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A comparative study of HLA-A and HLA-B antigens and haplotype distribution among donors of hematopoietic stem cells from Russian and German regions

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Summary

Genetic polymorphism in the HLA system is extremely high, thus rendering a search for individuals exhibiting identical genetic characteristics difficult. Meanwhile, successful allografting of unrelated donor hematopoietic stem cells (allo-HSCT) is determined mainly via genetic similarity between the recipient and donor. A Republican Register that unites the databases of HLA-typed donors from the Russian and Kirov Research Institutes of Hematology and Blood Transfusion, and the blood banks of Nyzhni Novgorod, Rostov-on-Don, Samara, and Pervouralsk has been cooperating for several years with the Stefan Morsch Registry in Germany, performing bilateral donor searches for patients with hemoblastosis.
The study has shown that the most pronounced differences in prevalence, both for certain antigens and their haplotypes, are observed between the general cohorts of the German and Russian Registers. Donors from St. Petersburg and Nyhzni Novgorod express maximal similarity in their genetic features. The donors from Samara Region are, for some characteristics, more related to German donors, whereas donors from Kirov possess some features that are typical to Northern folk. This data confirms an urgent need for expansion of the Russian Donor Registry, since the probability of finding a donor in the Russian population is sufficiently higher when performing the search in a local Registry.

Keywords: hematopoietic stem cells, allogeneic transplantation, donor registry, HLA antigens, typing, genotype, haplotype, Russian regions, Germany

Introduction

Human leukocyte antigens (HLA), or the major histocompatibility complex represents a genetic system which plays a central role in the immune response, recognizing “own” and “foreign” cells, and presenting processed antigens to immunocompetent cells. This system is characterized by extreme polymorphism. The HLA gene complex is located at chromosome 6 (short arm), includes over 4000 base pairs, and consists of three gene groups (Class I, II, and III). According to the Classification of 2005, it includes 47 loci, encoding over 2000 known allelic variants [7]. Such an extreme polymorphism is stipulated by a necessity to withstand a huge number of evolving environmental antigens, and to maintain the genetic individuality of the human organism. Therefore, a search for two individuals with identical genetic characteristics is prone to sufficient problems.

At the same time, successful transplantation of unrelated hematopoietic stem cells (HSCT) is primarily determined by the genetic similarity between recipient and donor [2]. This highly technological therapeutic approach is successfully employed worldwide to treat many diseases, including different leukemias, combined immune deficiencies, generalized autoimmune diseases and so on. To carry out a selection of donor-recipient pairs, a network of regional HSC donor registries is arranged throughout the world, under active cooperation within this community. For several years, the Russian Registry has been cooperating with the Stefan Morsch Foundation [1], in performing mutual searches for donors for patients with hemoblastosis.

The aim of our study was to evaluate immunogenetic characteristics among HSC donors, including the populations from various Russian regions, as well as comparisons with appropriate data from German Registries.

Materials and methods

Characteristics of the group under study

HLA typing for class A and B loci was performed on 1198 persons included in the Registry of potential bone marrow donors, based upon the Russian Research Institute of Hematology and Transfusion. Most of these cohorts were represented by regular blood donors. Small numbers of volunteers and relatives of the patients with hemoblastosis were also involved. In general, distribution for age and gender corresponds to appropriate parameters for blood donors, i.e., males prevailed in the samples (63%). With respect to age, the group mainly represents a cohort from 26 to 40 years (50%), followed by individuals of 40 to 50 years old (35.7%). The least numerous group was represented by persons 18 to 25 years old (14%). In Samara, Kirov, Rostov-upon-Don, and Nizhny Novgorod 161, 196, 347, and 817 persons respectively, were studied (Fig. 1). All these persons were active blood donors.

HLA Class I typing

Class A and B HLA typing was performed by serological techniques, in a standard microlymphocytotoxic test, using a panel of histotyping anti-leukocyte sera produced at Russian Institute of Hematology and Transfusion.

Statistical analysis

The prevalence of antigen (A) was calculated according to equation: **A=n/N**, where n is number of donors with given antigen, and N, total number of persons studied.

The prevalence of the gene (p) was determined by the following equation:

Formula 1.

$$p = 1 - \sqrt{1 - A}$$

where A is the prevalence of the appropriate antigen [4].

Haplotype frequency for the two genes (H) was estimated using the equation:

Formula 2.

$$H_{1,2} = p_1 \cdot p_2 + \Delta_{1,2}$$

where p_1 and p_2 are gene frequencies for HLA antigens, and $\Delta_{1,2}$ is an inter-allelic disequilibrium linkage.

The values of disequilibrium linkage ($\Delta_{1,2}$) were calculated accordingly, using a formula from a four-field table (2x2 tables) [5]

Formula 3.

$$\Delta = \sqrt{d / N} - \sqrt{b+d / N} \cdot \sqrt{c+d / N}$$

where a, b, c, and d comprise values in the fields of the tables;

N = a+b+c+d, the volume of sample;
a = + + (number of donors in the given sample in whom both antigens of the given haplotype are present);
b = + – (number of donors in the given sample in whom the first of two antigens of the given haplotype is present);
c = – + (number of donors in the given sample in whom the second of two antigens of the given haplotype is present);
d = – – (number of donors in the given sample in whom both antigens of the given haplotype are absent).

From: http://www.lib.utexas.edu/maps/commonwealth/russia.gif © public domain

To evaluate the statistical significance of differences for HLA antigen frequencies between the groups, we used χ^2 criterion [10].

Formula 4.

$$\chi^2 = \frac{(/a \cdot d - b \cdot c/ - 0,5 \cdot n)^2 \cdot n}{(a+b) \cdot (c+d) \cdot (a+c) \cdot (b+d)}$$

where
a is the number of donors in group 1 with a given antigen,
b is the number of donors in group 1 without this antigen,
c is the number of donors in group 2 with a given antigen,
d is the number of donors in group 2 without this antigen,
n, the numbers of persons in the group, /a•d – b•c/ – absolute value of difference, 0,5•n – correction for continuity in small samples.

The determination of the p level corresponding to the assessed χ^2 value was performed with computer software, taking into account one degree of freedom (df=1). With respect to the polyallelic nature of the HLA set, calculation of corrected p levels was carried out when testing the significance of differences for distribution of a given trait [8].

Formula 5.

$$P_{corr} = n \cdot p\,(1 - p)^{1-}$$

,where n is a number of traits studied.

χ^2 values exceeding 3,841 (corresponding to P<0,05), were regarded as a stable borderline index for significant difference between the frequencies in the groups compared.

Results

As seen in Table 1, the HLA class I study at the St. Petersburg Registry reveals that among locus A specificities, A2 and A3 (48.9% and 28.7% respectively) are the most common specificities. When analyzing distributions in locus B antigens for the same populations, B7 and B35 are frequently represented (26.8% and 20.9% resp.; see Table 2).

The common incidence of these genes is characteristic of the

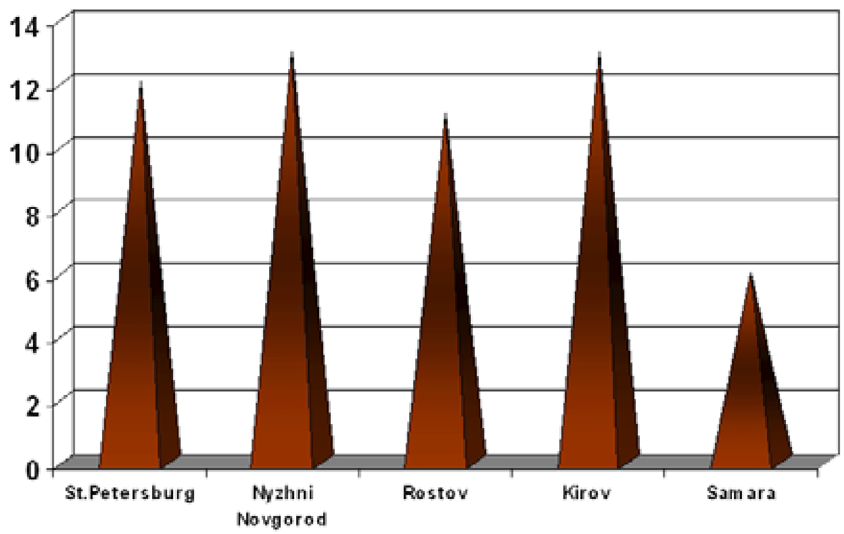
Table 1. Relative frequencies of HLA antigens (locus A) in various regions of Russia and Germany

Antigen	St. Petersburg n=1198	Germany n=13000	Samara n=161	Kirov n=196	Rostov-upon-Don n=347	Nizhny Novgorod n=817
A1	20.8²	28.9¹	28.8¹ ³	20.4² ³	20.2² ³	19.1² ³
A2	48.9	50.8	44.7	48.5	48.7	51.2
A3	28.7	27.9	28.6	30.1	25.6	26.4
A9* (A23,24)	23.7²	20.6¹	22.4	31.1¹ ² ³	26.2²	23.6² ³
A10* (A25,26)	19.7²	13.3¹	24.2²	18.9²	23.1²	19.2²
A11	11.8	10.3	14.9	17.3¹ ² ³	11.0³	11.9³
A19* (A29,30, 31,32,33)	16.4	22.3	19.3	22.8	15.9	17.7
A28	7.4	8.3	14.9¹ ² ³	2.5¹ ² ³	7.8³	10.8¹ ² ³

¹ - significant differences in antigen frequency between Russian cities and St. Petersburg (P<0.05),
² - significant differences in antigen frequency between Russian cities and Germany (P<0.05),
³ - significant differences in antigen frequency between Russian cities (P<0.05),
*- the data is provided for broad specificities, due to the incomplete nature of the split analysis data obtained from all the participating laboratories.

general European population [3]. However, if compared with the German Registry [6], which is among the largest in the Europe, one may see that in spite of a general similarity, the frequency of some genes differs quite significantly. It concerns such locus A specificities as A1 (20.8% in St. Petersburg vs 28.9% in Germany), A9 (23.7% vs 20.6%), and A10 (19.7% vs 13.3%).

Figure 2. Numbers of differences in frequencies of HLA-antigens between the donors from various regions of Russia, as compared with German donors



These differences are even more pronounced for locus B; for example B8, a quite common gene, occurs among Germans much more often than in the Russian Registry (20.3% and 12.4% resp.). Meanwhile, the frequencies of the B13 and B18 antigens that are rather common in St. Petersburg proved to occur half as often in the German population (11.7% vs 6.9%, and 14.9% vs 9.7%). The incidence of the B41 antigen was almost 3-fold higher in St. Petersburg than in Germany—thus suggesting a different distribution of some genes in Russia and Germany, in spite of the general similarity of both populations.

When studying haplotype incidence for A and B loci (Table 3), we have shown that the following haplotypes are the most common in the St. Petersburg Registry: A2-B7 (4.25%), A2-B35 (3.44%), and A3-B7 (3.31%); whereas A11-B35 and A25-B18 proved to be more rare (0.13% and 1.11% resp.).

A comparative study of haplotype frequencies for two genes from the A and B loci in the Russian and German Registries (Table 3) has shown that their incidence is sufficiently different: the most common in St. Petersburg, the A2-B7 haplotype, is at the 5th position in the German sample, whereas the A1-B8 haplotype, which occurs most commonly in Germany and in a majority of European countries, occupies only 9th position in the population of St. Petersburg.

The absence of certain highly prevalent haplotypes is another important feature of the Russian Registry, thus being indicative for a more pronounced genetic heterogeneity in the local population, i.e., neither of the most common haplotypes in St. Petersburg is over-represented, as compared to the German population with 8.29% for A1-B8, 5.73% for A3-B7, or 4.65% for A2-B44. In St. Petersburg, the most common A2-B7 haplotype was found in 4.25% of cases.

Our results suggest that the distribution of HLA genes and haplotypes of the MHC complex in the St. Petersburg Registry shows gross similarities to the gene distribution in Western Europe. The St. Petersburg Registry, however, displays some specific features due to an altered frequency due to some antigenic specificity and more pronounced genetic heterogeneity among the populations represented in the Registry.

We analyzed the opportunities for unrelated donor matching for 52 patients with oncohematological disorders treated at the Institute clinics. Comparisons of phenotypically determined HLA sets for the patients with those represented in the donor Registry showed that HLA-A,B-compatible donors were found for 48% of the patients. When based on the patient's phenotype, the ratio of class I-compatible donors for each patient proved to be 1 to 14, thus providing evidence in favor of finding a donor for the patients even within a relatively small registry of potential donors from the same region.

When comparing the frequencies of loci A and B specificities among the donors from Russian regions and Germany, the most pronounced differences, both for single antigens and haplotypes, were revealed between the general cohorts from the German Registry and the Russian Republican Registry (Fig. 2, Tables 2, 3).

That is, the most similar genetic characteristics are expressed in the donors from St. Petersburg and Nizhny Novgorod (Fig. 3).

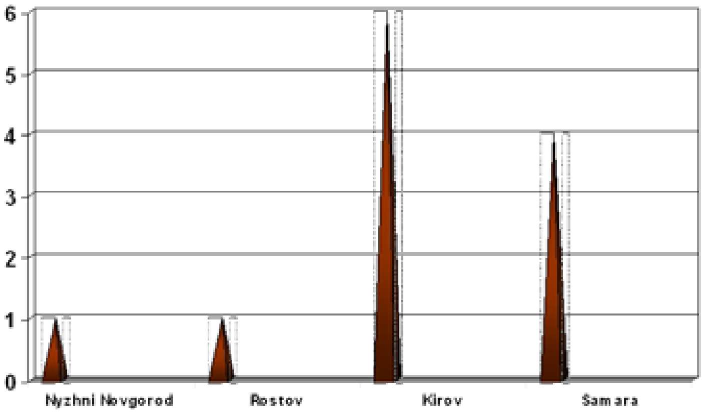
Donors from the Samara Region are, for some characteristics, closer to German donors (Fig. 2), whereas the persons from Kirov possess some markers that are typical to Northern folk. Donors from Rostov-upon-Don represent a highly heterogeneous

Table 2. Relative frequencies of HLA antigens (locus B) in various regions of Russia and Germany

Antigen	St. Petersburg n=1198	Germany n=13000	Samara n=161	Kirov n=196	Rostov-upon-Don n=347	Nizhny Novgorod n=817
B5* (B51,52)	12.6	13.1	16.8	13.7	16.7	15.3
B7	26.8	25.4	24.2	26.0	23.3	22.2
B8	12.4[2]	20.3[1]	27.3[1,2,3]	7.1[1,2,3]	12.1[2,3]	11.4[2,3]
B12* (B44,45)	18.0	23.6	14.3	17.3	18.7	21.5
B13	11.7[2]	6.9[1]	8.7[3]	17.9[1,2,3]	12.4[2]	11.8[2]
B14	3.8	4.7	2.5	4.1	4.9	5.6
B15* (B62,63)	11.8	16.0	8.7[2]	12.2	9.5[2]	8.3[2]
B16* (B38,39)	11.8[2]	8.0[1]	11.8[2]	16.8[1,2]	13.3[2]	10.4[2]
B17* (B57,58)	8.2	7.7	9.9	3.5[1,2,3]	8.1[3]	7.5[3]
B18	14.9[2]	9.7[1]	16.8[2]	13.2[2]	16.1[2]	12.5[2]
B21* (B49,50)	2.8[2]	4.8[1]	7.5[1]	4.6	5.2[1]	5.3[1]
B22* (B54, 55,56)	4.2	4.4	3.7	5.1	5.2	3.3
B27	10.8	8.7	11.2	15.8[1,2]	10.4	11.3
B35	20.9	18.5	19.3	20.4	18.7	23.1
B37	1.3[2]	2.6[1]	3.1[3]	1.0	0.9[2]	0.5[2,3]
B40* (B60,61)	11.2	13.9	14.9	9.7	10.4	10.2
B41	4.7[2]	1.8[1]	1.9[3]	4.6[2]	4.6[2]	5.6[2,3]

[1] - significant differences in antigen frequency between Russian cities and St. Petersburg (P<0.05),
[2] - significant differences in antigen frequency between Russian cities and Germany (P<0.05),
[3] - significant differences in antigen frequency between Russian cities (P<0.05),
*- the data is provided for broad specificities, due to the incomplete nature of the split analysis data obtained from all the participating laboratories.

Figure 3. Number of differences in frequencies of HLA-antigens between the donors from Russian regions and St. Petersburg



population with respect to their nationality, since this region is inhabited by Russians, as well as Ukrainians, Armenians, Greeks, etc. However, their immunogenetic features tend to be more similar to other representatives of the Russian Republican Register than to the German population. As seen in Table 3, containing a synopsis of 10 most common haplotypes, the A2-B7 haplotype is encountered most often in St. Petersburg, as well as in Rostov-upon-Don and Kirov. In Nizhny Novgorod, however, it holds 2nd position, whereas in Samara Region, a European A1-B8 haplotype is clearly prevalent (Fig. 4).

This data confirms the need for an urgent expansion of the Russian Register for potential HSC donors, since the probability of finding a donor for Russian patients becomes sufficiently higher when the search is performed in a regional Registry. Taking into account the significant rate of current migration to Germany, a closer working cooperation would be desirable between the Russian and German Registries.

Discussion

HLA polymorphism has evolved since the very origin of human beings. Its evolution was influenced by many factors, as the immune system has undergone its development for effective protection against the invasion of potential pathogens, i.e., from viruses to pathogenic worms.

The ability for a rapid response to single pathogens is mediated by the adaptive immune system, including an appropriate scale of host responses provided by the HLA molecules, together with T and B cell receptors. The type of pathogens existing in the various geographic regions inhabited by different human populations

Table 3. Relative frequency of HLA haplotypes in various regions of Russia and Germany

Haplotype	**St. Petersburg**	**Germany**	**Samara**	**Kirov**	**Rostov-upon-Don**	**Nizhny Novgorod**
A2-B7	**4.25**	3.67	2.99	**4.02**	**4.10**	3.39
A2-B35	3.44	1.32	1.45	2.53	1.83	3.30
A3-B7	3.31	5.73	3.15	3.10	2.78	2.47
A2-B44	3.08	4.65	1.64	3.37	2.55	**3.92**
A3-B35	2.78	3.02	1.88	2.87	1.23	2.28
A2-B18	2.32	1.27	1.46	1.56	2.37	2.05
A24-B7	1.99	1.34	2.14	2.54	1.63	1.37
A2-B62	1.88	4.23	1.27	2.02	1.65	1.51
A1-B8	1.83	**8.29**	**4.38**	1.08	2.18	1.32
A2-B27	1.83	1.87	1.97	2.60	1.63	1.99
A2-B13	1.79	1.17	2.04	2.79	3.09	1.68
A2-B51	1.75	2.04	2.11	1.85	1.91	1.71
A24-B44	1.15	1.12	0.68	1.80	0.83	1.21
A25-B18	1.11	1.46	1.19	0.61	0.92	0.57
A11-B35	0.13	1.86	1.05	1.26	0.28	0.82

may determine the selection of specific HLA gene products. Gene conversions, i.e., substitutions of existing nucleotide sequences of HLA genes with short segments from other HLA alleles or loci within the HLA complex, comprise the main source of biodiversity created within relatively short timelines (e.g., several centuries). This process reconstitutes an HLA gene repertoire for effective protection against pathogens that exist in steadily renewing environment.

Hence, the combined set of HLA gene products expressed in each human population is, mainly, a result of interactions with the flora and animals of the region inhabited by the population over centuries and millennia. Some cases of linkage disequilibrium observed with certain HLA genes reflect evolutionary advantages of appropriate gene-gene interactions.

A history of people inhabiting the region comprises the second important factor that determines the divergence of genetic features between the populations (the so-called „ancestor effect“). For example, A1-B8 is the most common haplotype in the German Registry. As suggested by G. Rodey [9], this genetic feature could be traced back to the Goths and ancient German tribes, whereas the A2-B7 haplotype (the most common in the St. Petersburg Registry) is typical for Vikings. This assumption seems quite probable, due to the close relations between North-Western Slaves and Vikings. The increased frequency of the A1, B8 haplotype in Samara may be due to the mass migration of Germans to the Volga Region, which has taken place since the times of Catherine the Great of Russia in XVIII century (Fig. 4).

As far as the broad phenotypic diversity that is characteristic of the Russian population is concerned, this could be directly connected with the vast habitation areas of Russian people and sharing these territories with other national groups.

Summarizing the above results, we may state that a search for donors for recipients living in other regions is associated with significant difficulties, irrespective of the Register size. However, such a search would be facilitated if the donor and the recipient originate from the same region.

Conclusions

1. When performing a comparative analysis of St. Petersburg and German Registries with other regional Russian Registries, we have revealed certain differences in frequencies of HLA genes and haplotypes (A and B loci).

2. The probability of a successful search for a histocompatible HSC donor is higher for a Registry in the country of recipients' origin.

3. There is a need for the urgent expansion of the Russian HSC Donor Registry.

4. International cooperation between the Registries is necessary, especially when searching for donors for migrants from abroad.

References

1. Bubnova L.N., Beliaeva E.V., Berkos A.S., Nikolenko V.N. A registry of bone marrow donors. Transfusion Medicine 1995, №5, p.14-18. (In Russian)

2. Cleaver S. Donor work-up and transport of bone marrow recommendations and requirement for a standardized practice. Bone Marrow Transplantation 1997;20:621-629.

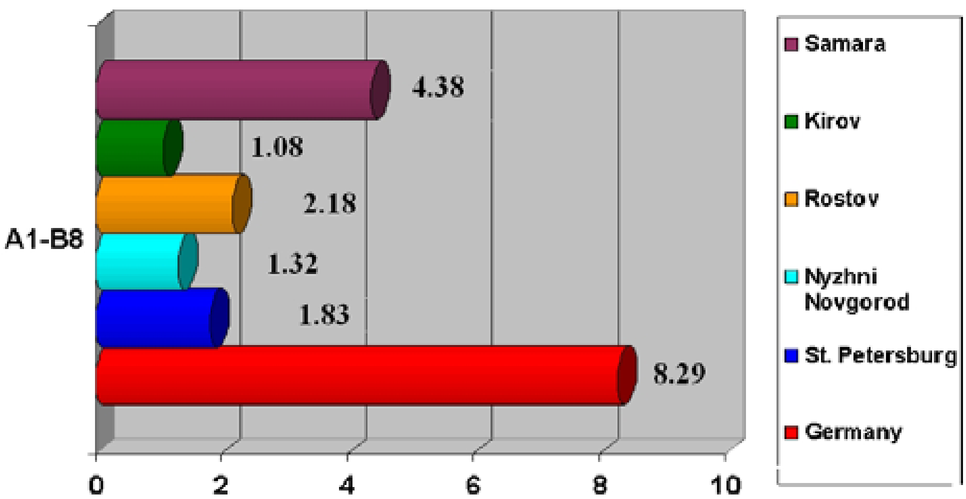
3. Goldman John M. Special report: Bone marrow transplants using volunteer donors-recommendations and requirements of a standardized practice throughout the world (1994 update). Blood 1994;84:2833-2839.

4. Haldane J.B. The estimation and significance of the logarithm of a ratio of frequencies. Ann Hum Genet 1956;20:309.

5. Mattius P.L., Ihde D., Piazza A., Cepellini R., Bodmer W.F. New approaches to the population genetic segregation analysis of the HLA system. In: Terasaki P.I. (ed): Histocompatibility Testing, 1970. Copenhagen: Munksgaard, 1970.

6. Müller C, Ehninger G., Goldmann S. Gene and haplotype frequency for the loci HLA- A, HLA- B, and HLA- DR based on over 13,000 German

Figure 4. Frequency of A1, B8 HLA haplotype among donors in Germany and various regions of Russia



Blood Donors. Human Immunology 2003;64,1:137-151.

7. Marsh S., Albert E., Bodmer W., et al. Nomenclature for factors of the HLA system, 2004. Tissue Antigens 2005:65,4:301-370.

8. Pevnitsky L.A. Statistical evaluation of associations between HLA antigens and diseases. Vestnik RAMS. 1998, №7, p.48-51. (In Russian)

9. Rodey G. HLA Beyond tears. introduction to human histocompatibility. Durango, CO : De Novo Distributed by Pel-Freez, 2nd edition, 2000.

10. Svejgaard A., Ryder L.P. HLA and disease association: Detecting the strongest association. Tissue Antigens 1994;43:18-27.

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Сравнение частоты распределения антигенов и гаплотипов локусов у доноров гемопоэтических стволовых клеток россиийских регионов и Германии

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Резюме

Полиморфизм генетической системы HLA исключительно высок, поэтому поиск двух индивидуумов, обладающих идентичными генетическими характеристиками, связан со значительными трудностями. В то же время, успех трансплантации аллогенных неродственных ГСК прежде всего связан с генетической близостью реципиента и донора. Республиканский регистр, объединяющий базы данных, содержащих сведения о типированных донорах Российского и Кировского НИИ гематологии и трансфузиологии, станций переливания крови Нижнего Новгорода, Ростова-на-Дону, Самары, Первоуральска в течение нескольких лет сотрудничает с немецким регистром Стефана Морша, осуществляя взаимный поиск доноров для больных гемобластозами. При исследовании установлено, что наиболее выраженные различия как частот встречаемости отдельных антигенов, так и гаплотипов, обнаружены между представителями Регистра Германии и Республиканского Регистра в целом. Наиболее близкими друг другу генетическими характеристиками обладают доноры Санкт-Петербурга и Нижнего Новгорода. Доноры Самарского региона по отдельным характеристикам ближе к донорам Германии, а доноры из Кирова обладают рядом признаков, характерных для северных народов. Эти данные подтверждают необходимость срочного расширения нашего Регистра потенциальных доноров ГСК, поскольку вероятность нахождения донора для жителя России значительно увеличивается, если поиск осуществляется в собственном регистре.

Ключевые слова: гемопоэтические клетки, регистр доноров, HLA, типирование, генотип, гаплотип, аллогенная трансплантация гемопоэтических клеток, регионы России, Германия