The -308 Promoter Polymorphism in the Tumour Necrosis Factor Gene in Patients with Lichen Planus

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The close chromosomal proximity of major histocompatibility complex and tumour necrosis factor genes may suggest the involvement of the latter in the pathogenesis of immunological diseases associated with HLA antigens. The major histocompatibility complex antigens may play a certain role in the pathogenesis of lichen planus. Therefore we examined the -308 promoter polymorphism of tumour necrosis factor gene in patients with lichen planus. Sixty-six patients and 66 age- and sex-matched controls participated in the study. Genomic DNA from patients and controls was typed for tumour necrosis factor (-308) polymorphism using an allele-specific polymerase chain reaction. We did not find any correlation between genotype distribution, allele frequency and lichen planus.

In our study we failed to observe any association between a genotype and mean level of tumour necrosis factor in plasma of our patients. Several polymorphisms have been identified in the TNF gene. All of them are single nucleotide substitutions (SNP) like T-C substitution at position -1031, C-A at position -863, C-T at position -857, G-A at -308 and G-A at -238 (7). In transfection studies it has been demonstrated that less frequent occurrence of TNF2 allele at position -308 is associated with an elevated level of both basic and induced TNF production (6, 8). However, other authors have not confirmed this relationship (9). TNF2 allele has been observed to be associated with susceptibility to a number of diseases including cerebral lesions in malaria (10), dermatitis herpetiformis (11), non-Hodgkin lymphoma (12), chronic bronchitis (6), acute heart-graft-rejection (13) and melioidosis (14). No such association has been found for diseases such as ankylosing spondylitis (15), rheumatoid arthritis (16) or psoriasis type I (17).

Taking into consideration the possible role of TNF and MHC antigens in the pathogenesis of lichen planus and the chromosomal proximity of TNF and HLA antigens, it still needs to be verified if TNF polymorphism correlates with the susceptibility to lichen planus and with the rate of production of this cytokine in this disease.

MATERIALS AND METHODS

Sixty-six patients with lichen planus and 66 age- and sex-matched controls participated in the study. The patients were divided into two subgroups. Group I comprised 33 patients with generalized skin lesions and group II comprised 33 patients with circumscribed skin lesions. Peripheral blood samples were collected from the antecubital vein in an EDTA-coated tube.

DNA isolation

DNA was extracted from 10-ml peripheral blood samples drawn into EDTA tubes according to the procedure described by Madisen et al. (18). DNA was stored at 4°C.

Amplification of DNA by means of polymerase chain reaction (PCR) with primers specific for the TNF and restriction analysis of the amplified fragment was conducted. The 345-bp fragment of genomic DNA containing a mutation at position -308, causing a G-A substitution (guanine-adenine) was amplified by PCR. Primers with the following sequences were used for the analysis of the TNF-gene polymorphism (6): sense, 5’- AGGCAATAGGTTTTGAGGGCCAT-3’; antisense, 5’-GAGCGTCTGCTGGCTGGGTG-3’. The 345-bp fragment of genomic DNA containing a mutation at position -308, causing a G-A substitution (guanine-adenine) was amplified by PCR. Primers with the following sequences were used for the analysis of the TNF-gene polymorphism (6): sense, 5’- AGGCAATAGGTTTTGAGGGCCAT-3’; antisense, 5’-GAGCGTCTGCTGGCTGGGTG-3’.
Table I. Distribution of genotypes and allele frequencies in the TNF polymorphism

<table>
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<tr>
<th>Study group</th>
<th>Genotypes, n (%)</th>
<th>Alleles, related frequency</th>
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<tr>
<td></td>
<td>TNF1/TNF1</td>
<td>TNF1/TNF2</td>
</tr>
<tr>
<td>Patients (n=66)</td>
<td>47 (71.2)</td>
<td>17 (25.8)</td>
</tr>
<tr>
<td>Controls (n=66)</td>
<td>47 (71.2)</td>
<td>15 (22.7)</td>
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DNA amplification was carried out in a thermal cycler (MJ Research – DNA Engine PTC 200); 50 µl of the genomic DNA (approximately 300 ng) were subjected to amplification using the following composition: 200 µl of each dNTP (deoxyribonucleotides), 10 mM Tris, 15 mM MgCl₂, 20 pmole of each primer and 2 U of Taq polymerase (all reagents from MBI Fermentas). The PCR conditions were as follows: initial denaturation 5 min at 94°C followed by 30 cycles of 1 min at 94°C (denaturation), 1 min at 60°C (annealing) and 1 min at 72°C (extension). The final elongation step was performed for 7 min at 72°C.

The PCR product (TNF gene) was digested at 37°C for 5–6 h with Nco I restriction enzyme (MBI Fermentas) and then the digested sample of the amplified DNA was subjected to electrophoresis in 2% agarose gel stained with ethidium bromide. Undigested 345-bp fragments corresponded to the digested sample of the amplified DNA was subjected to electrophoresis in 2% agarose gel stained with ethidium bromide. The PCR conditions were as follows: initial denaturation 5 min at 94°C followed by 30 cycles of 1 min at 94°C (denaturation), 1 min at 60°C (annealing) and 1 min at 72°C (extension). The final elongation step was performed for 7 min at 72°C.

The PCR product (TNF gene) was digested at 37°C for 5–6 h with Nco I restriction enzyme (MBI Fermentas) and then the digested sample of the amplified DNA was subjected to electrophoresis in 2% agarose gel stained with ethidium bromide. Undigested 345-bp fragments corresponded to TNF2 (-308A) allele, whereas TNF1 (-308G) allele was susceptible to digestion, which resulted in the formation of two fragments of 325-bp and 20-bp.

The plasma was collected and frozen in 1 ml aliquots at −70°C for measurement of TNF. Plasma TNF levels were measured by an ELISA method with Med System Diagnostic GmbH, Austria. The measurements were performed according to the instructions included in the assay. To measure the absorption, ELx800™ (BIO-TEK Instruments) was used.

Statistical analysis

The differences in the frequency of each genotype between patients and controls were tested using Pearson’s χ² test. The same statistic was used to test the differences in the frequency of each genotype between patients with generalized and circumscribed lichen planus. Comparisons of allele frequencies between patients and controls and between patient subgroups were made by means of Fisher’s two-sided exact test and odds ratios (relative risk, rr) with 95% confidence intervals were calculated. The differences in plasma TNF levels were tested between patients and controls within each genotype using analysis of variance (ANOVA). A significance level of 0.05 was assumed. All statistical analyses were carried out with Statistica 5.0 software.

RESULTS

The following three genotypes of the -308 polymorphism were observed: TNF1/TNF1, TNF1/TNF2, TNF2/TNF2. The distribution of genotypes and allele frequencies in the TNF gene for patients and controls is presented in Table I. No statistically significant differences were observed between these groups either with respect to genotype distribution (χ²=0.79, p=0.673) or allele frequencies (Fisher’s two-sided exact test: p=0.869; rr=1.12, CI 0.58–2.13).

The distribution of genotypes and allele frequencies in the TNF gene for two patient subgroups (with generalized and circumscribed lichen planus) are presented in Table II. Again, the two patient subgroups did not differ significantly in genotype distribution (χ²=3.06, p=0.216) or allele frequencies (Fisher’s two-sided exact test: p=0.152; rr=0.44, CI 0.17–1.18). In patients with generalized lichen planus homozygous genotype TNF2/TNF2 occurred relatively more frequently but with no statistical significance (6.1% vs 0.0%). Taking into consideration the extremely rare occurrence of this genotype and relatively small sample size, no definite conclusions could be drawn.

No statistically significant differences were observed in mean plasma TNF levels between patients and controls (ANOVA: F=1.90; p=0.171). Fig. 1 shows mean plasma TNF levels in patients and controls within each genotype. Patients and controls within TNF1/ TNF1 or TNF2/TNF2 polymorphisms did not differ significantly (F=0.03, p=0.866 and F=1.01, p=0.389, respectively). The difference between patients and controls with TNF1/TNF2 genotypes turned out to be statistically significant (F=5.91, p=0.021). The higher value was observed in the TNF1/TNF2 heterozygote in the control group.

DISCUSSION

The results of studies investigating the relationship between MHC and lichen planus are not clear and in some cases are even contradictory (19). Simon et al. (20) reported a significantly higher occurrence of HLA-B8

Table II. Distribution of genotypes and allele frequencies in the TNF polymorphism depending on the extent of lichen planus

<table>
<thead>
<tr>
<th>Study group</th>
<th>Genotypes, n (%)</th>
<th>Alleles, relative frequency</th>
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<tbody>
<tr>
<td></td>
<td>TNF1/TNF1</td>
<td>TNF1/TNF2</td>
</tr>
<tr>
<td>I</td>
<td>21 (63.6)</td>
<td>10 (30.3)</td>
</tr>
<tr>
<td>II</td>
<td>26 (78.8)</td>
<td>7 (21.2)</td>
</tr>
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</table>

Group I, patients with generalized skin lesions; group II, patients with circumscribed skin lesions.
antigen in patients with dermal lichen planus in a German population. Close chromosomal proximity of MHC and TNF genes may suggest the participation of the latter in the pathogenesis of immunological diseases associated with HLA antigens. Therefore, undertaking the task of studying one of the TNF gene polymorphisms in patients with lichen planus seems justified.

As the prognostic value of the -308 polymorphism has not been unequivocally determined yet, we made an attempt to define its role in the population of patients with lichen planus. The -308 allele has been reported to be in linkage disequilibrium with some HLA antigens such as HLA-A1,-B8,-DR3,-DQ2 in Caucasian populations (21, 22). These haplotypes are thought to be related to several autoimmunological diseases. HLA-DR3 haplotype is associated with the phenotype increasing the TNF expression (22). There is evidence for a positive correlation between the TNF2/TNF2 allele and systemic lupus erythematosus (23) as well as dermatitis herpetiformis (11), which may also be linked with the HLA-DR3 antigens. McGuire et al. (10) claimed that subjects homozygous for TNF2/TNF2 allele had a seven times higher risk of death or serious neurological complications following cerebral malaria. Ethnic differences may also account for the variability of frequency of genotypes and TNF allele, which is also believed to increase the discrepancies between results of the studies. For example, the frequency of the TNF2 allele in the European population is approximately 2% (11), whereas in Taiwan it is estimated to be 5.2% (6).

In our subjects the TNF2 allele was found in 3.0% of patients and 6.1% of controls. Similar results were reported by Buraczynska (24) both for peritoneal dialysis patients (5.3%) and controls (4.3%). While investigating the association between the TNF polymorphism and lichen planus we did not observe any statistically significant differences in genotype distribution or allele frequency between patients and the control group. When we took into consideration the extent of the disease process (generalized or circumscribed lichen planus), no statistically significant differences could be found in genotype distribution or allele frequencies between the two types of the disease. However, it should be pointed out that the homozygous TNF2/TNF2 genotype occurred more often in patients with generalized lichen planus (6.1% vs 0%). Therefore, the TNF2 allele can be suspected to intensify this disease process; however, the small sample size makes such a conclusion weaker and only a much larger subject group might allow demonstration of significant differences. As several different factors are involved in the pathogenesis of lichen planus, it is unlikely that a direct correlation between the TNF2 allele and the disease can be demonstrated.

According to some authors, TNF may be a relevant factor in the pathogenesis of the disease (5). Thus, it might prove useful to determine if TNF genotypes influence the potential TNF protein expression in patients’ plasma. Chouchane et al. (12) claim that the presence of TNF2 allele is accompanied by elevated basic and induced production of TNF protein. In our study, we failed to identify statistically significant differences in TNF plasma levels between patients and controls within genotypes TNF1/TNF1 and TNF2/TNF2. However, within genotype TNF1/TNF2 we found a significantly higher level of TNF in the control group than in patients. This finding is striking as we had expected the opposite tendency, namely that TNF level might be higher in patients than in controls. There is no direct evidence that the -308 polymorphism is associated with TNF production and its plasma level, but it may be in linkage disequilibrium with certain microsatellite alleles of this gene influencing the TNF expression (25).

In conclusion, the results obtained from the examination of TNF gene polymorphism at position -308 suggest that there is no direct link between genotype distribution, allele frequency and lichen planus. In our study we failed to observe any association between genotype and mean plasma TNF level in patients with lichen planus.

REFERENCES

3. Jacob CO, Fronzek Z, Lewis GD, Koo M, Hansen JA, McDevit HO. Heritable major histocompatibility complex class II-associated differences in production of tumor...


