Microreview

Macrophages: sentinels and regulators of the immune system

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Summary
The important role of macrophages in host defense against a variety of pathogens has long been recognized and has been documented and reviewed in numerous publications. Recently, it has become clear that tissue macrophages are not entirely derived from monocytes, as has been assumed for a long time, but rather show an ontogenetic dichotomy in most tissues: while part of the tissue macrophages are derived from monocytes, a major subset is prenatally seeded from the yolk sac. The latter subset shows a remarkable longevity and is maintained by self-renewal in the adult animal. This paradigm shift poses interesting questions: are these two macrophage subsets functionally equivalent cells that are recruited into the tissue at different development stages, or are both macrophage subsets discrete cell types with distinct functions, which have to exist side by side? Is the functional specialization that can be observed in most macrophages due to their lineage or due to their anatomical niche? This review will give an overview about what we know of macrophage ontogeny and will discuss the influence of the macrophage lineage and location on their functional specialization.

Introduction
Macrophages were discovered by Ilja Iljitsch Metschnikow in 1884 (Zalkind, 2001; Cavaillon, 2011) and named after their capability to engulf and digest cellular debris or foreign substances. In recent years, it has become increasingly clear that the term macrophage describes a heterogeneous group of cells diverse in terms of lineage and functionality (Yona et al., 2013; Varol et al., 2015). Different and highly specialized types of macrophages are found in nearly all tissues of the body (Gordon and Taylor, 2005; Davies et al., 2013). In the spleen alone, at least three different populations of macrophages and subsets of immature monocytes are present, all showing a divergent phenotype and having different functions (Mebius and Kraal, 2005; Kohyama et al., 2009; Miyake et al., 2007; Aichele et al., 2003; Backer et al., 2010).

Macrophages are widely considered important effector cells of the innate immune system that directly and indirectly participate in host defense against pathogens. As sentinel cells, they are equipped to sense danger signals from pathogens or tissue damage, as most macrophages express a plethora of pattern recognition receptors (PRRs), including the classical Toll-like-receptors, diverse C-type lectins or cytosolic sensors such as nucleotide-binding oligomerization domain (NOD)-like receptors (Fritz et al., 2006; Palm and Medzhitov, 2009). To make best use of this sensor array, macrophages are strategically positioned in tissues in a way that they can sense danger signals efficiently: in common epithelia such as those of capillaries or lymph vessels, macrophages are positioned along the basement membrane. In stratified epithelia such as those in the skin, macrophages are part of the epithelial layer (Gordon et al., 1986). Red pulp macrophages in the spleen reside in venous sinusoids in the spleen, where the blood of the whole body passes through and can be screened by these macrophages (Mebius and Kraal, 2005). When encountering a pathogen, macrophages participate in the induction of inflammation and also fight pathogens directly. In addition to these well-studied macrophage functions, they can also play regulatory roles, which comprise tissue repair, adaptive immune system regulation or checkpoint functions during immune cell migration (Schiwon et al., 2014). Especially, the last point is a complex process that integrates information extending far beyond the simple increase of endothelial permeability induced by tumour necrosis factor (TNF), which was described before (Friedl et al., 2002). To add a further
level of complexity, macrophage functions do not necessarily have to be related to immune functions: red pulp macrophages of the spleen, for example, are responsible for the phagocytosis and degradation of aged or damaged erythrocytes and play a central role in the iron metabolism of the body (Davies et al., 2013; Kohyama et al., 2009). During this process, they accumulate large amounts of heme-degradation products and iron, which bestows upon them with superparamagnetic properties that can hamper certain cell-sorting techniques (Franken et al., 2015).

Further examples of highly specialized macrophages are the microglia of the brain or the osteoclast in the bone. For microglia, it has been demonstrated that a primary deficit of these cells was sufficient to induce some autism-related behavioural and functional connectivity deficits (Zhan et al., 2013). Osteoclasts, on the other hand, are responsible for the maintenance, repair and remodelling of bone tissue, an essential process for the integrity of the vertebral skeleton (Zaidi, 2007). These few examples illustrate that macrophages can perform highly diverse functions. In this review, we will focus on functional specialization of macrophage subsets in infection and inflammation.

Ontogeny of tissue macrophages

Macrophages types have long been summarized as the constituents of the mononuclear phagocyte system (van Furth, 1992). This system comprises highly phagocytic cells whose progenitors arise from the bone marrow and enter tissues from the circulation to differentiate into the various tissue macrophage types (van Furth, 1992; Geissmann et al., 2010; Yona and Jung, 2009). These progenitors, the monocytes, enter the tissue with slower kinetics than the other main phagocyte population in the body, the polymorphonuclear cells or neutrophilic granulocytes, which differ by exhibiting a segmented nuclear morphology (Schiwon et al., 2014). Monocytes and neutrophils are derived from a common myeloid progenitor that is regulated by soluble factors such as colony stimulating factor (CSF)1, granulocyte macrophage CSF and Fli3L (Dai et al., 2009; Stanley, 2009). Mice lacking a functional CSF1 receptor show reduced numbers of many tissue macrophages (op/op mice) (Pollard, 2009).

Two types of monocytes have been described, which differ in both surface marker expression and function, referred to as ‘patrolling’ and ‘inflammatory’ monocytes. Patrolling monocytes are Ly6C<sup>low</sup> CX<sub>3</sub>CR<sub>1</sub><sup>high</sup> and protect and repair endothelial surfaces, in part by coordinating neutrophil recruitment. These cells may be considered as terminally differentiated blood macrophages, rather than bona fide monocytes (Ginhoux and Jung, 2014). Ly6<sup>high</sup> CX3CR1<sup>low</sup> inflammatory monocytes, on the other hand, are released from spleen and bone marrow into the blood and recruited to tissues by inflammatory stimuli in a CCL2-dependent manner and contribute to anti-infectious control, especially when tissue macrophages alone cannot fight of invading pathogens (Gordon and Taylor, 2005; Geissmann et al., 2010; Ingersoll et al., 2011; Geissmann et al., 2003). While there is much evidence supporting this model, recent findings have demonstrated that it might not tell the whole story (Yona et al., 2013; Davies et al., 2013; Hume et al., 2002).

Recent evidence indicated that not all macrophages are derived from circulating monocytes. Even in the absence of haematopoietic stem cells (HSCs), progenitors from the embryonic yolk sac were sufficient to give rise to most types of tissue-resident macrophages (Schulz et al., 2012). That study furthermore showed that this embryonic lineage is the main development for most tissue-resident macrophages, such as those in skin, pancreas, liver, spleen, brain and, in part, kidney macrophages. The yolk sac-dependent tissue-resident macrophages are characterized by a strong expression of the macrophage marker F4/80 (F4/80<sup>bright</sup>), while HSC-dependent macrophages, on the other hand, express lower levels of F4/80 and high levels of CD11b. In embryonic development, yolk sac macrophages are formed at day 8.5 and spread through the embryonic tissue as soon as the circulatory system is fully established (E8.5–E10), while HSC-derived monocytes initially appear in the fetal liver between day 11.5 and 12.5 (Ginhoux and Jung, 2014). Yolk sac macrophages may also colonize the fetal liver and from there on the body (Ginhoux and Jung, 2014), but for simplicity and for compliance with established literature, we will here refer to all macrophages formed during the embryonic state as yolk sac macrophages.

Interaction of tissue macrophages with pathogens

In concert with neutrophils, macrophages serve as the first line of defense against bacterial, viral and parasite infections, and their response against these pathogens often is critical in determining the outcome of the infection for the host. Regardless of the macrophage subtype, all macrophages can exhibit two generic types of behaviour when encountering pathogens: first, phagocytosis and lysosomal inactivation of the pathogens and, second, secretion of molecules that are either antimicrobial effector molecules or immune regulator messenger molecules.

Phagocytosis is the hallmark of the macrophages’ antibacterial host response and can occur via opsonization-dependent or opsonization-independent pathways (Schaible and Weiss, 2015): opsonized pathogens are taken up regardless of pathogen type by complement specific receptors (C1qR, CR3, CR4) or Fc-receptors, which recognize pathogen-bound opsonins (Ricklin et al., 2010). Macrophages are also equipped with PRRs, which
functions and polarization of tissue macrophages

Functionally, macrophages are very often forced into a system that classifies macrophages in one of two polarization states, termed M1 or M2 (hence the name ‘M1–M2 paradigm’) (Pieters, 2008; Cambier et al., 2014). M1-polarized macrophages are ‘pro-inflammatory’ macrophages, while M2-polarized macrophages are involved in tissue repair and wound healing. Unactivated tissue macrophages are thought of being more M2 polarized, expressing anti-inflammatory markers such as IL-10, transforming growth factor (TGF)β, IL-4 and IL-13 and being involved in tissue homeostasis (Italiani and Boraschi, 2014).

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The common response of macrophages to bacterial and viral infections is the upregulation of genes involved in M1 polarization. These include cytokines such as TNFα, IL-6, IL-12, IL-1β, the chemokine receptor CCR7, costimulatory markers like CD80 and CD86 as well as the enzymes indoleamine-pyrrole-2,3-dioxygenase and NO synthase 2 (Benoit et al., 2008). The M1 polarization is usually induced by binding of bacterial components to TLRs, such as lipopeptides (TLR2), lipopolysaccharides (TLR4) or flagellin (TLR5) (Beutler, 2009; Lu et al., 2008; Hayashi et al., 2001). Endosomal receptors such as TLR9 can detect low-methylated DNA, while intracellular receptors such as NOD1, NOD2 and stimulator of interferon genes (STING) can sense muramyl dipeptide or cyclic guanosine monophosphate (GMP)-adenosine monophosphate (AMP) generated from microbial DNA (Beutler, 2009; Moreira and Zamboni, 2012; Burdette and Vance, 2013). The M1 polarization also contains a positive feedback loop in which macrophage-derived TNFα and IL-12 induces production of interferon gamma (IFNγ) in lymphocytes, which in turn activates the macrophages and enhances their capacity to deal with intracellular bacteria (Benoit et al., 2008). During infections with Listeria monocytogenes, M1-polarized macrophages are able to prevent bacterial escape from their phagosomes. Mice that lack crucial M1 effectors, such as IFNγ or TNFα, die from L. monocytogenes infection (Harty and Bevant, 1995; Shaughnessy and Swanson, 2010). The M1 polarization increases resistance against intracellular bacteria by controlling the acute phase of infection. Therefore, intracellular bacteria, such as Salmonella or Mycobacterium, interfere with M1 polarization. The Sa. typhimurium-derived SPI-2, for example, inhibits the relocation of the nicotinamide adenine dinucleotide phosphate oxidase to the macrophage phagosome, thereby severely reducing the microbial activity of affected macrophages, while Salmonella dublin...
instances cannot be easily forced (Wang et al., 2014). Some macrophage subsets under certain circumstances can kill intracellular M. tuberculosis (Figure 1B), as the bacterium is unable to block phagosome maturation and is quickly destroyed as a consequence (Herbst et al., 2011; Gutierrez et al., 2004).

Macrophage polarization should, however, not be seen as a dichotomic decision, but rather as two of many extremes of a delicate immune balance, which require adjustment and refinement, depending on the situation: an excessive M1 programme can be deleterious for the host, because many macrophage-derived products, such as reactive oxygen species, can cause substantial tissue damage and an overshooting M1-polarization may lead to severe immune dysregulation. The latter case might be the reason that in an experimental peritonitis model tested with Escherichia coli in baboons, animals that showed a predominant M1 phenotype died from the infection, while animals showing a mixed M1/M2 phenotype survived. Also, in sepsis patients, high concentrations of M1-type cytokines in the serum are correlated with mortality (Bozza and Bozza, 2007; Mehta and Shnyra, 2004).

Contrary, chronic infectious diseases are often associated with an M2 polarization: chronic brucellosis is often accompanied by an IL-10-mediated M2 phenotype. When IL-4 and IL-10 are artificially neutralized, an M1 phenotype is reactivated, which allows the macrophages to kill the Brucella bacteria (Fernandes et al., 1996).

While this model has certainly been useful in guiding research and its interpretation in the last years, it now is thought be an oversimplification of the diverse nature of tissue macrophage subsets (Gautier et al., 2012; Xue et al., 2014). Some macrophage subsets under certain circumstances cannot be easily ‘forced’ into the M1–M2 paradigm. For example, resting splenic red pulp macrophages are much more prone to produce pro-inflammatory cytokines in response to exogenous stimuli as bone marrow macrophages (Wang et al., 2013). Although being more ‘M1 polarized’, RPM still express large amounts of the C-type lectin CD206, which is usually considered a marker for M2-polarized macrophages (Franken et al., 2015; Gordon, 2003). Because of the transcriptional diversity observed between the different macrophage subsets, it is of course not surprising that differences in the ability of these cells to react to exogenous inflammatory stimuli can be observed – it is clear that this is merely one aspect of the functional differences between these cells (Gautier et al., 2012). Whether the transcriptional diversity of macrophage subsets is due to the anatomical niche they reside in or whether these cells choose their niche because of their transcriptional programming is unclear. Quite likely, it will be a mix of both scenarios, as precursors need certain receptors to home to tissue, which would require at least a certain degree of preprogramming (Engel et al., 2008). On the other hand, circulating monocytes can replace yolk sac-dependent Langerhans cells or red pulp macrophages, which shows that macrophages exhibit a large degree of plasticity and that their phenotype is profoundly shaped by the micro-environment they reside in (Tussiwand et al., 2012).

A recent study by Gautier et al. has revealed how diverse and specialized tissue macrophages really are. The authors analysed the transcriptome of several tissue macrophage populations and tried to pinpoint the differences between macrophages and DC, as classical markers do not allow to reliably distinguish macrophages from DCs in most tissues (Gautier et al., 2012). The integrin CD11c, for example, which is often employed as a DC marker by scientists working with mouse models, is also expressed by splenic red pulp macrophages, alveolar macrophages and Langerhans cells (Franken et al., 2015; Probst et al., 2005; Romani et al., 2003), while the well-known macrophage marker F4/80, on the other hand, is also expressed at low levels by the most numerous splenic DC subset, the CD4+ CD11b+ cDCs (Leenen et al., 1998; Franken et al., 2015). While CD64 (FcRyRI) and MerTK were proposed as genes that represent a macrophage core signature, setting macrophages and DCs apart, it was initially hard for them to find genes that were expressed in all macrophage populations, but not in classical DCs. Different macrophage populations (for example, red pulp and peritoneal macrophages) were transcriptionally more diverse than different DC populations (for example, CD103+ versus CD11b+ DCs). The few genes that showed uniform expression in all macrophage subset were the beforementioned genes for the Mer tyrosine kinase receptor (MerTK – which is involved in the phagocytosis of apoptotic cells) and CD64 as well as the genes for TLR4, TLR7, TLR8 and TLR13. Furthermore, the authors could find specific gene expression profiles for the four analyses of macrophage subsets that corresponded well with their known physiological function and underline further how specialized tissue macrophages are: the transcriptome of microglia, for example, was enriched in mRNA for genes of oxidative metabolism, while red pulp macrophages expressed genes involved in iron metabolism and interferon signalling (Gautier et al., 2012). Table 1 presents a more detailed overview about the functional specialization and origin of tissue macrophages.

Replacement of yolk sac macrophages by circulating monocytes

Although HSC-derived and yolk sac-derived macrophages differ in terms of lineage and gene expression, both cell types very often coexist side by side in the same tissue at varying proportions (Gautier et al., 2012; Hashimoto et al.,
Table 1. Origins, functions and markers of tissue macrophages.

| Tissue                     | Cell type                | Origin                          | Function                                                                 | Marker                                      |
|----------------------------|--------------------------|---------------------------------|--------------------------------------------------------------------------|---------------------------------------------|
| Adipose tissue             | Adipose tissue macrophage| HSC (Weisberg et al., 2003)     | Insulin sensitivity, adaptive thermogenesis                              | F4/80, CD45 (Odegaard et al., 2007; Nguyen et al., 2011) |
| Blood                     | Ly6C<sup>hi</sup> monocyte| HSC (Yona and Jung, 2009)       | Replenishment of monocyte-derived tissue macrophage populations         | CXC3CR1<sup>low</sup>, Ly6C<sup>hi</sup>, F4/80, CSF1R, CCR2 (Yang et al., 2014) |
| Blood                     | Ly6C<sup>lo</sup> monocyte| HSC (Yona and Jung, 2009)       | Surveillance of the luminal side of the endothelium                     | CXC3CR1, Ly6C<sup>lo</sup>, F4/80, CSF1R (Carlin et al., 2013; Yona and Jung, 2009) |
| Bone                      | Osteoclast                | HSC (Yona and Jung, 2009; Udagawa and Suda, 1990) | Bone remodelling and resorption (Davies et al., 2013; Italiani and Boraschi, 2014; Udagawa and Suda, 1990) | Calcitonin receptor (Davies et al., 2013) |
| Bone                      | Bone marrow macrophage    | HSC (Davies et al., 2013; Yona and Jung, 2009; Haldar et al., 2015) | Support of erythropoiesis, maintenance of stem cells                   | F4/80, CD11b, CD169, CD45<sup>lo</sup> (Davies et al., 2013; Chow et al., 2013) |
| CNS                       | Microglia                 | YS (Schulz et al., 2012)       | Brain development, promotion of neuronal survival, removal of dead neurons, synaptic remodelling (Zhan et al., 2013; London et al., 2013; Paolicelli et al., 2011) | F4/80, CD11b, CD169, CD45<sup>lo</sup> (Prinz et al., 2011) |
| Gastrointestinal tract    | Intestinal macrophages    | HSC (Bain et al., 2013; Smith et al., 2011) | Tolerance to microbiota, maintenance of intestinal homeostasis         | CX3CR1, F4/80, CD11b, CD11c, CD64 (Bain et al., 2013; Zimon and Jung, 2013) |
| Liver                     | Kupffer cells             | YS (Schulz et al., 2012)       | Phagocytosis of blood-borne pathogens and debris, lipid metabolism, toxin removal (Klein et al., 2007; Ganz, 2012) | F4/80<sup>lo</sup>, CD11b<sup>lo</sup>, CD169, CD68 (Klein et al., 2007) |
| Lung                      | Alveolar macrophages      | YS/HSC (Schulz et al., 2012)   | Immune surveillance, clearance of surfactant                            | F4/80<sup>lo</sup>, CD11b<sup>lo</sup>, CD11c<sup>lo</sup>, CD68, Siglec-F, MARCO, CD206 (Davies et al., 2013) |
| Lymph nodes               | Subcapsular sinus macrophages | HSC (Gray and Cyster, 2012) | Cross-presentation, uptake of opsonized antigen and transfer to B-cell follicles (Gray and Cyster, 2012; Asano et al., 2011; Martinez-Pomares and Gordon, 2012) | F4/80<sup>lo</sup>, CD169<sup>lo</sup>, CD11b<sup>lo</sup> |
| Lymph nodes               | Medullary cord macrophage | Unknown                         | Clearance of apoptotic plasma cells, trophic support to plasma cells and plasmablasts (Gray and Cyster, 2012) | F4/80, CD11b, CD169<sup>eq</sup>, APRIL (Gray and Cyster, 2012) |
Table 1. Continued

| Tissue       | Cell type                  | Origin          | Function                                                                 | Marker                                                                 |
|--------------|----------------------------|-----------------|--------------------------------------------------------------------------|------------------------------------------------------------------------|
| Lymph nodes  | Medullary sinus macrophage | Unknown         | Antigen capture, removal of apoptotic granulocytes (Gray and Cyster, 2012) | F4/80, CD11b, CD169<sup>a</sup>, SIGN-R1, MARCO, LYVE1 (Gray and Cyster, 2012) |
| Skin         | Dermal macrophages         | HSC (Tamoutounour et al., 2013) | Immune surveillance (Chorro and Geissmann, 2010) | F4/80, CD11b, CD11c<sup>b</sup>, CD206, Dectin-2 (Davies et al., 2013; Hashimoto et al., 2011) |
| Skin         | Langerhans cells           | YS (Schulz et al., 2012) | Immune surveillance, interaction with T lymphocytes (Chorro and Geissmann, 2010; Merad et al., 2008) | F4/80, CD11b, CD11c, Langerin (Chorro et al., 2009) |
| Spleen       | Marginal zone macrophage   | Unknown         | Immune surveillance, suppression of immune responses to apoptotic cell-associated antigen (Miyake et al., 2007; den Haan and Kraal, 2012) | CD68, CD209b, MACRO, Dectin-2, Tim-4 (Davies et al., 2013) |
| Spleen       | Metallophilic macrophage   | Unknown         | Immune surveillance (den Haan and Kraal, 2012) | CD68, CD169 (Davies et al., 2013) |
| Spleen       | Red pulp macrophages       | YS > HSC (Schulz et al., 2012) | Erythrocyte recycling, iron metabolism, regulation of CD4<sup>+</sup> T cell responses (Kohyama et al., 2009; Franken et al., 2015; Ganz, 2012; Kurotaki et al., 2011) | F4/80<sup>c</sup>, CD206, VCAM-1, Spi-C (Franken et al., 2015) |
| Urinary tract | Urinary tract macrophages  | YS/HSC (Schiwon et al., 2014) | Immune surveillance, regulation of neutrophil migration (Schiwon et al., 2014) | F4/80, Ly6C (Schiwon et al., 2014) |

The origin is only stated if experimentally established. Many tissue macrophage populations show a heterogeneous origin. In this case, the origin is noted as YS and HSC are both lineages contributing equally or as X > Y if one lineage contributes significantly more to the tissue macrophage population than the other.

YS, yolk sac; CNS, central nervous system.

2011; Hashimoto et al., 2013). Of all tissue macrophages, only the macrophages of the brain – the microglia – seem to be solely derived from yolk sac progenitors. In all other tissues, at least some macrophages are derived from HSC progenitors (Gautier et al., 2012). This raises the question whether it is functionally relevant that the different macrophage subsets originate from different lineages. One possibility is that the origin of the macrophage subsets is just an expression of the time in which the tissue was seeded and that both subsets are functionally equivalent in response to the differentiation signals supplied by the micro-environment.

Indeed, a recent study demonstrated that at least one group of yolk sac-dependent macrophages can be replenished – or at least functionally substituted - by circulating monocytes. Furthermore, this study identified heme as a local cue that governs specialization of splenic macrophages as red pulp macrophages. These macrophages are responsible for the phagocytosis of damaged and aged erythrocytes in the spleen and accumulate significant amounts of heme and iron during this process. During certain conditions, such as pathological hemolysis however, large amounts of heme can be released, which are toxic to the very cell that is responsible for the degradation of the heme. Such a condition results in a severe decline in the number of red pulp macrophages and greatly reduces the ability of the organism to recycle aged erythrocytes. Under these circumstances, Ly6C<sup>+</sup> monocytes are able to replace the yolk sac-dependent RPM functionally (Fig. 2). It is, however, unclear if such a replacement would take place in a normal, physiological situation or if it only occurs in very severe cases of pathologic hemolysis. In normal, healthy mice, nearly 100% of the RPM were yolk sac derived, pointing towards the latter possibility (Gautier et al., 2012). Further analysis is certainly required to clarify how similar these two RPM populations are.
Cooperation between tissue-resident and monocyte-derived macrophages

Despite phenotypic similarities, there is evidence that yolk sac-derived and Ly6C+ monocyte-derived macrophages can perform distinct and synergistic functions. In the spleen, RPM usually are the largest macrophage population in the steady state (Mebius and Kraal, 2005; Kohyama et al., 2009; Cesta, 2006), and only immature Ly6C+ monocytes/macrophages can be found in the splenic tissue (Swirski et al., 2010). During pathologic hemolysis, however, the number of RPM was drastically reduced, and Ly6C+ monocytes were recruited into the spleen, to functionally replace the tissue-resident RPM. A very similar situation can be observed in the bladder: during steady state, tissue-resident macrophages form the largest cell population, whereas nearly no Ly6C+ macrophages can be found (Hung et al., 2009; Tittel et al., 2011). However, when the bladder epithelium is infected with uropathogenic E. coli, large numbers of neutrophils and Ly6C+ monocytes were recruited into the bladder (Tittel et al., 2011). Strikingly, very different from the situation in the spleen, where the recruited, monocyte-derived cells were modulated to perform the same functions as the tissue-resident cells, during a bladder infection, the monocyte-derived Ly6C+ and the tissue-resident macrophages performed different but highly coordinated and synergistic functions to clear the bladder infection (Schiwon et al., 2014).

To clear urinary tract infection with uropathogenic E. coli, the antimicrobial effector cells of the innate immune system—neutrophilic granulocytes—need to reach the site of infection. Besides their strong antimicrobial capabilities, activated neutrophils can also cause substantial collateral damage and compromise tissue integrity (Summers et al., 2010; Howard and Wang, 1987). Therefore, the access of neutrophils to sites of infection is strictly regulated, to guarantee that neutrophils are only...
allowed to enter the tissue if an infection is present (Miller et al., 2000; Parkos et al., 2010; Rowe and Weiss, 2008; Nielubowicz, 2010; Flores-Mireles and Hultgren, 2015). During urinary tract infection, this neutrophil response was coordinated by two subsets of macrophages: yolk sac-derived tissue-resident macrophages attracted circulating phagocytes by releasing CXCR2-binding chemokines, such as CXCL1 or MIF. Recruited monocyte-derived Ly6C+ macrophages did not fight the pathogens directly but instead performed a role that is very similar to that of helper cells during adaptive immunity: they ‘licensed’ the tissue-resident macrophages to produce the signals that granted neutrophil access to the front line of urinary tract infection. Such licensing was mediated by secretion of TNF, which was sensed by the tissue-resident macrophages. The resident macrophages in turn released CXCL2, which upregulated MMP-9 secretion in the neutrophils, granting them access to the infected uroepithelium (Schiwon et al., 2014) (Fig. 1C). Thus, the different CXCR2 chemokines performed distinct functions, identifying another example of biased chemokine agonism (Kenakin et al., 2012; Kohut et al., 2004; Scholten et al., 2015). These findings demonstrated that yolk sac-derived tissue macrophages and recently recruited monocyte-derived macrophages, although present in the same tissue, can fulfil different roles and complement each other’s functions: the former request a second opinion from the latter, and they jointly decide whether to unleash neutrophils into the front line of infection. This macrophage cross-talk may serve to reduce the likelihood of ‘false alerts’ that induce unnecessary neutrophil responses.

Conclusions

Macrophages are important components of the innate host defense against pathogens. They directly eliminate pathogens by phagocytosis and indirectly by regulating immune responses of other immune cell subsets. Additional to these universal functions, which are shared by most macrophage subsets, tissue macrophages are also highly specialized at performing various tissue-specific functions such as erythrocyte recycling, bone remodelling or modulation of neuronal signalling. Despite such diverse functional specializations, all macrophage subsets are derived from one of two distinct developmental lineages, originating either from HSC in the bone marrow or from primitive macrophage precursors in the embryonic yolk sac. Macrophages from both lineages coexist in many tissues, and either performs redundant, overlapping functions or are capable of a functional collaboration. These recent findings have deepened our understanding of macrophage biology but at the same time demonstrated that many open questions remain. We still do not fully understand what local cues initiate functional specialization of tissue macrophages and how these specializations are initiated and stabilized on a transcriptional level. Additionally, the interplay and consequences of interactions between embryonic and monocyte-derived macrophages in the adult organism is only beginning to emerge. Future studies undoubtedly will further broaden our understanding of the biology of this multifaceted Swiss-army knife of the immune cells.

Competing interests

The authors declare no competing financial interests.

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