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Early hematopoiesis and macrophage development

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ABSTRACT

The paradigm that all blood cells are derived from hematopoietic stem cells (HSCs) has been challenged by two findings. First, there are tissue-resident hematopoietic cells, including subsets of macrophages that are not replenished by adult HSCs, but instead are maintained by self-renewal of fetal-derived cells. Second, during embryogenesis, there is a conserved program of HSC-independent hematopoiesis that precedes HSC function and is required for embryonic survival. The presence of waves of HSC-independent hematopoiesis as well as fetal HSCs raises questions about the origin of fetal-derived adult tissue-resident macrophages. In the murine embryo, historical examination of embryonic macrophage and monocyte populations combined with recent reports utilizing genetic lineage-tracing approaches has led to a model of macrophage ontogeny that can be integrated with existing models of hematopoietic ontogeny. The first wave of hematopoiesis contains primitive erythroid, megakaryocyte and macrophage progenitors that arise in the yolk sac, and these macrophage progenitors are the source of early macrophages throughout the embryo, including the liver. A second wave of multipotential erythro-myeloid progenitors (EMPs) also arises in the yolk sac. EMPs colonize the fetal liver, initiating myelopoiesis and forming macrophages. Lineage tracing indicates that this second wave of macrophages are distributed in most fetal tissues, although not appreciably in the brain. Thus, fetal-derived adult tissue-resident macrophages, other than microglia, appear to predominately derive from EMPs. While HSCs emerge at midgestation and colonize the fetal liver, the relative contribution of fetal HSCs to tissue macrophages at later stages of development is unclear. The inclusion of macrophage potential in multiple waves of hematopoiesis is consistent with reports of their functional roles throughout development in innate immunity, phagocytosis, and tissue morphogenesis and remodeling. Understanding the influences of developmental origin, as well as local tissue-specific signals, will be necessary to fully decode the diverse functions and responses of tissue-resident macrophages.

1. Introduction

In the adult, all of the red blood cells, platelets, granulocytes, monocytes, and lymphoid cells that circulate in the bloodstream are continuously replenished from hematopoietic stem cells (HSCs). HSCs are functionally defined by their capacity to regenerate all of these circulating blood cells upon transplantation into a myeloablated recipient. Differentiating HSCs undergo restrictions in lineage fate to generate multipotential, and ultimately, unipotential progenitor cells that, in turn, generate mature blood cells. The capacity of hematopoietic progenitors to give rise to colonies of blood cells in semisolid media has revealed hierarchical lineage relationships within the hematopoietic system. Macrophage formation in the adult is characterized by progressive differentiation of bipotential granulocyte and macrophage (GM-CFC) progenitors to monocyte progenitors, and mature monocytes that differentiate into macrophages upon stimulation [1].

Studies of hematopoiesis in the adult organism led to the concept that all blood cells, including macrophages, are ultimately derived from HSCs. This widely held concept was also applied to the embryo, leading to the concept that HSCs arise in the yolk sac, the place where blood cells first emerge during development. However, functional transplantation studies in the mouse embryo two decades ago revealed the emergence of HSC beginning at embryonic day 10.5 (E10.5) in the AGM region of the embryo proper [2,3]. In contrast, the onset of hematopoiesis is characterized by the development of maturing primitive erythroid cells in the yolk sac beginning at E7.5, as well as macrophage cells and megakary-

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ocytes by E9.0. Analysis of functional hematopoietic progenitors, capable of colony-forming activity, has revealed a complex pattern of emergence in embryonic time and space, which is characterized by two distinct, but partially overlapping waves of erythroid progenitors initiating in the yolk sac prior to the onset of a functional vasculature and adult-repopulating HSC [4,5]. Macrophage colony-forming potential is associated temporally and spatially with each of these waves of primitive and definitive erythroid progenitors [4]. Recent cell marking studies have revealed that subsets of tissue-resident blood cells, including some tissue-resident macrophages, do not appear to be replenished by HSCs postnatally, and are instead regenerated by self-renewal of fetal-derived cells [6–11]. In this review, we consider the developmental origin of fetal-derived macrophages in the context of the various waves of hematopoietic potential that emerge during early embryogenesis.

2. Emergence of hematopoiesis in the fetus

2.1. Emergence of hematopoietic stem cells

The first adult-repopulating HSCs in mouse embryos are found associated with the appearance of cell clusters in the aorta, umbilical artery, and vitelline artery between E10.5 and E11.5 [2,12]. In both mouse and zebrafish, rounded hematopoietic cells form directly from the endothelium through an endothelial-to-hematopoietic transition [13–16]. Murine HSCs then colonize the liver, the site of fetal hematopoiesis, and begin to expand in numbers from E12.5 to E16.5 [3,17]. Similarly, at 5 weeks post-conception in the human embryo, aortic cell clusters arise along with HSC-like cells that can repopulate adult immune-deficient mice [18,19]. Studies in the mouse indicate a large discrepancy between the number of cell clusters and the number of functional HSC [2,3,20,21]. While the reasons for this discrepancy remain unclear, it is evident that bona fide adult-repopulating HSC activity is extremely rare in the embryo before E12.5 [3,17] and likely does not significantly contribute to fetal hematopoiesis until well after this time.

2.2. HSC-independent hematopoiesis

The first cells to circulate in the embryonic bloodstream consist of primitive erythroid cells that emerge in blood islands in the yolk sac of mammalian and avian embryos soon after the onset of gastrulation, well before HSC formation [22–24]. The several million primitive erythroid cells that mature in the murine embryo are subsequently replaced by hundreds of millions of smaller definitive erythrocytes produced in the fetal liver [25]. Primitive erythroid cells are distinguished from their later definitive counterparts not only by their large size, but also their expression of embryonic globin genes, for review see Ref. [26]. Foundational insights into the complexity of HSC-independent hematopoiesis have been chiefly derived from the analysis of erythropoiesis [4,27]. The first few primitive erythroid progenitors (EryP-CFCs) emerge in the nascent yolk sac of the late streak mouse embryo at E7.25, peak in number by E8.5, and subsequently differentiate into a wave of maturing erythroblasts in the bloodstream. At E8.25, just before the first cardiac contractions and the onset of circulation, the first few definitive erythroid progenitors (BFU-Es) begin to emerge, also in the yolk sac [4,27] and then increase in numbers by E9.5. Unlike the transient wave of EryP-CFCs, BFU-Es are also detected in the fetal bloodstream and in the early fetal liver by E10.5, which is prior to the liver colonization by adult-repopulating HSCs [4,17]. These studies of erythroid colony-forming progenitors initially raised the hypothesis that two distinct (primitive and definitive) waves of hematopoietic progenitors emerge in the yolk sac prior to HSC emergence.

2.3. The first wave of HSC-independent hematopoiesis: primitive

Coincident with primitive EryP-CFC, Meg-CFC and Mac-CFC also first emerge at E7.25 in the yolk sac and increase in numbers by E8.5 [4,28,29]. Subsequently, significant numbers of maturing megakaryocyte and macrophage precursors are found in the yolk sac contemporary with primitive erythroblasts, at a time when only the first few BFU-E have begun to emerge [4,5,30,31]. These include small acetylcholinesterase-positive and GP1bβ-positive megakaryocytes identified in the yolk sac of E8.5 and E9.5 mouse embryos, respectively [28,29] that have formed platelets by E10.5 [29,32]. As will be discussed in more detail below, the first immature embryonic macrophages have also been identified in blood islands of the early murine yolk sac by E9.0 [33–36].

In the adult bone marrow, the definitive erythroid and megakaryocyte lineages share a common bipotential progenitor (MEP). Interestingly, MEP that give rise to primitive erythroid cells and megakaryocytes are also present in the early murine yolk sac [29], and in human iPS cell cultures [37]. Unlike the bipotential primitive MEP, early macrophage potential is associated with monopotent progenitors [4,30], suggesting that if there is a common primitive hematopoietic trilineage progenitor, it must be rare and/or very transient in the embryo. Macrophage potential in the murine embryo is not a component of bi- and multilineage progenitors until E8.25 and these progenitors also contain definitive erythroid and/or granulocyte potential and can be immunophenotypically distinguished from the more abundant unipotential macrophage progenitors and primitive erythroid progenitors at this time [4,5,30]. Interestingly, in the zebrafish embryo, primitive macrophages first arise in the head, spatially distinct both from primitive erythroid potential and from the subsequent wave of definitive erythro-myeloid progenitors (see below) [38]. While it is not clear if these rapidly produced primitive hematopoietic progenitors possess the same hierarchal relationship as later waves of hematopoiesis, they are distinct from later definitive hematopoietic progenitors, and their timing of emergence and their rapid maturation link the primitive erythroid and coincident megakaryocyte and macrophage lineages as a “primitive” wave of hematopoiesis (Fig. 1A).

2.4. Definitive HSC-independent hematopoiesis: erythro-myeloid progenitors (EMP)

Analysis of hematopoietic progenitors in E8.25-E8.5 mouse embryos revealed not only the onset of definitive erythroid (BFU-E), but also the first occurrence of bipotential GM-CFC, mast cell progenitors (Mast-CFC), and multilineage high proliferative potential colony-forming cells (HPP-CFC), which give rise in vitro to macrophages, granulocytes, and mast cells [4,39]. This diverse myeloid potential appears to arise from a cohort of “erythro-myeloid progenitors” (EMPs) that express significant Kit and CD41 on their cell surface [40,41], distinguishing them from the $Kit^{l\circ}CD41^{lo}$ primitive erythroid and unipotential macrophage colony-forming activity at E8.5 [40]. By E9.5, EMPs can be prospectively identified as a $Kit^{+}CD41^{+}CD16/32^{+}$ cell population that can be distinguished from maturing populations of Kit^{neg} $CD16/32^{+}CD45^{hi}$ primitive macrophages and Kit^{neg} $CD41^{hi}$ $Gp1b\beta^{+}$ primitive megakaryocytes [5,31]. Consist with the emergence of HPP-CFC, clonal analyses have confirmed that single E9.5 EMPs have the capacity to generate multiple myeloid lineages in vitro, as well as definitive erythroid potential [5]. Unlike HSCs, EMP do not contain B-lymphoid potential [5]. Like HSCs, EMPs arise via an endothelial-to-hematopoietic transition, and do so in