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Hematopoietic regulation in the embryo

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A B S T R A C T

The aim of this report is to review briefly the ontogeny of hematopoiesis in mice and humans and to discuss accumulating evidence that hematopoietic stem cell activity arises from endothelial cells during embryogenesis. This overview summarizes information present in the PubMed online database and from experiments conducted in our laboratory. The major sites of hematopoiesis change throughout development in mice and humans. Hematopoietic cells are derived from mesoderm precursors within the embryo, as well as in the yolk sac, and recent evidence for direct development of blood cells from endothelium is compelling. The ontogeny of hematopoiesis is similar in mice and humans. Understanding the role of endothelium in producing blood cells may provide new strategies for augmenting hematopoiesis in patients undergoing stem cell transplantation.

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The primary sites of blood cell production change throughout embryonic and fetal development of most vertebrates.[1] In mice and humans, blood cells first emerge in the extraembryonic yolk sac.[2-4] Recent evidence indicates that hematopoietic cells are also found in clusters along the walls of certain arterial vessels in the early developing embryo. With the onset of systemic blood flow, hematopoietic progenitor cells seed the rudimentary liver and, soon afterwards, the liver becomes the predominant site of blood cell production for a major portion of in utero development. The spleen and marrow are subsequently seeded with liver-derived cells. In both mice and humans, bone marrow hematopoiesis sustains blood cell production for the life of the individual.

This review will discuss evidence for the site of emergence of hematopoietic cells in human and murine embryos and will highlight recent studies demonstrating a direct role of endothelial cells in the formation of hematopoietic stem and progenitor cells. In addition, evidence will be presented that vascular endothelial cells appear to play a major role in supporting stem and progenitor cell expansion, as the first embryonic sites of hematopoiesis appear to produce cells intravascularly. This is a unique feature since all other hematopoietic sites (fetal liver and bone marrow) produce the stem and progenitor cells in extravascular niches.

Yolk sac hematopoiesis

The murine yolk sac is a simple tissue composed of mesoderm and endoderm cells. Mesoderm cells migrate from the posterior portion of the primitive streak and move into the proximal yolk sac on embryonic day (E) 7–7.5.[4] In this site, mesoderm cells proliferate into clumps of cells. A long held theory states that mesoderm cells of the outer area of the clumps differentiate into endothelial cells, while the inner cells differentiate into primitive erythroblasts and these structures are called blood islands. More recent evidence indicates that the primitive erythroid progenitor cells and endothelial cells differentiate from mesoderm and that only near the time of the onset of circulation, do endothelial cells enclose the red blood cells to form an intact blood filled vascular network.[5] The close temporal and spatial development of endothelial cells and hematopoietic cells has led to the concept of a common *hemangioblast* precursor for the blood and endothelial lineages[6] and a colony forming cell with these properties has been identified in the murine yolk sac.[7]

On E8.25, progenitors for adult-type erythroid, myeloid and megakaryocytes can be isolated from the murine yolk sac.[8] These progenitors do not appear to mature in the yolk sac since no adult-type erythroid, myeloid or platelets are found in the yolk sac circulation until after the time that the liver becomes a primary site of hemato-

poiesis. High proliferative potential-colony forming cell (HPP-CFC) activity also emerges from the yolk sac on E8.25.[8,9] The concentration of HPP-CFCs increases significantly in the yolk sac over the next few days of development and these cells are soon detectable in the bloodstream. Few HPP-CFCs are present within the embryo proper.

Are the adult-type progenitors in the yolk sac derived from a hematopoietic stem cell? This is a controversial topic. Yolk sac cells do not repopulate lethally irradiated adult mice before the time that the liver also possesses stem cell repopulating activity. However, yolk sac cells isolated before the establishment of the circulation have been demonstrated to reconstitute lethally irradiated adult mice if injected into myeloablated newborn mice[10] or after co-culture with an endothelial-like cell line *in vitro*.[11] Thus, the murine yolk sac appears to be the first primary site of stem, progenitor and mature blood cell production, although it also appears that stem and progenitor cell maturation is not completed in the yolk sac.

In the human system, yolk sac development occurs in several phases.[12] A primary yolk sac is formed after implantation at 7–8 days postconception (DPC) but blood cells do not emerge until the secondary yolk sac is formed on DPC 12–15. Primitive erythroblasts completely surrounded by endothelial cells can be identified as early as DPC 16–19.[13] Hematopoietic progenitor cells expressing the cell surface marker CD34 are present in the day 18.5 yolk sac.[14] When placed in culture with an adult bone marrow stromal cell line, yolk sac cells are capable of generating erythroid, myeloid and natural killer cells for up to 6 wk *in vitro*.[14] However, under these culture conditions, yolk sac cells do not contribute to the formation of B or T lymphocytes. Thus, evidence to suggest that stem cell activity emerges in the human yolk sac is lacking.

Intraembryonic hematopoiesis

Identification of hematopoietic stem cells in murine embryos, before colonization of the fetal liver with stem cells, has been localized to the para-aortic splanchnopleure (P-Sp) tissue in the ventral aspect of the embryo which forms the region of aorta, gonad and mesonephros (AGM) development.[15-18] Injection of E10 AGM tissue into lethally irradiated mice gave evidence of some long-term hematopoietic repopulating activity.[15] One day later, such activity is present in yolk sac, liver and the AGM region, suggesting that stem cells are now circulating. Surprisingly, the AGM region does not support significant maturation of the stem cells into progenitor cells or mature blood cells *in vivo*.[19] However, when P-Sp or AGM tissue is explanted and cultured *in vitro* on an adult bone marrow stromal cell line with added hematopoietic growth factors, significant differentiation into a variety of hematopoietic progenitor cells, mature granulocytes, and B and T lymphocytes occurs. In fact, if the P-Sp tissue is removed from the embryo before initiation of systemic blood flow, hematopoietic stem and progenitor cells emerge autonomously *in vitro* under specific conditions.[20] This is not the case for explanted yolk sac tissue under the same culture conditions. These results have led some investigators to conclude that the P-Sp/AGM region is the sole site of hematopoietic stem cell emergence in the murine embryo.

In the human system, colonization of the fetal liver rudiment with hematopoietic elements occurs very soon after the first appearance of primitive erythroblasts in the yolk sac on days 16–19.[21] Hematopoietic precursor cells are first detectable in the aorta of the embryo on day 19. Since cardiac contractions do not begin until day 22–23, it appeared unlikely to the investigators that these cells had moved into the aorta from the yolk sac. If the aortic tissue was explanted and cultured over an adult bone marrow stromal cell line with added growth factors, hematopoietic cells including CD34-expressing cells were produced in the coculture for up to 4 wk in culture.[14] Unlike the cocultured yolk sac cells, the aortic tissue gave rise to B and T lymphocytes as well as natural killer and myeloid cells. These data are consistent with the presence of stem cell activity emerging from an intraembryonic vascular site.

Endothelial cells with blood-forming potential

In the murine system, multiple lines of evidence implicate vascular endothelial cells as immediate precursors for hematopoietic stem/progenitor cells. We have demonstrated that hematopoietic cells expressing distinct cell surface proteins emerge from endothelial cells comprising capillaries of the yolk sac vascular plexus in the proximal yolk sac.[22] Nishikawa et al.[23] isolated endothelial cells from the embryonic P-Sp region and generated lymphohematopoietic cells in vitro. Using transgenic mice in which one allele of the Runx1 gene was targeted with the lacZ gene permitted North *et al.*[24] to visualize Runx1 in the developing murine embryo. They reported that lacZ was expressed in primitive erythrocytes and endothelial cells in the E8.0 yolk sac. At E8.5, expression remains high in some yolk sac endothelial cells but now initiates in mesoderm and endothelial cells in the P-Sp region of the embryo. Between E9.5 and 11.5, Runx1 is expressed in endothelial cells of the vitelline, aortic (AGM region), and umbilical arteries in addition to yolk sac vessels. Almost simultaneously, clusters of hematopoietic cells with stem cell potential appear to be budding from the vitelline, aortic, and umbilical endothelium. Transplantation of endothelial cells from these sites, recon-

stitutes both lymphoid and myeloid lineages in lethally irradiated recipient mice, proving that certain endothelial cells possess the potential or give rise to hematopoietic stem cells *in vivo*.[24] A further elegant proof that endothelial cells expressing Runx1 are required for the production of hematopoietic stem cells in the murine embryo was recently published.[25]

The endothelial cells present in the capillaries of the yolk sac and P-Sp region appear to play additional roles in hematopoietic stem cell support. Recently, we demonstrated that certain cell surface integrin molecules are uniquely expressed in hematopoietic progenitor cells and not endothelium of the early yolk sac and P-Sp.[22,26] This feature permitted a detailed examination of the role of yolk sac and P-Sp endothelial cell (separated from endothelial cells with hemogenic potential) support of bone marrow hematopoietic stem cells *in vitro*. Surprisingly, co-culture of adult marrow stem cells with yolk sac and P-Sp endothelial cells resulted in a 2-9 fold increase in hematopoietic stem cells with competitive repopulating ability.[27] This kind of endothelial support is not displayed by various vascular endothelial cells isolated from adult mice.[28] Thus, early yolk sac and P-Sp endothelial cells possess both a direct capacity to form hematopoietic stem and progenitor cells (hemogenic endothelium) and the ability to expand stem cells in co-culture *in vitro*. These capacities do not appear to be properties of the same population of cells, but only further marking studies will permit clarification of this issue.

In the human system, Oberlin *et al.*[29] isolated endothelial cells (cells expressing CD31 and CD34 but not the common leukocyte antigen CD45) from the yolk sac, dorsal aorta, embryonic liver, and fetal bone marrow. The endothelial cells were co-cultured with a murine bone marrow stromal cell line (known to support multilineage hematopoietic progenitor cell expansion) and each site examined was reported to give rise to blood cells and establish long-term hematopoietic cultures. These data suggested that multiple sites of hematopoietic development in the embryo and fetus contain endothelial cells with hemogenic potential. Future studies will examine the precursor cells of the hemogenic endothelium and determine the molecules that are critical for endothelial-hematopoietic development.

Endothelium in the bone marrow stem cell niche

Exciting advances in the molecular dissection of the hematopoietic stem cell niche in the bone marrow have been accomplished.[30,31] The primary site for hematopoietic stem cell homing, self-renewal, and survival appears to reside in the endosteal region of bone with osteoblast cells and local calcium concentrations playing important regulatory roles. However, recent data also suggests that perivascular niches for stem cell homing and expansion may also be crucial to stem cell homeostasis.

Conclusions

Murine and human hematopoietic development share many similar features during embryonic and fetal development. Recent studies indicate that vascular endothelial cells within certain developmental sites, possess hemogenic capacity directly while other endothelium displays a unique supportive role for hematopoietic stem/progenitor cell expansion. Future studies aimed at isolating the molecules secreted by the endothelial cells that permit stem cell expansion may permit novel approaches to augmenting therapeutic human stem cell transplantation.

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