Placenta 47 (2016) 1–11

Contents lists available at ScienceDirect

Placenta

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

Localization of transient immature hematopoietic cells to two distinct, potential niches in the developing mouse placenta

Nathália Azevedo Portilho [a, c, *], Priscila Tavares Guedes [b], B. Anne Croy [c], Marcelo Pelajo-Machado [a]

[a] Laboratory of Pathology, Oswaldo Cruz Institute/Fiocruz, Rio de Janeiro, 21040-900, Brazil
[b] Department of Morphological Sciences, Federal University of the State of Rio de Janeiro/ UNIRIO, Rio de Janeiro, 20211-010, Brazil
[c] Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, K7L3N6, Canada

ARTICLE INFO

Article history:
Received 5 May 2016
Received in revised form
9 August 2016
Accepted 22 August 2016

Keywords:
Placenta
Mouse
Hematopoiesis
Erythropoiesis

ABSTRACT

Previous studies have shown that human and mouse placentas have hematopoietic potential during mid-gestation. In this investigation, we used histological and immunohistological approaches to visualize hematopoietic cells in mouse placenta between 9.5 and 12.5 days of gestation (gd), identifying their topography and niche. Putative hematopoietic foci were present on 10.5 and 11.5 gd but not 9.5 or 12.5 gd and was restricted to the placental labyrinth. Two major niches each with distinctive hematopoietic cell clusters were present. One type of hematopoietic cell cluster involved the chorioallantoic vasculature and fetal vessels near the chorionic plate. These clusters resembled the hematopoietic stem cells produced by large embryonic arteries such as aorta that persist in postnatal marrow. The other type of hematopoietic cell cluster identified was at the opposite side of labyrinth next to the junctional zone and was composed of erythropoietic foci. Our results suggest that mouse placenta not only produces hematopoietic stem/progenitor cells but also a second wave of primitive erythrocytes that may support a rapid, mid-pregnancy, fetal growth trajectory. Our data also point to a close relationships in the origins of hematopoietic and endothelial cells within placenta.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Hematopoiesis is a vital process that begins during embryonic development. Blood cell forming activity is sequential in different fetal organs and continues postnatally mostly in bone marrow. In human, mouse and other mammalian embryos, hematopoietic cells first appear in blood islands of the extra-embryonic yolk sac. Yolk sac progenitor cells give rise to transient nucleated erythrocytes, macrophages and megakaryocytes [1–3]. Because cells of these initial early lineages differ from their counterparts produced later in gestation or during adult life, this first wave of hematopoietic production is known as primitive [4]. Blood islands are comprised of masses of mesodermal cells known as hemangioblasts. In mice, hemangioblasts arise from visceral yolk sac cells around 7.5 gestational days (gd) and give rise predominantly to endothelial cells and primitive erythrocytes that mature semi-synchronously into new vessels [5,6]. As development progresses, primitive erythrocytes are gradually superseded by progeny from progenitor cells that are able to differentiate into more complex hematopoietic cell lineages. This second wave of hematopoiesis, called definitive hematopoiesis, takes place within large vessels of the embryo itself (such as dorsal aorta) and in the vitelline and umbilical arteries [7–11]. The hematopoietic progenitor cells from this latter wave migrate to fetal liver where they expand and differentiate. Mouse fetal liver is seeded by transient yolk sac vessel-derived erythro-myeloid progenitor cells at 8.5 gd and then by hematopoietic stem cell (HSC) from the large embryonic arteries about 11.5 gd. Fetal liver then provides the inductive niche for myelopoiesis and erythropoiesis during the second half of pregnancy. Definitive hematopoietic cells and non-nucleated erythrocytes from the latter hematopoietic wave appear in fetal blood by 12.5 gd and eventually replace primitive, nucleated erythrocytes. Towards the end of gestation (after 15.5 gd), HSCs migrate from the fetal liver to cavitating bones where they persist in bone marrow throughout postnatal life [12–15].

Placental hematopoiesis is also reported. Morphological analyses of human placentae recently identified first trimester

* Corresponding author. Laboratory of Pathology, Oswaldo Cruz Institute/Fiocruz, Rio de Janeiro, 21040-360, Brazil.
E-mail address: nath.portilho@hotmail.com (N. Azevedo Portilho).

http://dx.doi.org/10.1016/j.placenta.2016.08.081
0143-4004/© 2016 Elsevier Ltd. All rights reserved.

hemangioblast foci that resembled yolk sac foci of primitive erythropoiesis. These placental foci occurred in villous mesenchyme beneath trophoblasts. It was postulated that developing erythroid and endothelial cells of the placenta elongate towards the chorionic plate to integrate with components that arose within the embryonic circulation [16,17]. Human placenta is also believed to contribute to definitive hematopoiesis. Cells exhibiting hematopoietic progenitor and HSC markers can be isolated from human placentas by gestation week 6 and onwards [18,19]. *In vitro* assays for hematopoietic colony formation showed that $CD34^{++}CD45^{low}$ cells isolated from chorionic villi differentiate into myeloid cells, erythroid cells and some leucocyte lineages [19]. $CD34^{+}CD38^{-}$ human placental cells not only demonstrate multi-lineage, clonogenic potential but also engraft and differentiate in immune deficient mice, defining them as long-term HSC. Immunostaining of first trimester human placentas suggests that early HSCs are in close contact with vascular elements [18].

Mouse placental hematopoiesis is also documented via clonogenic and other functional assays. Multipotent, short-term hematopoietic progenitors and long-term repopulating HSC were identified at 9.0 gd and had expanded by 12 and 13 gd. These cells were highly proliferative and more abundant than in fetal organs. Immunohistochemistry, *in situ* hybridization and transgenic mice have been useful tools for characterization of hematopoietic cells and their niche in the mouse placenta [20–24]. Cells, including HSC, that express hematopoietic progenitor markers such as *Sca1*, c-KIT, and integrin alpha 2b (CD41), are observed within chorioallantoic mesenchyme and fetal vessels of the placental labyrinth over midgestation. Some of these cells co-express endothelial markers revealing close associations between these two lineages [22–24]. $CD41^{+}$ cells were identified in placental vessels of transgenic mice prior to onset of a fetal heartbeat, suggesting that at least some hematopoietic progenitors are intrinsic to placenta [23]. Multiple authors suggest that vessels of the placental labyrinth generate these multilineage progenitors and provide a suitable niche for lineage expansion [21–24]. In contrast to human placenta, earlier, primitive erythropoiesis has not been reported in mouse placenta.

The mouse placental labyrinth forms subsequent to fusion of the chorion (mesothelium and extraembryonic ectoderm) and allantois (mesoderm), that begins at 8.5 gd. Allantoic vessels undergo branching towards chorionic folds and by 9.5 gd, the interhemal membrane that separates fetal and maternal blood is present. Between 10.5 and 11.5 gd, the labyrinthine cell layers become functionally organized and are structurally constant throughout the rest of pregnancy [25,26]. Isolated chorion and isolated allantois have each been shown to contain cells capable of forming myeloerythroid colonies *in vitro*, indicating intrinsic hematopoietic potential of these two structures [27,28]. However, placental contributions to hematopoietic ontogeny remain poorly understood. In the present work, histological and immunohistological approaches were successfully used to seek evidence for earlier, primitive erythropoiesis in mouse placenta and to visualize and localize the sites of later, definitive hematopoiesis.

2. Materials and methods

2.1. Ethics statement

Experimental animal procedures conducted in Brazil were approved by Ethical Commission of Animal Experimentation of the Oswaldo Cruz Foundation (CEUA, Authorization Number LW-44/13) based on the principles of the Brazilian Society for Laboratory Animal Science (SBCAL). Procedures conducted in Canada used protocols approved by the Animal Care Committee, Queen's University and complaint with Guidelines of the Canadian Council on Animal Care.

2.2. Mice

Swiss Webster mice aged 2–3 months were provided by Animal Laboratory Center of the Oswaldo Cruz Foundation (CECAL). For some immunofluorescence studies, C57BL/6 (B6) females purchased from Charles River Canada, St. Constant, QU were mated to B6 males with ubiquitous green fluorescent protein (GFP) transgene expression that was incidental in the reported experiments. For all studies, males and females were housed under 12 h-light/dark cycles and received food and water *ad libitum*. The morning of copulation plug detection was considered 0.5 gd. Thirty-seven pregnant females were studied with 9.5 gd (7), 10.5 gd (20), 11.5 gd (8) and 12.5 gd (2). Mice were euthanized in the morning on each experimental day as described below.

2.3. Sampling and histology processing

Pregnant females received a lethal intraperitoneal injection of Ketamin-Xylazin (0.1 ml/12–15 g) (Syntec) at 9.5, 10.5, 11.5 or 12.5 gd. Each uterus was transected into implantation sites which were placed into Carson's Millonig formalin [29] for 48 h, then halved sagittally. Specimens were processed into paraffin using a Shandon Citadel 2000 tissue processor (Thermo, USA) according to standard methodology. Every implantation site in each pregnant female was analyzed using 5 μm serial sections. Every tenth section was de-waxed, hydrated and washed in distilled water prior to staining with Mayer's hematoxylin-eosin (H&E). Alternatively, the implant sites were embedded in OCT compound and flash frozen in liquid nitrogen. These samples were stored in a −80 °C freezer until 6 μm sectioning using a cryostat (Leica).

Characterization of putative hematopoietic foci used immunofluorescence. Paraffin sections were de-waxed, hydrated and incubated in sodium citrate buffer (pH 6.0) prior to antigen retrieval in a Pascal chamber (Dako, USA), according to the manufacturer's recommendations. The slides were incubated in a humid chamber overnight at 4 °C with primary antibodies (Table 1) or phosphate-buffered saline (PBS, pH 7.4) and isotype for negative controls. Slides were washed in PBS and incubated with secondary antibody for 1 h at 37 °C when necessary (Table 1). Some tissue sections were counterstained with 1:10,000 Evans Blue (Sigma, EUA) and 1:5000 DAPI (03571, Molecular Probes, USA) and mounted in ProlongGold (P36934, Life Technologies, USA). Others were mounted in ProlongGold with DAPI (P36931, Life Technologies).

H&E images were analyzed in an AxioObserver Z1 microscope (Zeiss, Germany) and documented in a digital AxioCam MRc5 or HRC cameras (Zeiss Vision, Germany). Some sections were digitally scanned using a VSlide imaging automation platform (Metasystems, Germany) mounted onto a motorized AxioImager Z1 (Zeiss, Germany). Immunofluorescent slides were analyzed in an LSM 510 META confocal microscope or AxioCam-equipped Zeiss M1 imager (Zeiss; Toronto, ON, Canada). Confocal set-ups including an Ar488 laser and BP510-530 filter (AlexaFluor488/FITC), HeNe543 laser and LP560 filter (Evans Blue), and 405 diode laser and BP420-480 filter (DAPI). The images were processed using Adobe Photoshop.

3. Results

3.1. Temporal assessment of the placenta for hematopoietic progenitor cells

Mouse placenta is composed of maternal and zygote-derived cells. Bordering maternal decidua is a thin layer of zygote-derived, parietal trophoblast giant cells (TGC). These giant cells and the adjacent spongiotrophoblast cells form the junctional zone

Download English Version:

https://daneshyari.com/en/article/5894095

Download Persian Version:

https://daneshyari.com/article/5894095

Daneshyari.com