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# ANALYSIS OF THE RELATIONSHIP OF THE HLA-PHENOTYPE WITH THE LEVEL OF IMMUNOREACTIVITY OF THE ORGANISM

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**Annotation**
An analysis of the relationship between the HLA phenotype of the studied individuals and the studied parameters of the immune status made it possible to establish the association of the HLA-DR4 and DR5 antigens with the level of the circulating pool of T-lymphocytes, A30- with the content of NCC in the peripheral blood, the presence of the HLA-antigen B35 in the phenotype of the studied individuals was associated with a reduced content and HLA-A30-with a high content of the main classes of serum immunoglobulins. The conducted studies indicate the role of HLA-associated genetic control in the level of immunoreactivity of the studied population. For the first time, HLA markers were identified that characterize the quantitative imbalance of the immune system.

**Key words:** HLA, markers, antigens, immune response, cytokines**.**

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## Introduction

As is known, HLA antigens of two classes, participating in the processes of immune response, not only provide immune homeostasis of the body, but also participate in broader biological processes, linking the immune system with other systems of the body, and thereby provide cellular interaction during any physiological processes [1, 3].

In this regard, studies of the population aspects of the HLA-associated genetically determined level of immunoreactivity seem to be very relevant, since they would contribute both to clarifying modern ideas about the biological role of Major histocompatibility complex system and to developing a number of regional issues of practical health care [4,5].

## MATERIAL AND RESEARCH METHODS

### Characteristics of the Examined Material

To achieve the set goals and objectives, we examined 86 practically healthy, unrelated residents of the city of Samarkand and the Samarkand region, Uzbek nationality, aged 17 to 52 years. Immunological, immunogenetic and biochemical research methods were carried out in 86 individuals in the city clinical AIDS center. Typing of HLA class II antigens (DR) was carried out in 82 examined individuals.

### Immunogenetic Research Methods

Immunogenetic research methods were carried out on the basis of methodological recommendations set out in the guidelines of Yu. M. Zaretskaya (1983) and V. I. Konenkov (1999) [9].

Therefore, in our work, the HLA phenotype was established in a standard microlymphocytotoxic test (Terasaki P. I., 1964) using a panel of HLA antiserum of the St. Petersburg Research Institute of Hematology and Blood Transfusion. When conducting the microlymphocytotoxic test, the recommendations of the NIH (National Institute of Health, USA) were taken into account, according to which, when digging lymphocytes into the wells of the Terasaki plate, the “shooting” technique was used, which largely ensured the purity of the “readable” wells.

### Immunological Research Methods

When studying the state of the human immune system, methods are used to quantify the main indicators of the immune status: the percentage and absolute content of total, T- and B-lymphocytes, the percentage of regulatory subpopulations of T-lymphocytes (T-helpers / inducers and T suppressors / killers, natural killers (NK)

and phagocytic activity of neutrophils (FAN). At the same time, the state of the immune system cannot be assessed without taking into account the functional activity of T- and B-lymphocytes. To study functional parameters, they use response of lymphocytes to T-cell mitogens (PHG-phytohemagglutinin or ConA) in the reaction of lymphocyte blast transformation (RLBT) or inhibition of leukocyte migration (RILM), as well as determination of the content of the main classes of immunoglobulins A, M and G in blood serum. Quantitative indicators of the immune status used in this study are included in the standard set of immune status assessment by tests of I and partially II levels, in accordance with the methodological recommendations developed by the Institute of Immunology of the Ministry of Health of the Russian Federation and the Institute of Immunology of the Academy of Sciences of the Republic of Uzbekistan [8].

Functional parameters were determined using the reaction leukocyte migration inhibition test (RLMIT) in micromodification [8]. The method for determining the number of T-lymphocytes, originally proposed by Jondal (1972), is based on the currently well-established ability of the pan-T-cell antigen (CD2) to interact with ram erythrocytes with the formation of the so-called rosettes, consisting of a central lymphocyte connected to ram erythrocytes. Relative content of immunoregulatory subpopulations T-lymphocytes were determined using monoclonal antibodies CD4, CD8, CD16 and CD19, produced by the Institute of Immunology of the Ministry of Health of the Russian Federation (Moscow), by the method of rosette formation with human erythrocytes of the 0 (I) blood group, sensitized in vitro with the corresponding monoclonal antibodies [9]. The phagocytic activity of peripheral blood neutrophils (PBN) was determined by their ability to absorb melamine latex particles 1.2-1.5 microns in size (produced by the All-Russian Research Institute of Biological Instrumentation, Moscow).

The assessment of the functional state of the T-system of immunity was carried out in the reaction of inhibition of leukocyte migration (RILM) by the micromethod in a closed capillary [6, 8].

Determination of the content of immunoglobulins in the blood serum of the main three classes A, M and G was carried out by the method of radial immunodiffusion according to Mancini (1963).

The assessment of the immune status of the general population group was carried out in a comparative aspect with the level of similar indicators typical for the population of Tashkent and Tashkent region (norm according to the Institute of Immunology of the Academy of Sciences of the Republic of Uzbekistan (1998-2001). Considering that immune protection is implemented by three effector links - natural protective factors,

cellular and humoral, the characteristics of the immune status of the examined population are given according to these conditional divisions. We investigated the distribution of HLA antigens, genes and haplotypes in a healthy Uzbek population of the Samarkand region using the most complete set of typing reagents.

The results of studies of the frequencies of HLA antigens and genes in the examined population of 86 practically healthy individuals are presented in (Table 1) As shown by the analysis, in locus A, antigens A2 (31.4%), A9 (32.4%), A19 (38.4%) are found with the highest frequency. The HLA-A10 antigens were detected in the phenotype in 25.6% and A28 in 19.8% of the examined individuals. HLA antigens, A1 (15.1%), A3, A24 (17.4%), A11, A26 (12.8%), A30 (11.6%), were often found. The low frequency of occurrence in the phenotype of the examined individuals is typical for HLA antigens - A23, A29, A31, A33 (2.3%), A36 (1.2%).

Of the split antigens, the A24 antigen occurs most frequently (17.4%), making the main contribution to the frequency of the A9 antigen. Another subtype of specificity A9-antigen A23 can be attributed to less common antigens (2.3%) of the A locus. Among the locus antigens in the population, the most common were HLA antigens B13 (26.7%), B5 (20.9%), HLA antigens B7 (17.4%), B35 (11.6%), B27 were often detected. (10.5%), less common (from 6 to 12%) antigens B8, B12, B17, B22, B27, B35, B40. The lowest frequency in the population is characteristic of HLA antigens B16, B39, B53, B56, B75.A rather high frequency of the blank in locus B (0.1624%) indicates the presence of unidentified antigens in these loci, which are rare antigens specific to Mongoloids.

Table 1 Distribution of HLA antigens in the examined population(n=86, DR, n=82)

| HLA-antigene | A% | G | HLA-antigene | A% | G | HLA-antigene | A% | G |
|---|---|---|---|---|---|---|---|---|
| A1 | 15,2 | 0,0787 | B15 | 4,7 | 0,0235 | DR1 | 28,1 | 0,1518 |
| A2 | 31,4 | 0,1717 | B16 | 1,2 | 0,0058 | DR2 | 22,0 | 0,1165 |
| A3 | 17,4 | 0,0914 | B17 | 9,3 | 0,0476 | DR3 | 18,3 | 0,0961 |
| A9 | 32,4 | 0,1660 | B18 | 4,7 | 0,0235 | DR4 | 13,4 | 0,0695 |
| A10 | 25,6 | 0,1373 | B21 | 4,7 | 0,0235 | DR5 | 18,3 | 0,0961 |
| A11 | 12,8 | 0,0661 | B22 | 9,3 | 0,0416 | DR6 | 9,8 | 0,0500 |
| A19 | 38,4 | 0,1224 | B27 | 10,5 | 0,0538 | DR7 | 37,8 | 0,2114 |
| A23 | 2,3 | 0,117 | B35 | 11,6 | 0,0599 | DR8 | 3,7 | 0,0185 |
| A24 | 17,4 | 0,0914 | B39 | 1,2 | 0,0058 | DR9 | 23,2 | 0,1235 |
| A26 | 12,8 | 0,0661 | B40 | 8,2 | 0,0416 | DR10 | 4,9 | 0,0247 |
| A28 | 19,8 | 0,1043 | B41 | 3,5 | 0,0176 | DR11 | 15,9 | 0,0827 |
| A29 | 2,3 | 0,0117 | B42 | 4,7 | 0,0235 | DR12 | 2,4 | 0,0123 |
| A30 | 11,6 | 0,0599 | B44 | 3,5 | 0,0176 | DR13 | 9,8 | 0,0500 |
| A31 | 2,3 | 0,0117 | B46 | 3,5 | 0,0176 | DR15 | 20,7 | 0,1097 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| A32 | 9,3 | 0,0476 | B48 | 1,2 | 0,0058 | DRbl | | 0,0420 |
| A33 | 2,3 | 0,0117 | B49 | 2,3 | 0,0117 | | | |
| A68 | 4,7 | 0,0235 | B50 | 2,3 | 0,0117 | | | |
| A69 | 7,00 | 0,0355 | B51 | 3,5 | 0,0176 | | | |
| Abl | - | 0,0560 | B52 | 2,3 | 0,0117 | | | |
| | | | B53 | 1,2 | 0,0058 | | | |
| B5 | 20,93 | 0,1108 | B55 | 2,3 | 0,0117 | | | |
| B7 | 17,44 | 0,0914 | B56 | 1,2 | 0,0058 | | | |
| B8 | 9,30 | 0,0476 | B60 | 5,8 | 0,030 | | | |
| B12 | 6,98 | 0,0355 | B75 | 1,2 | 0,0058 | | | |
| B13 | 26,74 | 0,1441 | Blb | | 0,203 | | | |
| B14 | 3,49 | 0,0176 | | | | | | |

Note: Abl - A-blank - the frequency of an unidentified, empty allele; A is the frequency of occurrence of the antigen, %; G is the frequency of occurrence of the gene.

In the DR locus, the most common antigen was HLA-DR7 (37.8%), DR1(28.196), DR2 (22.0%), DR9 (23.2%), DR15 (20.7%), DR8 and DR12 antigens were detected with the lowest frequency in the DR locus (2.4%). The data presented in Table 3.1 on the distribution of HLA antigens in the Uzbek population indicate the presence of both similarities and distinctive features in comparison with the distribution of HLA antigens inherent in the world populations of Caucasoids and Mongoloids.

Thus, in the surveyed population, a number of antigens occur with frequencies similar to the average frequencies in Caucasoid HLA populations - A9, A11, A19, A23, A24, A28, A33, B7, B22, B50, B53, B55, DR1, DR3. DR13. However, HLA antigens are more common than in Caucasoids: A10, A30, B42 and less often A1, A2, B8, B15, B39, B40, which is more typical for Mongoloids. An intermediate position between the average frequencies in Mongoloids and Caucasians is occupied by the frequencies of HLA antigens: A3, A19, A24, A30, A36, B41, B42, B52, B55, B60, DR8.

Thus, according to the nature of the distribution of HLA antigens, the Uzbek population has common features with both Caucasians and Mongoloids, and also has peculiar features of the distribution of HLA antigens. The most characteristic differences, both from the populations of Caucasoids and Mongoloids, are the reduced frequencies of occurrence of HLA antigens B12, DR6 and, on the contrary, the increased frequencies of B13, DR9 antigens. The cellular link is represented by the relative and absolute number of total T-lymphocytes (CD3), and their regulatory subpopulations CD4 (T-helpers/inducers), CD8 (T-cytotoxic/suppressors) and their ratio, i.e. immunoregulatory index. Although the parameters of these indicators are highly variable, most of them are in the range of the Tashkent norm, the differences are expressed within 10% and are unreliable. It can be noted only a reduced level of T-lymphocytes circulating in the peripheral blood in the surveyed residents of the

rural area (52.7±0.50% compared to 57.7±0.99% in the population of Tashkent, P<0.05)

Table 2 Comparative characteristics of the immune status of the surveyed Uzbek population of the Samarkand region (M±m)

| immune system parameters | General population (n=86) | Urban population (n=36) | Rural population (n=50) | Regulations for Tashkent |
|---|---|---|---|---|
| Lymphocytes % | 32,6±0,70 | 29,4±0,80 | 34,9±0,80 | 33,2±0,80 |
| Abs. | 1928±61 | 1835±55 | 2015±45 | 2090±80 |
| CD3, % | 54,1±0,40 | 55,9±0,50 | 52,7±0,50* | 57,7±0,99 |
| Abs. | 1039±16 | 1019±26 | 1054±20 | 1212±64 |
| CD4, % | 33,7±0,20 | 34,9±0,30 | 32,8±0,30 | 36,0±1,10 |
| CD8, % | 19,2±0,21 | 19,6±0,30 | 18,9±0,02 | 19,0±0,70 |
| CD4/CD8 | 1,76±0,02 | 1,79±0,02 | 1,74±0,05 | 1,79±0,05 |
| **Humoral parameters** | | | | |
| CD19,% | 16,4±0,40 | 16,0±0,60 | 16,8±0,40 | 15,2±0,90 |
| Abs. | 324±8 | 303±13 | 340±10* | 287±17 |
| IgA, мг % | 131±3 | 133±5 | 130±3 | 154±6 |
| IgM, мг % | 124±3 | 130±4 | 120±4 | 119±7 |
| IgG, мг % | 930±20 | 936±32 | 926±26 | 1072±46 |
| **Natural protective factors** | | | | |
| CD16,% | 9,6±0,30 | 9,8±0,40 | 9,3±0,40 | 10,0±0,50 |
| phagocytic activity of neutrophils % | 53,6±0,70 | 53,5±0,90 | 53,6±0,60 | 57,2±1,80 |
| spontaneous migration of leukocyte in peripheral blood | 52,6±1,14 | 51,6±1,30 | 9,3±0,40 | 10,0±0,50 |
| **Functional activity** | | | | |
| serum cytokines, migration index (MI), migration oppression index (MOI)% | 1,03±0,02-3% | 0,99±0,02+1% | 1,06±0,03-6% | 0,96±0,04+4% |
| leukocyte migration inhibition factor, migration index (MI), migration oppression index (MOI) % | 0,74±0,01*+26% | 0,73±0,01*+27% | 0,74±0,01*+26% | 0,58±0,05+42% |
| leukocyte migration stimulating factor, migration index (MI), migration oppression index (MOI) % | 1,17±0,04*-17% | 1,19±0,01*-19% | 1,15±0,01*-15% | 1,25±0,03-25% |
| FIM/MSF | 1,53 | 1,42 | 1,73 | 1,68 |

Note: * - significant in comparison with the normative data of the city of Tashkent (P<0.05)

The average indicators of the humoral link, compared with the norm, are characterized by a tendency to some increase in the absolute number of B-lymphocytes, while significant differences when compared with the population of Tashkent are recorded only in the group of rural residents (287 + 17 cells / μl and 340 + 10 cells / μl. respectively, P<0.05) and a slight decrease in the concentration of serum IgA and IgG. A decrease in the concentration of serum immunoglobulins with a high number of B-lymphocytes may be associated with a decrease in Th 2 activity, since Th 2 is responsible for the activation of humoral immunity with the formation of antibodies. Natural protection factors, represented by the relative content of natural killer-CD16+ cells, phagocytic (FAN) and spontaneous migratory activity of neutrophilic leukocytes- SMANL in periphery blood, also turned out to be slightly reduced compared to the norm, remaining in the range of its deviations. We determined the activation of the immune system using the LMIR- leukocyte migration inhibition reaction, in which biological testing was used to study spontaneous serum and mitogen-induced ConA optimal and suboptimal doses cytokines that affect the migration of leukocytes and macrophages - factors that inhibit the migration of leukocytes -FIML and its alternative activity - stimulating migration – FSML [6].

As is known, the imbalance of serum cytokines with FIML and FSML is one of the signs indicating a change in immune homeostasis. If the dynamic balance of the immune system is achieved by activity at IM = 1.0 ± 0.15, then IIM greater or less than 15% indicates an imbalance in the studied serum cytokines that affect in vivo the state of cellular immunity with blockade or stimulation [2]. The huge role of mediators - cytokines is also known. The processes of intercellular interaction in the immune response are the basis of immunity and are carried out by direct contact and indirectly with the help of mediators. One of them, the leukocyte migration inhibitory factor (FIML), produced by T-lymphocytes in the first 1-4 hours of activation by T-cell or specific antigens, is determined by biological testing in RIML [7].

As can be seen from table 1. although the activity of spontaneous cytokines in the general group of the population of the Samarkand region was within the normal range, however, ConA-induced production was changed. Thus, the study of the level of ConA-induced production of FIML and FSML revealed a decrease in the response of T-lymphocytes to activation of ConA by the T-cell mitogen at optimal and suboptimal doses (P<0.05). ConA-induced production of FIML was 26% with IM = 0.74±0.01 vs. 42% with IM = 0.58±0.05 and FSM - 17% with IM = 1.17±0.04 vs. - 25% with IM = 1.25 +0.03. Based on these data, it becomes obvious that the functional

activity of T-cell immunity in the population of this region is significantly reduced. However, the ratio of the activity of the helper and suppressor links does not seem to be disturbed, as evidenced by the calculation of the functional IRI equal to the ratio of ConA-induced production of FIML and FSML. Thus, despite the large variability in the parameters of the immune status of the Uzbek population of the Samarkand region, the differences between them in most cases were not significant, since they were in the range of average regional standards for Tashkent. This is reflected in the immunograms of the general group, as well as the urban and rural population.

Despite significant progress in fundamental research indicating the presence of HLA-linked Ig and Is genes that determine the strength of the immune response to specific antigens, there is still a lot of uncertainty in the problem of genetic control of human immunoreactivity. The available data, especially taking into account the immunogenetic heterogeneity inherent in different populations, are rather contradictory. In connection with the task of this study to determine the presence of genetically determined, associated with HLA-complex, features of immunoreactivity in the studied population, we analyzed the relationship of HLA-antigens with indicators of the immune status of the examined practically healthy individuals. For this, the average values of the studied parameters of the immune status were determined in subgroups of individuals, differing in the presence (antigen-positive) or absence (antigen-negative) of each of the identified HLA antigens.

As studies have shown, statistically significant associations of HLA antigens with the level of immunoreactivity of the organism among the individuals of the examined population were identified in four cases and related to such parameters of the immune status as the content of T-lymphocytes and the pool of natural killer cells, as well as the level of serum immunoglobulins of classes G and M.

As can be seen from the data presented in Table 1 HLA-DR4 positive individuals had significantly reduced values of the absolute content of T-lymphocytes (960.5±35.0·10 μl) compared to HLA-DR4 negative individuals (1118.2±16.6·10 μl, $P<0.001$) . Individuals in the phenotype of those who had the HLA-DR5 antigen, on the contrary, had a higher level of this indicator (1150.1±43.1·10 μl) compared with the opposite group of examined individuals without the HLA-DR5 antigen in the phenotype (924.0±26, 6 x 10 μl, $P<0.001$). Thus, according to our analysis, in the studied population of people of Uzbek nationality, the quantitative indicators of the pool of T-lymphocytes circulating in the blood are associated with HLA - markers that characterize an increased (DR5) or decreased (DR4) level of this parameter of the immune status.

When analyzing the content of cells that perform the functions of natural killers, in connection with the HLA phenotype of the examined individuals, intergroup differences were found in the average value of this indicator in individuals differing in the presence of the HLA-A30 antigen in the phenotype. As evidenced by the data presented in Table 5, the level of NKC in HLA-A30 positive individuals was significantly reduced compared to the values of the opposite group, amounting to 8.2±0.34% compared to 11.0±0.5% in the HLA-A30 group. negative faces ($p<0.02$). Thus, the data obtained indicate the presence of a relationship between the HLA-A30 antigen and the quantitative content of the NK pool in the peripheral blood.

In general, after analyzing the relationship between HL.A antigens and the characteristics of the cellular immunity in the studied population of people of Uzbek nationality, it can be concluded that different levels of circulating immunocompetent cells are associated with phenotypes including antigens DR4, DR5 (CD3), and A30 (CD16). It was of interest to conduct a similar analysis of the state of the humoral link of immunity, depending on the expression of various HLA specificities in the phenotype.

The average level of IgM in the serum of HLA-B35 positive individuals was 107.0±10.8 mg/% compared to 143.9±7.3 in the opposite group of examined people ($P<0.001$). When analyzing the characteristics of the production of class G serum immunoglobulins depending on the HLA phenotype, significant intergroup differences in the average values of this indicator were registered depending on the presence of HLA-A30 and HLA-B35 antigens in the phenotype. Thus, a high level of IgG was registered in the groups of HLA-A30-positive individuals (1045.3±21.6 mg% compared to 817.6±35.9 mg% in the opposite group, $P<0.001$) and, on the contrary, a lower level - for HLA-B35 positive individuals (858.0±43.6 mg% vs. 1004.5±21.9 mg%, $P<0.01$).

Thus, characterizing the relationship of HLA antigens with the humoral link of the immune status, it should be noted the association of the HLA-B35 antigen with a reduced level of production of serum Ig classes M and G, while the increased content of IgG was associated with the presence of the HLA - A30 antigen in the phenotype of the examined individuals. In general, the results of the study allow us to conclude that in the surveyed population of residents, groups of individuals with a certain HLA phenotype can be identified, which have significantly different from the average level of immune status indicators, detected in tests for assessing the immune status of level I.

The obtained data testify in favor of the existence of HLA-mediated mechanisms that determine the immunoreactivity of the organism in the studied human population.

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