SCIENTIFIC-ORGANIZATIONAL ASPECTS FOR DEVELOPING AN INVENTORY OF THE DONORS OF UMBILICAL CORD BLOOD WITH CCR5 DELTA32/DELTA32 GENOTYPE FOR HIV INFECTION TREATMENT

ABSTRACT

Projections of the using umbilical cord blood for HIV infection cure consist in the transplantation of umbilical cord blood hematopoietic stem cells from the donors of homozygous CCR5 delta32 mutation carriers.

This work presents the results of screening evaluation of umbilical cord blood samples from the donors, included in the public registry of the Pokrovsky stem cells bank, for identification of the homozygous CCR5 delta32 polymorphism carriers and their following HLA typing to see the perspectives for creating a public registry of CCR5 delta32/delta32 donors of the umbilical cord blood for treatment of HIV-infected patients. Total 2860 umbilical cord blood samples were examined from which 29 samples with CCR5 delta32/delta32 genotype were selected.

High frequency of the HLA alleles most prevalent in the North-West region of the Russian Federation has been found in the donors of umbilical cord blood with wild CCR5 gene and among CCR5 delta32/delta32 donors.

KEYWORDS: umbilical cord blood, HIV, CCR5 delta32, hematopoietic stem cells transplantation.
and heterozygous state [8, 9]. Lowering of CCR5 protein expression on the membranes of malignant and stromal cells in the hematological patients with CCR5 delta32 mutation brings about their reduced response on anti-inflammatory chemokines that may be the positive factor limiting tumor spreading at lymphoproliferative diseases [10].

In recent time CCR5 delta32 polymorphism has been studied actively in connection with its influence on the pathogenesis of Human Immunodeficiency Virus (HIV) infection. Earlier studies reported that the virus finds membrane CD4 receptor to be a suitable for its entry into cell [11, 12, 13]. However soon it became clear that for virus entry into cell it’s binding with only one receptor is not sufficient. In 1996 five different research groups independently made a discovery that along with CD4 the HIV virus uses chemokine receptor CCR5 for penetration through cell membrane. Simultaneous expression of CD4 and CCR5 receptors occurs on T-lymphocytes, monocytes, macrophages and dendrite cells.

Presence of CCR5 delta32 in homzygous state determines synthesis of defective CCR5 receptor non-expressing itself on cell membrane. For this reason homzygous carriers of study polymorphism possess practically full resistance to HIV infecting [14, 15, 16, 17, 18]. One successful case of transplantation of peripheral blood hematopoietic stem cells (HSC) was performed in 2007 year in Germany to a HIV-infected patient with acute myeloid leucosis from the donor with CCR5 delta32/delta32 genotype.

Following transplantation the highly active antiretroviral therapy was discontinued. Virus loading in blood plasma and bioplates of states that HSC of umbilical cord blood with CCR5 delta32/delta32 gene type could be used for transplantation to HIV-infected patients. In 493 samples (17.2 %) the CCR5 delta32 polymorphism was present in the heterozygous state (Table 1).

Comparison analysis was carried out to see the distribution of HLA alleles among cord blood donors, selected at random, with the wild CCR5 genotype and among cord blood donors with CCR5 delta32/delta32 genotype (Table 2). High frequency of the most prevalent HLA alleles in the North-West of Russia was established among the donors of both groups (Table 3).

Identification of the CCR5 delta32 polymorphism opened new possibilities for HIV-infection cure. Of today, transplantation of HSCs with CCR5 delta32/delta32 genotype has been the only method that allows eradicate HIV from the infected organism. However the use of bone marrow or peripheral blood samples from adult donors is practically inadmissible because of rare polymorphism occurrence in the population and observance of stringent compatibility conditions by HLA system in donor-recipient matching. At the same time cord blood HSCs could significantly more likely to be suitable for treating such patients.

Hence the aim of this work was to evaluate the scientific-organizational possibilities for developing a public registry of umbilical cord blood with CCR5 delta32/delta32 genotype for cure of HIV-infected patients.

**RESULTS AND DISCUSSION**

**Assessment of CCR5 delta32 polymorphism**

DNA was isolated from the cord blood samples frozen at -70°C using the PROTRANS kit (Protrans, Germany). Screening for CCR5 delta32 alleles was performed by means of the polymerase chain reaction (PCR) in the amplifier MyCycler, Ver. 1.065 (BioRad, USA). Polymorphism detection was done in 9%polyacrilamide gel using vertical electrophoresis. The length of PCR of fragments made 244 bp at wild type gene variant and 192 bp at homzygous CCR5 delta32 polymorphism.

**HLA typing**

HLA typing of umbilical cord blood specimens was performed by sequence-specific priming (SSP) method. DNA was isolated from 0.5-0.7 ml of umbilical cord blood using Protrans DNA Box 500 (Protrans, Germany). DNA concentration was estimated on spectrophotometer, mean value 70 µg/ml. Further we performed amplification by using Cyclerplate systems Protrans HLA-A*, -B*, -DRB1* (Protrans, Germany). Amplification was performed by using thermocycler MyCycler (BioRad, USA). Products of amplification placed in the gel wells and performed electrophoresis during 25 min at 170 V. In each of 96 wells there should appear control product for checking correct amplification. In some of the wells there should be the strip of specific product that defined the respective genotype by loci HLA-A, HLA-B and HLA-DRB1.

<table>
<thead>
<tr>
<th>CCR5 GENOTYPE</th>
<th>CORD BLOOD SAMPLES</th>
<th>TOTAL NUMBER</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT/WT</td>
<td></td>
<td>2338</td>
<td>81.8</td>
</tr>
<tr>
<td>WT/CCR5 delta32</td>
<td></td>
<td>493</td>
<td>17.2</td>
</tr>
<tr>
<td>CCR5 delta32/delta32</td>
<td></td>
<td>29</td>
<td>1.0</td>
</tr>
<tr>
<td>All</td>
<td></td>
<td>2860</td>
<td>100</td>
</tr>
</tbody>
</table>

Note: WT – wild type

**Table 1. CCR5 delta32 allele prevalence in cord blood samples of donors from North-West Federal region of Russian Federation (Pokrovsky Stem Cell Bank data)**

**MATERIALS AND METHODS**

Total 2860 umbilical cord blood samples taken from an Registry of Pokrovsky bank of stem cells (St. Petersburg) were examined. These samples were screened for the presence of CCR5 delta32 polymorphism and distribution of HLA alleles.
Table 2. HLA allele prevalence in donors with wild type genotype and genotype CCR5 delta32

<table>
<thead>
<tr>
<th>LOCUS</th>
<th>THE MOST COMMON ALLELE</th>
<th>ALLELE FREQUENCY IN INDIVIDUALS WITH WILD TYPE GENOTYPE, %</th>
<th>ALLELE FREQUENCY IN INDIVIDUALS WITH GENOTYPE CCR5 DELTA32/DELTA32, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-A</td>
<td>*02</td>
<td>37.5</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>*03</td>
<td>5</td>
<td>17.5</td>
</tr>
<tr>
<td></td>
<td>*01</td>
<td>10</td>
<td>7.5</td>
</tr>
<tr>
<td>HLA-B</td>
<td>*07</td>
<td>5</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>*35</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>*44</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>HLA-DRB1</td>
<td>*07</td>
<td>20</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>*15</td>
<td>7.5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>*13</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 3. The most common HLA alleles in Northwest region of Russian Federation

<table>
<thead>
<tr>
<th>LOCUS</th>
<th>THE MOST COMMON ALLELE</th>
<th>FREQUENCY, %</th>
<th>THE AVERAGE FREQUENCY OF THIS ALLELE FOR CAUCASIANS [35]</th>
<th>FREQUENCY RANGE [35]</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-A</td>
<td>*02</td>
<td>28.3</td>
<td>25.01</td>
<td>7.2-39.6</td>
</tr>
<tr>
<td></td>
<td>*03</td>
<td>15.8</td>
<td>6.87</td>
<td>1.6-25.6</td>
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<tr>
<td></td>
<td>*01</td>
<td>13.6</td>
<td>14.07</td>
<td>5.3-28.1</td>
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<tr>
<td>HLA-B</td>
<td>*07</td>
<td>13.6</td>
<td>8.67</td>
<td>1.0-16.0</td>
</tr>
<tr>
<td></td>
<td>*35</td>
<td>12.3</td>
<td>10.33</td>
<td>5.0-18.3</td>
</tr>
<tr>
<td></td>
<td>*44</td>
<td>8.8</td>
<td>11.19</td>
<td>4.6-21.7</td>
</tr>
<tr>
<td>HLA-DRB1</td>
<td>*07</td>
<td>15.1</td>
<td>13.7</td>
<td>5.3-28.9</td>
</tr>
<tr>
<td></td>
<td>*15</td>
<td>14.8</td>
<td>10.73</td>
<td>5.7-25.6</td>
</tr>
<tr>
<td></td>
<td>*13</td>
<td>13.7</td>
<td>11.11</td>
<td>4.5-26.2</td>
</tr>
</tbody>
</table>

**CONCLUSIONS**

1. FREQUENCY OF CCR5 DELTA32/DELTA32 OCCURRENCE IN THE NORTH-WEST FEDERAL REGION OF THE RUSSIAN FEDERATION MAKES NEARLY 1 %.

2. HIGH INCIDENCE OF HLA ALLELES, MOST WIDELY-SPREAD IN THE NORTH-WEST OF RUSSIA AMONG THE UMBILICAL CORD BLOOD DONORS WITH WILD CCR5 AND AMONG CCR5 DELTA32/DELTA32 GENOTYPE HAS BEEN ESTABLISHED.

3. THE CCR5 DELTA32/DELTA32 SAMPLES FOUND AS A RESULT OF THE SCREENING SPEAK IN FAVOR OF THE NEED OF CREATING A NATIONAL REGIONAL STORAGE OF UMBILICAL CORD BLOOD SAMPLES FOR HIV-INFECTION CURE.
REFERENCES


The authors indicate no potential conflicts of interest.

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