Developmental and Comparative Immunology 58 (2016) 18–29

Contents lists available at ScienceDirect

Developmental and Comparative Immunology

journal homepage: www.elsevier.com/locate/dci

Review

Development and differentiation of the erythroid lineage in mammals

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ARTICLE INFO

Article history:
Received 6 November 2015
Received in revised form
15 December 2015
Accepted 15 December 2015
Available online 19 December 2015

Keywords:
Erythropoiesis
Mammal
Hematopoiesis
Embryo
Progenitor

ABSTRACT

The red blood cell (RBC) is responsible for performing the highly specialized function of oxygen transport, making it essential for survival during gestation and postnatal life. Establishment of sufficient RBC numbers, therefore, has evolved to be a major priority of the postimplantation embryo. The "primitive" erythroid lineage is the first to be specified in the developing embryo proper. Significant resources are dedicated to producing RBCs throughout gestation. Two transient and morphologically distinct waves of hematopoietic progenitor-derived erythropoiesis are observed in development before hematopoietic stem cells (HSCs) take over to produce "definitive" RBCs in the fetal liver. Toward the end of gestation, HSCs migrate to the bone marrow, which becomes the primary site of RBC production in the adult. Erythropoiesis is regulated at various stages of erythroid cell maturation to ensure sufficient production of RBCs in response to physiological demands. Here, we highlight key aspects of mammalian erythroid development and maturation as well as differences among the primitive and definitive erythroid cell lineages.

Contents

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http://dx.doi.org/10.1016/j.dci.2015.12.012
0145-305X/

1. Introduction

Mammalian hematopoiesis produces approximately 10 distinct cell types, the most abundant of which belongs to the erythroid lineage (Seita and Weissman, 2010). Erythropoiesis results in the production of large numbers of RBCs that are responsible for supplying oxygen to the developing embryonic, fetal, and adult tissues. They also help maintain blood viscosity and provide the shear stress required for vascular development and remodeling (Baron, 2013; Lucitti et al., 2007).

In the developing mammalian embryo, hematopoiesis occurs in three sequential waves. The first wave emerges in the yolk sac (YS), with the development of progenitors committed primarily to the primitive erythroid lineage (EryP), as well as to the macrophage and megakaryocyte lineages (Baron et al., 2012). The second wave of hematopoiesis also arises in the YS, producing definitive erythroid, megakaryocyte, and myeloid lineages (Lux et al., 2008). These first two waves are transient and are eventually replaced by RBCs that are derived from a third wave of hematopoiesis, generated from HSCs that arise in the major arteries of the developing embryo, placenta, and YS (Dzierzak and Philipsen, 2013; Speck et al., 2002) and subsequently colonize the fetal liver, where they differentiate to the various hematopoietic cell lineages (Baron et al., 2012). Toward the end of gestation, hematopoiesis transitions to the bone marrow, which becomes the primary site of postnatal blood production in the adult.

The earliest erythroid progenitors, identified in clonogenic colony assays as burst-forming units (BFU-E), give rise to later progenitors known as colony-forming units (CFU-E) that undergo terminal differentiation to enucleated RBCs (reviewed by Hattangadi et al., 2011). In humans, the life span of an RBC averages approximately 120 days (Hattangadi et al., 2011). To maintain circulating RBCs at numbers necessary for sufficient oxygen distribution, approximately 2×10^6 RBCs must be generated every second (Palis, 2014). RBC production is regulated primarily by the peptide hormone erythropoietin (EPO) (reviewed by Fried, 2009). Dramatic reductions in RBC numbers lead to compensatory "stress" erythropoiesis through the expansion of BFU-Es (Paulson et al., 2011). This review describes the development of the RBC lineage and how RBC production is regulated in the adult. We highlight some of the key growth factors and genes that regulate mammalian RBC production, as well as differences between erythroid cells at different stages of their development.

2. Emergence of primitive erythroid progenitors in the yolk sac

In the mouse, EryP are first detected around embryonic day (E) 7.5 within the "blood islands" of the YS (Ferkowicz and Yoder, 2005). EryP arise from mesodermal progenitors found in close proximity with the visceral endoderm (Baron, 2005). *Gata-4* deficient embryonic stem (ES)-derived embryoid bodies cannot form a visceral endoderm and show defects in primitive erythropoiesis (Bielinska et al., 1996). Explant culture studies using mouse embryos suggested that soluble signals from the visceral endoderm, one of which may be Indian hedgehog, activate primitive hematopoiesis (Belaoussoff et al., 1998; Dyer et al., 2001). Co-culture of Bone Morphogenetic Protein (BMP)-stimulated extraembryonic endoderm (XEN) cells with EryP progenitors isolated using flow cytometry resulted in progenitor expansion (Artus et al., 2012). Two candidates for the XEN cell factors are Indian hedgehog and Vascular Endothelial Growth Factor (Vegf) (Artus et al., 2012). Together, these studies indicate that secreted signals from the visceral endoderm regulate primitive erythropoiesis.

The close temporal and spatial association of EryP and endothelial cells within the "blood islands" of the YS led to the hypothesis that these two lineages arise from a common progenitor termed the hemangioblast (Baron et al., 2012; reviewed by Ferkowicz and Yoder, 2005; Murray, 1932; Sabin, 1920, 1917). Experimental support for the existence of a hemangioblast came from studies of differentiating human and mouse embryonic stem (ES) cells (Choi et al., 1998; Zambidis et al., 2005) and, later, from mouse embryos (Huber et al., 2004). "Blast colony-forming cells" (BL-CFC), derived from ES-cell derived embryoid bodies (EBs), display properties expected of the hemangioblast and are thought to be its *in vitro* equivalent (Choi et al., 1998). However, it is now evident that BL-CFCs are not bipotent but multipotent, giving rise to hematopoietic, endothelial, and mesenchymal cells, including smooth muscle (Ema et al., 2003). Analyses of chimeric mouse embryos expressing four different fluorescent proteins identified polyclonal (not monoclonal) blood islands (Ueno and Weissman, 2006), consistent with the observation that BL-CFC are found primarily in the posterior primitive streak, not in the YS, and that the commitment of these cells takes place as they migrate into the YS (Huber et al., 2004). An early lineage tracing study (Kinder et al., 2001) and a recent clonal analysis (Padron-Barthe et al., 2014) also lend support to the idea that the earliest hematopoietic and endothelial cell populations in the mouse embryo arise from different progenitors. An analysis of antibody-stained embryos using confocal microscopy suggested that "blood bands" rather the "blood islands" form in the YS (Ferkowicz and Yoder, 2005).

EryP progenitors were initially identified by their ability to form red-pigmented colonies in cultures with semisolid medium supplemented with EPO (Palis et al., 1999). These progenitors are found in the YS only from E7.25 to E9.0 (Isern et al., 2011; Palis et al., 1999). They are the first functionally differentiated mesodermal cell and, at E7.5 and E8.5, represent a considerable proportion (15–20% and 40–50%, respectively) of all cells in the embryo (Isern et al., 2011). The primitive erythroid progenitor is thought to be a bipotent cell that can give rise to unipotent megakaryocyte and EryP progenitors (Tober et al., 2007). Isolation of these progenitors has been challenging, as cell surface markers (e.g. CD31, Tie-2, VE-cadherin, and CD41) that have been used for their enrichment are also expressed on endothelial cells and definitive hematopoietic progenitors (Ema et al., 2006; Ferkowicz et al., 2003). A transgenic mouse model expressing a nuclear histone H2B-GFP fusion protein driven by a human embryonic *ε-globin* gene promoter and sequences from the *β-globin* locus control region (LCR) has provided a means to isolate homogenous populations of EryP (Isern et al., 2008, 2010, 2011). In embryos from these mice, green fluorescence was detected as early as E6.75, around the time when the first erythroid cells are specified from mesoderm (Isern et al., 2011). EryP progenitor activity was found exclusively in the GFP-positive population of cells sorted from E7.5 and E8.5 embryos (Isern et al., 2011). Progenitor activity is lost as the cells enter the circulation (Isern et al., 2011; McGrath et al., 2003). Microarray analysis of GFP-positive populations of EryP isolated at different days of development revealed that several Wnt/β-catenin pathway genes are expressed in EryP progenitors and are subsequently down-regulated (Isern et al., 2011). Wnt/β-catenin signaling has been shown to regulate specification of the EryP lineage from mesoderm in differentiating cultures of mouse and human ES cells (Nostro et al., 2008; Sturgeon et al., 2014). Study of human EryP progenitors has proven difficult, as the time blood islands emerge in human development (day 16 and 17) is too early to be accessible from elective termination pregnancies (Tavian and Peault, 2005).

3. Maturation of primitive erythroid progenitors

By mid-gestation, the circulatory systems of the mouse embryo, the YS, and the placenta have connected (McGrath et al., 2003). As