Tumor necrosis factor-alpha polymorphism in inflammatory bowel disease

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ABSTRACT

The existence of an underlying genetic predisposition to ulcerative colitis and Crohn’s disease is beyond dispute, but the mechanism for this susceptibility is unclear. Because of the localization of the TNF-α gene within the central region of the Major Histocompatibility Complex on the short arm of chromosome 6 and the biological activities of TNF-α, we investigated the association between inflammatory bowel disease and a single base polymorphism located at position -308 of the TNF-α gene. The frequency of TNF-α alleles in 92 patients with ulcerative colitis and 70 patients with Crohn’s disease was found no significantly different compared with a group of 55 healthy controls (UC:p = 0.146 and CD:p = 0.396). In conclusion, from the existing data, the polymorphism in the TNF-α gene at position -308 does not appear to be associated with ulcerative colitis or Crohn’s disease but further study of this polymorphism in association with subgroups of patients, different haplotypes, and with the production of TNF-α may lead to the identification of subgroups and contribute to define the disease heterogeneity.

Key words: Crohn’s disease, genetics, major histocompatibility complex, polymorphism, tumor necrosis factor, ulcerative colitis.

INTRODUCTION

There is strong evidence that genetic factors play a significant role in the pathogenesis of ulcerative colitis (UC) and Crohn’s disease (CD). The observations supporting hereditary factors are, increased familial incidence, higher rates in monozygotic versus dizygotic twins and higher rates in first degree relatives versus spouses. The variant frequencies found in different ethnic groups and the association of inflammatory bowel disease (IBD) with genetic syndromes and diseases with known genetic predisposition also support this hypothesis.1-3 The use of complex segregation analysis in recent studies on large populations of patients has shown that up to 30% of cases of IBD may be the manifestation of a major gene effect. In Crohn’s disease, a major recessive gene and in ulcerative colitis a major dominant gene with incomplete penetrance have been postulated.4,5 However, the search for a specific genetic marker in IBD has been disappointing. So far only a weak association between ulcerative colitis and HLA DR25 and recently an association between this disease and the allele 2 of interleukin-1 receptor antagonist (IL-1ra)5,6 have been reported.

Tumor necrosis factor alpha (TNF-α) or cachectin, is a potent proinflammatory cytokine produced mainly by activated macrophages and monocytes and is thought to play a significant role in the initiation and regulation of the immune response.6 Interindividual differences in TNF production by peripheral blood mononuclear cells from normal volunteers have been observed.7 Moreover, the individual differences in TNF-α production have been found to be linked to HLA type and other polymorphic markers.8,9 These
observations have raised the possibility that a predisposition to increased TNF production may be associated with the development of autoimmune diseases.

There are several studies about TNF-α production in IBD patients but the results are not consistent due to the different methods used, different inducers of cell activation and patient selection. However, TNF-α is thought to play an important role as mediator of inflammation in IBD and initial studies with the therapeutic use of chimeric monoclonal antibodies of TNF in Crohn’s disease are encouraging.12

The gene encoding TNF-α lies approximately 1 Mb telomeric to the HLA-DR locus and 300 kb centromeric to the HLA-B locus.13 In view of the chromosomal localization, the biological effects and its implication in chronic inflammation, there has been much speculation that polymorphism in this gene may be implicated in the pathogenesis of IBD. Thus, research on TNF gene polymorphism in IBD is nowadays in progress.

A biallelic polymorphism within the 5’ DNA region of the TNF-α gene has been recently described.14 This polymorphism involves the substitution of guanine (G) by adenine (A) at position -308 of the TNF-α gene in the mutant allele. Its location seems to be important for the transcriptional control of TNF-α. A strong association of this polymorphism and HLA A1, B8 and DR3 alleles have been reported.15 TNF-α2 allele has been found to be associated with higher constitutive and inducible levels of transcription than TNF-α1 allele.16 In normal individuals TNF-α2 allele is uncommon. However, this allele has been found with increased frequency in autoimmune diseases such as systemic lupus erythematosus17 and is associated with susceptibility to complications of infectious diseases such as cerebral malaria.18

To test the hypothesis that this TNF-α polymorphism might be involved in the pathogenesis of inflammatory bowel disease we analysed the frequency of the TNF-α alleles in patients with ulcerative colitis and Crohn’s disease as well as in control subjects.

MATERIAL AND METHOD

Patients

A total number of 92 unrelated Dutch patients with ulcerative colitis and 70 with Crohn’s disease were recruited from the Department of Gastroenterology, Free University Hospital, Amsterdam. Moreover, 55 unrelated healthy local blood donors served as controls. All subjects were caucasians. The diagnosis of CD or UC was based on the conventional clinical, radiological, endoscopic, and pathological criteria, as described by Lennard-Jones et al.19 The localization of the disease was for UC: proctitis 21%, left side colitis 49%, and total colitis 30%; and for CD: ileum 22%, colon 25%, and ileum & colon 53%.

DNA extraction

Genomic DNA was extracted from EDTA anticoagulated peripheral blood by a conventional proteinase K digestion and phenol-chloroform extraction method and stored at -4 °C.

Typing of TNF-α polymorphism

A 107-bp stretch of the TNF-α promoter region was amplified by PCR (sense primer 5'-AGGCCACTAGTTTTGAGGGGC-3', antisense primer 5'-TCCCTGCCTGCTCGATTCCG-3') with conditions identical to Wilson’s et al description.20 PCR was performed on a Perkin Elmer Cetus Gene Amp 9600 PCR system. Typing of the biallelic RFLP at position -308 in the TNF-α promoter was performed after NcoI digestion of the PCR products. Finally, fragments of 107 bp, reflecting the undigested product (allele 2: nucleotide at position -308=A) were separated from the 87 bp and 20 bp digested products (allele 1:nucleotide at position -308=G) upon electrophoresis on a 4% Nu-sieve GTG agarose gel (stained with ethidium bromide).

Statistical analysis

The results are presented as genotypes and allelic frequencies. X² test for statistical significance has been performed on allelic frequencies. Odds ratios (OR) equal to approximate relative risk have been calculated for the disease in carriers of each allele. The 95% confidence intervals (CI) for the OR are shown in the text.

RESULTS

Table 1 shows the distribution of genotypes observed in CD and UC patients and in the control group. The frequency of each allele in the three groups is shown in table 2. In all groups the observed distribution of homozygotes and heterozygotes conformed to expectations based on Hardy-Weinberg equilibrium analysis.

By X² analysis, no significant differences were observed in the TNF-α allele frequencies between CD
Table 1. TNF-alpha genotypes in ulcerative colitis and Crohn’s disease patients and in healthy controls.

<table>
<thead>
<tr>
<th>Study group</th>
<th>TNF-α 1,1</th>
<th>TNF-α 1,2</th>
<th>TNF-α 2,2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD patients (n=70)</td>
<td>47</td>
<td>19</td>
<td>4</td>
</tr>
<tr>
<td>UC patients (n=92)</td>
<td>65</td>
<td>23</td>
<td>4</td>
</tr>
<tr>
<td>Controls (n=55)</td>
<td>32</td>
<td>19</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 2. Allelic frequency (%) in ulcerative colitis and Crohn’s disease patients and in healthy controls.

<table>
<thead>
<tr>
<th>Allele frequencies (%)</th>
<th>Allele 1 n (%)</th>
<th>Allele 2 n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD patients</td>
<td>113 (81)</td>
<td>27 (19)</td>
</tr>
<tr>
<td>UC patients</td>
<td>153 (83)</td>
<td>31 (17)</td>
</tr>
<tr>
<td>Controls</td>
<td>83 (75)</td>
<td>27 (25)</td>
</tr>
</tbody>
</table>

- CD vs controls $\chi^2=0.7197, 2\text{df}, p=0.3962$, Odds Ratio=1.361 (95% CI:0.7441-2.491)
- UC vs controls $\chi^2=2.113, 2\text{df}, p=0.1461$, Odds Ratio=1.606 (95% CI:0.8979-2.871)

and UC patients in comparison to healthy controls (CD: $p=0.3962$, UC: $p=0.1461$).

Furthermore, no significant differences were found with comparison of the allele frequencies in the different groups of disease localization (data not shown).

DISCUSSION

Ulcerative colitis and Crohn’s disease have well recognized familial tendencies but so far the genetic basis of this clinical observation remains under investigation. The genetic markers, studies have shown a lack of consistent association between these markers and ulcerative colitis or Crohn’s disease. The association of IBD (mainly UC) with major histocompatibility complex (MHC) via HLA-DR2 is notably weaker than the association of the MHC with other diseases (coeliac disease, rheumatoid arthritis and psoriasis).

Recently the research is focused on the cytokine genes and their known polymorphisms. A recent study has shown a significant association of allele 2 or IL-1ra with ulcerative colitis. This association has also been confirmed for Dutch population in our laboratory and moreover no association between IL-1ra and pANCA or HLA-DR2 was found.

Because of the localization of the TNF-α gene and the function of the product, a polymorphic TNF-α gene could be involved in the pathogenesis of IBD. However, this study in Dutch patients shows that there is no association between the TNF-α gene polymorphism at position -308 and inflammatory bowel disease. This is in accordance with a previous study from UK.

Therefore, from the existing data it is unlikely that this polymorphism plays a significant role in the predisposition to IBD.

It is known, that ulcerative colitis and Crohn’s disease are heterogeneous diseases and individual IBD patients may differ extremely in their course of the disease, prognosis, and response to medical treatment. Therefore, further study of this polymorphism of TNF-α in association with subgroups of patients, different haplotypes, and with the production of TNF-α may identify patient subgroups and contribute to define the disease heterogeneity. For example, in this study, patients with Crohn’s disease localized at the ileum had a tendency for more common carriage of the TNF-α 2 allele compared to colonic disease patients although the number of patients was small to draw firm conclusions (data not shown).

Recent studies in our laboratory have shown that 4 single base polymorphisms (including the polymorphism studied herein) at the TNF-α and TNF-β loci, are present in only 5 haplotypes in the Dutch population. It is interesting that some of the gene products were quantitatively related to these haplotypes. In conjunction with other markers, these TNF-α and TNF-β haplotypes may help to define subgroups in these clinically heterogeneous diseases.

Our aim is to study TNF and HLA polymorphism in patients with IBD from Greece and to compare them with the Dutch patient population in order to assess the strength of polymorphisms and patient subgroups.

Acknowledgements

I. Koutroubakis, M.D. is a gastroenterologist of the University Hospital of Heraklion, Greece, presently working for a year at the Laboratory for Gastrointestinal Immunogenetics, Free University, Amsterdam. We are grateful for the support received from the Greek Society of Gastroenterology.

We would also like to acknowledge the financial support received from Tramedico, B.V., The Netherlands and the Falk Foundation, Germany.
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