The development and maintenance of resident macrophages

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Development and maintainance of resident macrophages

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Abstract

The molecular and cellular mechanisms that underlie the many roles of macrophages in health and disease states *in vivo* remain poorly understood. The purpose of this Review is to present and discuss current knowledge on the developmental biology of macrophages, as it underlies the concept of a layered myeloid system composed of ‘resident’ macrophages that mostly originate from yolk sac progenitors and of ‘passenger’ or ‘transitory’ myeloid cells that originate and renew from bone marrow hematopoietic stem cells, and to provide a framework to investigate the functions of macrophages *in vivo*.

The current ‘mononuclear phagocyte system’ model [1] has guided many investigators studying the functions of macrophages over the past 30 years. It postulates that tissue macrophages are maintained from a constant supply of bone marrow derived circulating blood monocytes that extravasate into tissues [2]. This model was initially based on the observation that blood leucocytes recruited in the inflamed peritoneum differentiate into macrophages. Subsequent transplantation experiments in irradiated recipients demonstrated that bone marrow hematopoietic stem cells (HSC) and HSC-derived myeloid progenitors can indeed give rise to tissue macrophages or dendritic cells in mice and humans [3, 4, 5, 6, 7]. Therefore, investigations designed to study the molecular and cellular basis for macrophage phenotypic diversity and functions have largely focused on the activation, or polarization, of monocyte-derived macrophage by external cues, such as cytokines and microbe-associated signals [8, 910]. Nevertheless, it remains currently unclear whether polarization stages of monocytes are stable or represent transient states of activation, whether they account for the phenotypic and functional heterogeneity of macrophages *in vivo* in health or disease states, and how many such polarized states exist [10, 11].

A new model of macrophage development is emerging based on a number of independent observations that two distinct populations of macrophages, which can be distinguished by their progenitors, developmental history, turnover and mechanisms of maintenance, coexist within the tissues of an adult mouse. The macrophage system of a mouse can now be described as a ‘layered’ system where ‘resident’ macrophages that develop in embryos independently of HSC [12] persist in adult tissues independently of adult

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HSC [12, 13, 14, 15, 16, 17, 18], and coexist with ‘passenger’ leucocytes such as monocytes and classical dendritic cells that originate and renew from bone marrow HSCs and myeloid progenitors [4, 6, 19]. Resident macrophages include spleen red pulp macrophages, lung alveolar macrophages, epidermal Langerhans cells, brain microglia, liver Kupffer cells, large peritoneal macrophages, and F4/80^{bright} pancreatic, kidney and cardiac macrophages. Many ‘resident’ macrophages are long-lived in mice and can proliferate within their tissue of residence, a mechanism involved in their maintenance in adults [20, 21, 22, 23, 24, 25, 26, 27]. Nevertheless, bone marrow-derived progenitors also contribute to resident subsets in the lamina propria, spleen, brain, skin, heart, liver and kidney in a proportion that varies with the tissue considered, the age of the mice, and pathological processes[12, 13, 14, 15, 16, 17, 18, 28, 29].

Therefore, the purpose of this Review is to present and discuss current knowledge on the developmental biology of resident macrophages, as it underlies the concept of a layered myeloid system composed of resident macrophages distinct from passenger macrophages and myeloid cells, and provides a new framework and experimental tools to characterise the functions of macrophages *in vivo*, in health and disease settings.

The original concept of ‘primitive macrophages’

During ontogeny, macrophages were found in the mouse yolk sac and the embryo before the initiation of HSC-derived hematopoiesis and before monocytes could be detected in the fetal blood. These ‘primitive macrophages’ were described in a series of seminal works by M. Naito and K. Takahashi in the 1980's [20, 30, 31] that reported the presence of large cells with macrophage morphology in blood islands of the yolk sac at E9 and in the fetal liver at E10. They were described as immature because of the lack of detectable phagocytic activity and a weak immunoreactivity for F4/80, as detected by electron microscopy [20, 30]. In addition to the ‘immature macrophages’, these early studies also reported the existence of ‘fetal macrophages’ from E10 to E17, as slightly smaller cells with pseudopodia, phagocytic activity and frequent mitotic figures [31]. At E17, the ‘fetal macrophages’ were reported as more differentiated, as they acquired ultrastructural features similar to those observed in adult tissue macrophages [30, 32]. These early studies noted that the remaining difference between fetal and adult tissue macrophages was the presence of numerous mitotic figures in fetal macrophages throughout development. It was also noted that poorly differentiated myeloid cells are detected in the blood vessels of the yolk sac at E10 [20], where immature stages of the monocyte and neutrophil differentiation series can be detected from E12 onwards, and bona-fide neutrophils and monocytes, based on ultrastructural features and PO-cytochemistry, are present in the fetal liver at E15 and E16 respectively [30]. They therefore proposed that fetal macrophages develop without a monocytic intermediate, and in parallel with the monocyte and granulocyte lineage.

In the recent literature, this early work was sometimes reported as evidence for the early colonization of the fetus by ‘yolk-sac macrophages’ before their replacement by monocyte-derived macrophages. However, this body of work described the identification of proliferating non-phagocytic macrophage precursors (described as ‘immature macrophages’) that originate in the yolk sac, colonize the embryo and finish their differentiation *in situ* within the fetal tissues [20, 30, 31]. Their description did not correspond to a particular wave of

hematopoietic progenitors ('primitive' or 'definitive'), as discovered subsequently. However, the description of erythro-myeloid progenitors (EMP) [33, 34, 35, 36] (see below) and recent fate mapping data are in accordance with the authors original interpretation of their morphological data. Indeed, genetic pulse labeling of *Csfr1*$^{+}$ Kit^{+} CD45^{lo} AA4.1^{+} EMP present in the yolk sac at E8.5 is followed by the detection of labeled CD11b^{+} cells with a negative or faint immunoreactivity for F4/80 at E9.5 in both the yolk sac and embryo and then by the detection of F4/80^{bright} cells starting from E10.5 [18].

More recent works have identified three successive but overlapping waves of hematopoietic progenitors during development, all of which have the potential to give rise to fetal macrophage [34]. They can be distinguished by the anatomical site (extra-embryonic yolk sac versus intra-embryonic) where they are generated, expand and differentiate, their differentiation potential and their transcription factor dependency (**Table 1** and **Fig. 1**). Here we will focus on the myeloid lineages. Erythroid lineages have been reviewed in [37].

Primitive hematopoiesis is a source of macrophages in embryos

Primitive hematopoiesis is the source of the first hematopoietic cells detected in the developing embryo. It is is characterized by the emergence of progenitors with limited potential for erythrocytes and macrophages and which can be found in the extra-embryonic yolk-sac blood islands in mice [36]. These progenitors are thought to arise directly from the posterior plate mesoderm, and clonal analysis of the blood islands reveals that the endothelial cells lining the blood islands and the hematopoietic cells they contain do not share a common origin [38]. This distinguishes them from the subsequent 'definitive' hematopoietic waves, which are thought to arise from a hemogenic endothelium. Accordingly, primitive progenitors and their daughter cells are not affected when transcription factors required for the endothelial-hematopoieitic transition, such as RUNX1, are absent (Table 1).

In zebrafish embryos, the 'primitive' wave can give rise to erythrocytes and macrophages, as well as neutrophils, in the absence of *Runx1* [39, 40, 41]. In the mouse embryo, the *in vivo* characterization of the macrophage progeny of the primitive wave is still hampered by experimental constrains. However, (rare) progenitors with restricted macrophage potential *in vitro* can be found in the yolk sac at the neural plate stage between E7.5 and E8 [3634].

Erythro-Myeloid Progenitors constitute the first wave of definitive hematopoiesis and give rise to most resident macrophages

The first 'definitive' progenitors emerge in the yolk sac of mouse embryos at E8.25 [34, 36]. Termed erythro-pyeloid progenitors (EMP), these progenitors are phenotypically defined as Kit^{+}, AA4.1^{+} (CD93), CD41^{+}, VE-cadherin^{+} (VE-Cad), CD16/32^{+} (FCγII/III receptors) and CD45^{low} [33, 35] (Table 1). Examination of their erythroid progeny led to their classification as 'definitive' progenitors [42]. However, they can be distinguished from HSCs by the lack of lymphoid potential, both *in vitro* and *in vivo*, the lack of long-term repopulating potential [35] and the lack of cell-surface expression of Sca-1 [35]. The EMP-derived hematopoiesis is sufficient to support survival of embryos lacking HSCs until birth [43]. EMP emerge from the

yolk sac hemogenic endothelium in a Runx1-dependant endothelial to hematopoietic transition [44]. Yolk sac-EMP emergence does not require blood flow, as evidenced by NCX1-deficient and VE-CAD-deficient embryos [35, 45, 46]. They are generated from Tie2^{+} yolk sac ancestors from E7.5 to E10.5 [18]. EMP numbers in the yolk sac peak between E9.5 and E10.5 and they seed the fetal liver as soon as E9 [47]. Circulating EMP are still detected in peripheral blood at E14.5 [35] and EMP-derived Kit^{+} cells are found in the fetal liver at 16.5 [18]. EMP expand in the fetal liver and differentiate into erythrocytes, megakaryocytes, macrophages, monocytes, granulocytes and mast cells [18]. Yolk sac-EMP isolated from the E8.5 yolk sac and the E12.5 fetal liver have the same *in vitro* differentiation potential, but erythroid, monocyte and granulocyte and mast cell potential is only observed *in vivo* after seeding of the fetal liver [18]. Thus, the fetal liver niche provides critical cues or an environment for EMP to reach their full potential. Yolk sac-EMP express *Myb* [48] but their commitment and differentiation into the myeloid fate is unaltered in *Myb*-deficient embryos, although their erythroid potential is blocked [12, 18, 49, 50] (**Table 1**). Therefore *Myb* is required for the commitment and differentiation of EMP into the erythroid fate [50], but is dispensable for myeloid differentiation, and possibly redundant, because EMP express *Mybl2* (also known as *b-Myb*), a gene that can rescue hematopoiesis in *Myb*-deficient *Drosophila* [51].

HSCs, the second wave of definitive hematopoiesis, are developmentally distinct from EMPs

HSCs are distinguished from other progenitors by their capacity to perform long-term repopulation of all leukocytes lineages when transplanted into a conventional irradiated recipient mouse. HSC-derived hematopoiesis is described elsewhere [52, 53]. HSC and EMP arise from distinct hemogenic endothelial cells [43]. HSC emerge from the hemogenic endothelium of the aorto-gonado-mesonephros (AGM) region (starting at E10.5 in mice) [39, 54, 55] and of the umbilical and vitelline arteries [56] and migrate to the fetal liver, where they expand and differentiate from E12.5 till definitive hematopoiesis begins to shift to the BM. HSC colonize the embryonic bone marrow at E15 and active hematopoeisis starts there at E17 [47, 57]. Fetal and adult HSC require the transcription factor *Myb* for their self-renewal and maintenance, and loss of *Myb* expression leads to rapid HSC-derived hematopoiesis failure [12, 58, 59, 60]. In addition, HSC also require NOTCH1 for their emergence, in contrast to EMP, as *Notch1*$^{-/-}$ embryos have normal numbers of hematopoietic progenitors in the yolk sac but very few in the body of the embryo [61]. Several groups have attempted to pinpoint the specific site and time when HSC can be first detected within the embryo proper. Pioneering work by Dzierzak has demonstrated that the AGM region contained the progenitors endowed with LTR ability at E10.5 [62]. However, immature HSC have been detected at E9-9.5 in the para-aortic splanchnopleura (P-SP), a structure that later evolves into the AGM region. Such immature HSC migrate into the fetal liver at E10, mature into HSC and contribute to the adult HSC pool [47].

Genetic models distinguish the three progenitor waves

As described above, the emergence of different waves of hematopoietic precursors partially overlap in a short timeframe, which has made fate-mapping difficult. However these

precursors can be distinguished genetically, and overall the layering of the hematopoietic system and in particular of macrophage development appears to stand on a robust genetic basis. Primitive hematopoiesis occurs in the absence of *Runx1* and *Myb* [63, 64], while EMP are *Runx1*-dependent for their emergence [64] but *Myb*-independent for their myeloid differentiation [12, 18, 49] and Notch1-independent [61]. In contrast, fetal and adult HSC require *Runx1*, *Myb and Notch1* (**Table 1**) [39, 40, 58, 59, 61]. Based on these data, several reports have investigated the lineage of origin of fetal (primitive) and adult macrophages and the mechanisms that may account for their persistence in adults.

Resident macrophages originate in majority from yolk sac EMP

The development of fetal F4/80^{bright} macrophages is unaltered in *Myb*-deficient mice and adult F4/80^{bright} macrophages are largely maintained in adult mice in the absence of *Myb,* and independently of bone marrow progenitors [12]. In addition, adult tissue resident F4/80^{bright} macrophages were traced back to *Myb*-independent *Csf1r*-expressing (*Csf1r*$^{+}$) precursors present in the early mouse embryo at E8.5 using Cre-mediated hydroxy-tamoxifen (OH-TAM)-induced pulse labeling [65, 66] in *Csf1r-MeriCremer* mice [12] (see below).

These data suggested that F4/80^{bright} macrophages do not originate from HSC, but from yolk sac progenitors such as either primitive progenitors or EMP [48]. In accordance, brain microglia develop from yolk sac progenitors[14], later functionally identified as EMP [48]. Following pulse-labeling of *Csf1r*-expresing cells with OH-TAM at E8.5, the labeling efficiency of F4/80^{bright} macrophages was 30% at day 10.5, and remained high (~20%) in microglia, but progressively decreased from E12.5 onwards in other organs to reach a plateau at birth, maintained even in one year-old animals (5% for the liver Kupffer cells, 1% for epidermal Langerhans cells ([12,18, 67] and unpublished data from E. Gomez Perdiguero and F. Geissmann). This may be explained by the observation that pulse labeling with OH-TAM at E8.5 only labels the very first EMP to emerge in the yolk sac, while EMP continue to emerge in the yolk sac until at least E10.5 [18], and therefore the labeling percentage of the macrophages in embryonic tissues may reflect the time at which their colonization by fetal macrophages is completed. However, it is also possible that this “dilution” reflects a contribution of another progenitor, such as fetal HSC or HSC-derived monocytes to several fetal macrophage populations [17, 67, 68, 69]. A contribution of fetal monocytes is unlikely, as they also derive from EMP until E16.5 [18, 35]. However, a contribution of fetal HSC is still debated [17, 68, 69, 70].

The main argument against a significant contribution of fetal HSCs come from experiments using a *Tie2-MeriCreMer* fate mapping model, where the expression of Cre recombinase is inducible in *Tie2*-expressing cells [18, 71]. Tie2 (also known as Tek) is expressed in the hemogenic endothelium, endothelial cells and hematopoietic progenitors in the yolk sac [72], aorta-gonado-mesonephros region, fetal liver HSC and adult HSC [52]. Pulse labeling with tamoxifen at E7.5, E8.5, E9.5 and E10.5 resulted in labeling of adult HSC, lymphocytes, monocytes and neutrophils with the same efficiency, as expected. In contrast, fetal macrophages and adult resident F4/80^{bright} macrophages in all organs were labeled with a decreasing efficiency (high at E7.5 to very low at E10.5) indicating that fetal and adult

F4/80^{bright} macrophages originate in majority from a progenitor that does not express *Tie2* after E10.5, with a minor contribution of progenitors (such as HSC) expressing *Tie2* after E10.5.

Using fate mapping models where the expression of Cre recombinase is either inducible or constitutive in *Csf1r*-expressing cells (*Csf1r-MeriCremer* and *Csf1r-iCre* mice), the macrophage progenitor itself was identified as a CD45^{lo}Kit^{+}AA4.1^{+} EMP that appears in the yolk sac from E8.5 [18]. These EMPs migrate in the fetal liver, where they expand and persist as Kit^{+} progenitors until at least E16.5 [18]. These EMPs give rise to erythrocytes, monocytes, granulocytes and mast cells until late fetal development [18,35] in accordance with the earlier observation that HSC-independent hematopoiesis is not only necessary, but also sufficient to support survival until the birth of embryos lacking HSCs [43]. They also give rise to F4/80^{bright} fetal macrophages in all embryonic tissues from E10.5 onwards [18]. An EMP origin of the majority of resident macrophages was also favoured in an independent report [67].

Altogether these data support a major contribution from yolk-sac EMPs to fetal and adult resident macrophages (Fig.1 Fig.2). EMP-derived erythrocytes, monocytes, granulocytes and mast cells are ultimately replaced by HSC derived myeloid cells in the late embryo [18], while EMP-derived macrophages persist all through fetal and post-natal development as tissue-resident macrophages [12, 18] (**Fig. 2**). It was also speculated that a first wave of *Myb*-independent EMP give rise to microglia and a second wave of *Myb*-dependent EMPs give rise to other macrophages respectively [67]. However, there is no available data to support the existence of two genetically distinct populations of EMP, and this hypothesis is not in accordance with previous reports that *Myb* is expressed in early yolk sac-EMP [36, 48] and with the persistence of fetal resident macrophages in all *Myb*-deficient embryonic tissues [12].

The contribution of “primitive” hematopoiesis to microglia

Primitive hematopoiesis could also contribute to the “primitive” and fetal macrophages. Early (E7.5) pulse-labelling of progenitors using several different reporters such as *Runx1*, *Csf1r*, *Tie2* and *Kit* [12, 14, 18, 70], all result in a more efficient labelling of adult microglia in comparison with other adult tissue resident macrophages. As mentioned above, this may relate either to an earlier termination of brain colonization by macrophages in comparison to other organs, or the existence of several subsets of EMPs. However, it might also reflect the contribution of primitive hematopoiesis to microglia. It will be interesting to determine whether primitive progenitors are labelled in these models, and to investigate whether macrophages produced by primitive hematopoiesis can persist in adult mice. *Myb*-independency does not allow to genetically distinguish primitive from EMP-derived hematopoiesis and it is not yet known whether *Runx1* is required for the differentiation of macrophages in mice. Altogether, the lack of genetic dissection and the low temporal resolution of classical fate mapping approaches in the mouse has not yet allowed independent tracking of the respective progeny of the two progenitor populations. A better understanding of the molecular control of primitive progenitors and EMP commitment and differentiation is required to develop better tools in order to characterize the possible

contribution of “primitive” hematopoiesis to the development of fetal macrophages, and possibly adult macrophages in mice.

Contribution of HSCs to populations of resident macrophages in adult mice varies depending on organs and time

The analysis of HSC contribution to adult resident macrophages in the absence of bone marrow irradiation was performed by several groups using *Flt3-Cre* mice [12, 17, 18, 73] based on the observation that *Flt3* (encoded by *Flk2*) is expressed by HSC-derived progenitors[71, 74], but not by fetal macrophages [12]. HSC contribution was also studied in several independent models of bone marrow transplantation without irradiation such as *Mx-1*–Cre $Myb^{fl/fl}$ and $Kit^{W/Wv}$ $Rag2^{-/-}$ $\gamma c^{-/-}$ [12, 18] and in parabiotic mice [15, 75]. Overall, these analyses revealed that HSC contribution to resident macrophages was different between organs and frequently increased with age. HSC contribution to adult resident macrophages was either minor (<5%) in brain, liver, epidermis [12, 14, 48], small but increasing with age in lung, heart and spleen [18, 75], and sometimes predominant after weaning in the case of the gut lamina propria [28, 76] (**Fig.1** and **Fig. 2**). It is not yet known whether the precursor(s) of the HSC-derived resident macrophages are monocytes or earlier myeloid progenitors, except in the case of the gut, where $Ly6C^+$ monocytes are proposed to be the immediate precursor of gut lamina propria macrophages [28, 76].

Of note, a recent report investigated the origin of resident macrophages using a fate mapping model where the expression of the Cre recombinase was inducible in *Kit*-expressing cells (*Kit-MeriCreMer* mice) [70]. Importantly, this study represents an independent confirmation that most adult resident macrophages originate from embryonic and fetal progenitors and persist in adults independently of adult HSCs, as Kit^+ progenitors do not seem to contribute to resident tissue macrophages after E16.5 [70]. The authors also conclude that most resident macrophages, except microglia, are descendants of fetal HSCs and not of EMP, based on the observation that labeling of Kit^+Sca1^+ EMP at day E7.5 results in the labeling of microglia only, while labeling of Kit^+ progenitors at E8.5 or E9.5 results in contrast in the labeling of all adult resident macrophages and HSC-derived cells. However, EMP do not express Sca1 (**Table 1** and [35]), while EMP and fetal HSC both express Kit and co-exist in the fetal liver until E16.5 [18, 35]. Thus, *Kit* expression and *Kit*-based fate mapping may not allow to discriminate between EMP and fetal HSC.

Recent work in chicken suggests that in contrast to mice, yolk sac-derived macrophages are not retained after hatching and, surprisingly, that tissue macrophage in adult birds originate from a macrophage-restricted, self-renewing progenitor cell in bone marrow that does not give rise to any other lineage [73]. These findings, if confirmed, would indicate that myelopoiesis in birds is markedly different from its counterparts in mice, zebrafish and human, because a macrophage-restricted, self-renewing progenitor cell in the bone marrow or its equivalent was not identified in these species.

Maintenance of macrophages by local proliferation or recruitment

As indicated above, a proportion of alveolar macrophages, kidney macrophages and heart macrophages appear to be replaced during the normal ageing process [12, 18, 75]. A partial replacement of resident macrophages is also observed following gamma irradiation, bone marrow transplantation or adoptive transfer experiments [3, 4, 77] and in macrophage depletion studies, such as intravenous injections of clodronate liposomes. Liver Kupffer cells are also reported to be replaced by bone marrow derived progenitors following, for example, the death of Kupffer cells in severe experimental *Listeria* infection [29]. However, resident macrophages such as Langerhans cells, Kupffer cells, microglia, alveolar macrophages, peritoneal macrophages and splenic macrophages all have the potential to proliferate [15, 22, 23, 27, 78, 79, 80, 81, 82]. In some cases, resident macrophages can immediately replenish themselves following a severe depletion [15, 23, 82]. A growing body of evidence now clearly shows that fully differentiated resident macrophages can re-enter cell cycle and maintain their numbers by local proliferation [23, 24, 25, 26, 27]. Macrophage proliferation increases dramatically during inflammatory conditions, such as skin inflammation, where up to 30% of Langerhans cells express the proliferation marker Ki67 [22]. During an acute inflammatory response, tissue-resident peritoneal macrophages that survive undergo a transient and intense proliferative burst *in situ* to repopulate the tissue [23]. In response to helminthes infection, resident macrophages in the pleura and peritoneal cavities expand by local proliferation [81]. In glucan-induced granuloma formation in monocytopenic mice, Kupffer cells proliferate actively and contribute to granuloma formation [78]. After conditional ablation of microglia in adult mice, the microglial compartment is reconstituted within one week of depletion by CNS-resident cells, independent of bone-marrow-derived precursors [82]. However, it is yet unclear whether the tissue-resident macrophage populations contain a local ‘progenitor compartment’, or whether all macrophages have the capacity to undergo mitosis. The mechanisms that may underlie this ability of resident macrophages to self-maintain and to proliferate are still unclear. An important role of the Maf family of transcription factors is likely, as combined deficiency for the transcription factors MafB and c-Maf enables the KLF4- and c-Myc–dependent proliferation of differentiated macrophages in culture in response to Csf1 [83]. In the case of epidermal Langerhans cell, proliferation *in vivo* was shown to be driven by an (unknown) keratinocyte-derived signal [22]. In the case of nematode infection, macrophage proliferation was impaired in the absence of IL-4 [81, 84]. The cellular and molecular mechanisms that control macrophage maintenance and expansion in adult mice, and the consequences of ageing on these processes, are an area of active investigation, as their elucidation will shed light on the pathophysiology of inflammation.

Conclusions and perspectives on macrophage functions

A revised model for macrophage development and functions is therefore emerging, which distinguishes the resident macrophages that develop during embryogenesis from the passengers myeloid cells that originate and renew from bone marrow HSC. This distinction between passenger and resident macrophages is in line with earlier kinetic studies of the splenic-myeloid compartment in adult mice, which proposed a dual origin for myeloid cells, with half of myeloid cells renewing from blood and the other half being produced within the

spleen [85]. It also explains the observations that Langerhans cells do not exchange between parabiotic mice [13] and that fetal microglia persists in adult mice [14].

This distinction is relevant to disease pathogenesis, as it is quite clear that the functions of HSC-derived monocytes can be very different from those of tissue-resident macrophages within the same inflammatory environment. The two cell types have distinct expansion mechanisms and distinct functions in the brain during experimental auto-immune encephalitis (EAE) [86]. Infiltrating monocytes are recruited via extravasation from blood vessels and produce inflammatory mediators important for disease progression, but do not persist after the resolution of inflammation, while, in contrast, activated resident microglia proliferate locally, persist and return to quiescence following remission [86]. Following helminthe parasite infection, pleural resident macrophages adopt anti-inflammatory phenotypes, while passenger macrophages exhibit pro-inflammatory responses [81]. However, in a model of chronic neurodegeneration, proliferating resident microglial cells contribute to neuronal damage during the development of the disease [87]. Thus, it might be inaccurate to describe resident macrophages as always being ‘anti-inflammatory’ as opposed to passenger macrophages being ‘pro-inflammatory’. The respective roles of resident and passenger macrophages remain to be studied in detail, and most experimental models available, at present, do not easily elucidate the roles of resident and recruited macrophages in adult mouse tissues. Future work should help characterize the roles of (EMP-derived) resident macrophages, separate them from the function of HSC-derived passenger macrophages and characterize the underlying molecular mechanisms involved.

It is also important to recognize that the distinct subsets of resident macrophages associated with different tissues, for example microglia, Langerhans cells, Kupffer cells, peritoneal macrophages, red pulp macrophages or alveolar macrophages have different phenotypes, transcriptional profiles and chromatin landscape [88, 89], require specific growth factors and rely on distinct transcription factors for their differentiation or maintenance. For example, colony stimulating factor 1 (CSF1/M-CSF) is essential for Kupffer cells [90], TGFβ1 and IL-34 for microglia and Langerhans cells [88, 91, 92, 93], colony stimulating factor 2 (CSF2/GM-CSF) for the differentiation or maintenance of alveolar macrophages in mice and humans [94, 95, 96, 97, 98, 99], and retinoic acid and GATA6 for large peritoneal macrophages [88, 100, 101]. SPIC is important for the development of red pulp macrophages [102], while LXR-deficient mice are defective in the generation of maginal zone and metallophilic macrophages [103].

Tissue-specific environment are important for the maintainance of macrophages identities [88, 89]. Several studies also suggest that individual resident macrophage subsets follow a differentiation program during embryogenesis and early post-natal life that result in the establishment of stable phenotypic and functional subsets [22, 48]. Future works investigating the the respective contribution and interplay of lineage-specific ‘hard-wired’ differentiation programs and of the local tissue micro-environment for the differentiation, maintenance and activation of tissue-specific resident macrophages will contribute to the understanding of their functions.

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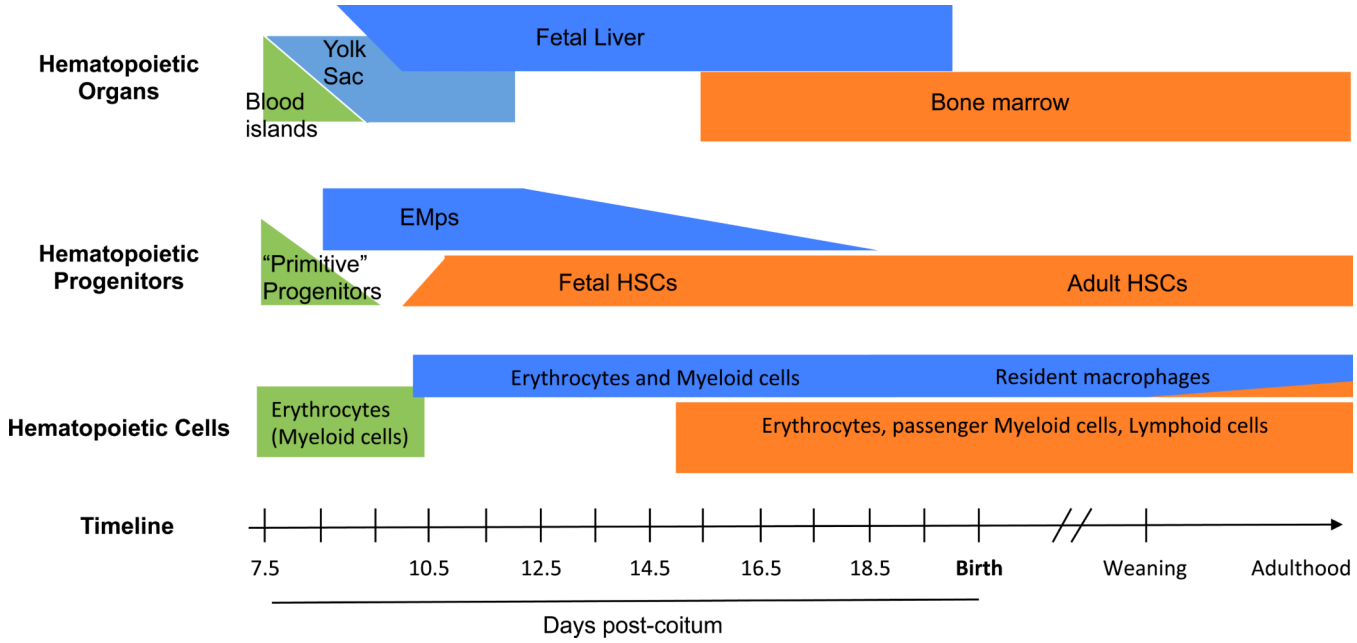

Figure 1.

Time scale of hematopoietic progenitors development in the mouse embryo.There are three successive but overlapping waves of hematopoietic progenitors during development, all of which have the potential to give rise to fetal macrophages. They can be distinguished by the hematopoietic niches exist where the progenitors expand and differentiate during development (upper panel), the yolk sac (primitive progenitors in green and EMP in blue), the fetal liver (EMP and HSC), and finally the bone marrow (HSC, orange). While primitive progenitors are only described in a small time window (middle panel), definitive progenitors (including EMPs and HSCs) co-exist during most fetal development in the fetal liver. Only HSC-derived hematopoiesis then shifts to the bone marrow niche. The three waves of progenitor can also be distinguished by their differentiation potential *in vivo* (lower panel). Primitive progenitors are restricted to the erythroid or the myeloid lineages, while EMP have both erythroid and myeloid potential. EMP-derived hematopoiesis gives rise to erythrocytes, macrophages, monocytes, granulocytes and mast cells and is sufficient to support survival of embryos lacking HSCs until birth. EMP-derived macrophages persist after birth in most tissues as resident macrophages, with the exception of the intestine lamina propria. HSCs are distinguished from other progenitors by their capacity to perform long-term repopulation of all leukocytes lineages when transplanted into a conventional irradiated recipient mouse.

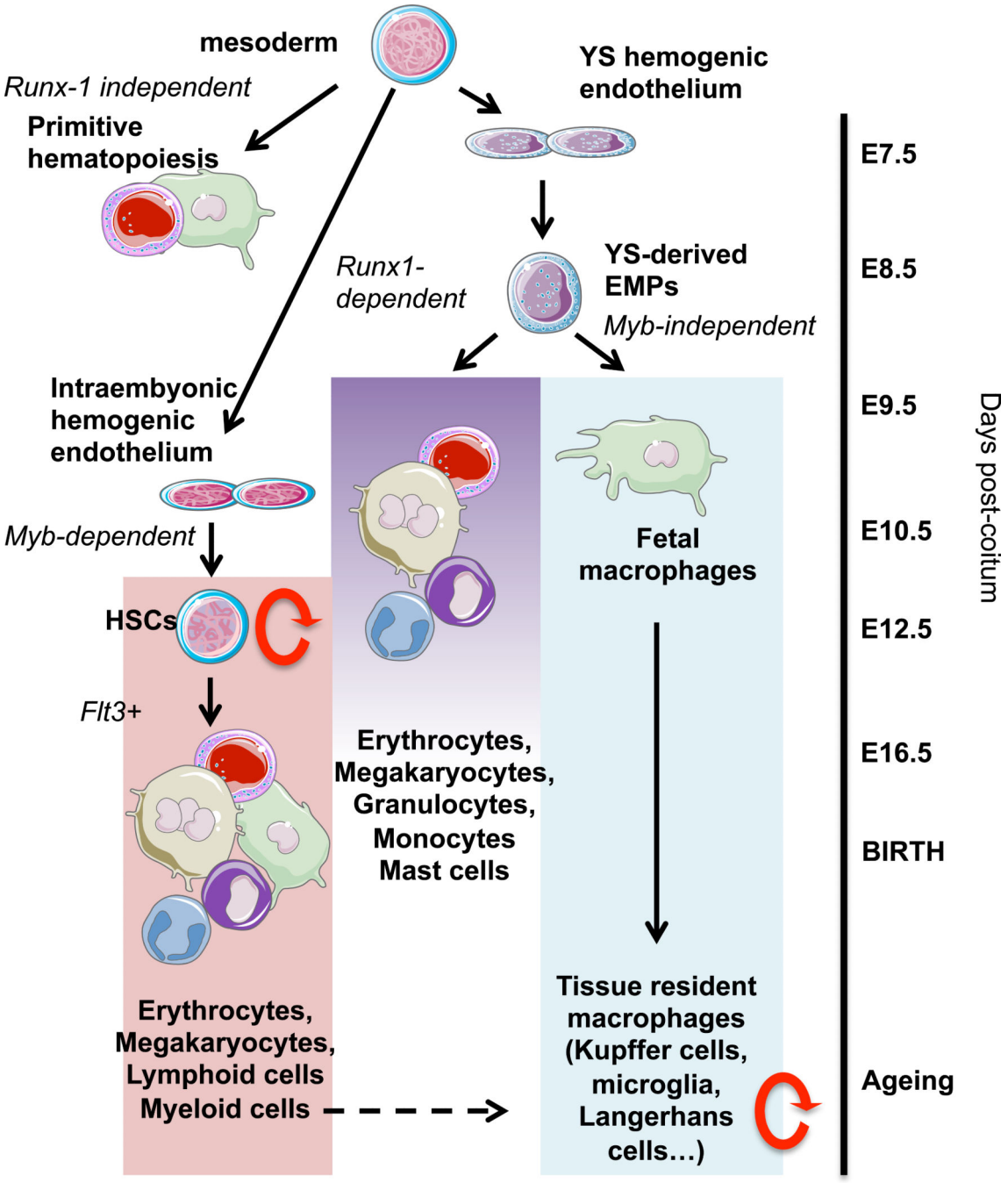

Figure 2.

Schematic of myeloid development and maintenance in the mouse embryo and adult (see text). Circular red arrows indicate self renewal potential. Primitive progenitors do not share a common origin with endothelial cells lining the blood islands in the yolk sac (YS) and they emerge the absence of *Runx1* and *Myb*. Within definitive hematopoiesis, HSC and EMP arise from distinct hemogenic endothelial cells through a *Runx1*-dependent endothelial to hematopoetic transition. Both EMP and HSC express the transcription factor *Myb,* and while no fetal or adult HSC-derived hematopoiesis can occur in the absence of *Myb*, EMPs are

Myb-independent for their myeloid differentiation. EMP-derived hematopoeisis gives rises to erythrocytes and short-lived myeloid cells (monocytes, granulocytes, mast cells) that are replaced by HSC-derived cells late during gestation. EMP-derived macrophages colonise all tissues during fetal development where they specialize to their tissue of residency after birth and can persist throughout adult life by local proliferation (red arrow). Depending on the age and environmental challenges, HSC-derived cells can contribute to adult tissue resident populations.

Table 1

phenotype, differentiation potential, and molecular features of hematopoietic progenitors of

		Primitive	Definitive	
	Progenitor name	**Mp**	**EMp**	**HSC**
	Origin	Posterior plate mesoderm	hemogenic endothelium	Arterial hemogenic endothelium
	Progenitor surface phenotype	Kit^{lo} $CD41^{lo}$	Kit^{+} $CD41^{+}$ VE-Cad^{+} $CD16/32^{+}$ $AA4.1^{+}$ $Tie2^{+}$ $CD45^{lo}$ $Sca1^{-}$	Lin^{-} Kit^{+} $Sca1^{+}$ $CD41^{+}$ VE−Cad^{+} $Tie2^{+}$ $CD45^{+}$
	time window of generation	E7.0-?	E8.25-E10.5	E9.5-E12.5
Anatomical location of	Generation	YS (blood islands)	YS	AGM (and large arteries)
	Expansion	YS	FL	FL
	Differentiation/committement	YS	YS and FL	FL, bone marrow
	Differentiation potential	Myeloid	Erythroid Myeloid	Erythroid Myeloid Lymphoid
	Macrophage lineage	Pu.1-dependent Runx1-independent Myb-independent	Pu.1-dependent Runx1 -dependent Myb-independent Notch1 independent	Pu.1-dependent Runx1 -dependent Myb-dependent Notch1-dependent
	Macrophage self-renewal	?	yes	
	progenitor long-term self-renewal	no (transient)	no (transient)	yes
TF expression	Pu.1 (spi1)	n/a	+	+
	Myb	n/a	+	+
	Gata2	n/a	+	+
	Gata3	n/a	-	+
	Lmo2	n/a	-	+
TF requirement	Pu.1 (spi1)	yes	yes	no *(**)*
	Myb	no	no *(*)*	yes
	Runx1	no	yes	yes
	CBFb	no	yes	yes
	Scl/tal-1	yes	yes	yes
	Notch1	no	no	yes

(*) red blood cell differentiation is compromised

(**) macrophage differentiation is compromised