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Association of Tumor Necrosis Factor-α (TNFα) Gene Polymorphisms with HLA Class II Alleles in HIV/AIDS Patients

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Authors' contributions

This work was carried out in collaboration between all authors. Author JE designed the study, wrote the first draft of the manuscript, DNA purification from blood samples, HLA and TNFα typing. Author IJ wrote the protocol. Author JG managed the literature searches and wrote draft of the manuscript. Author EU managed the literature searches and performed the statistical analysis. Author BR gathered and formed a group of patients. All authors read and approved the final manuscript.

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ABSTRACT

Aim of Study: To identify the factors of molecular genetic risks during the development of infection in HIV, based on the TNFα cytokine gene polymorphism in combination with HLA DRB1/DQA1/DQB1 genes, as well as to analyse their possible association with the progress of the disease. 185 HIV infected patients and 173 individuals control group have been analysed. The DNA was extracted from peripheral blood, by using QiagenQIAamp DNA kit reagents. The quality and quantity of DNA was checked by using *Qubit ® fluorometer* HLA typing for HLA DRB1/DQB1/DQA1* was performed by RT-PCR with sequence-specific primers (SSO). TNFα gene G–238A and G–308A polymorphic variant incidence was determined by RT-PCR analysis.

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Results: We have detected TNFα gene allele 308A in 11% HIV infected patients, whereas in control group this allele have been detected only in 4% patients. Although the incidence of the TNFα gene *–238A* allele was twice as high in the control group (6%) as in the HIV infected patients (3%), it did not prove to be statistically valid ($p = 0.253$). The incidence analysis of three-locus haplotypes DRB1-DQB1-DQA1 – in TNFα position-238A/G -308A/G showed that haplotypes 01:01/05:01/01:01-TNFα-238(GA)/308(GG) and 01:01/03:02/03:01 - TNFα-238(AA)/308(GG) are more frequent in the control group in comparison to the groups of infected patients. This means that these haplotypes have a protective function, which significantly affects the progress of infection. The association of *15:01/05:01/01:01 -TNFα-238(GG)/308(GG)* and *03:01/05:01/01:01- TNFα-238(GG)/308(GA)* genotypes indicates a high risk of developing a fulminant infection. The genetic factors of AIDS-related complex of syndromes development are associated not only with the HLA complex class II alleles, but also with the SNP polymorphism in the promoter region of cytokine genes.

Keywords: Tumour necerosis factor-a (TNFα); HIV; HLA; RT-PCR; polymorphism.

1. INTRODUCTION

HIV is a slow and chronically progressing disease [1]. HIV target cells are CD4+ T lymphocytes that are a very important part of the immune system [2,3]. Up to this date, the disease cannot be cured and its consequences are lethal. However, it may take several years until HIV infection develops into AIDS. Also contemporary pharmacological achievements prolong survival and improve the quality of life of HIV patients [4,5].

One of the most urgent medical problems is infectious diseases, of which HIV, tuberculosis (TBC), hepatitis B and C viruses (HBV, HBC) are the leading ones, according to the rapid expansion and an increase in mortality due to these diseases [6,7]. HIV, TBC and HBV, HCV, as well as any other infectious diseases have an imbalance of cytokines and their normal functions [8,9]. Cytokine imbalance is associated with a T-lymphocyte destruction, which in turn leads to a progression of immunosuppression and the consequences of its further development. The efficiency of cytokines depends also on the activation of overall and specific transcription genes, despite the fact that they are located on different chromosomes [10-12]. The research of the HLA phenotype is one of the immunogenic parameters to predict the development of infectious diseases in the infected patients. The research data indicate that HLA-D locus participates most actively in the development of the pathogenesis of tuberculosis. These antigens determine the tendency and intensity of the adaptive immunity in case of an infection. The most pronounced associations are connected with DRB1 allele, which is linked to HLA II class [13-15]. It is known that the development of individual immune response characteristics, as well as predisposition to various diseases, including infections, is associated not only with the HLA gene polymorphism, but also with single-nucleotide polymorphism (SNP), which is equally important for the implementation and regulation of the immune response [14,15].

Several recent studies have shown the connection between the hyper- and hypo-production of the genetically-determined cytokine and the quality of the immune response, which is also associated with the severity, development and consequences of an infectious disease [12,16,17]. So it should be possible to find out the regulating role of HLA DRB1 and other II class alleles in the cytokine activity response to various pathologies.

1.1 Aim of Study

To identify the factors of molecular genetic risks during the development of infection in HIV patients, based on the TNFα cytokine gene polymorphism in combination with major histocompatibility complex (HLA class II DRB1/DQA1/DQB1 genes), as well as to analyse their possible association with the progress of the disease.

2. MATERIALS AND METHODS

The research was carried out the ChromSword within the project managed by Environment, Bioenergetics and Biotechnology Competence centre and co-financed by ERAF.

Table 1. Demographical and clinical information on the study population of total research group

Characteristic	HIV positive group n*=185	Control group n*=173	*p* value
HIV positive (AI-III stage)	185 (100%)	-	
Male	129 (70%)	137 (79%)	0.041
Female	56 (30%)	36 (21%)	0.041
Age (years) (mean ± SD)	33.6 (±13,4)	26.44(±9.87)	0.39

** n=number of patients*

185 HIV infected AI-III stage patients have been analysed during the research and Control group consisted of 173 healthy blood donors. The average age of the patients was SD 33.6 (±13.4), *p=0.39* year (see Table 1.) Out of the 185 HIV infected patients, 56 (30%) were women and 129 (70%), *p=0.041* were men. The Control group consisted of 173 healthy individuals of which 36 (21%), *p=0.041* were women and 137 (79%) were men SD 26.44(+ 9.87), *p=0.39* (Table 1). HIV infection for all the patients (100%) was proved with the primary test by determining antibodies against HIV and Western Blot tests. Only the patients who have been infected for more than 10 years were included in the study group.

The study excluded patients who were younger than 18 years of age, pregnant women, patients who are in custody or pre-trial solitary confinement, patients who acquired the infection in vertical transmission path, HIV-2 infection patients and patients who are not Latvian citizens or are non-permanent residents of Latvia.

The DNA was extracted from peripheral blood, by using Qiagen QIAamp DNAkit reagents as per the manufacturer's protocol [18]. The quality and quantity of DNA was checked by using *Qubit* ® *fluorometer* (*Invitrogen* USA). HLA typing tests and group, data of healthy donors (n=173), were performed in the IK Elmedicum of Latvia

Low-resolution HLA typing for HLA DRB1*; DQB1*; DQA1* was performed by RT-PCR with amplification with sequence-specific primers (SSO). TNFα gene G–238A and G–308A polymorphic variant incidence was determined by RT-PCR analysis as described previously (DNA-technology, Moscow, Russia).

The amplification of TNF α gene fragment was done with DT Light Cycler instrument (DNA Technology, Russia). Reagents Amplification primers and Hybridization Probe combined in Tool Sets. Oligo Primer Analysis Software (Med Probe, Oslo, Norway).

The distribution of genotypes was tested by the Hardy-Weinberg equation in which defined criteria were used by the program Genepop version 4.2. [19]. To test the homogeneity of the results, two types of criteria were used: the chi-square (χ2) and the Fisher criterion (Fi). These two measurements were performed with StatXact-4 software (Cytel Software Corporation, Inc., USA) [20,21].

3. RESULTS

Research of HLA II class DRB1, DQA1, DQB1* genetic markers with HIV infected stage AIDS. The immunogenetic research shall be performed to find out the possible associations between the development risk of AIDS and the particular HLA II class gene genotypes – DRB1*/DQA1*/DQB1*. To compare frequency of HLA haplotype incidence a test was made between HIV infected patients in AIDS group and Control group (healthy blood donors) (Table 2).

This work is a continuation of the Research of HLA II Class DRB1, DQA1, DQB1 Genetic Markers in Patients with HIV Infection and AIDS. [13,14,15,22].

The incidence of the alleles and genotype of the TNF α gene polymorphisms *G–238A* and *G–308A* is shown in Table 3. The distribution of genotypes in all the groups based on the two polymorphisms were not statistically different from each other. Of the two TNFα gene polymorphisms in HIV patients, the incidence of genotype (G/G, G/A and A/A) alleles (G and A) differed from the control group only in the G–308A polymorphism (respectively, *p*=0.037 and *p*=0,012). Detailed analysis showed that mainly the homozygous G/G causes a heterogeneous distribution. Their incidence in the control group is slightly higher than in the infected patients (84% versus 59%) with the p-value of 0.03. The genotypes G/A and A/A in both groups form statistically valid results, i.e., in this case they can be viewed as a whole. It is considered that the TNFα gene allele in the position –308A is

associated with an increased production of TNFα. Thus, also in homogeneous group in which the genotypes *–308A/–308A*; *–308G/–308A* are combined, it appeared that the stimulating TNFα gene was dominant in the infected patients, however, in the control group — the gene that inhibited expression and synthesis of TNFα.

As for the G–238A dimorphism in the same gene, the genotype and allele frequencies are not statistically different from each other in the HIV patients and the healthy control group, (respectively, p = 0.062 and p = 0.25).

The incidence analysis of three-locus haplotypes DRB1-DQA1-DQB1 – in TNFα position-238A/G -308A/G showed that the haplotypes HLA-DRB1/DQA1/DQB1-01:01/01:03/06:02-8-TNFα-238(GA)/308(GG) and 13:01/01:02/06:02-8 -TNFα-238(AA)/308(GG) are more frequent in the control group in comparison to the groups of infected patients. This means that these haplotypes have a protective function, which significantly affects the progress of infection The association of DRB1-DQA1-DQB1-15:01/01:01/05:01 -TNFα-238(GG)/308(GG) and 15:01/01:02/03:02- TNFα-238(GG)/308(GA) genotypes indicates a high risk of developing a fulminant infection (Table 4).

4. DISCUSSION

The tumor necrosis factor-α (TNF-α)-308G/A and -238G/A polymorphisms influences the pathogenesis and evolution of HIV-1 disease. [23,24]. There is a lot of data that show the TNFα production changes in various different virus infections. However, an excessive TNFα production may be the cause of complications of acute inflammatory processes and of a septic shock, and it also has an important role in the pathogenesis of infectious and autoimmune diseases [25-27].

Hyperproduction of TNFα is one of the main mechanisms during the infection activation process, when it transits from the latent phase to clinical manifestations, as well as shows progression of a disease. Anelevated level of TNFα in plasma was found at the exacerbation phase of chronic diseases, which are induced by

Table 2. To compare frequency of HLA haplotype incidence a test was made between HIV infected patients in AIDS group and control group (healthy blood donors)

HLA- DQB1/DQA1/DQB1	Oddsratio (OR)	P	Group (Gf)	Control group (Gf)
Immunogenetic riskhaplotypes (predispositionhaplotypes)				
01:01/01:01/05:01	2.35	0.009	0.12	0.03
15:01/01:01/05:01	3.49	0.039	0.36	0.01
15:01/01:02/03:02	8.34	0.013	0.07	0.04
Protectivehaplotypes (resistancehaplotypes)				
01:01/01:03/06:02-8	0.31	0.030	0.51	0.33
13:01/01:02/06:02-8	0.21	0.0001	0.02	0.01

The numbers in brackets have been indicated in a successive order OR/ P<0.05; OR=odds ratio; gf=haplotype frequency

Table 3. Distribution of alleles and genotypes of TNFα gene polymorphisms G-308A and G-238A in the groups of HIV infected and healthy patients

Polymorphism	Genotypes and alleles	Patients *N* = 186		Control group *N* = 173		p-value
		n	gf	n	gf	
G–308A	*G/G*	109	0.59	145	0.84	0.019
	G/A	72	0.39	26	0.15	0.037
	A/A	5	0.03	2	0.01	0.017
	G	290	0.78	316	0.91	0.007
	A	82	0.22	28	0.08	0.012
G–238A	*G/G*	124	0.67	101	0.58	0.062
	G/A	58	0.31	72	0.42	0.048
	A/A	4	0.02	0	0.001	ND
	G	306	0.82	274	0.74	0.250
	A	62	0.17	72	0.21	0.253

n=number of people; OR=odds ratio, P=0.05, gf= gene frequency

Table 4. Associations of TNFα gene genotypes -308A/G, -238A/G in positions with HLA-DRB1, DQA1 and -DQB1 gene haplotypes between healthy individuals and HIV infected patients with AIDS

HLA-DRB1*/DQA1*/DQB1* haplotypes	Genotype TNFα G–238A	Genotype TNFα G–308A	Patients gf	Control group gf	OR	P
Immunogenetic risk haplotypes (predisposition haplotypes)						
15:01/01:01/05:01	G/G	G/G	0,159	0,83	2,15	0,023
15:01/01:02/03:02	G/G	G/A	0,062	0,05	7,61	0,008
01:01/01:01/05:01	G/G	A/A	0,011	0,048	0,92	0,028
Protective haplotypes (resistance haplotypes)						
13:01/01:02/06:02-8	A/A	G/G	0,045	0,116	0.27	0,031
01:01/01:03/06:02-8	G/A	G/G	0,087	0,161	0,19	0,041

OR=odds ratio, P=0.05, gf= haplotype frequency

HBV, HCV, HIV, herpes simplex virus (HSV), Epstein-Barr virus, cytomegalovirus, poliomyelitis, tick-borne encephalitis, etc [28-30]. The inhibition of the TNFα production in the chronic state of infection process is considered to be dysregulation of immune response. One of the main biological functions of TNFα is considered its participation in the regulation of apoptosis, including for/in/at cells damaged by viruses [31,32]. It is known that an increase in the TNFα — a programmed cell death inducer — production during the early stages of chronic HBV and HCV infection can act as a gateway for an increased hepatocyte apoptosis by promoting liver cell damage and as consequently lead to the development of malignant tumours [33-35]. The term “functional polymorphism” means a replacement of a single nucleotide or tandemic repeats of nucleotide regions in non-coded parts of genes (promoter, introns). Such changes do not affect the protein structure, but in some cases changes the mRNA transcription rate (speeding it up or slowing it down). The TNF gene is located on the locus of the sixth chromosome (6p21.3) which encodes the first (HLA-A, B, C) and the second (HLA-DP, DQ, DR) class of the major histocompatibility complex [36]. Its location in the middle of the genome determines the high variability of locus, especially promoter part of TNFα gene, which includes the eight polymorphic regions with a single-nucleotide replacement: -1031T/C, -863C/A, -857C/T, -575G/A, -376G/A, -308G/A, -244G/A, -238G/A. However, only two replacements are considered to be the most significant. They are a single-nucleotide replacement of guanine to adenine in the 308 (G/A) and -238 (G/A) position, which leads to changes in the level of TNFα production, and they are considered to be functional changes. Thepositions -308 and -238 are on the promoter part, which enables transcription factors to be linked with this part of a gene, and thus influence the transcription rate. These substitutions of nucleotides are quite common and frequent, e.g., 27-33% of the Caucasian genotype has the polymorphic (rare) allele -308*A and about 7-10% — a rare allele -238*A [37]. De Jager, et al. [38] measured the TNFα production by mononuclear substances of peripheral blood, which was stimulated by concanavalin A. The study showed that the donor cells, which according to the polymorphic allele -308 * A are homozygous, synthesize cytokines three times more actively than the cells of patients which have the -308 GG genotype. After the study, four independent groups of researchers used a chimeric structure, which consisted of the promoter part of the TNFα gene and the transcriptional activation protein, which is easy to measure (the enzyme chloramphenicol acetyltransferase or luciferase) [39,40]. As a result, it was proven that the nucleotide guanine replacement to adenine in the position 308 greatly increases the transcriptional activity and accelerates the production of the mRNA. Thus, the -308 polymorphism increases the transcription speed of the TNF α gene and, respectively, the cytokine production.The transcription speed increase rate depends on the substances which affect the cells, and cells of the same type.The speed increase of transcription rate depends on the substances which affect the cells, and on the type of a cell. In most cases, this is done in a way as described above, but there are several studies, which do not prove the impact of the -308 polymorphism on the effectiveness of the TNFα gene transcription [41,42]. The reason for the differences apparently lays in the fact that different types of

cells in which the plasmids are transfected were used in the studies. The transcription of the most active polymorphic gene TNFα (-308*A) occurs in macrophages where it is 5 times higher than the transcription of the normal -308*G gene. Taking into account the fact that macrophages are the main source of TNFα, their genetically determined ability to increase a proimflammatory cytokine production may influence inflammatory and immunological processes in the body.Theposition-238 is considered to be another TNFα gene polymorphic region that may affect the production of cytokine. However, in this case, the replacement of guanine to adenine decreases protein production not increases it. The whole blood cell stimulation with lipopolysaccharide showed that the cells with the genotypes -238GA synthesize 1.5 times less TNFα than the cells with the genotype -238GG. It has to be noted that the stimulation of monocyte fraction of peripheral blood with streptococcus superantigen increased this difference by 2.8 times [43]. It turned out that the change of the nucleotide -308 (G/A) and -238 (G/A) causes the opposite reaction on the TNFα production: -308 increases it, while the -238 reduces. Theoretically, co-location of these two polymorphisms in the human genotype should compensate for each other's actions and have no effect on cytokine production, but in practice these two polymorphisms are rarely found together.The high-producing allele -308*A is mostly inherited together with the normal -238*G allele, while the normal -308*G allele together with the low-producing -238*A allele [44].

New gene markers of the given cytokine alleles that are susceptible to virus infections have been researched very little in medicine. Polymorphic cytokine genes actively participate in the formation of the specific immune response. Separate allelic variants in the gene may be associated with the production of specific protein levels, which in turn affects the course of the disease and the various complications of the disease [41,45]. The immune cells secrete a variety of mediators (cytokines) which have high specificity. TNFα is one of this kind of cytokines which participates in formation of inflammatory response: initiates synthesis of IL-1, IL-6, which in turn activates neutrophilic granulocytes, macrophages, and stimulates proliferation of T and B lymphocyte. TNFα participates in the pathogenesis of most infectious and immunopathological diseases [42]. According to studies that have been conducted in the last few years, heritability factor has a role in the development of infection process. Genetically programmed increased or decreased synthesis of TNFα, affects the human immune system's ability to respond to different types of pathogens and the development of immunepathological processes [36].

Several polymorphisms have been detected in the TNFα gene promoter; one of them is located in the -308 position in which guanine defines the common (widespread) allele (TNF1; –308G).At the same time, the replacement of guanine to adenine presents itself as an unusual allele (TNF2 G–308A), which is considered to be quite a strong transcriptional activator with a 6-7 times higher induction of TNFα gene transcription. By contrast, another A polymorphic variant (G–238A) of the TNFα gene allele is associated with a decreased production of TNFα.

It is known that the TNFα levels in the blood correlate with the disease activity in polyarticular and oligoarticular JIA patients. Gene TNF coding TNFα is localized on the short arm of the 6^{th} chromosome, according to the data found in other studies, single-nucleotide polymorphisms (SNP) -308A/G, -238A/G and 244AA, which are localized in the gene promoter region may affect the quantity of the TNFα production. There is still conflicting data on the effect of SNP on the development of JIA, prognosis and therapy efficiency. Valid genotype -308A/G was found more rarely in seronegative and seropositive polyarthritis, persistent oligoarthritis and enthesitis-related arthritis patients (respectively, $p = 0.000$, $p = 0.002$, $p = 0.000$ and $p = 0.000$). The differences in the distribution of the -238A/G genotype between types and control of JIA were not valid ($p = 0,05$).

A valid difference was found between JIA and control groups in the -244AA genotype ($p = 0.023$), but the differences between the types of JIA were not found ($p = 0.05$). From the aforementioned study it can be understood that the data acquired confirm the role of the TNFα gene polymorphisms in the pathogenesis of JIA. The polymorphism -308A/G could have a protective role in the development of seropositive and seronegative polyarthritis, persistent oligoarthritis and enthesitis-related arthritis. The polymorphism 244AA genotype might have a protective role, but it has no association with any of the four types of JIA [46].

The first human infectious disease, which was linked with the TNFα gene variability, was

malaria. The studies were conducted in Brazil and more than a total of 263 healthy individuals (control group) and 216 individuals infected by *P. vivax* (malaria group) were genotyped for 33 single nucleotide polymorphisms (SNPs) in IL1B, IL2, IL4, IL4R, IL6, IL12B, and P2X7 genes were researched. The results of genotyping showed that the IL1B gene -5839C > T and IL4R 1902A > G polymorphisms and IL12RB1 -1094A/-641C and TNF -1031 T/-863A/-857 T/-308 G/-238 G haplotypes were associated with malaria susceptibility after population structure correction ($p = 0.04$, $p = 0.02$, $p = 0.01$ and $p = 0.01$, respectively) [47]. Similar studies have been conducted in Sri Lanka and the link between the allele -308*A and the risk of developing the cerebral form of malaria was confirmed [48]. Nasi et al HIV-1 infection study showed that IL-10 is able to inhibit virus replication in cases when the proimmflamatory cytokines TNFα and IL-1 stimulates replication of the virus [49].

This study analyses the possible correlation between the TNFα polymorphic alleles and the progression of the disease. The ability of the TNFα allele -238A to delay the progression of HIV infection can be considered as one of the most statistically valid results. Although the functional effect of the TNFα -238 A allele on transcriptional activity is not entirely clear, it may be considered as a beneficial factor in cases when viral replication was reduced. The study identified a genetic relationship between the TNFα -238 variants of alleles and the HLA class II genes, and a longer period of latent condition. The meaning of these differences in is not entirely clear, but the existing data may mean that the latent state in case of HIV infection could be from multiple, combined genetic variants which by merging slow the progression of the disease. The results even more emphasize the role of genetic determinants during the development of HIV infection. The obtained data also confirm the HLA loci ability to control the progression of the disease.

The main limitation of this study was the small sample of the selected group and adding of new patients to the study. However, the obtained results allow understanding the possible mechanisms that characterize the control of infection progression.

5. CONCLUSION

The incidence analysis of three-locus haplotypes DRB1-DQA1-DQB1 – in TNFα position -238A/G -308A/G showed that the haplotypes HLA-DRB1/DQA1/DQB1-01:01/01:03/06:02-8- TNFα-238(GA)/308(GG) and 13:01/01:02/06:02-8 - TNFα-238(AA)/308(GG) are more frequent in the control group in comparison to the groups of infected patients. This means that these haplotypes have a protective function, which significantly affects the progress of infection. The association of HLA- DRB1/DQA1/DQB1-15:01/01:01/05:01-TNFα-238(GG)/ 308(GG) and 15:01/01:02/03:02 genotypes indicates a high risk of developing a fulminant infection. The genetic factors of AIDS-related complex of syndromes development are associated not only with the HLA complex class II alleles, but also with the SNP polymorphism in the promoter region of cytokine genes. For the acquisition of statistically valid data, it is necessary to increase the number of patients in each of the research groups.

CONSENT

All authors declare that written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images.

ETHICAL APPROVAL

All the authors hereby declare that all the experiments have been examined and approved by the appropriate ethics committee No. A-14 and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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