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The placenta as a haematopoietic organ

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ABSTRACT The recent description of the placenta as a tissue rich in haematopoietic stem and progenitor cells has not only opened up a whole new line of investigation into how haematopoiesis is regulated in this unique mammalian tissue, but has also resulted in the revisiting of long-standing and yet unanswered questions about the significance of having multiple haematopoietic organs during development. Due to its remarkable capacity for haematopoietic stem/progenitor cell expansion, the study of placental haematopoiesis is also of obvious clinical interest. In the following pages, we summarise what is currently known about the haematopoietic regulatory processes in the murine placenta and describe our most recent data demonstrating that the human placenta, like its murine counterpart, is also a source of haematopoietic stem and progenitor cells throughout development.

KEY WORDS: *placenta, haematopoietic stem cell, HSC, development*

Introduction

The adult haematopoietic system is a model for the study of the basic processes of cell proliferation, differentiation and self-renewal. The many steps beginning with the initiating haematopoietic stem cell (HSC) and leading to the production of the various lineages and finally to the mature blood cell types have been described (Weissman and Shizuru, 2008). Cell surface marker characterisation by flow cytometry has made it possible to isolate many of the distinct intermediate progenitor cells of the haematopoietic hierarchy. Yet, new haematopoietic progenitors continue to be found, and challenges to the well-established adult hierarchy are still being made (Adolfsson *et al.* 2005; Bell and Bhandoola, 2008; Wada *et al.* 2008).

In contrast, little is known about the origins and differentiation hierarchy of the haematopoietic system during development. In the mouse and other vertebrate embryos, two separate waves of haematopoietic activity have been described, a primitive wave, consisting of primitive macrophages, megakaryocytes and nucleated erythrocytes, and a definitive wave, which includes a number of progenitors with varying degrees of complexity and differentiation potentials (Dzierzak and Speck, 2008). The lineage relationship between the cells of the primitive and definitive waves is unclear, and it is uncertain whether these cells or their descendents can be found in the adult system. It is only the definitive wave that culminates in the appearance of the first adult-type HSCs at midgestation. The appearance of mature macrophages, megakaryocytes and erythrocytes in the primitive wave precedes the appearance of HSCs in the definitive wave indicating that an alternative and reversed hierarchy exists in the embryo as compared to that in the adult. This reversed embryonic hierarchy suggests that there are no lineage relationships between HSCs and the earlier mature blood cells. It is only after midgestation that an adult-type hierarchy begins to be established.

Currently there is great interest in the origin of HSCs, i.e. the embryonic tissue in which they are first generated, and the precursor cell(s) from which they are derived. During development HSCs can be found in several different haematopoietic territories at overlapping and/or different time points (Fig. 1). The use of different assays and different criteria are further complicating factors in the study of HSC development in the embryo. Experiments carried out in the 1970s using mouse embryos seem to suggest that HSCs and other multipotent haematopoietic progenitors are first generated in the yolk sac (YS) (Moore and Metcalf, 1970; Weissman *et al.* 1978). This paradigm was challenged by quail-chick embryo grafting experiments, which demonstrated that adult blood derives from an intra-embryonic source (Dieterlen-Lievre, 1975). A similar intra-embryonic source for

Abbreviations used in this paper: AGM, aorta-gonad-mesonephros region; FL, foetal liver; HSC, haematopoietic stem cell; YS, yolk sac.

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multipotent progenitors and HSCs was eventually identified in mice (Cumano *et al.* 1996; Medvinsky and Dzierzak, 1996; Muller *et al.* 1994) and termed the AGM region, as it contains the developing aorta, gonads and mesonephros. Adult-type HSCs, i.e. cells that can repopulate a normal, irradiated adult recipient, are first detected in the AGM at embryonic day (E)10.5 (Medvinsky and Dzierzak, 1996; Muller *et al.* 1994), a day earlier than their appearance in the YS. HSCs reside in both tissues only transiently, and their numbers start to decline as stem cells begin to colonise the foetal liver (FL). For the remainder of foetal development, the FL remains the main haematopoietic tissue where HSCs expand in number and differentiate into the various mature blood cell types, before they relocate to the bone marrow (BM) around the time of birth (Fig. 1). More recently, another major source of HSCs in the murine embryo was discovered, the midgestation placenta (Gekas *et al.* 2005; Ottersbach and Dzierzak, 2005). It had been suggested that due to the large numbers of HSCs present in the FL after E12, HSCs from several sources contribute to the colonisation of the FL (Kumaravelu *et al.* 2002). The placenta was found to contain HSCs starting from E11, with numbers expanding dramatically at E12 and far beyond the number found in the AGM and YS at that point (Gekas *et al.* 2005; Ottersbach and Dzierzak, 2005). This exciting discovery then raised a number of questions: (1) are the placenta-derived HSCs phenotypically and functionally identical to the ones found in other embryonic tissues?; (2) does the placenta have the capacity to generate HSCs or is it colonised by cells from a different origin?; and (3) what are the stromal components responsible for the dramatic HSC expansion? These questions are only just starting to be tackled, and the following pages will discuss some of the initial findings.

Development of the placenta

The placenta is a unique mammalian tissue that forms the interface between the maternal and foetal environments. Placenta development is initiated when the allantois, a mesodermal structure that extends from the posterior end of the embryo starting at E7.5, fuses with the chorionic ectoderm at E8.5 (reviewed in Cross *et al*. 2003; Rossant and Cross, 2001). At this juncture major morphological changes take place that result in the formation of primary villi, which then through further growth and branching develop into the highly vascularised labyrinth region of the placenta. The foetal vessels, including the umbilical vessels, and the associated stroma are contributed by the allantois, while the three layers of trophoblast cells that surround the foetal capillaries and function as a barrier between the maternal and foetal circulation, are derived from the chorion (Simmons *et al.* 2008). Overlying the labyrinth is the spongiotrophoblast layer, which is thought to derive from the ectoplacental cone and which provides the developing placenta with structural support. The final interface between the foetal and the maternal tissues is formed by a layer of trophoblast giant cells (Fig. 2).

Interestingly, the two precursor tissues of the placenta, the allantois and the chorion, have recently been shown to possess intrinsic haematopoietic potential (Corbel *et al.* 2007; Zeigler *et al.* 2006). Already a number of years previously, the avian allantois had been demonstrated in grafting experiments to contribute to adult haematopoiesis (Caprioli *et al.* 1998; Caprioli *et al.* 2001). At the same time, similar grafting experiments in mouse embryos showed allantoic contribution to endothelial, but not haematopoietic cells in the host (Downs *et al.* 1998; Downs and Harmann, 1997). However, recent studies using an *in vitro* culture of pre-fusion allantois and chorion for 2 days, followed by methylcellulose assays, reveal the intrinsic potential of these tissues to give rise to clonogenic haematopoietic progenitors (Corbel *et al.* 2007; Zeigler *et al.* 2006). In addition, the expression of definitive haematopoietic markers can be detected in the early allantois and chorion (see below). Thus, the tissues giving rise to the placenta have haematopoietic progenitor potential, but whether they also possess potential for HSC generation still remains to be determined.

Placental haematopoiesis

The discovery of HSCs in the mouse midgestation placenta immediately raised the question whether the placenta-derived HSCs are functionally and phenotypically identical to HSCs from other sources. Such information could shed some light on the origin of these cells. We examined the expression of the HSC marker Sca-1 during placental development. Sca-1 has been shown previously to be expressed on all HSCs in the adult bone marrow and foetal liver (Huang and Auerbach, 1993; Spangrude *et al.* 1988). We generated a transgenic mouse line which expresses the green fluorescent protein (GFP) under the regulatory elements of the Sca-1 gene, *Ly-6A* (de Bruijn *et al*. 2002). This transgene was found to mark all the HSCs in the AGM, the FL and the adult BM (de Bruijn *et al.* 2002; Ma *et al.* 2002). When the expression of the transgene was examined at early developmental stages, we discovered GFP positive cells already at E6 within the extraembryonic ectoderm and the ectoplacental cone (Ottersbach and Dzierzak, 2005). Interestingly, the expression of GFP in the extraembryonic ectoderm was strongest in cells directly adjacent to the epiblast and gradually decreased with increasing distance from the epiblast (Fig. 2). This expression pattern may reflect the location of trophoblast stem cells, which

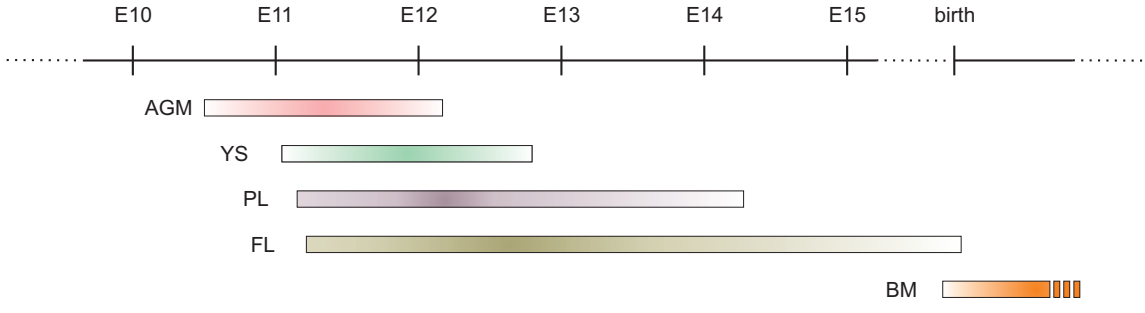

Fig. 1. Timeline depicting the emergence of HSCs in haematopoietic tissues during mouse development. *AGM, aorta-gonads-mesonephros region; YS, yolk sac; PL, placenta; FL, foetal liver; BM, bone marrow; E, embryonic day.*

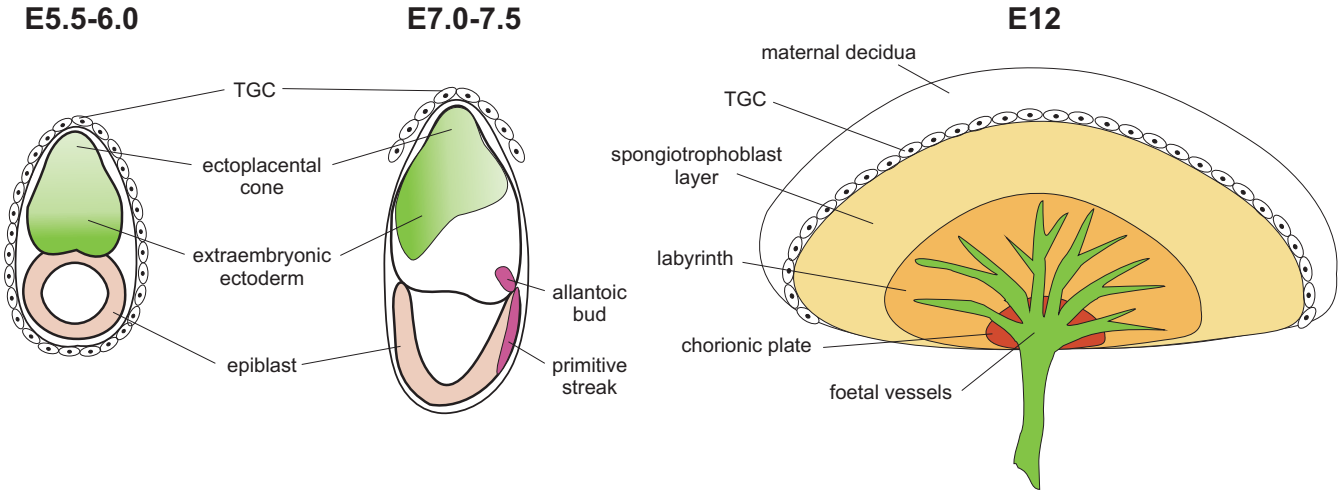

Fig. 2. Schematic diagram of various stages leading up to the development of the fully formed placenta. *Tissues that express the* Ly-6A GFP *transgene are shown in green. TGC, trophoblast giant cell; E, embryonic day.*

are thought to be maintained in a pluripotent state by signals from the embryo and start differentiating as they move towards the ectoplacental cone (reviewed in Rossant and Cross, 2001). Thus, the *Ly-6A GFP* transgene may also mark other types of stem cells. The GFP expression in the ectoplacental cone, the extraembryonic ectoderm and then in the chorionic ectoderm continues into E7 (Fig. 2). Zeigler and colleagues have since then also reported expression of the same transgene in the pre-fusion chorion and allantois following 24h of explant culture. While GFP is also detected in the freshly isolated chorion, it only appears in the allantois following this culture step (Zeigler *et al.* 2006).

The expression of the *Ly-6A GFP* transgene in the precursor tissues of the placenta prompted us to analyse its expression pattern in the fully formed placenta (Ottersbach and Dzierzak, 2005). Surprisingly, the expression of GFP was not maintained in the parts of the placenta that are derived from the extraembryonic ectoderm and the ectoplacental cone (Fig. 2). Instead, we found that the vascular tree of the placental labyrinth, including the umbilical vessels, was highly marked by GFP at all stages analysed (E9-E12, E18; Ottersbach and Dzierzak, 2005, and unpublished data). Furthermore, we found that as with HSCs from other sources, all of the repopulating activity was contained within the Ly-6A GFP$^+$ fraction at E12, the peak of HSC activity in the placenta. The majority of the E12 GFP$^+$ cells also expressed the HSC markers c-kit, CD31 and CD34, and just over 50% of the GFP$^+$ cells carried both c-kit and CD34 on their surface – the classic embryonic HSC signature (Sanchez *et al.* 1996). It was confirmed by Gekas and colleagues that indeed all of the stem cell activity in the placenta is within the CD34$^+$c-kit$^+$ population (Gekas *et al.* 2005). Although about two thirds of GFP$^+$ cells are positive for CD31 and/or CD34 (also expressed on endothelial cells), only one third co-expresses the pan-haematopoietic marker CD45. It is therefore likely that a considerable proportion of the Ly-6A GFP$^+$ cells are endothelial cells. Some of these are possibly haemogenic endothelial cells, the putative developmental precursors of HSCs (reviewed in Ottersbach and Dzierzak, 2006). This is partly supported by the observation that, as in the AGM, VE-cadherin, a marker for endothelial cells and early HSCs, is progressively downregulated on CD34$^+$c-kit$^+$ placental cells (Taoudi *et al.* 2005). However, whether there are haemogenic endothelial cells in the placenta needs to be examined, and such studies should provide insight into whether the placenta can autonomously generate HSCs.

Clusters of cells that express haematopoietic as well as endothelial markers (including Ly-6A GFP) and that seem to adhere to the luminal side of major blood vessels, are considered to be signs of HSC generation (reviewed in Ottersbach and Dzierzak, 2006). They appear in the locations and at the time when HSCs can be detected, and they are absent in embryos with genetically impaired HSC production (Cai *et al.* 2000; Ling *et al.* 2004). Despite the fact that haematopoietic clusters have been described in the umbilical artery, thus far they have not been detected within the placental vessels. CD41, an integrin of the α subunit family (α_{IIb}), has recently been suggested as a marker for nascent HSCs (Bertrand *et al.* 2008; Corbel and Salaun, 2002; Ferkowicz *et al.* 2003; Matsubara *et al.* 2005; Mikkola *et al.* 2003; Mitjavila-Garcia *et al.* 2002). We found CD41 expression on approximately one third of E12 Ly-6A GFP$^+$ placental cells, and immunohistochemical staining revealed that CD41$^+$ cells are located within the vessels of the labyrinth, with a few of them attached to the inner vessel wall (Ottersbach and Dzierzak, 2005). This expression pattern was subsequently confirmed by Rhodes and colleagues (Rhodes *et al.* 2008). CD41-expressing cells are first seen in the pre-fusion allantois as early as the 4-6 somite pair stage (Corbel *et al.* 2007). Interestingly, CD41 expression was analysed at E10 in embryos defective for the sodium/calcium pump 1 (*Ncx1*-/-), which results in the absence of a heart beat and therefore no blood circulation (Rhodes *et al.* 2008). Since CD41$^+$ cells were still detected in the mutant placentas, albeit in severely reduced numbers, it suggests that the placenta is capable of generating at least some haematopoietic progenitors. The early death of the *Ncx1*-/- embryos (E10.5) precluded examination of HSCs in the placenta, which normally appear at E11. The reduced number of CD41+ cells in mutant placentas suggests that a majority of progenitors in a wild-type placenta originate elsewhere or, alternatively, that circulation and shear stress trigger the generation of haematopoietic cells in the placenta. Recent reports have indeed highlighted the role of blood flow-induced sheer stress in

haematopoietic stem and progenitor cell emergence in the aorta (Adamo *et al.* 2009; North *et al.* 2009).

Recently, we have set out to compare the cell surface phenotype of HSCs from different embryonic sources, concentrating in the first instance on members of the integrin family (C. Robin, K. Ottersbach and E. Dzierzak, manuscript in preparation). Integrins are composed of one α and one β subunit. Depending on the combination, integrins can interact with different soluble ligands, receptors or extracellular matrix proteins (reviewed in Danen and Sonnenberg, 2003; Kumar, 1998). CD41 (α_{IIb}) exclusively pairs with CD61 (β_3), which then allows it to interact with several ligands, including von Willebrand Factor and fibronectin. A $CD41^-$ $CD61^+$ phenotype has recently been described for adult BM HSCs (Ferkowicz *et al.* 2003; Umemoto *et al.* 2008). CD61 can also interact with another α subunit, CD51 (α_V), thus opening up studies on another repertoire of ligands that include osteopontin and CD31. Our preliminary results suggest that CD41 is a variable marker of HSCs during development. We are also examining the expression of the other subunits and ligands. Differences in integrin expression may reflect differences in the protein composition of the supportive microenvironment in the various haematopoietic territories of the embryo and/or may also represent different stages of HSC maturation and migration.

The placental expression pattern of a number of haematopoiesis-associated transcription factors has also been examined. Gata2 is essential for HSC generation, as embryos deficient for Gata2 die at E10.5, displaying severe anaemia and a complete absence of HSCs (Ling *et al.* 2004; Tsai *et al.* 1994). We initially looked at the expression of this transcription factor in embryos containing a Gata2 lacZ transgene (Zhou *et al.* 1998). As previously reported (Ma *et al.* 1997; Ng *et al.* 1994), we detected Gata2 lacZ expression in some trophoblast giant cells. Gata2 is known to regulate the transcription of the placental hormones placental lactogen I and proliferin and thus, it may influence neovascularisation within the conceptus as well as the adjacent decidual tissue (Ng *et al.* 1994). However, a much more pronounced expression of Gata2 lacZ was seen within the labyrinth, with levels being particularly high on the border to the chorionic plate. We have subsequently confirmed this expression pattern by *in situ* hybridisation (Fig. 3 B,C white arrow), but have also seen additional expression in cells of the trophoblast lineage within the labyrinth (Fig. 3 B,C). These cells have the appearance of tightly packed cuboidal cells that have been theorised to be labyrinth progenitors and that are preferentially located near the chorionic plate (Fig. 3C, white arrowhead), although evidence in support of this hypothesis is still scarce (Simmons *et al.* 2008; Wu *et al.* 2003). Whether Gata2 is also involved in hormone synthesis within these cells and whether this could support vascularisation within the labyrinth or even influence the haematopoietic environment is currently unknown. However, as within the AGM, we also saw endothelial-specific expression of Gata2 in the placenta. It remains to be shown whether this can be taken as evidence for the presence of haemogenic endothelium in the placenta.

The expression of another haematopoietic transcription factor, Gata3, was examined, using a lacZ knock-in mouse line (van Doorninck *et al.* 1999). Deletion of Gata3 results in embryonic lethality at E11.5, with FL haematopoiesis also being affected (Pandolfi *et al.* 1995). Like Gata2, Gata3 is known to be expressed in trophoblast giant cells where it also regulates the transcription of trophoblast-specific genes (Ma *et al.* 1997; Ng *et al.* 1994). Accordingly, we detected lacZ expression in a few trophoblast giant cells. However, further expression of Gata3 in trophoblast cells within the labyrinth was only revealed by subsequent *in situ* hybridisation analysis (Fig. 3A). The highly similar expression pattern of Gata2 and Gata3 suggests that they may perform overlapping functions in the placenta.

The transcription factor Runx1 is essential for HSC generation during development, and Runx1-null embryos die at E12.5 due to a complete disruption of definitive haematopoiesis (Okuda *et al.* 1996; Wang *et al.* 1996). Its expression during development has been mainly studied in a lacZ knock-in mouse line (North *et al.* 1999). Expression from this allele can already be seen in isolated mesodermal cells in the chorion at the early bud stage (Zeigler *et al.* 2006). A limited number of Runx1-expressing cells can also be observed in the distal and proximal regions of the pre-fusion allantois, although explant cultures of sub-divided allantoic tissues demonstrated that Runx1 expression can be initiated in cells throughout the entire allantois. After chorio-allantoic fusion, $Runx1^+$ cells were detected at the fusion interface and also in the proximal allantoic vasculature (Zeigler *et al.* 2006). The expression of Runx1 by RT-PCR analysis of the fully formed placenta was first reported by Alvarez-Silva and colleagues (Alvarez-Silva *et al.* 2003). Using the $Runx1^{lz}$ allele, we subsequently assigned the expression to cells in the chorionic plate and, within the labyrinth, to endothelial cells, individual cells and clusters of cells in the circulation and to cells just underneath the placental vessels (Ottersbach and

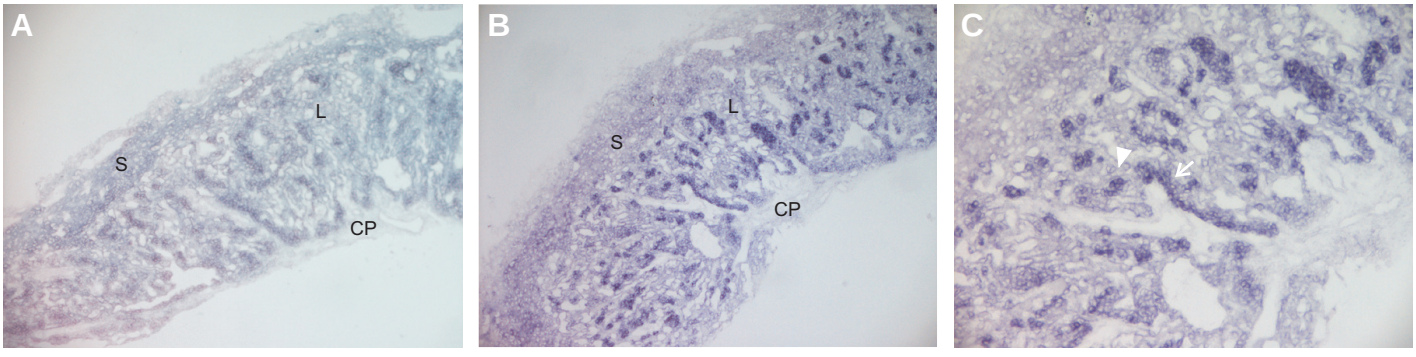

Fig. 3. *Gata2* and *Gata3* expression in the placenta. In situ hybridisation *on E11 placenta sections with riboprobes for* **(A)** Gata3 *and* **(B,C)** Gata2. *(C) is a close-up of (B). White arrow, strong* Gata2 *expression near chorionic plate; white arrowhead, Gata2+ cuboidal cells. CP, chorionic plate; L, labyrinth; S, spongiotrophoblast layer.*

Dzierzak, 2005). This pattern is similar to the one found in the AGM where Runx1 is known to be essential for the formation of HSCs (Cai *et al.* 2000; North *et al.* 1999; North *et al.* 2002). This pattern of Runx1 expression in the placenta was subsequently confirmed by Rhodes and colleagues (Rhodes *et al.* 2008). They also reported that the Runx1$^+$ cells within the placental vessels were more mitotically active than the ones in the AGM, thus supporting the notion that the placenta acts as a site of HSC expansion. While deletion of Runx1 results in a complete lack of HSCs, a half dose of Runx1 results in a spatial and temporal shift of HSC emergence (Cai *et al.* 2000). HSCs appear earlier in both AGM and YS of Runx1+/- embryos as shown by direct transplantation of freshly isolated tissues. However, when these tissues are subjected to an explant culture step, it becomes apparent that HSCs in the AGM cannot be maintained, with numbers already severely reduced at E10 and then disappearing thereafter (Cai *et al.* 2000). In contrast, haploinsufficient YS explants show increased HSC numbers at E10 and E11. We have recently included the placenta in this analysis and found that the phenotype is similar to the YS: HSC numbers are increased in E11 Runx1+/- placenta explants as compared with wild-type tissues (Robin *et al.* 2006). We have also observed an early appearance of HSCs in E10 Runx1+/- placentas (Robin *et al.* 2006; unpublished data). Therefore, a full dose of Runx1 has a negative effect on HSC appearance in the YS and placenta, while promoting HSC activity in the AGM. Interestingly, placental HSCs are indistinguishable from YS and AGM HSCs (Runx1+/+ and Runx1+/-) in their response to the growth factor Interleukin-3 (IL-3), with numbers increasing dramatically after explant culture in the presence of recombinant IL-3 (Robin *et al.* 2006). These results perhaps indicate important differences in the intraembryonic haematopoietic microenvironment.

In summary, placental HSCs share the common phenotype of Ly6A$^+$CD34$^+$c-kit$^+$ with AGM HSCs. However, there are some differences in HSC growth in different parts of the conceptus which are revealed by the analysis of embryos haploinsufficient for pivotal hematopoietic transcription factors. In many aspects placental HSCs are more similar to YS HSCs. Finding a functional explanation for these differences and uncovering further dissimilarities between embryonic haematopoietic sites, should clarify the significance of having multiple haematopoietic sites during development.

Placenta microenvironment

The main function of the placenta is the exchange of nutrients, gases and waste products between the foetal and maternal circulation. This takes place within the placental labyrinth, and mutations that affect the development of this vascularised part of the placenta, where HSCs are known to reside (Ottersbach and Dzierzak, 2005), can have serious consequences for the foetus, ranging from growth retardation to midgestational lethality (reviewed in Watson and Cross, 2005). The placenta is also an endocrine organ that produces an array of hormones from its trophoblast cells (reviewed in Linzer and Fisher, 1999). Some of these hormones are known to influence vascularisation in the maternal as well as the foetal part of the placenta (Ma *et al.* 1997; Ng *et al.* 1994). Such hormones may impact on placental haematopoiesis, as haematopoietic and endothelial development are tightly linked (reviewed in Ottersbach and Dzierzak, 2006). Other hormones are also likely to affect haematopoiesis directly (reviewed in Linzer and Fisher, 1999). It was reported many years ago that the human placenta releases haematopoietic growth factors that promote haematopoietic colony formation (Burgess *et al.* 1977), thus indicating that the placenta supports haematopoiesis. In addition to hormones and growth factors being directly produced and secreted by placental trophoblast cells, it is also likely that maternally derived factors enter the placenta via a number of transporters found at the junction of the maternal and foetal circulation. It was recently demonstrated that maternal loss of the transcriptional repressor Tgif results in a defect in foetal placental vasculature development (Bartholin *et al.* 2008). The molecular details of this observation are currently unclear, but it clearly proves that maternal processes can indeed affect the formation of foetal-derived placental structures.

The first indication that the placenta has a microenvironment suitable for the maintenance and expansion of a wide range of haematopoietic progenitors came from work carried out by Françoise Dieterlen-Lièvre's group (Alvarez-Silva *et al.* 2003). These authors demonstrated that clonogenic progenitors appeared in the placenta as early as E9, slightly later than in the embryo proper and the YS. Up to E12, total colony numbers in the placenta are even more numerous than in the FL. Their frequency remains above that found in the FL for the rest of development, and the progenitors in the placenta seem to have a more immature phenotype. However, after E13, there seems to be a change in the supportive environment since the number of progenitors and HSCs in the placenta declines (Gekas *et al.* 2005). Even an attempt to enrich for HSCs by sorting Ly-6A GFP$^+$ cells from E18 (term) placentas did not yield any HSC activity (K. Ottersbach and E. Dzierzak, unpublished data). It is currently unclear what causes the decline in placental haematopoietic support, but an identification of the components that make up the haematopoietic microenvironment is likely to offer some explanations.

A candidate cell lineage providing haematopoietic support in the placenta niche may be mesenchymal stem/stromal cells, as these have been linked to haematopoietic support in other tissues (Mendes *et al.* 2005; Sacchetti *et al.* 2007). Indeed, Zhang and colleagues have isolated mesenchymal progenitor cells from the human placenta that are capable of expanding long-term culture-initiating cells from cord blood-derived CD34$^+$ cells (Zhang *et al.* 2004). We also have derived mesenchymal stromal cell lines from the human placenta (Robin *et al.* 2009) and mouse placenta (I. Lauw, Master of Science thesis) from several stages of development. Some of these showed an extensive mesenchymal differentiation potential and also displayed a pericyte/perivascular cell phenotype similar to cells that have recently been reported to be the *in vivo* counterpart of mesesenchymal stromal/stem cells (Crisan *et al.* 2008). Furthermore, these stromal cells also had the capacity to expand haematopoietic progenitor cells from human cord blood in coculture experiments (Robin *et al.* 2009). Thus, the placenta appears to contain stromal cells indicative of a haematopoietic niche throughout gestation, suggesting that *in vivo* the microenvironment of the placenta is conducive to haematopoietic maintenance and growth.

The human placenta

Many aspects of HSC development are conserved between the mouse and the human conceptus. Since the mouse placenta

was shown to generate haematopoietic progenitors early in development, and from midgestation on to contain an abundance of haematopoietic progenitors and HSCs, it is of great interest to determine whether the human placenta is a potential source of haematopoietic progenitors and HSCs. The human placenta, as a resource for human cells and factors, has thus far found little medical application. It is a rest tissue and is usually discarded at birth. Recently, we examined the haematopoietic potential of the human placenta at a series of developmental time points, including term (Robin *et al.* 2009). As measured by *in vitro* clonogenic activity, foetal-derived haematopoietic progenitors are present in the placenta as early as week 6 in gestation. Initially progenitors are in both $CD34^-$ and $CD34^+$ fractions, but by week 15 all progenitors are $CD34^+$. The presence of $CD34^+$ progenitor cells in the human placenta from week 8 has also been reported by Barcena *et al.* (Barcena *et al.* 2009 a,b). Moreover, using NOD-SCID mouse transplantation assays, we also found potent multilineage, high level repopulating cells to be present in the human placenta (Robin *et al.* 2009). HSCs are detected beginning at week 6 of gestation and throughout trimesters 1 and 2 and also in term placenta. These cells are foetal-derived and based on the extraction method, some are with the vascular fraction. Considering the lack of HSCs in the term mouse placenta (Gekas *et al.* 2005; K. Ottersbach and E. Dzierzak, unpublished data), the finding of HSCs in the human term placenta is surprising and suggests that this normally discarded rest tissue has potential medical value.

The presence of HSCs in the human placenta now focuses attention on how the haematopoietic growth properties of this tissue can be exploited. As mentioned above, efforts should concentrate on identifying the factors that are responsible for the remarkable ability of the placenta to expand HSCs. Such factors can be utilised to increase human HSC numbers *ex vivo* for cell replacement therapies. It is also likely that placental HSCs are similar to cord blood-derived HSCs. Cord blood HSCs are known to be more naive than adult bone marrow HSCs, thus allowing less stringent criteria for HLA matching and resulting in availability of these cells to a much wider population. These cells also appear to have a lower transmission rate of infectious and genetic diseases (Benito *et al.* 2004; Cohen and Nagler 2004 a,b; de Vries *et al.* 2004). Thus, human placental HSCs together with umbilical cord blood cells, could offer a quantitatively higher number of such cells for replacement therapies. Finally, the placenta as a HSC niche may also be important in the context of leukaemia aetiology, since transplacental chemical agents have been put forward as possible causes for acute infant leukaemia (reviewed in Greaves, 2006). As there is some evidence suggesting that infantile hemangioma may be of placental origin (Barnes *et al.* 2005), placental HSCs may represent the initial target for these transforming agents.

Concluding remarks

Often in seminars the question arises 'why there are HSCs in the placenta in the first place'. Non-mammalian vertebrate species, such as chicken, zebrafish and frog, in which blood development has also been intensely studied, have a well-functioning haematopoietic system in the absence of a placenta. Yet the processes of blood development are highly conserved amongst all these vertebrate species (reviewed in Cumano and Godin, 2007; Dzierzak and Speck, 2008). It has been suggested that more than one source is required to provide sufficient numbers of HSCs for the colonisation of the FL (Kumaravelu *et al.* 2002). Therefore, has the placenta evolved as an external HSC territory to provide larger quantities of HSCs and progenitors than the YS and AGM are capable of producing? Perhaps the growth factor- and hormone-rich, vascularised placental niche encourages the proliferation of HSCs migrating from the AGM via the umbilical vessels to the FL. This possibility is supported by the observation in the mouse that HSCs become detectable in the placenta nearly a day after they emerge in the AGM and their numbers decline again rapidly a day after HSC production in the AGM stops (the AGM ceases to exist as an entity after E12). Therefore, as HSC production and migration in the mouse embryo is terminated and the FL is fully colonised, the flow of HSCs through the placenta also stops and HSCs can no longer be detected there. Also, the gross morphology of the placenta does not seem to change dramatically after HSC numbers decline at E13. Thus, it may not be a change in the haematopoietic environment in the placenta that causes HSCs to disappear. Indeed, mouse placenta-derived haematopoietic progenitors remain numerous after E13 (Alvarez-Silva *et al.* 2003), and haematopoietic supportive stromal cell lines have been isolated from human placenta throughout development. However, in the mouse it would be interesting to examine if stromal cell lines derived from the placenta at later developmental stages have the same supportive capacity as stromal cell lines derived at the peak of HSC expansion in the placenta.

Another possibility is that HSCs arise in the placenta. $CD41^+$ haematopoietic cells were found in the placenta in the absence of cellular exchange between different tissues via the circulation (Rhodes *et al.* 2008), and at the early stages of normal placenta development the allantois and chorion possess haematopoietic potential (Corbel *et al.* 2007; Zeigler *et al.* 2006). The fact that some placental HSCs have a phenotype suggestive of an endothelial origin (Ottersbach and Dzierzak, 2005; Taoudi *et al.* 2005) supports the possibility that some HSCs are derived from placental haemogenic endothelium. Thus, whether the placenta is another example of the recurrent theme of HSC generation along the major vasculature of the conceptus awaits lineage tracing experiments and *in vivo* imaging.

The placenta as a HSC niche was only reported in the mouse five years ago and little is currently known about the cellular and molecular mechanisms regulating haematopoietic activity within this tissue, particularly in the human. Once some of the niche components have been identified, it may be possible to utilise this information to specifically disrupt placental haematopoiesis and hence reveal the relative importance of the placenta in foetal haematopoiesis.

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