The Influence of Tumor Necrosis Factor–α and Interleukin-10 Gene Promoter Polymorphism on the Inflammatory Response in Experimental Human Endotoxemia


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In this study, we show that there is no correlation between tumor necrosis factor–α gene promoter polymorphism at position −308, interleukin-10 gene promoter polymorphism at position −1082, and the cytokine levels they produce in the human endotoxemia model.

There is a genetic influence on the inflammatory response in humans. Recent discoveries of genetic variation have provided insight into susceptibility to and outcomes of a wide range of diseases. The significance of genetic variations for septic patients remains uncertain.

The polymorphism of the TNF-α gene at position −308, which is in the promoter region of this gene, located on chromosome 6, has been associated with outcome of infectious diseases. The more-common TNF1 allele has a guanine (G) residue, whereas the less-common TNF2 allele has an adenine (A) at position −308. In vitro studies with human peripheral blood monocytes have demonstrated that the TNF2 allele, whether homozygous or heterozygous, is associated with a higher production of TNF-α after stimulation with endotoxin [1]. In meningococcal disease in childhood, heterozygous children had a higher relative risk of death than children who were homozygous for the TNF1 allele [2]. In addition, an increased occurrence of the TNF2 allele has been found among patients with septic shock, compared with healthy, unrelated blood donors [3], which suggests that mortality and morbidity may be increased among high producers of TNF-α. In contrast, in a study of first-degree relatives of patients with meningococcal disease, TNF polymorphism at −308 was not associated with outcome or TNF production, whereas families in which low TNF production was common had a 10-fold increased risk for fatal outcome [4]. In addition, in adult patients with sepsis, no correlation was demonstrated among allele and TNF-α production [3], multiple organ dysfunction, and mortality [5]. The data therefore appear to be contradictory. One explanation could be that the incubation period of ex vivo stimulation with endotoxin might have influenced the amount of TNF-α production [4]. It has been suggested that, in patients with sepsis, variation in the moment of sampling was responsible (lead-time bias) [6].

IL-10 gene promoter polymorphism at position −1082 has not been analyzed in patients with sepsis or in subjects during experimental endotoxemia. Clinical data on the relationship between IL-10 response and IL-10 gene polymorphism are contradictory. Most reports have indicated that the G→A mutation at −1082 was associated with a low level of IL-10 production [7], whereas one study reported the opposite [8]. The anti-inflammatory cytokine IL-10 could play a significant role in the clinical outcome in some groups of patients. High IL-10 levels are associated with higher mortality among patients with infectious diseases [9].

Administration of iv endotoxin is a unique and safe procedure for the evaluation of the pathophysiology of inflammation and sepsis in healthy human volunteers and for avoiding lead-time bias. We evaluated the influence of both TNF (−308) and IL-10 (−1082) gene promoter polymorphism on the inflammatory response in experimental human endotoxemia.

PATIENTS AND METHODS

Patients. Twelve healthy male subjects, mean age 28.8 years (range, 19–43 years), took part in this study. Medical history and physical, hematologic, and biochemical examinations were unremarkable for all subjects. A radial artery catheter was placed for blood sampling; heart rate and blood pressure were continuously monitored. At time point 0, endotoxin (Escherichia coli, batch EC-6; US Pharmacopeia) was administered at a dose of 4 ng/kg of body weight (10,000 units of endotoxin per μg) as a 1-min infusion. Blood for genetic investigation was drawn once, and blood for the determination of cytokines was drawn...
before the dose and at 0.25, 0.5, 1, 2, 3, 4, 6, 8, and 24 h after endotoxin infusion, via an indwelling catheter. The latter samples were put immediately on ice, centrifuged (1500 g for 15 min at 4°C), and stored at −80°C until assay.

Cytokines. TNF-α and IL-10 plasma concentrations were determined by use of MEDGENIX EASIA kits (BioSource Europe SA) [10].

Isolation of DNA and IL-10 and TNF-α gene promoter polymorphism. DNA was isolated from peripheral vein blood samples by use of a QIAamp Blood Kit (Qiagen). For the IL-10 promoter (−1082) genotyping, 2 PCR reactions were performed. One detected the −1082A allele, and the other detected the −1082G allele. Similarly, one PCR reaction was performed to detect the TNF1 allele (−308G), and another was performed to detect the TNF2 allele (−308A). Human growth hormone primers were included in all reactions as internal controls. The IL-10–specific primers yielded a product of 578 bp, and the TNF primers yielded a PCR product of 104 bp. PCR reactions were done using thin-walled tubes in a total reaction volume of 10 μL in a GeneAmp PCR System 9600 (Perkin-Elmer). The complete reaction mixture consisted of 5% glycerol (Merck), 0.2 μg/μL of cresol red (Sigma Aldrich Chemicals); deoxyribonucleoside triphosphates, 200 μM each (Boehringer Mannheim); 1× PCR buffer that included 1.5 mM of MgCl2 (Perkin-Elmer); specific primers at 2.14 μM each and internal control primers at 0.07 μM each; 0.23 U of AmpliTaq (Perkin-Elmer); and 75 ng genomic DNA. The PCR profile consisted of a denaturation step (2 min at 94°C), 10 two-temperature PCR cycles (denaturation, 10 s at 94°C, and annealing and extension, 60 s at 65°C), and 20 three-temperature PCR cycles (denaturation, 10 s at 94°C; annealing, 50 s at 61°C; and extension, 30 s at 72°C). The PCR products were separated by electrophoresis on a 2% agarose gel and visualized by ethidium bromide staining.

Statistical analyses. All data are expressed as mean ± SEM. The Mann-Whitney U test was used to compare cytokine levels from different groups. Correlation between parameters was analyzed by the Spearman rank correlation test. A 2-tailed \( P \) value <.05 was considered to be statistically significant.

RESULTS

TNF gene promoter polymorphism (−308) and TNF-α response. The 6 volunteers who were homozygous for TNF1 demonstrated peak TNF plasma concentrations of 5989 ± 1568 pg/mL, and peak TNF plasma levels were 6147 ± 775 pg/mL in the 6 volunteers who carried the TNF2 allele (1 homozygous) (figure 1). No significant difference was found (\( P = .93 \)).

IL-10 gene promoter polymorphism (−1082) and IL-10 response. Four volunteers who were homozygous for G demonstrated peak IL-10 levels of 278.5 ± 54 pg/mL. Five heterozygous volunteers had IL-10 plasma levels of 491.8 ± 157 pg/mL, and 2 volunteers homozygous for A had IL-10 levels of 173.5 ± 11 pg/mL. No significant difference was observed among these groups (figure 2).

TNF gene promoter polymorphism (−308) and IL-10 response. Six volunteers who were homozygous for TNF1 demonstrated the lowest peak IL-10 plasma concentrations (203.8 ± 38 pg/mL). Five heterozygous subjects had higher IL-10 plasma levels (398.6 ± 128 pg/mL) (figure 3), and 1 homozygous TNF2 carrier had the highest IL-10 peak plasma levels (843 pg/mL). TNF gene promoter polymorphism was correlated with the IL-10 response (\( r = .62, \ P = .03 \)). A nonsignificant trend was seen in individual ratios of IL-10 to TNF-α (\( r = .53, \ P = .07 \)).
FIGURE 3. Course of IL-10 plasma levels in a 24-h period after endotoxin infusion, related to the TNF gene promoter polymorphism.

DISCUSSION

Our results show that polymorphism of the TNF-$\alpha$ promoter region at $-308$ does not lead to clinically significant difference in levels of TNF-$\alpha$ in experimental human endotoxemia. Our findings may explain the inconclusive results of clinical studies [3, 4]. Another explanation has to be sought for the relationship between TNF2 and the incidence and severity of sepsis. One explanation could be the linkage of other disadvantageous genes on the same chromosome. The genes for TNF-$\alpha$ and TNF-$\beta$ (lymphotoxin-$\alpha$) are arranged in tandem within a 7-kb region in the major histocompatibility complex (MHC). The location of TNF within the MHC has prompted much speculation about the role of TNF genes in the etiology of MHC-linked diseases, in particular those with an inflammatory or autoimmune component [11].

We also showed that IL-10 gene promoter polymorphism at position $-1082$ is not correlated with concentrations of IL-10 in plasma after endotoxin challenge. Although the numbers are small, the lack of a linear relation between the number of high-producer alleles and IL-10 production makes it unlikely that increasing the number of study subjects will result in discovery of a clinically significant relationship. The fact that we did not find a significant association between IL-10 gene promoter polymorphism and IL-10 production might be an explanation for the conflicting data reported in the literature.

Furthermore, we demonstrated that IL-10 plasma levels were associated with the TNF gene promoter polymorphism $-308$. The highest IL-10 levels were observed in subjects who carried the TNF2 allele. This might lead to a second explanation for TNF2-related mortality. A high ratio of IL-10 to TNF-$\alpha$, an anti-inflammatory cytokine profile, is associated with fatal outcome in febrile patients [12]. Although we failed to demonstrate a significant correlation between this ratio and TNF gene polymorphism ($P = .07$), probably because of the small sample size, a nonsignificant trend was shown. Transregulatory mechanisms might be responsible for the correlation between TNF2 and IL-10 levels.

In conclusion, polymorphisms in the TNF-$\alpha$ and IL-10 gene promoters do not lead to alteration in the respective gene products. The influence of genotype on sepsis outcome, which is evident, is far more complicated. The conclusions of this single-hit endotoxemia model in healthy volunteers may help explain why data reported in clinical studies of this relationship are conflicting.

REFERENCES