Secretion of Tumour Necrosis Factor α and Lymphotoxin α in Relation to Polymorphisms in the TNF Genes and HLA-DR Alleles. Relevance for Inflammatory Bowel Disease

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The genes for tumour necrosis factor alpha (TNFa) and lymphotoxin alpha (LTα; TNFβ) are tandemly arranged in the central region of the MHC. They may, therefore, be of importance for the aetiology of MHC-associated diseases. The authors have prospectively studied the secretion of TNFa and LTα in relation to polymorphisms at positions -308 and -238 in the TNFa gene (TNFA), and two polymorphisms in the first intron of the LTα gene (LTA), as well as HLA-DR in 30 patients with chronic inflammatory bowel diseases (IBD) and 12 healthy controls. In the Dutch population, the alleles of these four polymorphisms are present in only five combinations, called TNF-haplotypes: TNF-C, -E, -H, -I, and -P. Significant associations between TNF haplotypes and TNFa and LTα secretion were found when PBMC were cultured with T-cell activators, irrespective of disease. Mean TNFa secretion of individuals carrying the HLA-DR3 associated TNF-E haplotype was significantly higher, as compared to individuals without this haplotype (26441 pg/ml versus 19629 pg/ml; P = 0.014). Individuals carrying the TNF-C haplotype produced the lowest amount of TNFa (17408 pg/ml; P = 0.022). The TNF-C and TNF-E haplotypes differ only at position -308 in the promoter of TNFA. Individuals carrying the HLA-DR1 associated TNF-I haplotype produced significantly less LTα when compared to those who lack this haplotype (1979 pg/ml versus 3462 pg/ml; P = 0.006). As the TNF-I haplotype is also associated with low TNFa secretion, this haplotype thus defines a 'low secretor phenotype'. In conclusion, this is the first study to show associations between TNF haplotypes and TNFa and LTα secretion when T-cell stimulators are used. These findings will contribute to define disease heterogeneity in IBD and may be of relevance for understanding the pathogenesis of autoimmune diseases.

INTRODUCTION

The aetiology of the chronic inflammatory bowel diseases (IBD), Crohn's disease (CD) and ulcerative colitis (UC) is still unknown. However, there is overwhelming evidence that genetic factors play a role in the predisposition to suffer from these diseases. Several associations between alleles of the major histocompatibility complex (MHC) and IBD have been observed in different groups of IBD patients, in particular the association between HLA-DR2 and UC [1–5].

Tumour necrosis factor alpha (TNFa) and lymphotoxin alpha (LTα or TNFβ) play a central role in the initiation and
regulation of the immune response. The genes for these cytokines are tandemly arranged in the central region of the MHC, between the HLA-B and HLA-D loci, at the short arm of chromosome 6. This location has prompted much speculation about the role of TNFα and LTα in the etiology of MHC-associated diseases [6]. In support of this, MHC-restricted differences in TNFα and LTα inducibility have been demonstrated in several studies. Jacob et al. showed that HLA-DR2 positive individuals produce less TNFα than individuals that are HLA-DR3 positive [7]. Polymorphisms in the genes for TNFα (TNFA) and LTα appear to be responsible for differences in secretion. Messer et al. found that a NcoI restriction fragment length polymorphism (RFLP) in the first intron of LTα influences LTα secretion, and Pociot et al. found an association between this polymorphism and the production of TNFα [8-10]. An AspHI RFLP, also in the first intron of LTα, was described by Ferencik et al. [11]. Wilson et al. described a polymorphism at position -308 in the promoter region of TNFα that is strongly linked to the HLA-A1,-B8,-DR3 ancestral haplotype, and suggested that variations at this position might be important in the regulation of TNFα production, although so far we know of no data that have been published to support this hypothesis [12, 13]. A second polymorphism, at position -238 in the promoter region of TNFα has been described by D’Alfonso & Momigliano Richiardi [14]. In 200 Dutch individuals the alleles of these four polymorphisms occur in only five combinations, TNF-haplotypes: TNF-C, -E, -H, -I, and -P (Fig. 1) [15].

We recently observed large interindividual differences in the in vitro production of TNFα and LTα in IBD patients [16]. Therefore, to determine whether variations in the genes encoding these cytokines are responsible for differences in the intrinsic capacity of peripheral blood mononuclear cells (PBMC) to produce TNFα and LTα, we performed a prospective study in which we determined the production of these cytokines in vitro in relation to polymorphisms in the TNFα and LTα genes, as well as HLA-DR alleles in patients with IBD and healthy controls.

**MATERIALS AND METHODS**

Blood samples were obtained from 30 IBD patients, 15 with CD (2 female/13 male, mean age 40 years, range: 22-66 years), and 15 with UC (1 female/14 male, mean age 52 years, range: 24-78 years), as well as from 12 healthy male controls (HC; mean age 56 years, range: 24-51 years). As TNFα production may be influenced by female sex hormones [7], only men or postmenopausal women were included. Diagnosis of CD or UC was based on the conventional clinical, radiological, endoscopic and pathological criteria, as described by Lennard-Jones [17]. UC disease activity was assessed using the Sutherland score [18]. The Crohn’s disease activity index (CDAI), according to Best and co-workers, was used for CD [19]. Of the CD patients, 13 were in remission at the time of study, with a CDAI <150. Two patients had active disease and were on prednisone therapy. In the group of UC patients, 13 were in remission at the time of the study with a Sutherland score <8. Two patients had a higher score and used prednisone.

As our initial study design was to evaluate the relation between the LTα NcoI polymorphism and TNFα and LTα secretion, selection of individuals was based on this gene polymorphism. Six individuals (3 CD, 2 UC and 1 HC) were homozygous for allele 1, 14 individuals (6 CD, 4 UC and 4 HC) were heterozygous, and 22 (6 CD, 9 UC and 7 HC) were homozygous for allele 2.

**TNFα and LTα polymorphisms.** Genomic DNA was extracted from anti-coagulated blood by a conventional proteinase K digestion/phenol-chloroform extraction method. Typing of the di-allelic polymorphism at position -308 in the TNFα promoter (TNFα -308) was performed by PCR amplification using a 5’ primer 5’-AGGCATAAGTTTAGGAGGCCAT-3’ and 5’-TCCCTCTGTCTCCGATTCTCGC-G3’ as the 3’ primer) and NcoI digestion as described by Wilson et al. [12]. A single-strand conformation polymorphism method was optimized for the detection of di-allelic polymorphisms at both positions -308 and -238 in the TNFα gene. PCR fragments spanning sequences from position -396 to -69 were generated using a 5’ primer 5’-TCCCTCTGTCTCCGGATTCTCGC-3’ and a 3’ primer 5’-CAGCGGAAACTCTCTTGGT-3’ as described by D’Alfonso & Momigliano Richiardi [14]. The 328 base pair (bp) fragments were denatured and run on pre-cast non-denaturing...
12.5% polyacrylamide PhastGels. Horizontal electrophoresis and silver staining were performed semi-automatically with the PhastSystem™ (Pharmacia LKB Biotechnology AB, Uppsala, Sweden).

PCR amplification with primers located in the 5' untranslated region and third intron of the LTA gene (5' primer 5'-CGCTTGTTGGCTGGAGAT-3' and 3' primer 5'-AGAGCTGTGGGGGACATGCTG-3') resulted in 740 bp fragments that were either cut (alleles LTA NcoI*) or remained intact (alleles LTA NcoI*) following incubation with the restriction enzyme NcoI [8]. For typing the LTA AspH1 RFLP, as described by Ferencik et al., the 740 bp PCR fragments were digested with the isoschizomer BstHI [11]. This resulted in 415 and 315 bp fragments (alleles LTA AspH1*) or the undigested 740 bp fragment (alleles LTA AspH1*2). Fragments from individual digests were analysed by electrophoresis in 1% ethidium bromide stained 1.5% agarose gels.

DNA isolation, PCR amplification, and dot-blotting for HLA typing. Amplification of the second exon of the HLA-DRB1 gene was performed by PCR. Dot-blot analysis of amplified DNA was carried out using the procedures [20] and biotin-labelled sequence-specific oligonucleotide probes (SSO) as described previously [21].

PBMC isolation and culture. PBMC were isolated immediately after collection of blood by density gradient centrifugation of heparinized blood on Ficoll-Hypaque (Lymphoprep) and cultured for 48 h at 37°C under 5% CO2/humidified air at a concentration of 1.10^6/ml. PBMC were cultured in RPMI 1640 with 25 mM Hepes, 100 U/ml, streptomycin, 100 µg/ml, and 10% pooled human serum (the same batch was used for all experiments). Since TNFα is produced by activated monocytes and T-, B- and NK cells, and LTA by activated T-cells, we stimulated the PBMC with T-cell activators. In order to achieve maximal stimulation we activated the T-cells with both 1 µg/ml anti-CD3 (monoclonal 15E8) and 1 µg/ml anti-CD28 (monoclonal 16A9, kindly provided by Dr van Lier, CLB, Amsterdam, the Netherlands) [22]. Under these circumstances, a maximal and reproducible secretion of TNFα and LTA was achieved. The supernatants were harvested after 48 h in culture by gentle centrifugation, aliquoted and stored at −80°C, and were not thawed until assay.

TNFα and LTA measurements. TNFα and LTA were measured by commercially available ELISA systems, as described by the manufacturers. For TNFα measurements, the TNFα EASIA was used (Medgenix Diagnostics SA, Fleurus, Belgium), and for LTA, the Quantikine LTA ELISA was used (R&D Systems, Minneapolis, USA). According to the manufacturers, the sensitivity of the TNFα ELISA is 7 pg/ml, with an intra- and interassay specificity of <4% and <8%, respectively.

Statistics. The relation between genotypes and TNFα or LTA production was calculated by one-way and two-way analysis of variance for the comparison of groups, using the BMDP software package [23]. Subsequently, individuals were stratified for disease. P values after stratification for disease are designated as Pr.

A P value <0.05 was considered statistically significant. Where appropriate we tested for linear trend by specifying a linear contrast within the analysis of variance. In order to check normality of the distributions, we examined histograms of the data, both before and after logarithmic transformation. P values reported are from analyses without the logarithmic transformation because, in general, this transformation did not improve normality. When comparing two groups we used Wilcoxon's non-parametric two-sample test.

RESULTS

TNFα and LTA production in relation to disease

The mean TNFα and LTA production upon stimulation with anti-CD3 and anti-CD28 is shown in Table 1. In this selected study group, as defined in Materials and Methods, the mean TNFα and LTA production did not differ statistically significantly between UC patients, CD patients and HC (P > 0.05).

TNFα production in relation to the TNF polymorphisms

The TNFα production in relation to the TNF polymorphisms is shown in Tables 2a and b. As the infrequent haplotype TNF-H was not found in our study group, the relevance of the polymorphism at position -238 of the promoter region of TNFα could not be investigated.

When studying the polymorphism at position -308 in the promoter region of the TNFα gene, it was found that individuals homozygous for the TNFα -308*1 allele produced significantly less TNFα upon stimulation than individuals homozygous for the TNFα -308*2 allele, whereas heterozygous individuals produced intermediate amounts (P = 0.014). As the number of individuals homozygous for the TNFα -308*1 allele was small, it was not possible to stratify for disease. Therefore, carriers of the allele 2 were compared with non-carriers. After stratifying for disease, a statistically significant difference was found between carriers

<table>
<thead>
<tr>
<th></th>
<th>CD (n = 15)</th>
<th>UC (n = 15)</th>
<th>HC (n = 12)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFα (pg/ml)</td>
<td>24 888 (10 409–38 813)</td>
<td>20 719 (11 970–32 821)</td>
<td>21 344 (21 199–46 364)</td>
<td>0.410</td>
</tr>
<tr>
<td>LTA (pg/ml)</td>
<td>3432 (1314–8162)</td>
<td>2648 (618–5979)</td>
<td>2910 (449–4363)</td>
<td>0.433</td>
</tr>
</tbody>
</table>

TNFα and LTA secretion are expressed as means; range between parentheses.

CD = Crohn's disease patients; UC = ulcerative colitis patients; HC = healthy controls.

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of allele 2 and individuals without this allele ($P_s = 0.032$) (Fig. 2).

No association was found between the LTA Ncol polymorphism and TNFα secretion, neither when individuals were compared ($P = 0.521$), nor after stratifying for disease ($P_s = 0.756$). No associations were found between the LTA AspHI polymorphism and TNFα secretion ($P = 0.656$, and $P_s = 0.654$).

**LTα production in relation to the TNF polymorphisms**

The production of LTα in relation to the TNF polymorphisms is shown in Tables 2a and 2b. As can be seen from these tables, a statistically significant association was found between the TNFA -308 polymorphism and LTα secretion ($P = 0.012$). However, when carriers of allele 2 were compared with non-carriers, this difference did not reach statistical significance ($P_s = 0.169$).

There was no statistically significant association between the LTA Ncol polymorphism ($P = 0.098$; $P_s = 0.271$) or the LTA AspHI polymorphism ($P = 0.656$, and $P_s = 0.654$) and the secretion of LTα.

**TNFα production in relation to TNF haplotypes**

The production of TNFα in relation to the TNF haplotypes is
Table 4. LTα production in relation to TNF haplotypes

<table>
<thead>
<tr>
<th>TNF haplotype</th>
<th>LTα production in individuals carrying the haplotype</th>
<th>LTα production in individuals not carrying the haplotype</th>
<th>P value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C (n=12)</td>
<td>2594 ± 1387</td>
<td>3166 ± 1753</td>
<td>0.320</td>
<td>0.399</td>
</tr>
<tr>
<td>E (n=17)</td>
<td>3570 ± 1841</td>
<td>2617 ± 1439</td>
<td>0.067</td>
<td>0.169</td>
</tr>
<tr>
<td>I (n=13)</td>
<td>1979 ± 1274</td>
<td>3462 ± 1625</td>
<td>0.006</td>
<td>0.006</td>
</tr>
<tr>
<td>P (n=31)</td>
<td>2956 ± 1473</td>
<td>3135 ± 2187</td>
<td>-0.763</td>
<td>0.652</td>
</tr>
</tbody>
</table>

LTα secretion in pg/ml ± SD.

n = number of individuals carrying the TNF haplotype.

shown in Table 3. Individuals carrying the TNF-C haplotype were low producers of TNFα (P = 0.022 and P* = 0.045 as compared to individuals without this haplotype). Individuals carrying the TNF-E haplotype (i.e. carriers of allele 2 of the TNFα -308 polymorphism) are high producers of TNFα (P = 0.014 and P* = 0.032). The TNF-C haplotype and the TNF-E haplotype differ only at position -308 in the promoter region of the TNFα gene. Individuals carrying the TNF-C haplotype differed statistically significant in TNFα secretion from those carrying the TNF-E haplotype (mean 17473 pg/ml versus 27050 pg/ml; P = 0.007 and P* = 0.024). Individuals carrying both the TNF-C and TNF-E haplotypes were excluded from this analysis.

With regard to the TNF-I haplotype, there was a trend towards lower TNFα secretion when carriers were compared with non-carriers of this haplotype (P = 0.062). After stratification for disease, this difference reached statistical significance (P* = 0.024). No association was found between the TNF-P haplotype and secretion of TNFα (P = 0.746 and P* = 0.829).

LTα production in relation to TNF haplotypes

The production of LTα in relation to the genotypes is shown in Table 4. Individuals carrying the TNF-I haplotype showed a statistically very significant lower production of LTα as compared to individuals who lack this haplotype (P = 0.006; P* = 0.006).

TNFα and LTα secretion in relation to HLA-class II alleles

In a previous study of IBD patients we showed that the TNF-E haplotype is in linkage disequilibrium with the HLA-DR3 allele, whereas the TNF-I haplotype was found to be associated with the HLA-DR1 allele [24]. Therefore, we also studied TNFα and LTα secretion in relation to the HLA-DR alleles in 30 IBD patients and 11 HC. Carriage of the HLA-DR alleles and TNF haplotypes is shown in Table 5. We observed a strong association between the TNF-E haplotype and the HLA-DR3 allele. In this study group all HLA-DR3 positive individuals also carried the TNF-E haplotype, whereas 12 out of 17 (71%) haplotype TNF-E positive individuals were also HLA-DR3 positive. TNFα secretion in relation to HLA-DR3 and TNF-E is shown in Table 6. As can be seen from the table, no differences were found between HLA-DR3*, TNF-E* individuals and HLA-DR3*, TNF-E* individuals, but secretion was significantly higher than in HLA-DR3*, TNF-E* individuals (P = 0.028 by testing for linear trend).

The frequency of the HLA-DR1 allele was increased in individuals carrying the TNF-I haplotype as, of the 13 TNF-I* individuals, six were DR1* (Table 5). LTα secretion in

Table 5. Carriage of the HLA-DR alleles and TNF haplotypes in 41 individuals. For comparison, the HLA-DR distribution in 2400 Dutch controls is given

<table>
<thead>
<tr>
<th>Carriers of</th>
<th>DRB1*01</th>
<th>DRB1*02</th>
<th>DRB1*03</th>
<th>DRB1*04</th>
<th>DRB1*05</th>
<th>DRB1*06</th>
<th>DRB1*07</th>
<th>DRB1*08</th>
<th>DRB1*09</th>
<th>DRB1*10</th>
</tr>
</thead>
<tbody>
<tr>
<td>C (n=11)</td>
<td>2/11</td>
<td>5/11</td>
<td>1/11</td>
<td>4/11</td>
<td>5/11</td>
<td>1/11</td>
<td>1/11</td>
<td>1/11</td>
<td>1/11</td>
<td>1/11</td>
</tr>
<tr>
<td>E (n=17)</td>
<td>3/17</td>
<td>4/17</td>
<td>17/17 (71%)</td>
<td>3/17</td>
<td>2/17</td>
<td>1/17</td>
<td>6/17</td>
<td>3/17</td>
<td>0/17</td>
<td>0/17</td>
</tr>
<tr>
<td>I (n=13)</td>
<td>6/13 (46%)</td>
<td>1/13</td>
<td>2/13</td>
<td>2/13</td>
<td>2/13</td>
<td>2/13</td>
<td>1/13</td>
<td>0/13</td>
<td>0/13</td>
<td></td>
</tr>
<tr>
<td>P (n=30)</td>
<td>8/30</td>
<td>10/30</td>
<td>3/30</td>
<td>3/30</td>
<td>4/30</td>
<td>6/30</td>
<td>1/30</td>
<td>1/30</td>
<td>1/30</td>
<td></td>
</tr>
<tr>
<td>Dutch controls</td>
<td>(n=2400)**</td>
<td>20%</td>
<td>22%</td>
<td>25%</td>
<td>28%</td>
<td>19%</td>
<td>34%</td>
<td>19%</td>
<td>5%</td>
<td>2%</td>
</tr>
</tbody>
</table>

** Serologically determined.

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Table 6. Secretion of TNFα and LTα in relation to TNF haplotypes and HLA-DR alleles in 30 IBD patients and 11 controls

<table>
<thead>
<tr>
<th>HLA-DR3/TNF-E</th>
<th>+/+ (n = 12)</th>
<th>+/- (n = 12)</th>
<th>-/+ (n = 5)</th>
<th>-- (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFα (mean pg/ml)</td>
<td>26 555*</td>
<td>absent</td>
<td>26 166*</td>
<td>19 557</td>
</tr>
<tr>
<td>HLA-DR1/TNF-I</td>
<td>+/- (n = 6)</td>
<td>+/- (n = 3)</td>
<td>-- (n = 7)</td>
<td>-- (n = 25)</td>
</tr>
<tr>
<td>LTα (mean pg/ml)</td>
<td>1660**</td>
<td>2253</td>
<td>2525</td>
<td>3597</td>
</tr>
</tbody>
</table>

*Significantly increased as compared to HLA-DR3'/TNF-E' (P = 0.028).
**Significantly decreased as compared to HLA-DR1'/TNF-I' (P = 0.006).

DISCUSSION

Recent observations have shown that the polymorphism at position -308 in the promoter region of TNFA is involved in the susceptibility for at least two diseases: cerebral malaria and mucocutaneous Leishmaniasis [25, 26]. Individuals carrying the allele 2 have a highly increased risk of developing these severe diseases. Wilson et al. already suggested that this position may be of importance for the regulation of TNFA secretion [13, 27], but, to our knowledge, no data exist to support this hypothesis. There is one abstract reporting that the TNF2 allele in gene assays directs higher levels of transcription [28], and another finding no differences in a similar assay [29]. In this study we have evaluated the relationship between polymorphisms in the genes encoding TNFA and LTα and the intrinsic capacity of PBMC to produce these cytokines when T-cell stimulators are used. Although our study population was small, we present, for the first time, four lines of evidence to support the idea that this position is associated with differences in TNFA secretion.

1. Individuals homozygous for the TNFA NcoI*1 allele produced significantly less TNFA than individuals homozygous for the TNFA NcoI*2 allele, whereas heterozygous individuals produced intermediate levels of TNFA. However, the number of homozygous TNFA NcoI*2 patients was too low to allow stratification for disease. Therefore, we compared carriers of allele 1 with non-carriers of allele 1, and showed that the carriers of allele 1 are high producers of TNFA.

2. When TNFA secretion was studied in relation to the TNF haplotypes, strong associations were found. High TNFA production was found in individuals carrying the TNF-E haplotype, whereas low TNFA production was found in individuals with the TNF-C haplotype. These haplotypes differ only at position -308 in the promoter region of TNFA.

3. In a previous study on the immunogenetics of TNFA, Jacob et al. showed that HLA-DR2 positive individuals produce less TNFA than HLA-DR3 or DR4 positive individuals [7]. Therefore, it might theoretically be that the HLA class II genes influence the regulation of TNF secretion. In the present study, however, we found no differences between DR3', TNF-E+ individuals and HLA-DR3*, TNF-E+ individuals, but a significant lower secretion in HLA-DR3*, TNF-E+ individuals, suggesting that the TNF haplotype is stronger associated with the increased TNFA secretion than the HLA-DR3 allele.

4. In support of our interpretation we can add another observation. Abraham et al. studied spontaneous TNFA secretion in B-lymphoblastoid cell lines and found large differences in secretion of TNFA between cell lines [29]. We have typed two of the high TNFA producing cell lines (numbers 9022 (COX) and 9023 (VAVY), according to the 10th International Histocompatibility Workshop) for the TNF haplotypes, and found that both are homozygous for the TNF-E haplotype [30].

Since TNFA is mainly produced by macrophages, LPS and PMA are frequently used stimulants to study the relationship between gene polymorphisms and production [8,10]. Messer et al. reported a correlation between the LTα NcoI polymorphism and LTα production [8]. In concordance with Pociot et al. we found no statistically significant correlation between this polymorphism and LTα production [10]. The differences observed between these studies may possibly be due to different study designs. As T cells are thought to be the essential cells in the pathogenesis of autoimmune diseases [31], we activated the PBMC with T-cell specific activators. Interestingly, TNFA secretion after 24 h LPS stimulation was also found to be significantly higher in TNFA -308*2,2 individuals as compared to those homozygous for allele 1 (results not shown).

We found no significant associations between any of the polymorphisms studied and LTα production (Tables 2a & b). However, when we studied LTα production in relation to the TNF haplotypes, we found a very strong association between...
LTα production and the TNF-1 haplotype. Individuals carrying this haplotype produce significantly less LTα and TNFα upon stimulation than individuals who lack this haplotype, irrespective of disease status. The HLA-DR1 associated TNF-1 haplotype thus seems to define a low-secretor phenotype for these cytokines. As the TNF-1 haplotype differs in at least two positions from the other TNF haplotypes, our findings suggest that there must be an additional factor(s) in the TNF-1 haplotype that down-regulates the production of these cytokines. Furthermore, these observations demonstrate that the study of haplotypes, rather than individual gene polymorphisms, may occasionally provide additional information, and should be taken into account when studying the immunogenetics of cytokines.

What is the importance of the present findings to the predisposition to IBD? TNFα facilitates inflammatory cell infiltration by promoting adhesion of neutrophils and lymphocytes to endothelial cells and up-regulates the expression of HLA molecules in human colonic cell lines and small intestinal epithelial cells [32,33], all being important features of inflammatory bowel disease. In this respect, it is interesting that there is recent evidence that a monoclonal anti-TNFα antibody induces active CD into remission [34]. In a previous study, we investigated the distribution of the four polymorphisms and TNF haplotypes in a large group of IBD patients and HC. We found a weak, but statistically significant, association between the TNFA NcoI polymorphism and UC. The infrequent allele 2 was found to be decreased in UC patients as compared to HC [24]. The strength of the association indicates, however, that these genes are not markers for disease predisposition. They may, however, be markers of subsets of patients with UC and CD. From the clinical point of view, UC and CD are heterogeneous diseases [35, 36]. Individual CD or UC patients can differ extremely in their course of the disease, prognosis and response to medical treatment; some patients respond well to standard treatment while others are therapy resistant. In some patients the disease is restricted to a small part of the intestinal tract while in others extended intestinal inflammation can be found. In a subgroup of patients, extra-intestinal manifestations, such as arthritis, are found. These clinical observations have led to investigations to determine whether IBD may actually represent a set of diseases that display a similar clinical picture, but have a wide variety of aetiologies. Recent data from animal models of intestinal inflammation support this hypothesis, showing that very different mutations can induce diseases with remarkably similar phenotypes [37]. As has been suggested for other diseases, subgroups of patients may therefore have a heterogeneous genetic background [38, 39]. For example, in patients with systemic lupus erythematosus (SLE), there is an increased incidence of nephritis in HLA-DR2*, DQ1* patients, but not in DR3* individuals [40].

In conclusion, the results of the present study support the concept that a different immunogenetic background may determine the height of the immune response. High and low secretor phenotypes are present in IBD patients, and may determine the number and severity of relapses in an individual patient. These clinical features will be studied in a prospective manner in relation to the genetic markers. Our study suggests that studying cytokine genes may help to understand the biological basis of disease heterogeneity in IBD, and may be helpful to design a more rational therapeutic approach for patients with these diseases.

ACKNOWLEDGMENTS

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