PHENOTYPIC HETEROGENEITY OF HEMATOPOIETIC PROGENITOR CELLS FROM PLACENTAL TISSUE: COMPARATIVE ANALYSIS WITH UMBILICAL CORD BLOOD AND FETAL LIVER

ABSTRACT

The study of placental hematopoietic progenitor cells (HPCs) and comparison of their properties with other fetal and adult HPCs is necessary for assessing of their possible clinical application. It has been shown that HPCs from placenta are heterogeneous by phenotype: placental tissue contains three populations with different level of CD34 expression such as $CD34^{++}CD45^{low/-}$, $CD34^{++}CD45^{low/-}$ and $CD34^{+/low}CD45^{low/-}$. Similar to fetal liver placenta contains both, population of $CD34^{++}CD45^{low/-}$ and $CD34^{+/low}CD45^{low/-}$cells, suggesting hematopoiesis in placental tissue. $CD34^{++}CD45^{low/-}$ population also expressed CD133, almost negative for lineage markers, and had lymphocyte-like morphology conforming the presence of primitive HPCs in this population. Additionally, we found later progenitors with phenotype $CD34^{+/low}CD45^{+}$ in placental tissue as the majority of these cells expressed hematopoietic lineage markers. Population with phenotype $CD34^{++}CD45^{low/-}$ was observed in the placenta that may evidence for their generation in the placental tissue or migration from the other sites of hematopoiesis and changing phenotype under placental microenvironment.

KEYWORDS: hematopoietic progenitor cells, placental hematopoiesis, CD34, umbilical cord blood.

Among the major issues of hematology is the lack of donor-derived hematopoietic progenitor cells (HPCs) transplanted for treatment of hematologic diseases and congenital hematopoiesis disorders. Thus, a search of new additional sources of HPCs is needed. It has been shown that human placenta plays an important role in embryonic hematopoiesis [3, 9]. At the same time, the immune phenotype of placental HPCs and their multipotency have not been completely studied as yet. Investigation of placental HPCs and comparison of their properties with the properties of fetal and adult HPCs are important for assessment of their possible clinical application. The purpose of this study has been to compare the phenotypes of HPCs from placenta, umbilical cord blood and fetal liver.

MATERIALS AND METHODS

OBTAINING OF MONONUCLEAR CELLS FROM UMBILICAL CORD BLOOD AND PLACENTAL TISSUE

Placenta was received after full-term delivery (physiological or by cesarean section) in 39-41 weeks of pregnancy from 23-36 year old women according to their informed consent. Cord blood was obtained by standard methods of umbilical blood sampling. All samples were tested for the aerobic and anaerobic microorganisms and a fungal infection.
Mother’s blood was tested for HIV-1/2, HCV, HBV, CMV and Treponema pallidum. Placental tissue was additionally tested for the Chlamidium trachomatis, Mycoplasma genitalis, Ureaplasma urealyticum and Ureaplasma parvum, HSV-1/2, CMV. The umbilical cord was cut; the placenta was purified from amnion and decidua. Placental tissue was minced with sterile scissors into small fragments and intensively washed with Hanks’ solution for blood removal. Then the tissue was treated with a 0.2% collagenase I (Serva, Germany), 0.35 mg/ml hyaluronidase (Sigma, USA), 100 U/ml DNase I (Sigma, USA) with 1 mg/ml bovine serum albumin (BSA) within 30-50 minutes at +37 °C. Placental cells were filtered through a 70 micron cell strainer (Becton Dickinson, USA). At the 2nd stage tissue was incubated with a fresh portion of the enzymes for 30-60 minutes at +37 °C. After fermentation cells were washed in phosphate buffered saline (PBS) with 1 mg/ml BSA. Mononuclear cells from placenta and umbilical cord blood were isolated using a Ficoll density centrifugation method (1.077 g/ml) (Biochrome, Germany), twice washed, and filtered through a 40 micron cell strainer. Cord blood mononuclear cells were treated with a mixture of such enzymes as placenta tissue for 50 min at 37°C and were washed in PBS with 1 mg/ml BSA.

FLOW CYTOMETRY
For immunophenotyping of cells the fluorochrome-labeled monoclonal antibodies (Becton Dickinson, USA) were used: anti-CD34 APC, anti-CD90 FITC, anti-CD45 APC-Cy7, anti-CD105 PerCP-Cy 5.5, anti-CD7 PE, anti-CD14 Pacific Blue, anti-CD31 PE, anti-45RA FITC, anti-CD7 PE, anti-CD19 PE-Cy7, anti-CD33 FITC, anti-CD235a PE. Phenotyping was performed on the cell sorter BD FACSaria (Becton Dickinson, USA), using FACS Diva 6.1 software. Control samples were used to adjust the compensation of fluorochrome overlap: unstained control, single stained control and FMO control.

STATISTICAL ANALYSIS
Data analyzed by nonparametric statistics (Mann-Whitney U test). p < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION
In our previous study we have shown that ISHAGE protocol is suitable for FACS analysis of HPCs derived from native and cryopreserved placental tissues [2]. Analysis according to such protocol have showed that the content of HPC with the phenotype CD34^+CD45^- and lymphocyte-like morphology (SSClow) among the viable CD45^- cells of placental tissue amounts 0.6% (0.39–0.86%, n = 13). Frequency of CD34^-CD45^-SSClow cells among the CD34^-CD45^- was 78.5% (70.5–85.6%, n = 13). We showed that placental tissue contains three populations, which differ in the level of expression of CD34 and identified them as: CD34^-/CD45^-/SSClow, CD34^-/CD45^-/SSCmed and CD34^-/CD45^-/SSChigh.

Two populations, CD34^-/CD45^-/SSClow and CD34^-/CD45^-/SSCmed, were present in the placental tissues, cord blood, and fetal liver (Fig. 1, a-c). CD34^-/CD45^-/SSCmed cells and a part of the CD34^-/CD45^-/SSCmed population...
The CD34 expression level on placental tissue as well as in the cord blood were positive for CD133 (Fig. 1, d). The CD14 expression on the placental cells with the phenotype CD34++CD45low/ - and CD34++CD45low/ - was present in the placental tissue, but at the same time it was practically not observed in the cord blood and fetal liver (Fig. 1, a-c).

The number of CD34++CD45low/- cells among all of Ficoll-purified cells was 0.28% (0.05–0.7) in the placental tissue and 0.006% (0.003–0.01) in the cord blood. FASC analysis showed that the CD34++CD45low/- cells derived from placental tissue, in general, had a lymphocyte-like morphology (FSClowSSClow). CD34+CD45low/ - and CD34++CD45low/ - cells were highly heterogeneous in morphology. The increase of the CD45 expression on CD34+ cells led to their increased size and granularity. Flow cytometry showed that CD34++CD45low/ - cells had the following phenotype CD33-/lowCD14-/lowCD235-CD19-CD7-/lowCD45RA-. The level of CD14 on CD34++CD45low/ - placental cells was 4.25% (1.5–8.4%, n = 4). The CD14 expression was increasing along with the reducing of the CD34 expression level on CD45low/ - placental tissue cells. Similarly, CD7 and CD19 expressions were increasing along with the reducing of the CD34 expression level on CD45low/ - cells of placental tissue and cord blood (Fig. 2).

In contrast to cord blood and fetal liver, the placenta also contained CD34+CD45+ cells. This population was characterized by a high expression level of linear markers compared to the population with a lower expression of CD45 and a higher level of CD34. The frequency of CD14 and CD33 on CD34++CD45+ cells was 73.5% (54.2–88.4%, n = 4). The level of CD19 and CD7 expression in the same population was 14.2% (8.4–21.2%, n = 4) and 3% (1.4–5.2%, n = 4), respectively. Moreover, we noted that the number of myeloid progenitors (CD14-CD33-) were higher among placental HPCs with a higher level of CD45 and lower level of CD34 (Fig. 3).

The phenotypic analysis has confirmed that placental tissue contains hematopoietic progenitor cells. Percentage of CD34+CD45low/ -SSClow cells among CD34+CD45low/ -HPCs, which is lower than in the cord blood, demonstrates the need for gating cells for their morphology as has been described in our previous research [2]. Placental tissue contains HPCs with different CD34 expression. Possibly, it can testify to different stages of immaturity. Similar to fetal liver, the placenta contains CD34+CD45low/ - and CD34+CD45low/ - cell populations. It may evidence for the hematopoiesis in placental tissue. Among placental cells there are more mature HPCs with CD34+CD45low/ - phenotype unlike in fetal liver and cord blood. Most of such cells express hematopoietic lineage markers. This fact allows us refer them to later precursors. We showed that lineage markers expression on HPCs, such as CD14, CD19, CD7, increased along with the decrease of CD34 expression. We found progenitor cells of various differential levels in mature placental tissue. Such cells continue to be formed in the placenta and/or migrate to the placental tissue and do not disappear by the moment of their delivery.

Besides, there has been shown that CD133 is mostly expressed by CD34+CD45low/ - placental and umbilical cord blood cells, evidences for the primitiveness of this cell population. This has been confirmed by the fact that expression of the lineage markers CD33, CD235, CD19, CD7 and CD45RA was almost absent, but a part of this cell population expressed CD14. Interestingly, the placenta contains CD34+CD45low/ - cells, which, like CD34+CD45low/ -, do not express lineage markers but unlike CD34+CD45low/ -, they do not express CD133 stem cell marker and have heterogeneous morphology. The absence of such cells in the cord blood and fetal liver is indicative of their possible formation in the placental

![Fig. 2 Expression of CD7 (a) and CD19 (b) in different population of HPCs: CD34+CD45low/ - , CD34+CD45dim, CD34+CD45++. * - p < 0.05, n=6.](image)

![Fig. 3 Myeloid progenitors (CD33-CD14+) in different population of HPCs in placental tissue: CD34+CD45low/ - , CD34+CD45dim, CD34+CD45++. * - p < 0.05, n=6.](image)
The study has shown that the above cells have a great proliferative potential in vitro [4].

Thus, the placenta contains primitive HPCs, i.e. potentially stem cells with phenotype CD34+CD45low-high. We have found that cells with high CD34 expression contain primitive HPCs, including stem cells. At the same time, the primitiveness of progenitor cells is closely related to the level of CD34 [5, 6, 7, 8, 10]. Barcena et al. found two populations of CD34+CD45low and CD34+CD45high in chorial villi and chorioamniotic membrane at different stages of placental development. CD34+CD45high cells express markers of multipotent primitive HPCs and hematopoietic stem cells, and demonstrate myeloid and erythroid potential in vitro. They also create CD56+ NK-cells and CD19+CD20+ sIgM+ B-cells in polyclonal cultures, while CD34+CD45low cells contain more committed progenitors [3].

Fetal bone marrow cells also contain cell population with different CD34 expression levels (CD34high and CD34low), while only CD34hi cells have the phenotype of the most primitive hematopoietic cells: Thy-1+, HLA-DR+, CD38low, CD45RA+. They also express a low level of CD13 and CD33 antigens, and do not have surface antigens of more mature cells (CD2, CD10, CD14, CD15, CD16, CD19, glycoporphin). CD34+ cells support prolonged B-lymphopoiesis and myelopoiesis in vitro and initiate T, B and myeloid repopulation of human tissues implanted in SCID mice [6]. In the mature bone marrow CD34 expression decreases along with the maturing of cells and increases during CD38 expression [10]. It was shown that fetal liver contains cells with high level of CD34, and they also express Thy-1, CD117, CD123, HLA-DR, CD7, CD38, CD45, CD71, CD115 and are able to restore of hematopoiesis in vivo.

**CONCLUSIONS**

Phenotypic heterogeneity is characteristic of the HPCs isolated from placental tissue in contrast to the umbilical cord blood and fetal liver. Placental tissue contains three cell populations which differ in the level of CD34: CD34highCD45low-high, CD34+CD45low and CD34+CD45high. Similar to fetal liver, the placenta contains populations of CD34+CD45low and CD34+CD45high cells. Owing to this, one can mention the presence of hematopoiesis in placental tissue. The CD34+CD45low population expresses CD133 and does not practically express lineage markers and has the lymphocyte-like morphology. This evidences for the presence of primitive HPCs in such population (potentially stem cells). Late progenitors with CD34lowCD45+ phenotype are also present among placental cells, because most of such cells express hematopoietic lineage markers. Population with phenotype CD34++CD45low is only observed in the placenta, which is possibly formed in the placental tissue or migrates from other hematopoietic sites and acquire of such phenotype under the placental microenvironment.

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