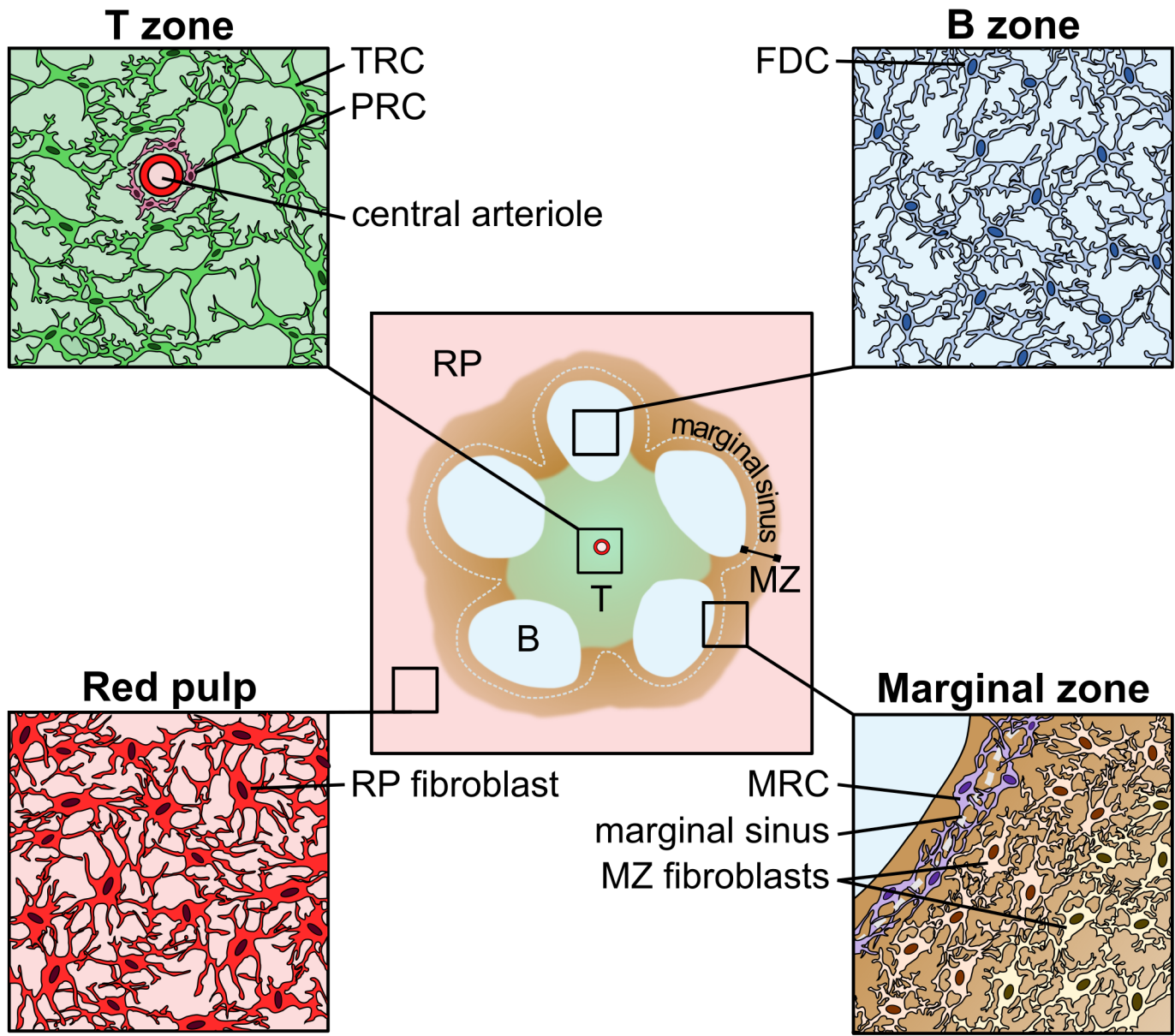


Figure 2

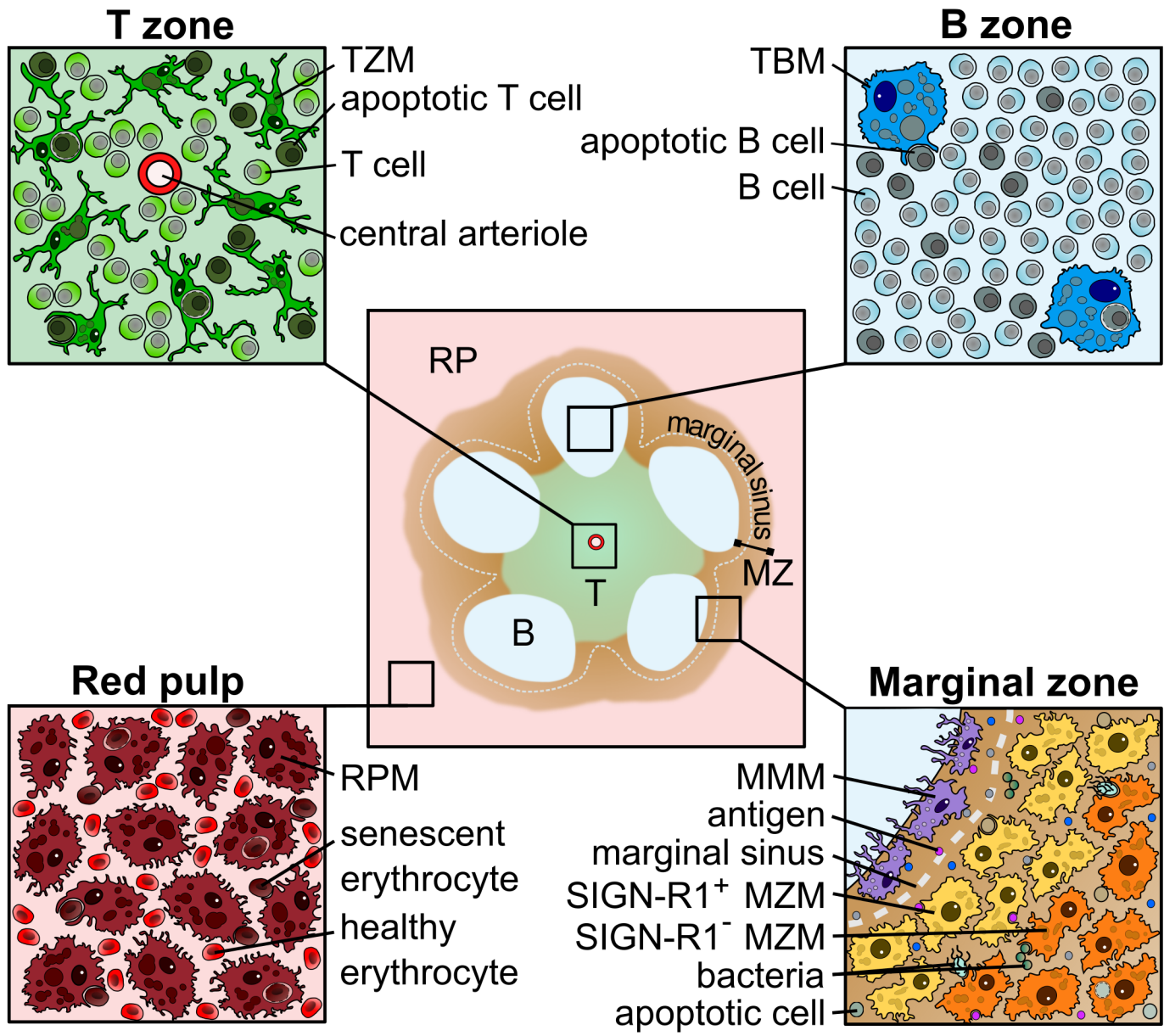
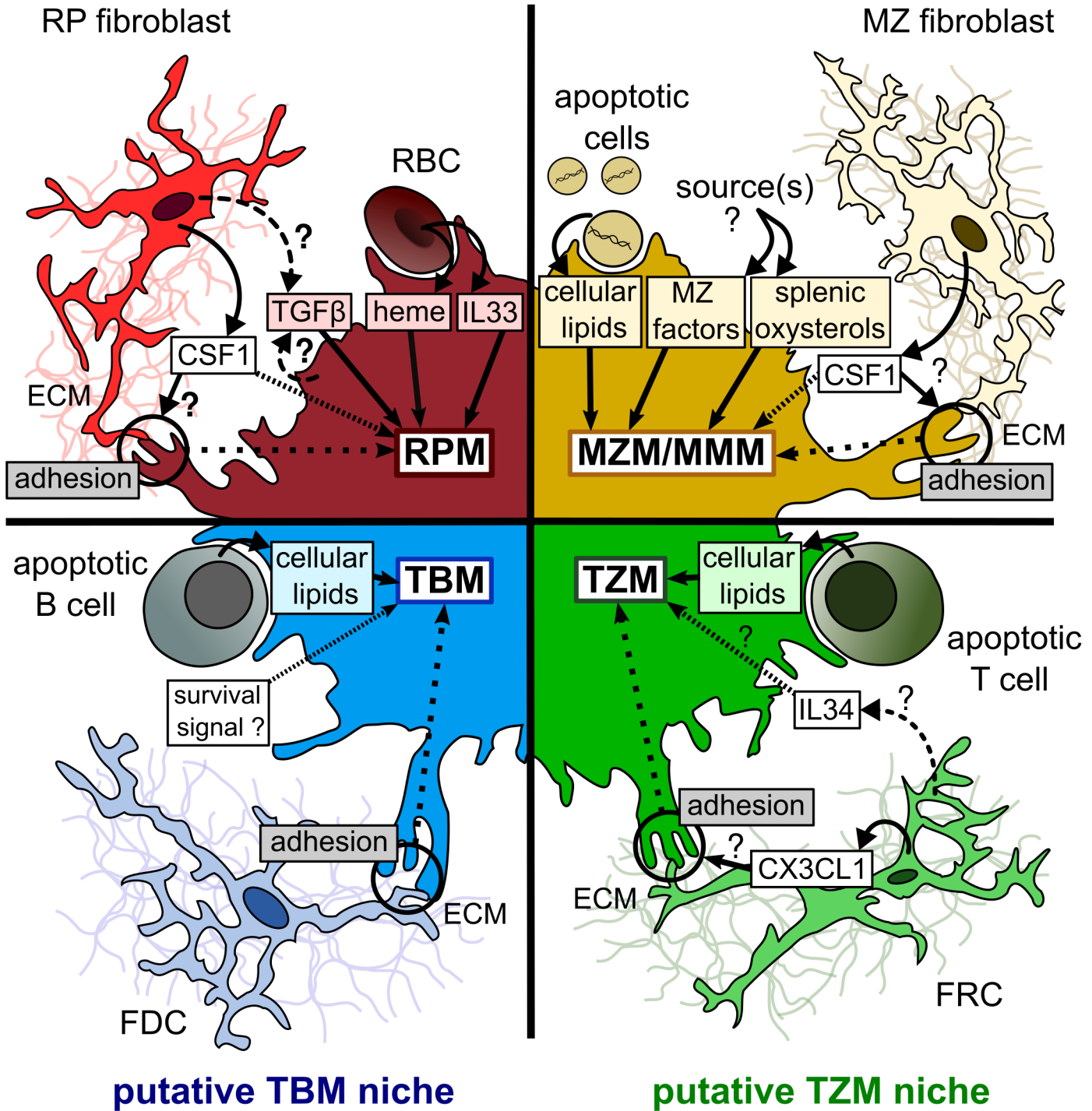


Figure 3

RPM niche

putative MZM/MMM niche



niche cues

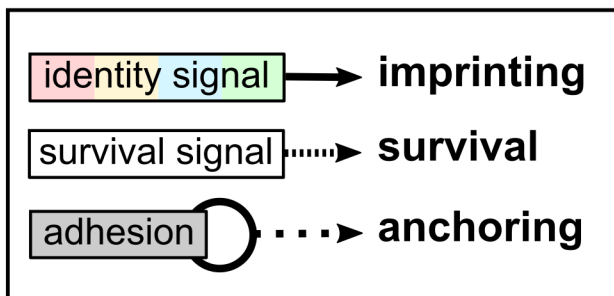
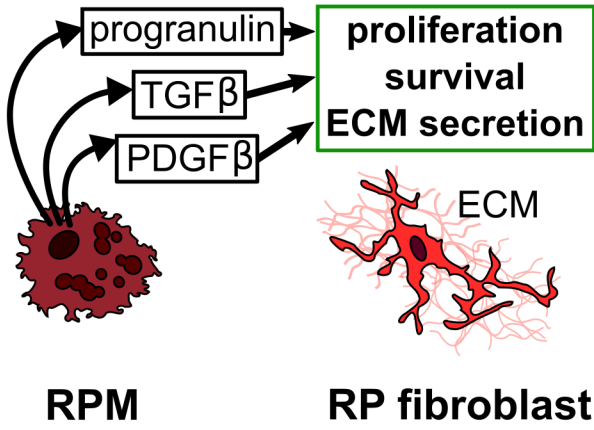
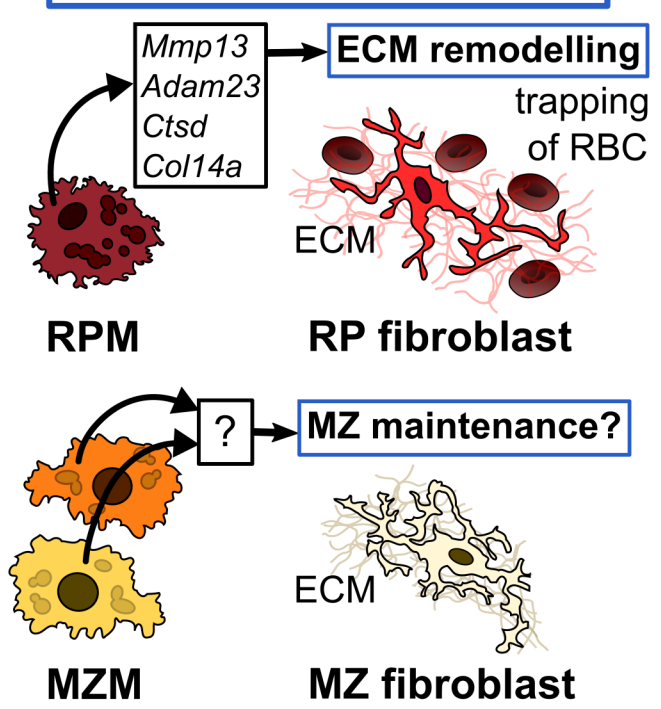


Figure 4

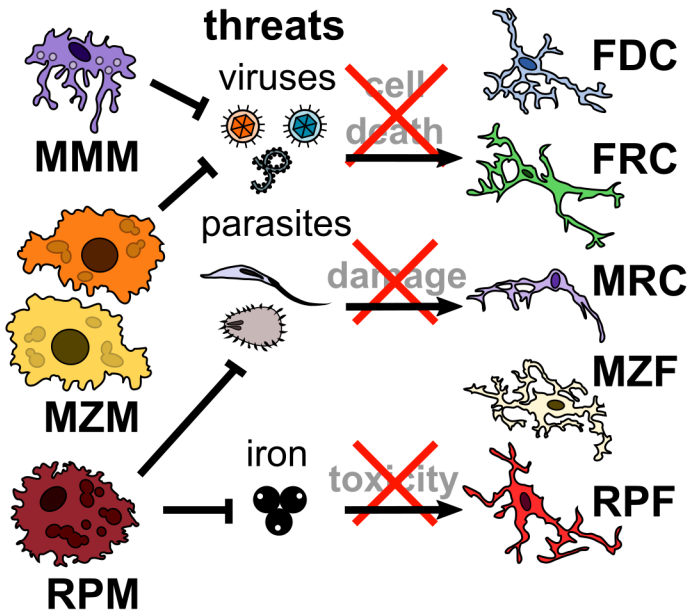
trophic function



maintenance of splenic architecture



protective function



stress erythropoiesis

