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**IMMUNOGENETIC PECULIARITIES AND PREVALENCE CARRIERS OF THE VIRUS
EPSTEIN-BARR, CYTOMEGALOVIRUS, INFECTION HELICOBACTER PYLORI
AMONG THE POTENTIAL DONORS OF HEMATOPOIETIC STEM CELLS**

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Summary: The article presents the results of research immunogenetic and population characteristic of bone marrow donors kirghiz nationality from the National Registry of hematopoietic stem cells Kirghizia in comparison with healthy residents in the North-Western region of the Russian Federation of the Russian Register of bone marrow.

These studies are being conducted for the first time and at potential donors kirghiz nationality and revealed significant HLA-markers characteristic of the kirghiz nation, as well as in the study of prophylactic virus have been identified Epstein -Barr 26% and 39% of cytomegalovirus in the cases and found elevated titers of immunoglobulin Ig E, Ig A (k, λ) and Ig G (k, λ) light chains. And have a highly significant difference.

Thus, the detected distinctions of genetic features major histocompatibility complex, should take into account of occurrence the frequency genes of HLA-loci (A and B) and locus DRB1* compared with the control population group. Extremely important, detection of immune-selective methods donor on the antigens complex HLA-alleles 1 and II classes, where identity is of the bone marrow of the donor and the patient testifies to their full compatibility. In the presence of HLA-identical donors is applying allogeneic hematopoietic stem cell transplantation in oncohematologic pathology, autoimmune diseases. Given, the hide carriers of the Epstein-Barr virus, cytomegalovirus, elevated titers of isotopes of heavy and light chains of immunoglobulins raises the question of the need for timely quality selection of potential donors bone marrow. When planning unrelated, related transplantation, it is necessary study of modern features the health status bone marrow donors.

For the qualitative selection of the donor - to improve the security algorithm bone marrow donor.

At the present stage, of the bone marrow transplantation gives the best results for the survival increase in the treatment malignant diseases.

The multinational people of Kirghizia, he is one and united, but for the treatment of oncohematological malignancies, autoimmune diseases, and in the planning of a bone marrow transplant must to find qualitative a compatible donor and make the selection based since blood group, Rh factor and ending with on ethnic, genetic indicators. On the basis genetic and ethnic characteristics is easier to find a donor, especially from whence arrived (by birth) patient.

Key words: HLA, hemopoetic stem cells, viruses, immunoglobulins, donors, kirghiz nation.

Introduction

Currently, bone marrow transplantation finds is more widely used and is one of the high-tech methods of therapy, both abroad and in our country in the treatment of oncohematological malignancies, with a generalized of autoimmune diseases, as well as immunodeficiency states.

It is well known, for a bone marrow transplant, the choice of a related donor (HSC) hematopoietic stem cells has substantially limited and antigens in distribution system HLA- alleles varies considerably among ethnic peoples and nationalities.

However, not all ethnic peoples can find a compatible donor hematopoietic stem cells in the European

and American registers, that they required the creation of their own registers of bone marrow donors, such as for example of China, Japan [1].

Since HLA-antigens are the recognized immunogenetic markers of many diseases and genetic the indicator of criteria predisposition to oncological diseases, identification of factors affecting the course of the disease and the prognosis - it is an urgent task for current medical science.

In [6], described the disease with the formation of the immunoglobulins heavy chains on clinical picture resembles lymphoma. It usually men, the peak incidence occurs in the period from 60 to 70 years. The first manifestations is the swollen lymph nodes, fever and anemia. In the bone marrow cells found lymphocytes the combination with plasma cells, frequently observed eosinophilia, serum protein electrophoresis reveal M gradient in zone α or b-globulins.

And also by data the author [2] observes, that in Kirghiz Republic the incidence cancer of stomach greatly is high and he went to first place in the structure of cancer among the of Countries Independent States.

In this connection, on the basis of these data and the opportunity consider to hidden the currents of similar diseases, it is important to consider when planning for unrelated transplantation and for quality transplant, where potential donors should to conduct research on the isotypes of heavy and light chains immunoglobulins, infection *Helicobacter pylori* with prophylactically aim.

It is well known that one of the main factors of poor prognosis of the underlying disease is the joining infections.

Infections play a key role in the development of whole series of diseases, and represent is the topical to for study the prevalence of herpes - viral infections, in particular caused by Epstein-Barr virus and cytomegalovirus

Performing bone marrow transplantation requires strict adherence to safety standards in accordance with government regulatory documents governing the activities of the center of a single republican register of bone marrow donors, where the conducted for of the necessary list of studies using specific and high-tech methods of the laboratory diagnostics [3].

In this regard, the urgent task for receiving monitoring data is required create modern information tests with obtain reliable information and to explore modern features states health of donors in preparation for the planning unrelated bone marrow transplantation.

It should be borne in mind that the information on the incidence of antigens in the systems of HLA-alleles, the content of free chains immunoglobulins and the prevalence of carriage of herpes - virus infection among different ethnic nationalities and ethnic nationalities is scarce and contradictory.

The aim of our study was to study of immunogenetic characteristic and prevalence the hidden

carriage Epstein-Barr virus and cytomegalovirus, infection *Helicobacter pylori* among healthy population - potential donors of hematopoietic stem cells Kirghiz nationality.

MATERIALS AND STUDY METHODS:

In the group study included 127 healthy potential donors Kirghiz nationality from the National Register of hematopoietic stem cells Kirghizia in St. Petersburg to study the immunological, genetically and population characteristics, typing of was performed in the laboratory immunohaematology Russian Research Institute of Hematology and Transfusiology with 1998 on 2009 years on system HLA-alleles I class loci (A and B), of them 78 donors typing of on antigens HLA -alleles II class locus DRB1*, aged from the 18 to 45 years.

The obtained data were compared with a control group - 4232 donors of bone marrow from register Russian Research Institute of Hematology and Transfusiology [5]

As well as with 2004 on 2015 years 80 healthy donors Kirghiz ethnic population investigated at the of hide carrier virus Epstein-Barr and cytomegalovirus in laboratory of molecular microbiology, St. Petersburg Institute of Epidemiology and Microbiology named after L.Pasteur.

The control group was -79 healthy residents in the North-Western region of the Russian Federation of St. Petersburg.

Typing of the 80 healthy donors Kirghiz nationality with 2004 on 2015 years in the laboratory hybridoma technology TSNIRRI on the isotypes of heavy and light chains of immunoglobulins. At donors with elevated titers of immunoglobulin identified antibodies Ig G to infection *Helicobacter pylori* in laboratory bacterial drop infections St. Petersburg Institute of Epidemiology and Microbiology named after L.Pasteur.

TYPING OF HLA-ANTIGENS I CLASS:

Determination of HLA- loci (A and B) phenotypes of surveyed persons was carried out serological method using a panel of sera glistotipiruyuschih antilekotsitarnyh Russian Research Institute of Hematology and Transfusiology, allowing to define the locus of antigens 17 "A" and 27 "B" the locus of antigens.

TYPING OF HLA-ANTIGENS II CLASS:

Molecular typing of HLA- genes a locus DRB1* was performed by the polymerase chain reaction using a set of primers domestic firm "DNA-technology"(Moscow) allows to select 13 groups of alleles HLA-DRB*1 (basic resolution). Genomic DNA was isolated of peripheral blood mononuclear cell (fresh or frozen at -20°C), stabilized with sodium citrate or EDTA(final concentration 0.5% of anticoagulant), by using a set reagents "NPF DNA technology"(Moscow), or using immunomagnetic method recruiting firm «Dynal» All stages of amplification were conducted on a thermocycler

"TERTSIR" DNA technology", Moscow). The product obtained in the amplification determined by the method horizontal electrophoresis in 3.2% of agarose gel with visualization under ultraviolet light $\lambda = 320$ nm. The specificity of the amplification product were compared with standard marker of DNA lengths PUC-09.

THE RESEARCH OF IMMUNOGLOBULINS:

Achieved the by direct immunoassay, paraproteins were immobilized on a solid phase of sera diluted 1:105 and 1:106 in 0.1 M carbonate-bicarbonate buffer pH-9,5. Isotypy types of heavy chains and light chains of myeloma immunoglobulins conjugates were determined in using monoclonal antibodies (mAbs) horseradish peroxidase.[4].

DETERMINATION OF THE AMOUNT ANTIBODIES OF IgG TO HELICOBACTER PILORI:

In order to determine the amount of Ig G to Helicobacter pylori by enzyme immunoassay, analysis expressed in international units, was used in this test-system "ImmunoKomb II H. pylori Ig G». The results were evaluated on a scale attached to the test system. On antibody levels serum distributed by the respective groups. 0-19 U / ml - negative, 20-39 U / ml - a low level of antibodies, 40-90 U / ml - the average (middle) levels of antibodies, 91-120 or more U / ml - high levels of antibodies

DEFINITION OF VIRUS EPSTEIN-BARR AND CYTOMEGALOVIRUS :

Investigation of blood plasma virus Epstein - Barr and cytomegalovirus of the method (PCR) polymerase chain reactions in the diagnosis used "AmpliSens™" PCR test system of production Institute for Epidemiology Public Health Ministry (license №15 / 002-L / 02) Ministry of Industry and Technology of the Russian Federation on the production of immunobiological drugs. Typing of was performed by polymerase chain reaction in the laboratory of molecular biology FBUN NIEM St. Petersburg behalf of the Pasteur.

Statistical processing of the results included the analysis of standard criteria χ^2 -square, which was used to assess the significance of differences in the occurrence of certain characteristics between the control group and the test group. Determination of the "p", the corresponding value found. χ^2 -square performed considering one degree of freedom.

Statistical analysis of the studies were carried out using the application package for the spreadsheet - "Microsoft - Excel™ version 7.0 for Windows 95, for Windows-based 2010, Statistica-5.

RESULTS AND DISCUSSION:

Donors have regarded themselves as healthy people, they said, that at me nothing does not hurt, but at the poll we noted in anamnesis a lot of chronic disease: the most common as chronic bronchitis

(smoker) in remission, chronic gastritis in remission, chronic tonsillitis, chronic otitis media and so forth. In connection with what we explore and on immunogenetic indicators and on virus infections.

Our studies among a small group of volunteer potential donors of hematopoietic stem cells kirghiz nationality in comparison with healthy donors residents of the North-West region of the Russian Federation from the Russian register of bone marrow [5], on the results of data revealed significant and important differences on the frequency occurrence of antigens HLA-alleles I and II class.

Table 1

The frequency occurrence of antigens HLA-alleles I class in healthy donors kirghiz nationality and of the healthy residents North-Western region Russian Federation from the Russian register of bone marrow donors.

HLA-loci (A and B)	Russian register of bone marrow donors $n = 4232$	donors kirghiz nationality $n = 127$
A11	11,6%	28,6% ***
A24	20,9%	53,1% ***
A26	10,1%	20,4% **
B37	1,3%	8,2% ***
B48	0,0%	4,1% ***
B51	9,3%	30,6% ***
B57	4,5%	26,5% ***
B63	0,2%	2,0% ***

note ** — $p < 0,05$, *** — $p < 0,001$

From the Table 1 shows that for healthy ethnic populations kirghiz nationality characteristically is more high occurrence of antigens HLA-A24, HLA-A11, HLA -A26, HLA-B51, HLA -V37, HLA-B48 and HLA-B57, HLA-V63. In this case, the allele HLA-B37, HLA-B48 and HLA-V63 is extremely rare in the comparison group.

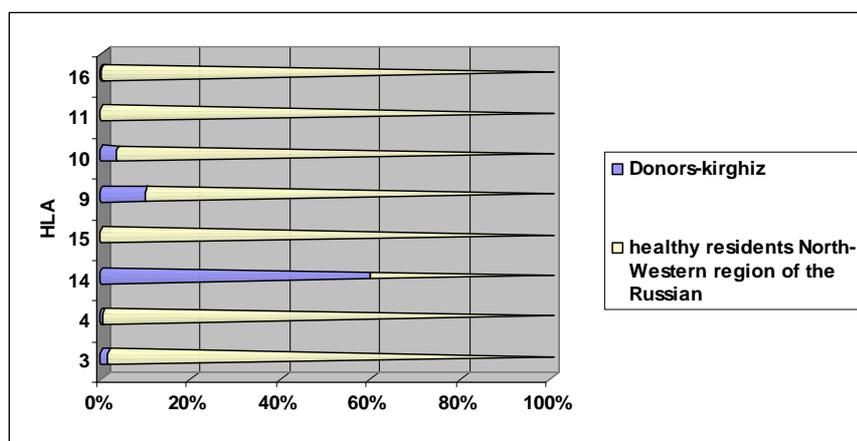


Figure 1. Comparative analysis of the occurrence frequency the antigens HLA- locus DRB1* II class healthy donors kirghiz nationality and healthy residents in the North-Western region of the Russian Federation from the Russian register of bone marrow donors.

According to the results of our study by comparing the incidence of the antigens alleles HLA- locus DRB1 * II class in healthy donors kirghiz nationality and healthy residents in the North-Western region of the Russian Federation from the Russian register of bone marrow donors as shown in Figure1, that in healthy donors kirghiz nationality rarely detected gene HLA-DRB1*10, almost in a few cases, revealed gene HLA-DRB1* 03 and the most common antigens HLA-DRB1*14, HLA-DRB1* 09 and there are highly statistically of significant differences $p < 0,001$.

It should be noted, that most important antigens HLA-DRB1*04, HLA-DRB1*15, HLA-DRB1*11, HLA- DRB1*16 for a healthy population of kirghiz nationality are rarity.

Table 2

The content of free chains immunoglobulins in serum blood at healthy donors kirghiz nationality

Donors- kirghizs n =80	пол	Age	κ-цепи (N=6-13mk/ml)	λ-цепи (N=0,2-1,3 mk/ml)	higher indicators content Ig (mlg %)	higher antibodies IgG in Helicobacter pylori (N= 0 -19 U/ml)
1	male	29		2,0 mk/ml	IgA-445 (N= 44-440)	negative result
2..	male	36		1,4 mk/ml	IgG- 1659	negative result
3. .	male	34	16 mk/ml		IgG -1643 (N=600-1640)	120 U/ml- высокий уровень антител
4. .	male	45	19 mk/ml		IgA-454	270 U/ml- высокий уровень антител
5.	male	22			Ig E-448	110 U/ml- высокий уровень антител

By us also investigated 80 of health of potential donors of hematopoietic stem cells kirghiz nationalities, which consider themselves practically healthy people on content of free chains of immunoglobulins in the blood serum. Were detected between the ages of 29 - 45 years of higher titers of immunoglobulins type IgE at one man and type of Ig A (κ, λ) chains in 2 men and in 2 men Ig G (κ, λ) chains. Additionally donors conducted common clinical studies: complete blood count with platelet count counting, urinalysis, total protein and its fractions, radiography of the skull, liver function tests, transaminase (ALT, AST), as well as on Epstein - Barr virus, cytomegalovirus and on the infection Helicobacter pylori. Is established that 2(two) of men with high titers of immunoglobulin type Ig Aλ - 445 мЛг% (Normaly 44-440 мЛг%)λ chain -2,0 mk/ml and type of Ig Gλ-1659мЛг% (Normaly 600-1640)λ chain -1,4 mk/ml(Normaly 0,2-1,3 mk/ml), as well as had elevated titers transaminases, Epstein - Barr virus and at 3-x men with elevated of immunoglobulin type Ig Aκ- 454 мЛг% κ chain -19 mk/ml (Normaly 6 -13 mk/ml), type Ig Gκ -1643мЛг% κ chain-16 mk/ml and type Ig E-448 mk/ml(Normaly

1-90 mk/ml), positive results is it the high levels of antibodies 100 to 270 U/ml (Normaly1-19 U/ml) on the infection *Helicobacter pylori*.

Herewith in anamnesis at 2(two) of donors detected chronic enterocolitis and earlier moved in childhood viral hepatitis, at men with increased titles of type IgE found food allergy and atopic dermatitis. All four donors on the moment of the survey suffered from chronic pyelonephritis and in the general analysis of urine showed traces of protein.

On this basis, that to from this it can be said that among the healthy donors in prevention studies in analysis detected high titers immunoglobulins type of Ig A, Ig G with elevated (k, λ) chains (Table 2). Nevertheless donors are in the database of the National Register of hematopoietic stem cells Kirghizia in St. Petersburg, but relieved of donation and are under observation a therapist, gastroenterologist with the passage of esophagogastroduodenoscopy (EGDscopy) with biopsy (from different plots a maximum with 5(five) seats of the biopsy), at nephrologists, of the allergist, dermatovenerologa. The results of the study on carriage of Epstein -Barr and cytomegalovirus donors kirghiz nationality and residents of the North-West region of the Russian Federation presented in table 3.

Table 3.

Comparative evaluation of the incidence of carriers of the virus Epstein - Barr virus, cytomegalovirus in healthy donors kirghiz nationalities and healthy residents in the North-Western region of the Russian Federation.

carriers infection	donors kirghiz nationality n = 80	residents North-Western region of Russian Federation n =79
virus Epstein-Barr	26 %**	58 %
cytomegalovirus	39 %**	62%

From the table 3.shows that, the frequency of carriers of the virus Epstein - Barr virus and cytomegalovirus at the healthy donors kirghiz nationality and healthy residents in the North-Western region of the Russian Federation significantly different among donors kirghiz ethnic Epstein-Barr virus detected in 26%, and cytomegalovirus in 39% cases. Clinically of carriers of the virus was not shown. The received reliable significant differences ($p < 0.001$) between donors Kirghiz nationality and the control groups.

It should be noted, that the findings is the basis for the recommendations in-depth survey of donors with purpose of right their selection at the planning of unrelated bone marrow transplantation.

CONCLUSION:

Thus, as a result of the research, in the distribution of HLA- alleles 1 class found, that most are often found at the donors kirghiz nationalities antigens HLA locus A- HLA -A11, HLA-A24, HLA-A26, in HLA locus B- HLA- B51, HLA- B57, HLA- B37, HLA- B48, HLA-B63 and the most

common in the distribution HLA -alleles of locus DRB1* is genes HLA -DRB1*14, HLA- DRB1*09, somewhat less gene HLA-DRB1*10 and practically in a few cases the gene HLA- DRB1 * 03.

Identified the differences in the distribution of antigens HLA-alleles, elevated titers of immunoglobulin (k, λ) light chains, the presence of a carrier of pathogens of viral infection, you need to consider when planning for closely related, unrelated bone marrow transplantation at malignant diseases.

Potential donors bone marrow, addition for of selection criteria of necessary, determine the presence of a carrier of the virus Epstein -Barr, cytomegalovirus and the content of free chains of immunoglobulins in the blood serum, in order to prevent complications in post transplantation periods. At the present stage, for a successful bone marrow donor search is necessary to expand and develop a database of about potential donors hematopoietic stem cells.

However, close cooperation with registers bone marrow other countries - this is the most effective the direction of conversion bone marrow transplantation in one of the high-tech methods of treatment.

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