Allantois and placenta as developmental sources of hematopoietic stem cells

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ABSTRACT While the aortic region, the para-aortic splanchnopleura/aorta-gonads-mesonephros (P-Sp/AGM) is currently considered as the source of definitive hematopoietic stem cells during development, the mouse placenta has been found to generate large numbers of these cells and to remain functional in this respect for a longer period than the P-Sp/AGM. The fetal component, which derives from the fused allantois and chorion, is responsible for this activity. We and others have shown that the pre-fusion allantois (before the stage of 6 pairs of somites) is able to yield clonogenic progenitors, provided that it is pre-cultured in toto before it is dissociated into single cells and seeded in semi-solid medium. Thus placental hematopoiesis can be concluded to derive from intrinsic precursors. It is similar in this regard to the yolk sac which both produces hematopoietic progenitors and supports their multiplication and differentiation. Hematopoietic activity, detected by in vitro colony assays, has also been recently uncovered in the human placenta. According to the data available, this newly identified source probably provides a large number of HSC during development and must play a foremost role in founding the definitive hematopoietic system.

KEY WORDS: allantois, placenta, mouse, hematopoietic stem cell

Introduction

During vertebrate development hematopoiesis is sequentially carried out by distinct organs, some of which allow the differentiation of common lineages, while others promote unique lineages. Thus yolk sac, mammalian fetal liver, spleen and bone marrow ensure the differentiation of myeloid lineages while T and B lymphoid lineages become amplified and instructed respectively in the thymus and in the bone marrow or the avian-specific bursa of Fabricius. All these organ rudiments include a mesodermal stroma which provides the specific microenvironment responsible for differentiation of the hematopoietic lineages. Some rudiments are purely mesodermal (bone marrow, spleen) while in others endoderm is also a component of the stroma (yolk sac, fetal liver, thymus, bursa).

Hematopoietic Stem Cells (HSC), the central players in the activity of the hematopoietic system, though mesodermal in origin, do not derive from the mesoderm of the stroma, except in the case of the yolk sac, for many years the only site in the embryo known to produce its own HSC (Moore and Owen, 1965; Le Douarain and Jotereau, 1973; Le Douarain et al., 1975; Pardanaud et al., 1989). As these cells are endowed with extensive migration properties, their ontogenic origin has been actively investigated since the 1960ies and still is a controversial issue (Yoshimoto et al., 2008). Indeed intra-embryonic hematopoietic organs become seeded by HSC, each at a precisely specified stage of ontogeny, as established by explanting early rudiments either in vivo or in vitro. The extrinsic origin of hematopoietic cells was diagnosed in vivo by means of appropriate markers, while in vitro, rudiments explanted before the stage of colonization did not become hematopoietic. The mammalian fetal liver for instance initiates its colonization by the stage of 28 to 32 pairs of somites (Johnson and Moore, 1975; Houssaint, 1981; Cudennec et al., 1982). This applies to all the organs which carry out hematopoiesis, with the exception of the yolk sac, so that, when these questions were first

Abbreviations used in this paper: BFU-E, burst forming unit-erythroid; CFC, colony forming cells; EPC, ectoplacental cone; Ery-CFC, erythroid colony forming cells; GFP, green fluorescent protein; HSC, hematopoietic stem cells; HPP-CFC, high proliferation potential-colony forming cells; ICM, inner cell mass; LTR-HSC, long term repopulating-hematopoietic stem cells; P-Sp/AGM, para-aortic splanchnopleura/aorta-gonads-mesonephros.
submitted to experimentation, all HSC were assumed to have a common origin and the yolk sac was proposed as the unique source of these cells (Moore and Owen, 1967).

Later investigations in the avian, amphibian, and finally murine models (reviews in Dieterlen-Lièvre et al., 2001; Turpen, 2001; Dzierzak and Oostendorp, 2001; Dzierzak and Medvinsky, 2008) were to show that the yolk sac progenitors contribute mainly to the primary erythroid lineage and marginally to the next red cell lineage (Beaupain et al., 1979; Palis et al., 1999) then become extinct (with some species-specific exceptions in amphibians).

The region of the aorta (or, in amphibians, the so-called dorsal compartment) was next identified as a site producing HSC in embryos with 18 to 40 pairs of somites (review in Durand and Dzierzak, 2005). This region, considered as the ultimate source of HSC, is the focus of many current investigations in the mouse and human embryo. In the mouse embryo, cells fated to hematopoiesis in this region were shown to evolve within a narrow developmental time frame, from short-term self-renewability (so-called progenitors) to long-term renewability, the latter capacity being the hallmark of true HSC. The peri-aortic tissues, which give rise sequentially to these two types of progenitors, became known as Para-aortic Splanchnopleura (P-Sp) (Godin et al., 1993) and Aorta-Gonad-Mesonephros (AGM), (Medvinsky et al., 1993), the two terms designating the same anatomical region at two successive steps of development (0-25 somites and 25-40 somites). As a matter of fact, now that the role of the aortic endothelium in the production of HSC is well established (Jaffredo et al., 1998; Zovein et al., 2008; Dieterlen-Lièvre and Jaffredo, 2009), as well as the more general notion of “hemogenic endothelium” (Eilken et al., 2009; Lancrin et al., 2009; Dieterlen-Lièvre and Jaffredo, 2009) it appears timely to revise this terminology.

More recently the placenta has been added to the list of organs which sustain hematopoietic activity during development of the fetus (Alvarez-Silva et al., 2003; Gekas et al., 2005; Ottersbach and Dzierzak, 2005). While the presence of reconstituting HSC (Till and McCulloch, 1961; Dancis et al., 1968; Dancis et al., 1977) and of B lymphoid progenitors (Melchers 1979) in the placenta has been reported many years ago, a contribution of the placenta to fetal hematopoiesis was not seriously considered then. These reports were published at a time when little was known about the ontogenic emergence of HSC in mammals and a possible role of the placenta in this process was not anticipated. Melchers (1979) for instance, interpreted B progenitors present in the placenta as resulting from circulating HSC derived from the yolk sac, according to the dominant theory of the time.

We have shown that the placenta is rich in clonogenic progenitors (Alvarez-Silva et al., 2003). The presence of reconstituting HSC was thereafter established (Gekas et al., 2005; Ottersbach and Dzierzak, 2005). Clearly this new development raises the question of the origin of these stem cells: are they extrinsic or intrinsic? Other results (Ziegler et al., 2006; Corbel et al., 2007) now indicate that clonogenic progenitors become committed in situ, rather than colonize the placenta. The progress of this novel issue will be reviewed here.

**Developmental assembly of the mouse placenta**

The fetal component of the placenta results from the fusion of two independent structures (Fig. 1). One derives from the early embryo outermost layer, the trophoderm. The other is the allantois, a mesodermal appendage emitted by the caudal end of the primitive streak, as the embryo undergoes gastrulation. At E3.5 the trophoderm encloses the inner cell mass (ICM), the structure that will give rise to the chorionic plate. The polar region of the trophoderm, in contact with the ICM, proliferates, and becomes the ectoplacental cone. The latter structure delaminates giving rise to the chorionic plate, which faces the developing embryo. Meanwhile the allantois grows out...
form the caudal end of the primitive streak into the exocoelomic cavity. By the stage of 6 to 8 pairs of somites (depending on individual embryos), the allantois contacts the chorionic plate and attaches to it. In the next step, ruffles, expressing the transcription factor-encoding gene Glial cell missing (Gcm 1), appear in the chorionic plate. Endothelial buds are emitted by the allantois at the points of contact with these ruffles. Gene expression antedates the formation of the ruffles, suggesting that ingrowth of the endothelial digits is induced by the contact with Gcm1-expressing focal points in the chorionic plate (Anson-Cartwright et al., 2000).

The endothelial buds emitted by the allantois thereafter interdigitate with maternal endothelia within a structure called the labyrinth, where fetal and maternal endothelia are separated by two layers of chorionic plate-derived synciotrophoblasts. Interestingly the process known as vasculogenesis (Risau and Lemmon, 1988) i.e., commitment of mesoderm to angioblasts, occurs in the allantoic mesoderm (Downs et al., 1998). Thus the endothelia of cord blood vessels (umbilical artery and vein) and of fetal blood vessels in the placenta derive from the allantois.

The avian lead: hematopoietic and angioblastic potential of the allantois

The avian model previously contributed several major concepts about the ontogeny of the hematopoietic system, which were later validated in other classes of vertebrates. The multiple steps involved in blood formation could be clearly defined because the avian embryo is conveniently accessible to experimental manipulations, and because the quail/chicken system (Le Douarin, 1973) allows to trace the origins and fate of cell lineages. Intra-embryonic HSC were thus discovered in chimeras constructed between a quail embryo and a chicken yolk sac (Dieterlen-Liévre, 1975; review in Dieterlen-Liévre et al., 2001; see also Dzierzak and Medvinsky, 2008). In the early analyses of these hematopoietic chimeras, the origin of cells was determined by means of the quail/chicken nuclear difference. The MB1 and QH1 antibodies, which recognize endothelium and blood cells (with the exception of the erythroid lineage) (Péault et al., 1983, Pardanaud et al., 1987) in the quail and not the chicken, subsequently made cell tracing in these blood chimeras remarkably efficient.

As the avian allantois (Fig. 2A) produces a large amount of erythrocytes when explanted in liquid medium (Dieterlen-Liévre, unpublished data), a possible hematopoietic potential was explored. Interestingly, endothelium-contained collections of red cells, very similar to yolk sac blood islands, were present in the allantoid bud prior to circulation closure (Caprioli et al., 1998, 2001). Pre-circulation quail allantoic buds were grafted ectopically in the coelom of stage-matched chicken. Cells from the bone marrow of E15-16 hosts, by then fully functional, were sorted by cytofluorimetry on the basis of QH1 expression (Fig. 2B). A significant proportion of quail cells was found.

These experiments modulate the former conclusion drawn from the study of yolk sac chimeras, namely that HSC come from the embryo proper. The allantois, a late forming structure in birds, grows out from the posterior intestinal portal of the grafted embryo, and is thus quail in yolk sac chimeras, so that blood cells produced in these chimeras by the aortic region or the allantois cannot be distinguished from each other. Nonetheless as various experimental approaches in several classes of vertebrates have
firmly substantiated the generation of HSC by the aorta (review in Durand and Dzierzak, 2005), the role of this blood vessel cannot be questioned. However the finding that the allantois is involved in hematopoietic cell production indicates that the process of HSC emergence is more extensive time-wise and space-wise than previously thought.

Finally it is interesting to point out that the cytological study of the bone marrow of quail allantois-engrafted chicken hosts uncovered a mix of quail hematopoietic and endothelial cells. As both lineages are known to derive in the bone marrow from extrinsic progenitors (Jotereau and Le Douarin, 1978; Pardanaud et al., 1989), the question of the nature of the colonizing cells is raised, eliciting the attractive hypothesis that these cells might be hemangioblasts, i.e., common progenitors for both lineages (Eichmann et al., 1997; Choi et al., 1998; Huber et al., 2004; Zovein et al., 2008; Chen et al., 2009; Eilken et al., 2009; Lancrin et al., 2009).

Hematopoietic activity of the mouse placenta

We proposed the hypothesis of a hematopoietic role of the mouse placenta (Alvarez-Silva et al., 2003), on the basis of the above-described blood forming potential of the avian allantois (Dieterlen-Liévre et al., 2001).

The first approach we implemented to support this hypothesis consisted in detecting short-term progenitors (Colony Forming Cells, CFC) by means of in vitro clonogenic assays (Alvarez-Silva et al., 2003). The fetuses were obtained from a cross between wild type females and males carrying one allele of a GFP transgene making it possible to diagnose whether CFC derived from the fetal or the maternal component of the placenta (Fig. 3).

Testing began at the stage of 5 pairs of somites. The first non-maternal progenitors were found in the placenta of embryos with 20 pairs of somites (E9), antedating by about 48 hours the colonization of the liver which is initiated between the stages of 28 and 32 somites according to Houssaint (1981). With respect to the production in the para-aortic splanchnopleura which begins at the 18 pairs of somites stage, placental HSC appearance was slightly delayed. Progenitors were still present at E17, the time limit of the study, their number actually peaking at that stage. Thus the myelo-erythroid potential of the placenta is sustained much longer than that of the AGM, in which the short-lived production of progenitors stops by the stage of 42 somites (Medvinsky and Dzierzak, 1996).

Table 1 compares the quantitative aspects of progenitor output from E10 to E17 in different organs. Within this time course, yolk sac supports a modest amplification (x1.7) as well as placenta (x3.8). In contrast, liver is a site where clonogenic progenitors multiply vigorously: 35-fold when E17 is compared to E12 (at E10 it can be considered that colonization has hardly begun). However, the proportion of colony types is strikingly different in placenta and liver at all stages, the former being richer in early progenitors. While the majority of the colonies detected in this assay reach their maximum development by day 14 of culture, a few colonies of the so-called ‘High Proliferation Potential’ (HPP) type were found in cultures from E12-E15 placenta and liver, with twice higher frequency in the placenta. These HPP-CFC were very large (more than 0.5 mm in diameter), multilineage and replatable for at least 60 days. Thus, while the liver supports both progenitor amplification and commitment to differentiation, the modestly growing hematopoietic population in the placenta re-

![Fig. 3. Hematopoietic potential of mouse placenta detected by clonogenic tests (Alvarez-Silva et al., 2003). Experimental scheme. In GFP-embryos carrying one allele of the Green Fluorescent Protein encoding transgene, the fetal component of the placenta fluoresces in green, while the wild type maternal component is visible in photonic light in the double exposure, bottom left. Note that some erythroid colonies, faint red in the phase contrast photograph, have lost GFP expression (stippled circles in the two bottom right boxes). All, allantois; Em, embryo; EPC, ectoplacental cone; Mat. blood, maternal blood.](image-url)
Allantois and placenta as developmental sources of HSC

Allantois and placenta as developmental sources of HSC contains an immature character all along. A recent study demonstrated that multipotent as well as myeloid- and erythroid-committed progenitors were present in the human placenta (Barcena et al., 2009).

Long term repopulating (LTR)-HSC were monitored by means of in vivo reconstitution assays (Fig. 4A) (Gekas et al., 2005; Ottersbach and Dzierzak, 2005). The results of our own endeavor were reviewed in detail elsewhere (Mikkola et al., 2005). Briefly fetal cells were traced using heterozygotes for the alleles of the CD45 pan-hematopoietic antigen. The kinetics of LTR-HSC, which were analyzed from E10.5 to E18.5, have some common points with that of CFC plus some distinctive traits (Fig. 4A). HSC, capable of contributing to the blood of irradiated adults, appeared simultaneously in the placenta and AGM, and 24 hours later in the fetal liver. The AGM numbers remain low at all times. The fetal liver soon overtakes the placenta. The caudal half of the embryo (below the umbilicus) was used at E10.5, the AGM was dissected at other stages. The cephalic half of the embryo (above the umbilicus), or the caudal half minus the AGM, or the uterine decidua were used as negative controls.

By comparison with placenta and fetal liver, LTR-HSC numbers found in the AGM were very small (Fig. 4C). Medvinsky’s group raised the problem of the major increase in HSC numbers which occurs in the liver between E11 and E12, stating that it could hardly be explained by incoming cells from the AGM, whose output is small, or by mitotic expansion within the liver in such a short time (Kumaravelu et al., 2002). The placental source may answer this issue.

The placenta reconstituting cells were shown to be multipotent, they displayed a CD34+, c-Kit high phenotype, and they were able to reconstitute secondary hosts (Gekas et al., 2005). They were Sca1+ as well (Ottersbach and Dzierzak, 2005).

Are placental hematopoietic stem cells born in situ?

In view of the diverse role of hematopoietic sites during development, it appeared crucial to find out whether placental HSC arise in situ, and hence to carry out tests prior to blood circulation. The latter event occurs at E8.5 (Palis et al., 1999), around the stage of 7 somites at about the time when the allantois fuses to the chorionic plate. If indeed the placenta is able to produce HSC, the component likely to be involved is the allantois, which undergoes vasculogenesis, i.e., tissue-autonomous endothelium development (Downs et al., 1998). However when Downs et al. (1998) looked for signs of erythropoiesis in pre-fusion allantois either ex vivo or after short term in toto culture, they reported, at the 6-somite pair stage, only a few benzidine-positive cells, whose number slightly increased in culture. Progenitor activity in cell populations isolated from 1 to 16 sp allantoises was

AGM harbours RU until E13.5 only. The organ specific differences in the early stages (hardly visible in the graph) can be appraised from the table below.

Table: Numbers of RU/ee at early stages are displayed. In brackets: number of reconstituted animals/number of injected animals. RU appear simultaneously in the placenta and AGM, and 24 hours later in the fetal liver. The AGM numbers remain low at all times. RU are specific for each organ. In placenta and fetal liver the values display a sudden early increase. Liver remains rich in RU until E18.5, at the end of the experiment. Placenta peaks at E12.5 and loses all RU by E15.5. AGM as well as yolk sac and blood retain low numbers at all times.

Fig. 4. In vivo reconstitution experiments (Gekas et al., 2005). (A) Experimental scheme. CD45.1/2 heterozygous fetal placental cells were injected into homozygous CD45.2 irradiated adults, along with supportive adult bone marrow CD45.1 cells. Six months later CD45.1/2 cells from the primary recipients were transplanted into secondary recipients. After each reconstitution, cells from bone marrow, spleen and thymus were phenotyped and tested for colony forming potential. Cells of placental origin proved capable of repopulating primary and secondary hosts. (B,C) Developmental reconstituting potential compared between different organs (Gekas et al., 2005, Mikkola et al., 2005). (B) the evolving profiles of RU/ee - Reconstituting units per embryo equivalent calculated as [reconstituted recipients/total recipients]/transplanted doses - are specific for each organ. In placenta and fetal liver the values display a sudden early increase. Liver remains rich in RU until E18.5, at the end of the experiment. Placenta peaks at E12.5 and loses all RU by E15.5. AGM as well as yolk sac and blood retain low numbers at all times.
also searched unsuccessfully by others (Palis et al., 1999). As we had found that the first progenitors appeared in the placenta at the 20 somite stage (Alvarez-Silva et al., 2003), we reasoned that, like in the P-Sp experiments (Cumano et al., 1996), their commitment from much earlier rudiments might be elicited only through the culture protocol implemented earlier, in which in toto preculture is followed by dissociation into single cells and by seeding in a semi-solid medium for the identification of clonal progenitors (2-step culture) (Fig. 5A).

In these conditions, cells from the pre-fusion allantoises did give rise to hematopoietic colonies that belonged to different types, erythroid, myeloid and mixed (Fig. 6). In the case of the erythroid lineage, clusters and colonies deriving from Erythroid (Ery)-CFC were identified as well as colonies derived from burst forming unit-erythroid (BFU-E). In the case of the myeloid lineage, huge macrophage colonies developed and mixed erythro-monocytic colonies were also observed. These various clonal hematopoietic progenitors could be obtained from pre-fusion allantoises as early as the 1 sp stage, i.e., long before the establishment of circulation (Corbel et al., 2007). While all 2-step cultured allantoises gave rise to colonies, neither the number nor the assortment of colony types were related to the embryonic stage of the buds, indicating that the commitment must be a random event. This is easily explained by the small number of cells in the allantoic buds at these early stages (0 to 6 somites).

The hematopoietic potential of the early allantoic bud is further indicated by the presence of CD41+ cell clusters, which were detected by immunocytology at the 4 sp stage. CD41 (also called GPIIb-IIIa or αIIbβ3) is an integrin now considered as a hallmark of embryonic and fetal hematopoietic progenitors (Corbel and Salaün, 2002; Corbel et al., 2005; Emanbokus and Frampton, 2003; Mikkola et al., 2003; Ferkowicz et al., 2003). At that time CD41+ cells were very few, 3 to 10 cells per allantois (Fig. 5B). These CD41+ clusters enlarged significantly during the 3D culture period, as the clonogenic potential emerged (Fig. 5C).

The hematopoietic potential of the pre-fusion allantois was examined by Ziegler et al., (2006). The experimental approach was similar, using an in toto culture period, in which the whole allantoic buds were explanted in liquid medium on an OP9 feeder layer for 2 days prior to dissociation for clonogenic assays. Myeloid and erythroid potential was demonstrated and the erythroid colonies were shown to synthesize adult β major hemoglobin. The Runx1 transcription factor, whose expression is required for HSC emergence (Chen et al., 2009), was expressed weakly in the allantoic bud at explantation and increased after in toto culture. Importantly Runx1-/- allantoic buds did not give rise to colonies. Moreover the authors demonstrated that the chorionic plate mesoderm expertly isolated from the ectoplacental cone (EPC) has a hematogenic potential. In our own experiments, we were

![Fig. 5. Detection of the hematogenous potential of the pre-fusion allantois (Corbel et al., 2007). (A) Experimental scheme. (B,C) CD41 expression. (B) Non-cultured 5sp allantois (Al) and yolk sac (YS) in a transverse section of the conceptus. (C) 4sp allantoises cultured in toto for 5 days.](image)

![Fig. 6. Erythroid, myeloid and multipotent progenitor potential in pre-fusion allantois. (A) Erythroid colonies inside a large myeloid colony from 1sp allantoises. In toto culture, 5 days; semisolid culture, 7 days. (B) BFU-E colony from 3-4sp allantoises. In toto culture, 5 days; semisolid culture, 16 days (Corbel et al., 2007). (C) Mixed colony from 5sp allantoises. In toto culture, 5 days; semisolid culture, 7 days (Corbel et al., 2007).](image)
able to obtain clonogenic progenitors from the EPC but did not pinpoint their origin. Using analogous methodological approaches, the two pieces of work converge to attribute a hematogenic potential to mesoderm from two distinct components of the future placenta.

In order to monitor in vivo whether HSC found in the placenta are generated in situ or are merely imported via circulation, Rhodes et al. (2008) utilized the Ncx1 knockout mouse model. Ncx1-/-/embryos have no heartbeat due to a defect in the sodium-calcium exchange pump and do not survive beyond E10.5 (Koushik et al., 2001). Rhodes et al.'s analysis showed that HSC development is initiated in the placenta vasculature independently of blood flow. Thus these authors confirm that the placenta is not only a niche for HSC but a true site of HSC generation.

Conclusion and perspectives

The body of experiments described here point to the placenta as a major player in fetal blood formation. Compared to the peri-aortic region, its activity as a provider of progenitors and of HSC is initiated at about the same time, but lasts longer and is much more important. Thus while the HSC put out by the AGM region certainly contribute to the initial colonization of the liver, placental HSC must participate in parallel to the beginning of the colonization process and ensure most of it later. Samokhvalov et al. (2007) generated Runx1/LacZ inducible mice to label the hematopoietic lineage at selected stages of development. They found that cells committed in the yolk sac colonized the umbilical cord, concluding that vessels of this structure may be a niche where to extrinsic blood progenitors migrate and differentiate. In our own experiments the allantois was retrieved prior to circulation, so that at least part of the progenitors found in this rudiment are born in situ.

The sites previously identified as hematogenous have a common feature: yolk sac and P-Sp are composed of mesoderm associated to endoderm. The latter germ layer is known to produce ‘ventralizing’ signals which are involved, among other processes, in commitment to hematopoiesis. Indeed the presence of endoderm appears to be an absolute requirement for blood formation (Miura and Wilt, 1969; Risau and Flamme, 1995; Pardanaud et al., 1996; Pardanaud and Dieterlen-Lièvre 1999). However the mouse allantois, which according to our data is at the origin of a large part of the placental HSC, is purely mesodermal (Inman and Downs, 2006). As an explanation for this apparent paradox, we propose that the endodermal contact experienced by allantoic mesoderm, as it emerges from the primitive streak, may allow the signaling event to occur (Belaoussof et al., 1998). The allantois and, in its wake, the placenta clearly belong to a continuum which includes P-Sp/AGM and the fetal arteries in the region of the umbilicus, where hematopoiesis was described (Tavian et al., 1999; de Bruijn et al., 2000).

The description of these novel sites for hematopoiesis and commitment opens a number of perspectives. For instance P-Sp cells do not display long term reconstituting potential of irradiated recipients, while AGM cells do. It is likely that as development proceeds cells produced by the allantois/placenta undergo the same phenotypic maturation underlying this change in potential, from weak to adult type expression of MHC class I antigens (Cumano et al., 2001). In other words, when early allantois cells will be probed for reconstitution potential as the next step in their characterization, they will probably, like P-Sp cells (Cumano et al., 2001), prove unable to reconstitute immunologically competent irradiated adults since they should be rejected by natural killer cells.

Other prospects concern the 3D cell interactions required for the commitment to hematopoiesis described above. The molecular signals involved are currently studied in the embryonic stem cell-derived embryoid body model (Robertson et al., 2000). It will be important to explore this aspect in the embryo/fetus proper. The allantois may be a particularly well suited structure for the analysis of this signaling process at the cellular level, not only because it is easy to retrieve, but also because in these early stages one of its main functions may be the hematogenous one.

It is essential to probe whether the EPC plays a role in the evolution of this activity after fusion. The placenta being a factory for growth factors, fusion is likely to promote HSC output, and the trophoblastic lineage may be involved.

The release of HSC by the placenta into the cord blood needs to be studied until birth, hopefully allowing to identify the origin of cord blood cells. The circulatory pathways responsible for the hypothesized traffic between placenta and fetal liver (Mikkola et al., 2005) open up another line of research. Other preferential pathways of migration and homing may also be at work, possibly due to privileged circulatory links and differential attractiveness of rudiments for HSC. In this respect, the transgenic mouse model devised by Iruela-Arispe’s group (Zovein et al., 2008) may yield further critical information since it allows permanent labeling of blood cells produced at a selected developmental stage. This model may be the mammalian equivalent of the quail/chick tracing system.

To sum up the placenta is now established as a new player in developmental hematopoiesis. As recently reviewed hematopoietic development occurs in different sites: yolk sac, P-Sp/AGM and chorion-allantoic placenta (Dzierzak and Speck, 2008). The most recent experiments show that some of the HSC involved become committed in situ in one of the placenta’s component structures adding this site to the few able to support this event.

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References


BEAUPAIN D., MARTIN C. and DIETERLEN-LIÈVRE F. (1997). Are developmental hemoglobin changes related to the origin of stem cells and site of erythropoie-
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