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Review

The biochemistry of hematopoietic stem cell development☆

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ABSTRACT

Background: The cornerstone of the adult hematopoietic system and clinical treatments for blood-related disease is the cohort of hematopoietic stem cells (HSC) that is harbored in the adult bone marrow microenvironment. Interestingly, this cohort of HSCs is generated only during a short window of developmental time. In mammalian embryos, hematopoietic progenitor and HSC generation occurs within several extra- and intraembryonic microenvironments, most notably from 'hemogenic' endothelial cells lining the major vasculature. HSCs are made through a remarkable transdifferentiation of endothelial cells to a hematopoietic fate that is long-lived and self-renewable. Recent studies are beginning to provide an understanding of the biochemical signaling pathways and transcription factors/complexes that promote their generation.

Scope of review: The focus of this review is on the biochemistry behind the generation of these potent long-lived self-renewing stem cells of the blood system. Both the intrinsic (master transcription factors) and extrinsic regulators (morphogens and growth factors) that affect the generation, maintenance and expansion of HSCs in the embryo will be discussed.

Major conclusions: The generation of HSCs is a stepwise process involving many developmental signaling pathways, morphogens and cytokines. Pivotal hematopoietic transcription factors are required for their generation. Interestingly, whereas these factors are necessary for HSC generation, their expression in adult bone marrow HSCs is oftentimes not required. Thus, the biochemistry and molecular regulation of HSC development in the embryo are overlapping, but differ significantly from the regulation of HSCs in the adult.

General significance: HSC numbers for clinical use are limiting, and despite much research into the molecular basis of HSC regulation in the adult bone marrow, no panel of growth factors, interleukins and/or morphogens has been found to sufficiently increase the number of these important stem cells. An understanding of the biochemistry of HSC generation in the developing embryo provides important new knowledge on how these complex stem cells are made, sustained and expanded in the embryo to give rise to the complete adult hematopoietic system, thus stimulating novel strategies for producing increased numbers of clinically useful HSCs. This article is part of a Special Issue entitled Biochemistry of Stem Cells.

1. Ontogeny of hematopoietic stem cells

1.1. Multiple waves of de novo hematopoietic generation in the embryo

To understand the biochemistry behind HSC development, the cells that make up the vertebrate embryo blood system need some introduction. Blood cell specification occurs at least three separate times in the mammalian embryo — resulting in three *de novo* waves of hematopoietic cell production (reviewed in [1]). While it seems strange for embryos to establish the hematopoietic system multiple times, this in fact is a recurrent theme during ontogeny. For example, the mouse excretory system is generated first as the transient pronephric kidney, a secondary transient mesonephric kidney and finally as a third long-lived metanephric kidney that functions throughout adult life. The three distinct wave-like generations of the hematopoietic system provide a means by which the embryo can be temporarily supplied with rapidly produced hematopoietic cells, while generating a highly complex adult hematopoietic system with long-lived self-renewing hematopoietic stem cells (HSC) at its foundation. Hematopoiesis in the embryo occurs in several tissues that include the yolk sac, aorta–gonad–mesonephros (AGM) region, placenta and liver (Fig. 1A).

The first wave of blood generation produces short-lived primitive erythrocytes that are necessary to carry oxygen through the rapidly growing conceptus and also primitive macrophages and megakaryocytes. Primitive erythrocytes are generated from aggregates of mesodermal precursors or 'hemangioblasts', in the yolk sac blood islands. Described over 100 years ago, the overlapping ontogenic appearance of both erythroid and endothelial cells indicates a common mesodermal precursor with at least bi-lineage potential [2,3]. This is further supported by the overlap in genetic programs for the two lineages

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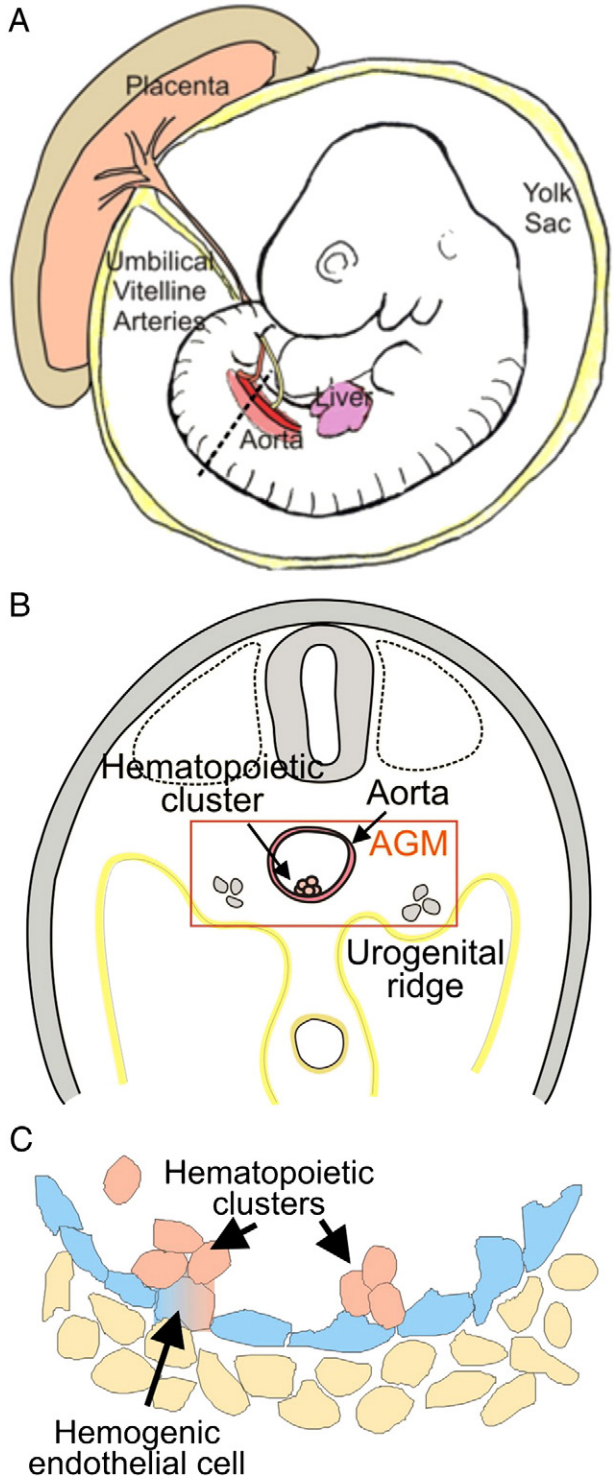

Fig. 1. Hematopoietic stem cell development in the mouse embryo. A) Depiction of a mouse embryo at day 10.5 at the time when the first hematopoietic stem cells are generated in the aorta. Sites harboring (and/or generating) hematopoietic cells are shown: the extraembryonic yolk sac and placenta, the intraembryonic aorta and liver, and the umbilical and vitelline vessels that respectively connect the placenta and yolk sac to the aorta. The dotted line through the trunk of the embryo indicates the transverse section shown in panel B. B) Depiction of a transverse section through an E10.5 mouse embryo with the AGM (aorta–gonad–mesonephros/aorta and urogenital ridges) region in the red rectangle. The AGM is flanked on the dorsal side by the neural tube and the somites, and on the ventral side by the gut and peritoneum. A hematopoietic cluster is indicated on the ventral wall of the dorsal aorta. Hematopoietic stem cells are localized in the clusters. C) A close up of the ventral wall of the aorta showing cluster formation. A hemogenic endothelial cell is undergoing the transition from endothelial cell to a hematopoietic cell.

(*i.e.* expression of Flk-1 (KDR), Scl (Tal1) and CD34) and the lack of both lineages in embryos deficient for some of these genes [4–6]. Surprisingly, hemangioblasts *in vivo* are localized not in the yolk sac but in the posterior primitive streak [7]. As they migrate to the yolk sac they begin their commitment to endothelial and hematopoietic progenitors, with several of these cells contributing to the formation of each blood island [8]. The first wave of primitive hematopoietic cell generation begins at embryonic day (E)7.5 in the mouse conceptus and is highly conserved across vertebrate species, including man (at 16–20 days of gestation [9].

In the mouse embryo the second wave of hematopoietic cell generation begins at E8/8.5, and overlaps with the first wave [10]. Definitive hematopoietic progenitors are *de novo* generated and some clusters of hematopoietic cells begin to appear in the major vasculature at E9.5. These hematopoietic progenitors are functionally more complex than primitive progenitors — they have multilineage potential (producing erythroid, myeloid and/or lymphoid cells), but they are not long-lived or self-renewing HSCs. *De novo* definitive progenitor generation occurs in the yolk sac, chorio-allantoic/placenta and the intraembryonic region around the aorta, as revealed by mouse embryo explant cultures and the *Ncx1*$^{-/-}$ mouse model (embryos lack circulation due to no heartbeat [11,12] (reviewed in [13]). Thus, 'definitive hematopoietic progenitors' constitute the second wave of hematopoietic specification.

The third wave of hematopoietic cell specification provides for the generation of adult type HSCs. Grafting studies in avian embryos provided unequivocal proof that the adult blood system is not derived from the yolk sac, but instead from an intraembryonic source of cells localizing to the dorsal aorta (reviewed in [1,13]). Clusters of hematopoietic cells consistently found on the ventral wall of the dorsal aorta and major arteries of the chick embryo, led to the proposition that HSCs for the adult hematopoietic system arise from vascular endothelial cells (Fig. 1B). Work in the mouse embryo showed that the first adult-type HSCs are generated in the intrabody AGM region (Fig. 1A and B). These transplantable HSCs (as potent as adult bone marrow HSCs) are generated beginning at E10.5 and are thought to be contained within the vascular clusters within the aorta and vitelline and umbilical arteries [14–16]. The real-time generation of hematopoietic cells from 'hemogenic endothelial cells' lining the aorta (Fig. 1C) has been demonstrated by vital confocal imaging in the mouse and zebrafish embryo [17–19]. The third wave of hematopoietic cell (HSC) generation is what generates the long-lived self-renewing HSCs that migrate, colonize and reside in the bone marrow throughout adult life.

1.2. Hemogenic endothelium as a source of definitive hematopoietic progenitors and HSCs

The generation of definitive hematopoietic progenitors (wave 2) and HSCs (wave 3) parallels the appearance of vascular hematopoietic clusters in the aorta, vitelline and umbilical arteries (Fig. 1C). Histologic/immunostained sections through the midgestation embryo AGM region show that 'hemogenic' endothelial cells express some hematopoietic markers and some hematopoietic cluster cells express endothelial markers [20]. Cluster numbers peak at E10.5, when HSCs first appear. However, not all hematopoietic cells in the clusters are HSCs and not all clusters contain HSCs. There are many more cluster cells in the aorta than there are HSCs at this time point. Genetic studies using Cre–Lox recombination methods for deletion of pivotal intrinsic regulatory molecules show that HSC generation occurs only during a short window of developmental time [21,22]. It is unclear as yet whether all HSCs/cluster cells emerge from hemogenic endothelium, whether larger clusters form by proliferation of the emerging cell or through the recruitment of circulating cells. Recently, it was suggested that the already hematopoietic committed cells (perhaps coming from the

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