ADAPTIVE IMMUNITY AND GENETIC ASPECTS OF TUBERCULOSIS IN CHILDREN

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Cell-mediated immunity and the cytokine interferon gamma (IFNγ) have an important role in promoting host resistance against tuberculosis-causing mycobacteria (TBM), but the exact mechanism of developing immunity against tuberculosis (TB) is unknown. In this work we evaluate the immune response in TB and the association between *IFNG* gene polymorphism *rs2069705* (T-1488C) and the intensity of specific immune reactions in children. The study was conducted in 310 children below 18 years distributed into 3 groups: the TB group included 110 children with TB confirmed by medical evaluation; the LTB group consisted of 156 children with latent infection; and the NTB group was represented by 44 non-infected children. A few immunoassays and molecular-genetic tests were performed; specifically, we evaluated the immune status of patients and the distribution of genotypic frequencies of the studied polymorphism, in the context of previous vaccination against TB. The cell-mediated immune response was mild in children with LTB, while in children with TB inflammation showed signs of chronicity due to the lack of functional activity of immune cells ($p < 0.05$). We also measured IFN-γ synthesis induced by specific mitogens (PPD-L, CFP32B, Rv2660c, ESAT6, 85a and ESAT6-CFP10), only to detect attenuation of the immune response in patients with TB, which was associated with the heterozygous *rs2069705* variant ($p < 0.05$). Children with homozygous TT and CC genotypes demonstrated a more pronounced immune response. Low effectiveness of the TB vaccine was shown to be associated with the heterozygous genotype (50 %), while its high effectiveness was associated with the homozygous T genotype (40 %), possibly indicating the protective role of the latter. Our findings suggest that the studied polymorphism (specifically, its heterozygous variant) can be a predictive marker of TB in children.

Keywords: Mycobacterium tuberculosis, tuberculosis, immune response, interferon gamma, antigen, PPD-L, CFP32B, Rv2660c, ESAT6, 85a, ESAT6-CFP10, *IFNG*, polymorphism, children

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АДАПТИВНЫЙ ИММУНИТЕТ И ГЕНЕТИЧЕСКИЕ АСПЕКТЫ ПРОГРЕССИРОВАНИЯ ТУБЕРКУЛЕЗНОЙ ИНФЕКЦИИ У ДЕТЕЙ

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Клеточный иммунитет и цитокин интерферон гамма (ИФН-γ) имеют важное значение для формирования устойчивости организма к микобактериям туберкулеза (МБТ), но точный механизм появления иммунитета к туберкулезу (ТБ) неизвестен. В исследовании оценивали иммуный ответ при развитии ТБ и связь полиморфизма *rs2069705* (T-1488C) гена *IFNG* с выраженностью специфических иммунологических реакций у детей. Участниками исследования стали 310 детей до 18 лет, распределенные по 3 группам: группа ТБ — 110 детей с установленным по результатам комплексного обследования ТБ; группа ЛТИ — 156 детей с установленной латентной туберкулезной инфекцией; группа НТ — 44 ребенка, не инфицированные МБТ. Проводили иммунологические и молекулярно-генетические исследования, в частности, оценивали иммунный статус пациентов и распределение частот генотипов по изучаемому полиморфизму, в том числе в контексте эффективности вакцинирования против ТБ. Иммуный статус детей с ЛТИ характеризовался лишь слабо выраженной активацией клеточного иммунитета, а детей с ТБ — появлением у воспалительного процесса черт хронического за счет недостаточной функциональной активности клеток иммунной системы ($p < 0,05$). При оценке иммуного ответа по уровню синтеза ИФН-γ, индуцированного специфическими митогенами (PPD-L, CFP32B, Rv2660c, ESAT6, 85a и ESAT6-CFP10), установили снижение ответа у больных ТБ, которое было ассоциировано с гетерозиготным генотипом по полиморфизму *rs2069705* гена *IFNG* ($p < 0,05$). При гомозиготных генотипах TT и CC ответ усиливался. Также установили, что низкая эффективность противотуберкулезной вакцинации также связана с гетерозиготным генотипом (50 %), а высокая — с генотипом, гомозиготным по аллелю T (40 %), что может свидетельствовать о его протективной роли. Полученные результаты указыают на то, что изучаемый полиморфизм (гетерозиготный генотип) можно рассматривать в качестве маркера развития туберкулезной инфекции у детей.

Ключевые слова: микобактерии туберкулеза, туберкулез, иммунный ответ, интерферон гамма, антиген, PPD-L, CFP32B, Rv2660c, ESAT6, 85a, ESAT6-CFP10, *IFNG*, полиморфизм, дети

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The research efforts of the recent years have proved the important role cellular immunity and interferon gamma (IFNγ) play in protecting human body from mycobacterium tuberculosis (MBT), but the mechanisms of development of immunological competence against tuberculosis (TB) remain poorly understood [1]. It is known that proteins secreted by MBT at early stages of infection support proliferation of lymphocytes producing IFNγ [2]. A number of studies states that TB patients with nonresistant *IFNG* genotype have the level of synthesis of this cytokine correlated to the severity of the disease [3, 4].

The search for candidate genes causing congenital and adaptive immunity disorders in the presence of TB is also underway [1, 5–9]. Previously, *IFNG* gene polymorphism in development of various diseases, including tuberculosis, received much attention [3, 10–13].

Studying immunological and genetic factors affecting antituberculous immunity ensures better understanding of the underlying pathogenesis of the disease and, what is more, allows optimization of the prevention approaches. This research aimed to investigate the immune response to TB development and the relationship between polymorphic variant *rs2069705* (T-1488C) of *IFNG* gene and the expression of specific immunological reactions in children.

METHODS

This prospective study was conducted in 2014–2016. 310 patients, all under 18 years of age, took part in it. They were divided into 3 groups depending on the TB status: TB group — 110 children, confirmed TB, mean age of 9.5 ± 0.5, every second child (46.6 %) under 8 years of age; LTB group — 156 children, latent TB confirmed (tuberculin diagnostics), mean age of 6.6 ± 0.3, 70.5 % of children under 8 years of age; NTB group — 44 children, no TB infection, mean age of 3.1 ± 0.4, 95.5 % of children under 8 years of age. Genderwise, the groups were not different ($p > 0.05$).

All children underwent specific immunoassays; 169 of them were also subjected to molecular-genetic tests. To make the comparison valid, only the immune status data describing children under 8 were compared.

TB diagnosing included various laboratory, bacteriological, molecular-genetic tests, radiological examinations. The results of tuberculin diagnostics were also taken into account: Mantoux tests with 2 tuberculin units (TU) of tuberculin (PPD-L) and recombinant tuberculosis allergen tests (RTA) Diaskintest (Generium, Russia), which contain 0.2 μg of CFP10-ESAT6 recombinant protein. A central medical-control commission checked and confirmed diagnoses.

In the TB group, 23 children were diagnosed with infiltrative pulmonary tuberculosis, 38 — with primary tuberculosis, 46 — with intrathoracic lymph nodes tuberculosis (one patient had it combined with the left proximal bronchus lesion and there were cases of focal pulmonary TB, disseminated pulmonary TB and pleuresia tuberculosa, one of each). In 4 cases, TB also affected other organs: peripheral lymph nodes, humerus, intestine. Also in 4 cases, TB caused complications (atelectasis, exudative pleurisy, pulmonary dissemination, hemoptysis). 60.9 % (n = 67) of patients had TB at the infiltration stage, 10.9 % (n = 12) showed disintegration and seeding phase, 0.9 % (n = 1) — sealing and resorption, 26.4 % (n = 29) — calcination phase. Bacterioexcretion was registered in 12 cases (10.9 %); 6 of them proved resistant to the basic antituberculosis drugs.

In the LTB group, 75 children were diagnosed with early primary tuberculosis (EPT), and 81 children had MTB for more than one year.

In the NTB group, 21 child had post-vaccination allergy and 23 showed positive tuberculin anergy.

TB group participants had the infiltrate measure the average of 12.6 ± 0.4 mm (95 % CI 11.8–13.5 mm) for the Mantoux test and 14.95 ± 0.5 mm (95 % CI 13.9–16.0 mm) for the RTA test. The mean values for the same tests in the LTB group were 10.3 ± 0.3 mm (95 % CI 9.7–10.9 mm) and 1.9 ± 0.4 mm (95 % CI 1.2–2.65 mm) respectively. As for the NTB group children that had postvaccinal allergy, the Mantoux test there brought the average results of 4.1 ± 0.4 mm (95 % CI 3.2–5.0 mm); 9 (42.9 %) cases were confirmed positive reactions, the rest were questionable. All children in this group returned negative reaction to the RTA test.

We had the data on TB vaccination of children. BCG administration site scar and Mantoux test a year after vaccination helped determine its effectiveness: when the scar was 4–10 mm long and tuberculin response positive, the vaccination was regarded a success, in the absence of the scar and negative reaction to tuberculin it was considered ineffective, all other cases fell into minimally effective category. In the TB group, 108 children (98.2 %) were vaccinated, but only for 31 of them the vaccination was effective. In the LTB group, 155 children (99.4 %) received vaccination and for the most of them (n = 97) it was effective. 36 children (81.8 %) of the NTB group were vaccinated; it was effective in every fourth case.

Patients went through immunoassays and molecular-genetic tests when admitted to the special in-patient department of the TB dispensary. The tests were made at the Omsk R&D Institute for Natural Focal Infections (Russia).

Immune status was assessed with the standard level I immunological screening tests: measurement of immunoglobulin levels in the blood serum (IgG, IgA, IgM and IgE) through enzyme-linked immunoassay; evaluation of neutrophil function through measurement of their ability to absorb inert latex particles and two variations of nitroblue tetrazolium (NBT) test, spontaneous and stimulated; evaluation of subpopulation composition of T-cells (CD) using a panel of monoclonal antibodies (DAKO, Denmark). We have also measured the content of spontaneously synthesized interferon gamma and IFNγ the synthesis of which was induced by specific antigens (PPD-L, CFP32B, Rv2660c, ESAT6, 85a, ESAT6-CFP10) for 72 h. Enzyme immunoassay system by Vector-Best (Russia) was used for the purpose. Antigens were isolated by the translational biomedicine lab of the Department of genetics and molecular biology of bacteria of N. F. Gamaleya Scientific Research Institute of Epidemiology and Microbiology (Moscow, Russia) [14].

DNA-blood reagent kit (TestGen, Russia) helped isolate DNA from blood serum; iQ5 amplifier (BioRad, USA) and a set of polymerase chain reaction reagents (FLASH format, by TestGen) were used to identify polymorphic marker *rs2069705* of *IFNG* gene, all following instructions provided by the manufacturers. Allelic Discrimination software supplied by the manufacturer of the amplifier enabled analysis of genotypes. The frequency distribution of genotypes at the examined loci was checked for compliance with the Hardy–Weinberg law.

OpenEpi v3.0 software calculated the minimal sample size ensuring conclusiveness of the empirical data obtained. The reliability of differences between groups was determined through nonparametric Kruskal–Wallis (H), Mann–Whitney (U) and χ^2 tests. The differences were considered significant at $p < 0.05$. We have also calculated the odds ratio (OR): if the chance (risk) was above 1, development of the disease was considered statistically significant. OpenEpi v3.0 and Statistica v6.0 software were used to process the data obtained.

The study got the approval of Omsk State Medical Academy ethics committee (Minutes no. 51 of October 10, 2012). Parents or legal representatives have signed voluntarily informed consent forms and thus approved participation of their children in the study.

RESULTS

Table 1 shows the comparative analysis of results of clinical and laboratory examinations/tests of children from TB and LTB groups. Children from the LTB group suffered from intoxication syndrome less often (9 %) than the TB group patients (19.1 %) ($p = 0.008$), but they had more of nasal breathing disorders caused by adenoid vegetations (16 % vs. 1.8 %, $p = 0.00008$), chronic infection foci (18.6 % vs. 10 %, $p = 0.027$), respiratory allergies manifestations (15.4 % vs. 0 %, $p = 0.000008$) and excess weight (10.3 % vs. 4.5 %, $p = 0.044$). Such clinical symptoms as hepato- and splenomegaly (OR 2.583 and 3.800, respectively) and body mass deficiency (OR 1.898) were significant in the TB group. Lab tests have also shown significance of anemia (OR 1.872), accelerated ESR (OR 2.255), lymphocytosis (OR 1.634) and eosinophilia (OR 5.371). It should be noted that only 4 cases of eosinophilia (out of 40) resulted from parasitic invasions.

Table 2 shows the results of assessment of the immune status of all children that participated in the study. There were no significant differences in immunological parameters describing the status of children constituting LTB and NTB groups ($p > 0.05$). The exception here was the content of spontaneously synthesized IFNγ ($p < 0.05$). In all groups, the numbers of leukocytes have increased slightly (compared to the reference values). Leukocytes reflect the state of cellular immunity, so the increase can signal of its activation due to nonspecific processes. At the same time, the differences were significant for CD16, HLA DR, and spontaneously synthesized IFNγ. In the TB group, activation of humoral immunity was observed: within the reference values, the volumes of IgG, IgA have grown, and IgE have shown a significant increase to 306.6 ± 130.7 IU/ml. Phagocytic activity of cells has also increased significantly, while neutrophils' reserve capacity has decreased. The changes of all the aforementioned values were statistically significant compared to those seen in the NTB group.

Thus, the immune status of children from the LTB group was the same to that of children not hosting MTB; at that, cellular immunity has activated slightly. In the TB group, no immunodeficiency development signs were observed, but the changes that did manifest themselves were those peculiar to an inflammatory process associated with TB. The process had features of a chronic one, primarily due to insufficient functional activity of cells and insufficient production of interferon gamma.

To better understand the contribution IFNG gene makes to the immunological defense against MBT, we investigated its polymorphism *rs2069705*. 51.9 % of children from the TB group had a heterozygous genotype, so it was associated with the disease (OR 1.885, 95 % CI 1.019–3.487). Be it secondary (65 %) or primary (47.5 %) TB, the genotype was there, which suggests a higher risk of the disease development regardless of its genesis. Heterozygous genotype was also associated with the development of infiltrates (OR 1.737), signs of lung tissue destruction (OR 1.458), dissemination (OR 1.75) and pleural effusion (OR 1.9), bacterial excretion (OR 1.458) and such clinical manifestations of tuberculosis as paraspecific reactions (OR 2.059), peripheral lymphadenopathy (OR 2.4), bodyweight deficit (OR 1.429), hepato- and splenomegaly (OR 5.5), anemia (OR 2.059), accelerated ESR (OR 3.4).

Given the association of *IFNG* gene polymorphism *rs2069705* with adaptive immunity to tuberculosis, we evaluated the genotype-wise effectiveness of BCG vaccination in children affected by primary TB at its early stage (n = 32). In 12 cases, the vaccination was minimally effective or ineffective; half of those patients had heterozygous genotype. The probability of minimal effectiveness of vaccination was 59.38 % (95 % CI 42.23–74.62 %). Homozygous genotype (T allele) was more common (40 %) among children for whom the vaccination was effective (n = 20).

Table 1. Results of clinical and laboratory examinations/tests of children from TB and LTB groups

Symptoms	TB group (n = 110), abs. (%)	LTB group (n = 156), abs. (%)	χ^2 criterion; p-value	OR
Intoxication syndrome	21 (19.1)	14 (9)	5,778; 0.008	2,393
Bronchopulmonary syndrome	10 (9.1)	12 (7.7)	0.166; 0.342	1,2
Paraspecific reactions	21 (19.1)	36 (23.1)	0.609; 0.218	0,787
Peripheral lymphadenopathy	18 (16.4)	19 (12.2)	0.943; 0.166	1,411
Hypertrophy of palatine tonsils, I-II degree	26 (23.6)	42 (26.9)	0.366; 0.273	0,84
Adenoids	2 (1.8)	25 (16)	14.28; 0,00008	0,097
Caries	6 (5.5)	14 (9)	1.149; 0.143	0,585
Hepatomegaly	7 (6.4)	4 (2.6)	2.349; 0.063	2,583
Splenomegaly	10 (9.1)	4 (2.6)	5,512; 0.009	3,8
Chronic infection foci	11 (10)	29 (18.6)	3,726; 0.027	0,487
Respiratory allergies	0	24 (15.4)	18.6; 0.000008	0
Body weight deficiency	24 (21.8)	20 (12.8)	3.783; 0.026	1,898
Excess body weight	5 (4.5)	16 (10.3)	2.894; 0.044	0,417
Anemia	16 (14.5)	13 (8.3)	2.563; 0.054	1,872
ESR acceleration	25 (22.7)	18 (11.5)	5.959; 0.007	2,255
Leukocytosis	16 (14.5)	28 (17.9)	0.541; 0.231	0,778
Lymphocytosis	43 (39.1)	44 (28.2)	3.473; 0.031	1,634
Monocytosis	19 (17.3)	25 (16)	0.073; 0.394	1,094
Eosinophilia	40 (36.4)	15 (9.6)	28.14; <0.0000001	5,371

Table 2. Results of immunoassay of blood of children from TB, LTB and NTB groups

Parameter	Reference value	TB group		LTB group		NBT group	
		n	M ± SEM	n	M ± SEM	n	M ± SEM
CD3. %	54–82	15	65.8 ± 1.3	16	66.2 ± 1.3	10	69.1 ± 1.7
CD3, abs.	1.65	15	1.8 ± 0.1	9	2.2 ± 0.3	8	2.1 ± 0.2
CD4, %	30–50	15	40.4 ± 1.5	16	39.0 ± 1.3	10	40.8 ± 1.0
CD4, abs.	0.92	15	1.1 ± 0.1	9	1.2 ± 0.15	8	1.2 ± 0.1
CD8, %	18–38	15	29.4 ± 1.4	16	26.9 ± 1.2	10	28.9 ± 1.4
CD8, abs.	0.6	15	0.8 ± 0.06	9	0.9 ± 0.15	8	0.9 ± 0.09
CD16, %	6–18	14	11.4 ± 0.5**	20	13.4 ± 0.6	10	17.1 ± 2.7
CD16, abs.	0.31	14	0.3 ± 0.03**	15	0.4 ± 0.04	10	0.6 ± 0.1
CD20, %	6–22	15	16.2 ± 1.5	12	16.8 ± 1.75	3	14.0 ± 1.2
CD20, abs.	0.2	15	0.5 ± 0.07	5	0.6 ± 0.1	-	-
HLA DR, %	14–25	15	25.9 ± 1.4**	20	23.7 ± 1.6	9	21.8 ± 1.1
HLA DR, abs.	0.33	15	0.7 ± 0.07	15	0.7 ± 0.09	9	0.7 ± 0.07
IFNγ sp., Pg / ml	0.16–10	50	21.1 ± 5.7**	110	20.5 ± 3.0***	43	12.9 ± 1.7
IgG, g / l	8.12–16.14	19	11.7 ± 0.6*.**	110	9.7 ± 0.2	43	8.9 ± 0.3
IgA, g / l	0.75–3.17	19	1.6 ± 0.1*.**	110	1.2 ± 0.06	43	1.0 ± 0.08
IgM, g / l	0.69–3.00	19	1.4 ± 0.2	110	1.15 ± 0.05	43	1.2 ± 0.08
IgE, IU / ml	< 150	19	306.6 ± 130.7*	108	79.3 ± 12.6	23	81.9 ± 25.5
Phagocytosis with latex, %	52–95	15	68.5 ± 3.1**	107	65.7 ± 1.4	33	53.3 ± 2.7
HCT test:							
– spontaneous, %	6–12	16	14.4 ± 1.9**	109	20.9 ± 1.3	41	24.4 ± 1.6
– stimulated, %	–	16	37.8 ± 3.2**	109	46.5 ± 2.2	41	56.5 ± 3.3

Note. * — $p < 0.05$ when comparing results in TB and LTB groups (Mann–Whitney test, U); ** — $p < 0.05$ when comparing results in TB and NTB groups (Mann–Whitney test, U); *** — $p < 0.05$ when comparing results in LTB and NTB groups (the Mann–Whitney test, U).

Ozhegova et al. [7] have established that polymorphism T-1488C of *IFNG* gene defines its expression level. Therefore, we assumed there is a connection between the genotypes studied and the level of interferon gamma synthesis. Analysis of the content of spontaneously synthesized IFNγ did not reveal any significant differences dependent on genotype; this is true for both LTB ($H = 1.663$, $p = 0.435$) and TB ($H = 4.810$; $p = 0.090$) groups. Analysis of the content of IFNγ synthesized through induction by specific antigens [15] showed that in the development of TB, there is a relationship between genotype and level of cytokine synthesis: with heterozygous genotype, the content of IFNγ decreased significantly under the influence of CFP32B, Rv2660c, ESAT6, Ag85a antigens (Table 3). With this in mind, we have also analyzed the frequency of negative results — no response to induction in children with TB (Table 4) — and established the relationship with heterozygous genotype. Its association with decelerated IFNγ synthesis when induced with PPD-L, CFP32B, Rv2660c, ESAT6, 85a and ESAT6-CFP10 antigens was confirmed.

DISCUSSION

The vast majority of people carrying MTB show no symptoms of tuberculosis, and although they are not contagious, they run a risk of developing an active form of TB. According to experts, the risk of TB reactivation during the lifetime of an LTB patient is 5–10 %. Often, the switch from latent to active happens within 5 years from the day of infection [16, 17]. Nevertheless, a number of researchers believes the level of this risk depends on several factors, the most important of which is the body's immune status [18–21]. The results of our study suggest presence of a specific inflammatory process and absence of secondary immunodeficiency.

We studied IFNγ, the main function of which is immunoregulation, including macrophages activation, enhancement of Th-1 mediated response, induction of expression of MHC class II antigens on antigen-presenting cells, etc. [22]. With cellular immunity activation in the background, IFNγ producers are activated Th1-lymphocytes (the main activation marker is HLA DR) and natural killer cells (CD16). Therefore, reduced numbers of those natural killer cells in the presence of TB could lead to lowered levels of cytokine synthesis, and IFNγ deficiency could cause a decrease in activity of cytotoxic cells. This could explain the growing volume of spontaneously synthesized IFNγ seen even when TB is latent ($p < 0.05$); at the same time, we can assume that the antigen load was not sufficient to trigger hyperactivation of cellular response. During the period of development of TB, this indicator remained at the level typical of LTB ($p > 0.05$). Given the increased volume of spontaneously synthesized IFNγ, one could expect a lower content of IgE, as IFNγ, being the product of Th1-lymphocyte, inhibits proliferation of Th2-lymphocytes and IL4-induced Ig to IdE synthesis switch and supports IgG2 synthesis instead [23]. However, we have witnessed high levels of IgE synthesis during TB development, which may indicate inadequate IFNγ production and onset of chronic inflammation. A number of researchers have also established a direct relationship between the appearance of chronic bacterial or fungal infection foci and hyperproduction of IgE [24–26].

IFNγ is also considered to be the most important factor in macrophage activation [22]. Macrophages lyse MTB and provide antimycobacterial protection; in particular, they regulate synthesis of pro- and anti-inflammatory cytokines [27, 28]. However, the balance between cells and mediators required to destroy MTB and prevent lung pathology is still unclear; the issue is subject of other research papers in progress [29]. In

Table 3. Level of interferon gamma synthesis induced by specific antigens, depending on the genotype (polymorphic variant *rs2069705* (T-1488C) of *IFNG* gene) in TB group children

Antigen	*IFNG* gene polymorphic variant *rs2069705* (T-1488C)		Mann–Whitney test (U); p-value
	TS	TT and CC	
	Me (Q25%; Q75%), n = 42	Me (Q25%; Q75%), n = 39	
PPD-L	1001.6 (698; 1200)	1380.7 (741.5; 1294.5)	703.5; 0.275
CFP32B	69.8 (10.2; 68)	95.8 (21; 122.5)	554.5; 0.018
Rv2660c	102.6 (12.2; 83)	149.6 (37.5; 195.5)	513; 0.006
ESAT6	101.5 (10.9; 57.4)	112.8 (26; 173)	523.5; 0.005
85a	65 (0.2; 40)	90.3 (6.5; 101)	549.5; 0.016
ESAT6-CFP10	440.4 (139; 761)	549.5 (187; 1133)	768; 0.630

Table 4. Frequency of negative reactions to specific antigens depending on genotype (polymorphic variant *rs2069705* (T-1488C) of *IFNG* gene) in TB group children

Antigen	*IFNG* gene polymorphic variant *rs2069705* (T-1488C)				p-value	OR	95 % CI
	TS (n = 42)		TT and CC (n = 39)				
	abs.	%	abs.	%			
PPD-L	3	7.1	1	2.6	0.202	2.923	0.291–29.35
ESAT6-CFP10	8	19	3	7.7	0.068	2.824	0.691–11.53
ESAT6	40	95.2	24	61.5	0.0001	12.5	2.628–59.47
Rv2660c	37	88.1	30	76.9	0.092	2.22	0.672–7.33
CFP32B	34	81	19	48.7	0.0012	4.474	1.656–12.08
85a	34	81	19	48.7	0.0012	4.474	1.656–12.08

our study, we saw a decrease in phagocytic activity of cells (decrease in reserve capacity of neutrophils) against the backdrop of developing TB, which may also point to inadequate production of IFNγ.

Averbakh et al. proposed a hypothesis stating there are genes in lymphocytes that control activation of cells synthesizing mediators and that a depression of one part of the genome can mean a depression of its another part, which may lead to disruption of intercellular interaction in the presence of TB [30]. The researchers are trying to establish genetic risk factors affecting TB infection and development [8, 9]. In particular, they are actively studying the *IFNG* gene associated with production of IFNγ cytokine [3,9] and T-1488C polymorphism that affects the synthesis of regulatory protein [7]. In the context of our study, the marker of high TB risk was its heterozygous genotype at the studied genetic locus (OR 4.667, 95 % CI 1.236–17.62, p = 0.008), regardless of the disease genesis (primary or secondary). Low effectiveness of BCG seen in the group with primary tuberculosis infection at its early stage is an indirect proof thereof. Some researchers also believe the low effectiveness of vaccination is a TB risk factor [18, 31]. We have also determined that the studied *IFNG* gene polymorphism (heterozygous genotype) influences the immune response to individual mycobacterial antigens when TB is developing: we have seen a significantly weaker response to early stage TB proteins — CFP32B, Rv2660c, ESAT6, 85a [15], and a slightly decreased level of IFNγ synthesis with PPD-L and ESAT6-CFP10 induction, which gives a yet another reason to look at these antigens in the context of TB. We have also established that homozygous genotype (T allele) means better protection against TB and found it affects development of antituberculous immunity in children.

The results of this study allow taking the *IFNG* gene polymorphism (T-1488C) as an additional genetic risk factor contributing to the development of tuberculosis infection in children and one of the reasons behind inadequate functional activity of cells regulating synthesis of IFNγ.

CONCLUSIONS

We studied the immune response to development of TB and seen activation of cellular immunity and insufficient functional activity of cells when TB turns from latent to active. The main adaptive immunity cytokine considered was IFNγ.

We have established that *IFNG* gene polymorphism T-1488C affects the severity of specific immunological reactions. Heterozygous genotype implies inadequate production of cytokine at the early stage of TB development. Homozygous genotype (T allele) means better protective immunity against the disease.

We have established that heterozygous genotype of the *IFNG* gene's polymorphic version (*rs2069705*) bears relation to TB switching from latent to active in children, which allows taking this genotype as an additional risk factor.

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