The Effect of Human Leukocyte Antigens-DRB1 Alleles on Development of Different Tuberculosis Forms in Children

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Abstract

Background: Nowadays, there is no doubt that the development of infectious process is determined not only by individual features of a human, but also by features of infection agent. It is commonly known that ability to form an adequate immune response is based on immunogenetic peculiarities of macroorganism. Methods: The immunogenetic study was performed in 228 children from 1 to 15 years old with different manifestations of tuberculosis (TB). Control group was consisted of 446 adult healthy donors-residents of the Northwestern region of Russia. Human leukocyte antigens (HLA)-DRB1* allelic genes were assessed in all individuals. Results: HLA-DRB1 alleles *01, *03, *11, *13, *07, and *15 were observed significantly rare in children with TB in comparison with healthy donors that may indicate their protective role in the development of the disease. It was also noticed that DRB1 *07 and *15 alleles were observed significantly rare in children with lung TB in comparison with other forms of disease that allows to assume a protective function of these alleles for lung TB with no influence on development of generalized TB. This assumption requires further researches. Conclusion: As a result of the study, statistically significant differences in the distribution of HLA-DRB1* alleles in children with TB in comparison with a control group for *01, *03, *11, *13, *07, *15, and *16 alleles were found. It may indicate their protective role in the development of TB. DRB1 *07 and *15 alleles were observed significantly rare in children with single TB than in children with generalized TB and healthy controls.

Keywords: Children, genotyping, HLA alleles, immunogenetic diagnosis, tuberculosis
influence of HLA-DRB1 alleles on development of the disease. For example, the HLA system has been suggested to play an important role in the mechanism of sexually transmitted infections. The study on the distribution of HLA-DRB1 alleles in Chinese population was performed to identify protective ones for development of syphilis. The authors proved that the frequency of HLA-DRB1*14 allele was significantly higher in patients with syphilis than in control group. It was found that HLA-DRB1*14 has a close correlation with syphilis.[7]

Impact of HLA-DRB1 alleles may correlate with particular ethnic groups. It has been proved that HLA-DRB1*04, *09, *10, *15, and *16 genes may contribute to the risk of TB development, especially in Eastern Asia.[8-11]

The interrelation of HLA-DRB1 alleles with the characteristic of immune response was proved for patients with lung TB.[12] It was revealed that HLA-DRB1* specificities predispose and protect teenagers and adults with lung TB.[13,14] Generalization of infection may be also facilitated by an immune response that is determined by genetic markers. A question about the significance of predisposing immunogenetic factors on the development of different forms of TB is still open.

Up to the present moment, there is a lack of knowledge about the relevance of HLA-DRB1 alleles’ in development of TB. However, a detection of alleles that determine the development of different forms of TB in children can be useful for prevention of the disease in groups of the high risk that was a rationale for the presented study.

Objective of this research was to detect an influence of HLA-DRB1 alleles on the development of single lung TB or generalized forms of TB in children.

**METHODS**

Immunogenetic study was performed in the period of 2008–2015 at Federal State Institution “St. Petersburg Research Institute of Phthisiopulmonology”, of Ministry of Health of Russian Federation (department of children lung TB and surgery of osteoarticular TB in children and teenagers) and Russian Research Institute of Hematology And Transfusiology of Russia and enrolled 228 children with TB (1–15 years old) of European race, residents of North-Western region of Russia. Among them, 188 children were diagnosed with single lung TB (i.e., with only lung TB or TB of intrathoracic lymph nodes) (Group I) and 40 children with generalized TB – meaning TB with two or more localizations (Group II). Inclusion criteria were as follows: the presence of clinical-radiological characteristics of TB and informed consent for study participation. Exclusion criteria were as follows: age above 16, a presence of HIV, and comorbid oncologic pathology.

For all children before starting in the study, informed consent has been received from parents. The form of informed consent document for the study was reviewed and approved by the Independent Ethics Committee. The study was financially supported by OAO “Sberbank of Russia”.

Control group for comparison of HLA was consisted of 446 healthy adult citizens of Russian Northwest region (healthy donors of blood) of European race with negative Mantoux test with 2 TU (MT/TST), without TB in medical history, and without a risk factor for the development of TB. The study was approved by Local Ethics Committee at FSBI “SPB NIIF” of Russia Ministry of Health (Approval No 2 2008).

Overall characteristic of children with TB is displayed in Table 1.

Numbers of boys and girls were balanced. In the overwhelming, majority of the cases, children were Bacille Calmette-Guérin...
vaccinated at birth. Each third child had contact with a patient with TB, and in half of the cases, these patients had mycobacteria with multiple drug resistance.

In 84.2% children, TB of intrathoracic lymph nodes was diagnosed that in 40.0% was accompanied by TB of other localizations. Most often were observed tuberculous spondylitis (8.8%) and tuberculous osteitis (5.7%). About 15.7% patients have had lung TB, while only 1.3% – military lung TB. Children provided complaints only in 27.2% cases.

Immunological tests were positive in 96.5% – Mantoux test (2 TE) and in 95.2% with using of recombinant TB allergen (Diaskintest) manufactured by GENERIUM JSC, Russia, is a recombinant fusion protein CFP10-ESAT6 produced by Escherichia coli BL21(DE3)/pCFP-ESAT.

In a diagnostic material, Mycobacterium tuberculosis complex was detected by polymerase chain reaction (PCR) DNA in 92.5% cases that coincided with the results of histological diagnosis.

**Study methods**

All children passed chest computed tomography (CT), laboratory methods (bacterioscopy assessment of respiratory material (sputum) and bacteriology (BACTEC MGIT 960), real-time PCR method (manufacturer - “Syntol”, Russia) of sputum and biopsy material inoculation on dense nutrient media and for detection of TB bacteria.

**Intradermal tests**

Performing a Diaskintest is similar to the tuberculin skin test. The injections were performed intradermally; the results were recorded after 72 h by measuring the papule diameter at the injection site.

According to the guidelines, in the presence of a papule of any size, the Diaskintest results were considered positive. The presence of hyperemia in the absence of a papule was considered as an inconclusive test. A cutoff of ≥5 mm was established in this study for an objective assessment of the presence of a papule.

According to the Russian legislation, tuberculin skin test (TST) was performed with the use of PPD-L tuberculin (Linnikova purified protein derivative) with two tuberculin units (Russia, Pharmstandard JSC). The TST results were evaluated as follows: positive-a papule of 5 mm and more, inconclusive-a papule up to 4 mm or hyperemia of any size. The absence of a papule and hyperemia in both tests was a negative parameter.

Control group for HLA typing results was consisted of 446 healthy adult individuals from Russia of the Northwest region.

Typing of HLA alleles (basic resolution) was undertaken in the Republic center of an immune tissue typing (European Federation of Immunogenetics accredited laboratory). Genomic DNA was extracted from white blood cells of peripheral blood with the use of microcentrifuge column with a commercial kit of reagents (DNA BOX) by “PROTRANS” (Germany). An evaluation of amount and quality of extracted DNA was done by spectrophotometry (SmartSpec Plus spectrophotometer, BioRad, disposable cuvettes Truviet Cuvette, BioRad). A measurement of each sample’s DNA optical density (OD) was done on 260 nm and 280 nm. A concentration of extracted DNA was 30–70 ng/mcl. Quality of extracted DNA was evaluated by OD ratio 260/280 and equal to 1.6–1.8 for extracted samples. Typing was carried out with the use of commercial kits sequence-specific primers (Cyclerplate System Protrans).

Visualization of products obtained as a result of PCR was done by electrophoresis in a horizontal agarose gel. Photographic recording of electrophoresis products and archiving of electrophoregrams was accomplished by photographic registration system GelDoc (BioRad). The phenotype was extracted by interpretation tables supplemented to kits.

Typing of HLA-DRB1* was accomplished with specification of *01, *03, *04, *07, *08, *09, *10, *11, *12, *13, *14, *15, and *16 alleles. Samples were analyzed by the methods of parametric and nonparametric statistic, and the strength of association was estimated by calculating of relative risk; reliability of differences between groups was assessed by Chi-squared criterion. Statistical analysis was carried out using SPSS software (version 11.5; IBM SPSS, Chicago, IL, USA) and Statistica 7.0, with the variation statistics methods. The quantitative data were calculated in the form mean ± standard deviation. The degrees of associations between proportions were estimated using confidence intervals, as well as the Chi-squared criterion with Yates correction. For variable values, <5, Fisher’s exact test was used. Differences or communication rates were considered statistically significant at a level of P < 0.05.

**Results**

HLA-DRB1* distribution was analyzed for children with TB in comparison with a control group. Results are displayed in Table 2.

HLA-DRB1 alleles *01, *03, *11, *13, *07, *15, and *16 were identified in children with TB significantly rare than in a control group: *01, *03, *11, *13, *07, *15, and *16 that characterizes these alleles as protective [Table 2].

Comparison of genotyping HLA-DRB1* alleles in children with single lung TB (I group) with generalized TB (II) and a control group was performed [Table 3].

It was found out statistically significant differences in children with single TB and a control group for HLA-DRB1*07 (14.3% vs. 28.2%, χ² = 33.26, P < 0.001) and HLA-DRB1*15 (18.4% vs. 26.7%, χ² = 8.3, P < 0.01). These results may evidence for a protective role of these alleles in development of single TB. As the next step, we compared the results of distribution HLA-DRB1* gene alleles in patients with generalized TB (II group) and a control group. No statistically significant difference in children with generalized TB and in control group was observed [Table 3]. Comparison between two groups
Table 2: Distribution human leukocyte antigen-DRB1* gene alleles in patients with tuberculosis and in control group

| HLA alleles | TB (n=228) | Control group (n=446) | \(\chi^2\) | P | RR |
|-------------|------------|----------------------|--------|---|----|
| DRB1*01    | 14.5 (33)* | 25.7 (115)           | 11.26  | <0.001 | 0.22 |
| DRB1*03    | 4.7 (17)*  | 15.2 (68)            | 8.3    | <0.01  | 0.20 |
| DRB1*04    | 19.7 (45)  | 19.7 (88)            | 0.00   | >0.1   | 0.33 |
| DRB1*07    | 12.3 (28)* | 28.2 (126)           | 21.82  | <0.001 | 0.18 |
| DRB1*08    | 4.3 (10)   | 6.5 (29)             | 0.76   | >0.1   | 0.25 |
| DRB1*09    | 3.1 (7)    | 2.7 (12)             | 0.079  | >0.1   | 0.36 |
| DRB1*10    | 1.3 (3)    | 2.5 (11)             | 0.98   | >0.1   | 0.21 |
| DRB1*11    | 14.9 (34)* | 20.9 (95)            | 3.97   | <0.05  | 0.26 |
| DRB1*12    | 3.5 (8)    | 4.9 (22)             | 0.71   | >0.1   | 0.26 |
| DRB1*13    | 15.7 (36)* | 25.6 (114)           | 8.32   | <0.01  | 0.24 |
| DRB1*14    | 1.3 (3)    | 3.1 (14)             | 2.03   | >0.1   | 0.17 |
| DRB1*15    | 11.8 (27)* | 26.7 (119)           | 19.57  | <0.001 | 0.18 |
| DRB1*16    | 3.5 (8)*   | 8.3 (37)             | 5.54   | <0.05  | 0.17 |

*P<0.01 - in comparison of children with TB and a control group. HLA: Human leukocyte antigen, TB: Tuberculosis, RR: Relative risk

Table 3: Distribution human leukocyte antigen-DRB1* gene alleles in patients with lung tuberculosis (single), with generalized tuberculosis (II) and in control group

| HLA alleles | Single TB (I) (n=188) | RR | Generalized TB (II) (n=40) | RR | Control group (n=446) |
|-------------|-----------------------|----|---------------------------|----|----------------------|
| DRB1*01    | 28.6 (28)             | 0.19 | 12.5 (5)                  | 0.04 | 25.7 (115)           |
| DRB1*03    | 11.2 (11)             | 0.13 | 15.0 (6)                  | 0.08 | 15.2 (68)            |
| DRB1*04    | 36.7 (36)             | 0.29 | 22.5 (9)                  | 0.09 | 19.7 (88)            |
| DRB1*07    | 14.3 (14)*            | 0.10 | 25.0 (10)                 | 0.07 | 28.2 (126)           |
| DRB1*08    | 6.1 (6)               | 0.17 | 10.0 (4)                  | 0.12 | 6.5 (29)             |
| DRB1*09    | 4.1 (4)               | 0.25 | 7.3 (2)                   | 0.11 | 2.7 (12)             |
| DRB1*10    | 1.1 (1)               | 0.08 | 5.0 (2)                   | 0.15 | 2.5 (11)             |
| DRB1*11    | 22.4 (22)             | 0.18 | 30.0 (12)                 | 0.11 | 20.9 (95)            |
| DRB1*12    | 6.1 (6)               | 0.21 | 5.0 (2)                   | 0.08 | 4.9 (22)             |
| DRB1*13    | 28.6 (28)             | 0.19 | 20.0 (8)                  | 0.06 | 26.5 (114)           |
| DRB1*14    | 0                    | 0    | 7.5 (3)                   | 0.17 | 3.1 (14)             |
| DRB1*15*   | 18.4 (18)*            | 0.13 | 22.5 (9)                  | 0.07 | 26.7 (119)           |
| DRB1*16    | 7.1 (7)               | 0.16 | 2.5 (1)                   | 0.02 | 8.3 (37)             |

*P<0.01 - in comparison of children with single TB, generalized tuberculosis, and control group. HLA: Human leukocyte antigen, TB: Tuberculosis, RR: Relative risk

As a result of the study statistically significant differences in the distribution of HLA-DRB1*, alleles in children with TB in comparison with a control group for *01, *03, *11, *13, *07, *15, and *16 alleles were found. It may indicate their protective role in the development of TB. DRB1*07 and *15 alleles were observed significantly rare in children with single TB than in children with generalized TB and healthy controls.

**Conclusion**

Data collected in the study may characterize immunogenetic features in children with TB – residents of the Northwestern region of Russia. High prevalence of protective alleles of HLA-DRB1* in children of this region may explain relevantly low incidence rate of severe and generalized forms of TB on the background of high TB burden. The presence in the genotype of children with single lung TB *07 and *15 alleles of HLA-DRB1* predicts the favorable course of the disease. The absence of protective alleles HLA-DRB1* in children with generalized TB requires further studying of relationships between features of genotype and parameters of the cell and humoral response.

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**Conflicts of interest**

There are no conflicts of interest.

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**Discussion**

HLA-DRB1 plays a central role in the immune system with the highest polymorphism in the HLA antigen system.\[15,16\]
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