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Research of the genetic polymorphism of Toll-like receptors in the patients suffering from tuberculosis of respiratory organs

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ABSTRACT

Development of tuberculosis is caused by many reasons, but the main is depression of efficiency of functioning of the immune system. The purpose of the work was to identify the frequency of occurrence of alleles and genotypes of TLR2 (Arg753Gln), TLR3 (Phe412Leu), TLR4 (Asp299Gly), TLR6 (Ser249Pro) genes in patients suffering from tuberculosis. The research is performed on the basis of the analysis of clinical findings of 120 patients suffering from tuberculosis and 30 healthy peoples. For studying of a polymorphism of genes of Toll-like receptors (TLRs) we used the method of specific allele polymerase chain reaction with electrophoretic detection.
Crucial importance for development of small forms and infiltrative tuberculosis has genetic predisposition in the form of a carriage of mutant genotypes of TLR3 (Phe412Leu) and TLR6 (Ser249Pro) genes. At the same time in pathogenesis of disseminated tuberculosis an important role is played by such factors as an alcoholism, an abuse of narcotics, contact to extractors, etc. The influence of external environment and genetic predisposition in the form of mutant variant of a polymorphism of a gene of TLR6 (Ser249Pro) are characterized by the fibrous and cavernous tuberculosis. The risk ratio of development of tuberculosis in carriers of mutant variants of polymorphism of TLR3 (Phe412Leu) and TLR6 (Ser249Pro) genes is higher in comparison with other variants of polymorphism of the same and other genes.

Introduction

Tuberculosis (TB) is the chronic infection of lungs proceeding imperceptible or with a picture of an acute sepsis [1]. According to literature the development of tuberculosis is caused by many reasons, but the main is depression of efficiency of functioning of immune system [2].

Interaction of the congenital and acquired immunity provides the effective inflammatory answer referred on elimination of infectious agents [3, 4]. An important role in effective work is played by Toll-like receptors. Toll-like receptors (English toll-like receptor, TLR; German toll — remarkable) — a class of cellular receptors with one transmembrane fragment which distinguish conservative structures of microorganisms and activate a cellular immune response. They are presented on different types of cells from epithelial to immunocompetent [5, 6]. Today it is known 13 TLRs among which TLR1–9 are taped in mice and the person; TLR10 — only in the person; TLR11–13 — only at mice [7].

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The mechanism of action of TLRs consists in signal transmission in a core of the cell and a dimerization which is followed by change of conformation of the TIR domain which is bound to the adaptor molecule MyD88 (myeloid differentiation protein 88) necessary for attraction of kinases of the IRAK family (IL-1 receptor associated kinase) [8]. The mechanism of MyD88-independent transfer of activation signals from TLRs is possible. At the same time the TIR domain interacts with the adaptor molecule TRIF (TIR domain-containing adaptor inducing IFN-β) with the subsequent activation of an intracellular factor of IRF-3 (interferon regulatory factor 3) inducing an expression of genes of interferons α and β (IFN-α and IFN-β) which are molecules for a differentiation of T-lymphocytes [9].

Thus, Toll-like receptors are the first signal molecules distinguishing ligands of microorganisms and coordinating an immune response at infectious and noninfectious diseases. Until now in literature there were no data about a complex research of Toll-receptors at tuberculosis. Therefore researches which will allow to establish a role of signal receptors in maintenance of normal reaction of an immune response at a pulmonary tuberculosis and also their genetic polymorphism are actual.

Aim of the Research

To determine the frequency of occurrence of alleles and genotypes of genes of TLR2 (Arg753Gln), TLR3 (Phe412Leu), TLR4 (Asp299Gly), TLR6 (Ser249Pro) in patients with different clinical forms of tuberculosis of respiratory organs in Zabaykalsky Krai.

Materials and Methods

The research is conducted on the basis of therapeutic department of the Transbaikal regional clinical phthisiopulmonology center of Chita. The program of the research included 120 patients with the established diagnosis of tuberculosis of respiratory organs at the age of 35 to 56 years old. DNA samples served as material for a research. DNA was extracted from the nucleated cell of the peripheric blood by means of reagent "DNA, an express blood" (Litekh, LLC STP, Moscow). In parallel with a sample of the extracted DNA two reactions of amplification with two pairs of the allele-specific primers were carried out in real time and gel electrophoresis with detection in ultra-violet light. The received results allowed to give three types of the conclusions: normal homozygote, heterozygote, mutant homozygote.

All patients were divided into the following groups, depending on a clinical form of tuberculosis. 11 patients with small clinical forms of tuberculosis (a pleuritis, a tuberculoma, focal tuberculosis) were included into the first group. The second group is the group with infiltrative tuberculosis (54 people), the third group is group with disseminated tuberculosis (34 patients), and the fourth group were made by 21 patients with the fibrous and cavernous tuberculosis (FCT) [10]. We compared each of groups with the group of control by a set of the same polymorphic variants of genes of Toll-like receptors. Populational control made 30 almost healthy people who never had tuberculosis and followed-up in the center.

The patients with a diabetes mellitus of the I type and bronchial asthma were not included in the research; the persons drinking to excess and smokers were not excluded from the program.

The clinical picture was different in order to a tuberculosis form. At small forms the clinic was most often erased; tuberculosis was determined by passing radiological examination (professional examination). Patients didn't complain, there were no physical changes [10].

The intoxicating and bronchopulmonary syndromes acted as leaders at infiltrative tuberculosis in clinic. Physical data were typical for infiltrative tuberculosis (harsh respiration, multifarious moist rales over a lesion zone, a dullness of the percussion sound in the same zone) [10].

In clinic of disseminated tuberculosis at auscultation the respiration is weakened in lower portions, throughout multifarious rales, insignificant pain at a palpation of a thorax [10].

There are destructive changes at fibrous and cavernous tuberculosis in a pulmonary tissue. The intoxicating and bronchopulmonary syndromes and a syndrome of a respiratory disturbance are leading in a clinical picture of a disease. The expressed cachexia, paleness of integuments, a cyanosis of a nasolabial triangle, a retraction supraclavicular fossa and infraclavicular fossa, asymmetry of a thorax (a retraction on the side of a lesion) are expressed at the examination. Physical data corresponded to fibrous and cavernous tuberculosis [10].

The criterion χ^2 was used for a statistical assessment of differences of qualitative signs during the study of a polymorphism of genes. Differences were considered statistically significant at $p < 0.05$. Degree of the risk of events development was estimated according to size of the relation of chances (Odds Ratio (OR)) with calculation for it by 95% of a confidential interval (CI). OR = 1 was interpreted as absence of associations; OR > 1 was interpreted as positive association (the increased risk of development of pathology); OR < 1 was interpreted as negative association of a genotype with tuberculosis (the lowered risk of development of pathology). Mathematical processing of the received results was carried out by methods of nonparametric statistics on the personal computer by means of Statistica 10 and with use of the online-calculator (http://gen-exp.ru/calculatoror.php).

Results and Discussion

All required alleles in homozygotic and a heterozygotic state were found out during the research. Distribution of single nucleotide polymorphism of genes of cytokines in the studied groups corresponded to Hardy's — Weinberg's distribution.

In groups of small forms of tuberculosis and control of a homozygote according to allelic variant Arg/Arg of a gene of TLR2 (Arg753Gln) were determined approximately in equal amount and were made 100% (11 TB patients) and 94% (28 healthy people) respectively (Table 1). The heterozygous Phe/Leu variant of a gene of TLR3 (Phe412Leu) met more than in 50% of cases (6 patients and 17 healthy people respectively) irrespective of group of investigated. The carriership of allele in a mutant condition of a gene of TLR3 Leu/Leu was revealed in group of small forms in 9% of observations ($p < 0.05$) ($OR_1 = 2.82$, CI 95% 0.36–171.72). The normal genotype of a polymorphism of Asp/Asp prevailed at a gene of TLR4 (Asp299Gly) in both groups in 81% (9 patients) and in 97% (29 healthy) cases respectively. A polymorphism of a gene of TLR6 (Ser249Pro) was characterized by prevalence of normal homozygotes of Ser/Ser — 64% (7 patients in group of small forms of a tuberculosis), and in group of control — 80% (24 investigated). The mutant variant of a polymorphism Pro/Pro of a gene of TLR6 was observed only in a group of persons having tuberculosis and it made 27% (3 patients) ($p < 0.05$) ($OR_1 = 11.54$, CI 95% 0.56–236.25) (see Table 1).

Statistically significant differences were not received in distribution of genotypes of a gene of TLR2 (Arg753Gln) in group of control and in group of patients with an infiltrative form of tuberculosis (Table 2). The carriership in a heterozygous state allele Phe/Leu prevailed in a polymorphism of a gene of TLR3 (Phe412Leu) in both groups. Existence of mutant allele Leu/Leu in a gene of TLR3 was characteristic only to infiltrative forms of tuberculosis. The normal homozygotes of Asp/Asp prevailed in 69% (37 patients) and in 97% (29 healthy people) of cases respectively in a polymorphism of a gene of TLR4 (Asp299Gly) in both groups. The normal homozygotes of Ser/Ser prevailed in group of control — 80% (24 people) in a polymorphism of a gene of TLR6 (Ser249Pro). The normal homozygotes and heterozygotes made the equal amount on 45% (on 24 patients) respectively in group of patients with infiltrative tuberculosis (see Table 2).

Differences in a polymorphism of a gene of TLR2 (Arg753Gln) were not found out in group of patients with disseminated tuberculosis of essential ($p > 0.05$): normal homozygotes of Arg/Arg prevailed in both groups — on 94% (32 patients and 28 healthy people) respectively (Table 3). Prevalence of heterozygous Phe/Leu was estimated in a polymorphism of a gene of TLR3 (Phe412Leu) in both groups more than in 50% of cases (20 patients and 17 healthy people). Mutant homozygotes of Leu/Leu were found out in none of groups. Normal homozygotes of Asp/Asp prevailed in a polymorphism of a gene of TLR4 (Asp299Gly) in both groups in 85% (29 patients) and 97% (29 healthy

Table 1

Frequency of a polymorphism of genes of TLR2, TLR3, TLR4, TLR6 is in groups of small forms of tuberculosis and control

Genotypes	Patients (n = 11), abs. (%)	Control (n = 30), abs. (%)	χ^2	p	OR_1	CI 95%
		TLR2 (Arg753Gln)				
Arg/Arg	11(100)	28(94)	—	—	1.43	0.12–16.86
Arg/Gln	0(0)	2 (6)	0.77	0.6	0.70	0.06–8.26
Gln/Gln	0 (0)	0(0)	—	—	0.00	0.00
		TLR3 (Phe412Leu)				
Phe/Phe	4(36)	13(43)	—	—	0.80	0.26–2.51
Phe/Leu	6(55)	17(50)	2.83	0.002	0.84	0.27–2.58
Leu/Leu	1(9)*	(0)	—	—	2.82	0.36–171.72
		TLR4 (Asp299Gly)				
Asp/Asp	9(81)	29(97)	—	—	0.11	0.01–1.03
Asp/Gly	2(18)	1(3)	2.62	0.27	6.82	0.70–66.16
Gly/Gly	0(0)	0(0)	—	—	0.00	0.00
		TLR6 (Ser249Pro)				
Ser/Ser	7(64)	24(80)	—	—	0.50	0.14–1.79
Ser/Pro	1(9)	6(20)	9.03	0.001	0.94	0.23–3.85
Pro/Pro	3(27)*	0(0)	—	—	11.54	0.56–236.25

* Differences are statistically significant ($p < 0.05$).

Table 2

Frequency of a polymorphism of genes of TLR2, TLR3, TLR4, TLR6 is in groups of patients with an infiltrative form of tuberculosis and control

Genotypes	Patients (n = 54), abs. (%)	Control (n = 30), abs. (%)	χ^2	p	OR_1	CI 95%
		TLR2 (Arg753Gln)				
Arg/Arg	45(83)	28(94)	—	—	0.36	0.07–1.77
Arg/Gln	9(17)	2 (6)	1.69	0.43	2.80	0.56–13.91
Gln/Gln	0 (0)	0 (0)	—	—	0.00	0.00
		TLR3 (Phe412Leu)				
Phe/Phe	19(35)	13(43)	—	—	0.71	0.28–1.77
Phe/Leu	31(58)	17(50)	2.56	0.08	1.03	0.42–2.54
Leu/Leu	4(7)*	0 (0)	—	—	5.44	0.28–104.5
		TLR4 (Asp299Gly)				
Asp/Asp	37(69)	29(97)	—	—	0.08	0.01–0.60
Asp/Gly	16(30)*	1(3)	9.09	0.001	12.21	1.53–97.48
Gly/Gly	1(1)*	0 (0)	—	—	1.71	0.07–43.30
		TLR6 (Ser249Pro)				
Ser/Ser	24(45)*	24(80)	—	—	0.20	0.07–0.57
Ser/Pro	24(45)	6(20)	10.83	0.004	3.20	1.13–9.08
Pro/Pro	6(10)*	0 (0)	—	—	8.18	0.44–150.36

* Differences are statistically significant ($p < 0.05$).

people) cases respectively. Mutant homozygotes of Gly/Gly were found out only in group of patients in 3% of cases (1 people). The homozygotic Ser/Ser variant prevailed in a polymorphism of a gene of TLR6 (Ser249Pro) in 85% of patients (29 people) and in 80% in control group (24 people) respectively ($p > 0.05$). Mutant homozygotes of Pro/Pro of the studied gene were found out in group of patients in 3% of cases (1 people), in control group they were absent (see Table 3).

Table 3

Frequency of a polymorphism of genes of TLR2, TLR3, TLR4, TLR5, TLR6 is in groups of patients with disseminated tuberculosis and control

Genotypes	Patients (n = 34), abs. (%)	Control (n = 30), abs. (%)	χ^2	p	OR_1	CI 95%
		TLR2 (Arg753Gln)				
Arg/Arg	32(94%)	28(94)	—	—	1.14	0.15–8.654
Arg/Gln	2(6%)	2 (6)	0.02	0.99	0.88	1.12–6.63
Gln/Gln	0 (0)	0 (0)	—	—	0.00	0.00
		TLR3 (Phe412Leu)				
Phe/Phe	14(41)	13(43)	—	—	0.92	0.34–2.47
Phe/Leu	20(59)	17(50)	0.03	0.98	1.09	0.40–2.95
Leu/Leu	0 (0)	0 (0)	—	—	0.00	0.00
		TLR4 (Asp299Gly)				
Asp/Asp	29(85)	29(97)	—	—	0.20	0.02–1.82
Asp/Gly	4(12)	1(3)	2.54	0.11	3.87	0.41–36.69
Gly/Gly	1(3)	0 (0)	—	—	2.73	1.11–69.60
		TLR6 (Ser249Pro)				
Ser/Ser	29(85)	24(80)	—	—	1.45	0.39–5.34
Ser/Pro	4(12)	6(20)	1.63	0.44	0.53	0.13–2.11
Pro/Pro	1(3)	0 (0)	—	—	2.73	0.11–69.60

Table 4

Frequency of polymorphism of genes of TLR2, TLR3, TLR4, TLR6 in groups of the patients suffering from the fibrous and cavernous tuberculosis and control group

Genotypes	Patients (n = 21), abs. (%)	Control (n = 30), abs. (%)	χ^2	p	OR_1	CI 95%
		TLR2 (Arg753Gln)				
Arg/Arg	20(83)	28(94)	—	—	1.43	0.12–16.86
Arg/Gln	1(17)	2 (6)	0.08	0.96	0.70	0.06–8.26
Gln/Gln	0 (0)	0 (0)	—	—	0.00	0.00
		TLR3 (Phe412Leu)				
Phe/Phe	8(38)	13(43)	—	—	0.80	0.26–2.51
Phe/Leu	11(52)	17(50)	2.99	0.002	0.84	0.27–2.58
Leu/Leu	2(10)*	0 (0)	—	—	7.82	0.36–171.72
		TLR4 (Asp299Gly)				
Asp/Asp	16(76)	29(97)	—	—	0.11	0.01–1.03
Asp/Gly	4(19)	1(3)	5.13	0.08	6.82	0.70–66.16
Gly/Gly	1(5)	0 (0)	—	—	4.46	0.17–115.03
		TLR6 (Ser249Pro)				
Ser/Ser	14(67)	24(80)	—	—	0.50	0.11–1.79
Ser/Pro	4(19)	6(20)	4.59	0.001	0.94	0.23–3.85
Pro/Pro	3(14)*	0 (0)	—	—	11.54	0.56–236.25

* Differences are statistically significant ($p < 0.05$).

The essential changes were not revealed in group of the patients suffering from fibrous and cavernous tuberculoses at an assessment of polymorphism of a gene of TLR2 (Arg753Gln). The normal variant of polymorphism Arg/Arg of the studied gene was more often observed in 83% (20 patients) and in 94% (28 people) of cases respectively ($p > 0.05$) in both groups (Table 4). The heterozygotic Phe/Leu variant prevailed in polymorphism of a gene of TLR3 (Phe412Leu) in both groups: 52% in group of the patients with fibrous and cavernous tuberculosis (11 people) and 50% in group of monitoring (17 people) ($p < 0.05$) ($OR_1 = 0.84$, CI 95% 0.27–2.58). Prevalence of genotypes in a homozygotic status was revealed in polymorphism of a gene of TLR4 (Asp299Gly) in 76% in group of the patients with fibrous and cavernous tuberculosis (16 people) and in 97% of cases in a control group (29 people) respectively. The mutant homozygote of Gly/Gly was found out in one patient. The normal Ser/Ser variant in polymorphism of a gene of TLR6 (Ser249Pro) the in group of healthy made 80% (24 people) and in group of the patients with fibrous and cavernous tuberculosis it made 67% (14 people) of cases respectively. The mutant homozygote of Pro/Pro of the researched gene was revealed only in group of patients in 14% (3 people) ($p < 0.05$) ($OR_1 = 11.54$, CI 95% 0.56–236.25) (see Table 4).

Thus, analyzing results of the research, it is possible to consider that the carriership of a mutant polymorphism of genes of Toll-like receptors is an essential predictor of development of some forms of tuberculosis of respiratory organs.

Conclusion

Crucial importance has genetic predisposition in the form of a carriership of mutant genotypes of genes of TLR3 (Phe412Leu) and TLR6 (Ser249Pro) for development of small forms of tuberculosis and infiltrative tuberculosis. At the same time in a pathogenesis of disseminated tuberculosis an important role is played by such factors as an alcoholism, a narcomania, contact to extractors, etc. Development of fibrous and cavernous tuberculosis is caused both by influence of factors of the external environment, and genetic predisposition in the form of mutant variant of a polymorphism of a gene of TLR6 (Ser249Pro). The relative risk of development of tuberculosis in carriers of mutant variants of a polymorphism of genes of TLR3 (Phe412Leu) and TLR6 (Ser249Pro) is higher in comparison with other variants of a polymorphism of the same and other genes.

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