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PHENOTYPIC HETEROGENECITY OF HEMATOPOIETIC PROGENITOR CELLS FROM PLACENTAL TISSUE: COMARATIVE 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 $CD34^{+}CD45^{low/-}$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 caesarean 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*.

OBTAINING OF CELLS FROM FETAL LIVER

The source of the fetal liver was aborted human embryos 5-12 weeks of gestation obtained after voluntary pregnancy termination according to the women's informed consent. Fetal liver cells were obtained by non-enzymatic method. They were obtained by homogenization in the Potter homogenizer in Hanks' solution, followed by filtration through a 100 microns cell strainer. Cryopreservation of liver cells was conducted by a three-stage program according to the method of *Lobyntseva G. S.* [1]. The samples were stored in liquid nitrogen at -196°C. Fetal liver cells were thawed in a water bath at +40°C.

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-CD73 PE*, *anti-CD14 Pacific Blue*, *anti-CD31 PE*, *anti-CD133 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 $CD34^{+}CD45^{dim}$ and lymphocyte-like morphology (*SSClow*) among the viable $CD45^{+}$ cells of placental tissue amounts 0.6% (0.39–0.86%, n = 13). Frequency of $CD34^{+}CD45^{dim}SSC^{low}$ cells among the $CD34^{+}CD45^{dim}$ 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: $CD34^{+/low}CD45^{low/-}$, $CD34^{++}CD45^{low/-}$ and $CD34^{+++}CD45^{low/-}$.

Two populations, $CD34^{+/low}CD45^{low/-}$ and $CD34^{++/low}CD45^{low/-}$, were present in the placental tissues, cord blood, and fetal liver **(Fig. 1, a-c)**. $CD34^{++}CD45^{low/-}$ cells and a part of the $CD34^{+/low}CD45^{low/-}$ population

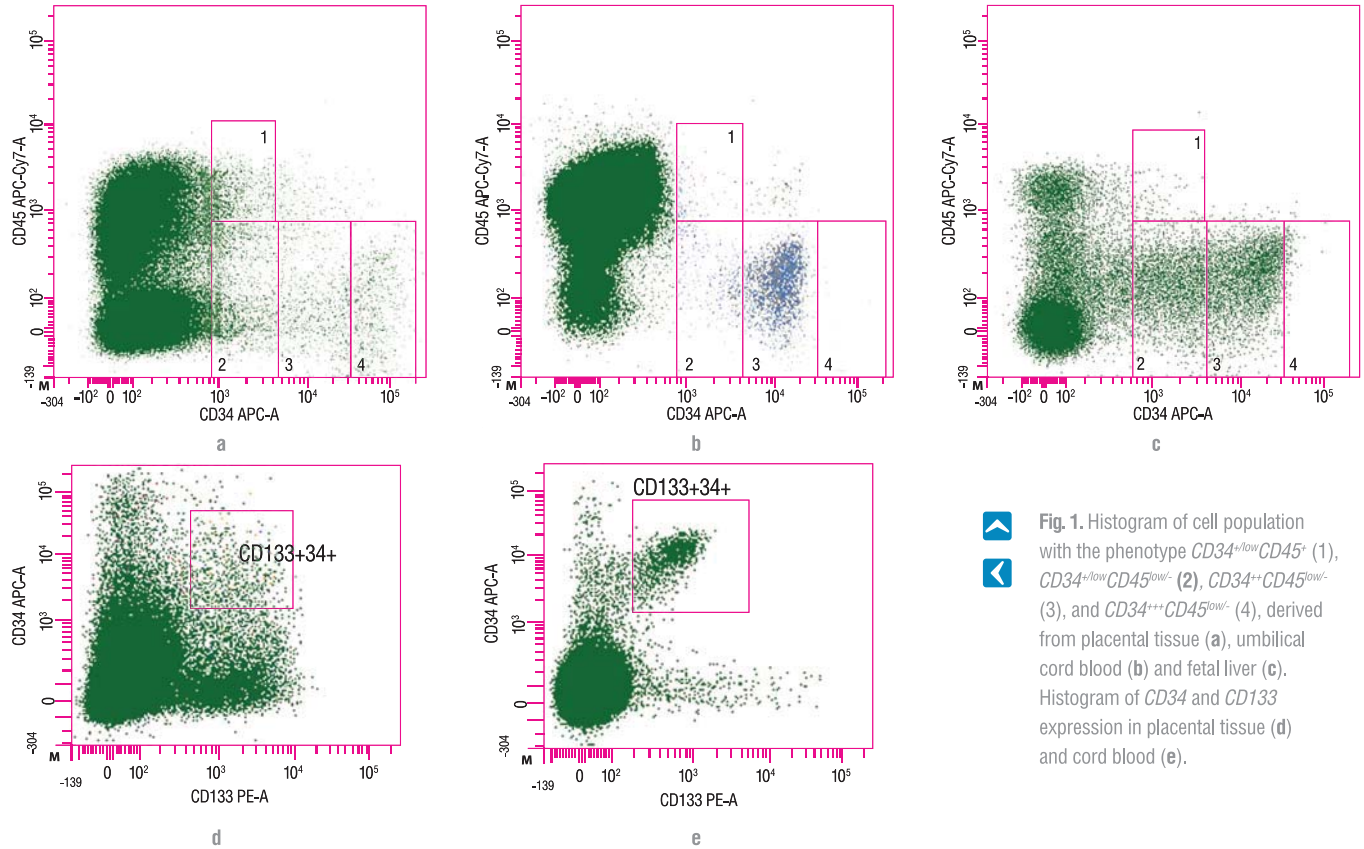

Fig. 1. Histogram of cell population with the phenotype $CD34^{+/low}CD45^{+}$ (1), $CD34^{+/low}CD45^{low/-}$ **(2)**, $CD34^{++}CD45^{low/-}$ (3), and $CD34^{+++}CD45^{low/-}$ (4), derived from placental tissue (**a**), umbilical cord blood (**b**) and fetal liver (**c**). Histogram of *CD34* and *CD133* expression in placental tissue (**d**) and cord blood (**e**).

in the 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 $CD34^{+/low}CD45^{low/-}$ and $CD34^{++}CD45^{low/-}$ rate was 7.2% (3.3–12.6%, n = 4) and 3% (0.5–7.6%, n = 4), respectively, unlike those in the population of cord blood cells, where it was not almost expressed. The population of cells with the phenotype $CD34^{+++}CD45^{low/-}$ 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 $CD34^{+++}CD45^{low/-}$ 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 $CD34^{++}CD45^{low/-}$ cells derived from placental tissue, in general, had a lymphocyte-like morphology ($FSC^{low}SSC^{low}$). $CD34^{+}CD45^{low/-}$ and $CD34^{+++}CD45^{low/-}$ 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 $CD34^{+++}CD45^{low/-}$ cells had the following phenotype $CD33^{-/low}CD14^{-/low}CD235^{-}CD19^{-}CD7^{-/low}CD45RA^{-}$. The level of *CD14* on $CD34^{+++}CD45^{low/-}$ 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 $CD45^{low/-}$ placental tissue cells. Similarly, *CD7* and *CD19* expressions were increasing along with the reducing of the *CD34* expression level on $CD45^{low/-}$ cells of placental tissue and cord blood **(Fig. 2)**.

In contrast to cord blood and fetal liver, the placenta also contained $CD34^{+/low}CD45^{+}$ 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 $CD34^{+/low}CD45^{+}$ 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 $CD34^{+}CD45^{dim}SSC^{low}$ cells among $CD34^{+}CD45^{dim}$, 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 $CD34^{++}CD45^{low/-}$ and $CD34^{+}CD45^{low/-}$ cell populations. It may evidence for the hematopoiesis in placental tissue. Among placental cells there are more mature *HPCs* with $CD34^{+/low}CD45^{+}$ 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 $CD34^{++}CD45^{low/-}$ 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 $CD34^{+++}CD45^{low/-}$ cells, which, like $CD34^{++}CD45^{low/-}$, do not express lineage markers but unlike $CD34^{++}CD45^{low/-}$, 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

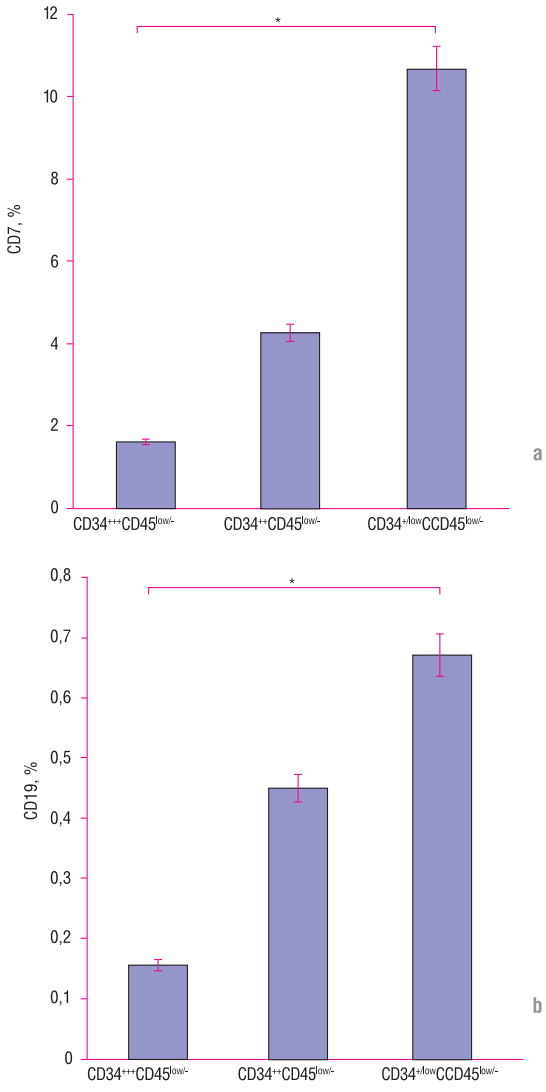

Fig. 2 Expression of *CD7* (**a**) and *CD19* (**b**) in different population of HPCs: $CD34^{+++}CD45^{low/-}$, $CD34^{++}CD45^{low/-}$, $CD34^{+/low}CD45^{low/-}$, * - $p < 0.05$, n=6.

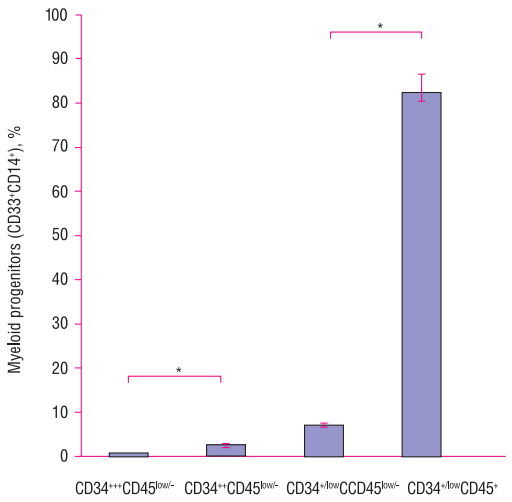

Fig. 3. Myeloid progenitors ($CD33^{+}CD14^{+}$) in different population of *HPCs* in placental tissue: $CD34^{+++}CD45^{low/-}$, $CD34^{++}CD45^{low/-}$, $CD34^{+/low}CD45^{low/-}$, $CD34^{+/low}CD45^{+}$. * - $p < 0.05$, n=6.

tissue or of their migration from the other sites of hematopoiesis and their alteration under the placental microenvironment.

The population of placental *HPCs*, which have a very high level of intercellular adhesion protein *CD34* ($CD34^{+++}CD45^{low/-}$), can interact with placental cell niche. On the cell level we there was studied the population of $CD34^{+++}$ hematopoietic progenitor cells of human umbilical cord blood. 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 $CD34^{++}CD45^{low/-}$. 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 $CD34^{++}CD45^{low}$ and $CD34^{+}CD45^{low}$ in chorionic villi and chorioamniotic membrane at different stages of placental development. $CD34^{++}CD45^{low}$ 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 $CD34^{+}CD45^{low}$ cells contain more committed progenitors [3].

Fetal bone marrow cells also contain cell population with different *CD34* expression levels ($CD34^{hi}$ and $CD34^{low}$), while only $CD34^{hi}$ cells have the phenotype of the most primitive hematopoietic cells: $Thy\text{-}1^{+}$, $HLA\text{-}DR^{low}$, $CD38^{low}$, $CD45RA^{-}$. 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*, glycophorin). $CD34^{hi}$ 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 of *CD38* expression [10]. It was shown that fetal liver contains cells with high level of *CD34*, and they also express $Thy\text{-}1^{+}$, $CD117^{+}$, $CD123^{+}$, $HLA\text{-}DR^{+}$, $CD7^{-}$, $CD38^{-}$, $CD45^{-}$, $CD71^{-}$, $CD115^{-}$ 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*: $CD34^{+/LOW}CD45^{LOW/-}$, $CD34^{++}CD45^{LOW/-}$ AND $CD34^{+++}CD45^{LOW/-}$. SIMILAR TO FETAL LIVER, THE PLACENTA CONTAINS POPULATIONS OF $CD34^{++}CD45^{LOW/-}$ AND $CD34^{+}CD45^{LOW/-}$ CELLS. OWING TO THIS, ONE CAN MENTION THE PRESENCE OF HEMATOPOIESIS IN PLACENTAL TISSUE. THE $CD34^{++}CD45^{LOW/-}$ 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 $CD34^{+/LOW}CD45^{+}$ PHENOTYPE ARE ALSO PRESENT AMONG PLACENTAL CELLS, BECAUSE MOST OF SUCH CELLS EXPRESS HEMATOPOIETIC LINEAGE MARKERS. POPULATION WITH PHENOTYPE $CD34^{+++}CD45^{LOW/-}$ 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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