Effects of Tumor Necrosis Factor Gene Polymorphisms on Patients With Congestive Heart Failure

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Background—Tumor necrosis factor-α (TNF-α) is known to be elevated in patients with congestive heart failure (CHF). Two biallelic polymorphisms have been identified in the TNF gene locus: one in the promoter region of TNF-α (TNFA1/2), and the other in the first intron of TNF-β (TNFB1/2). Both TNFA2 and TNFB2 alleles are associated with high TNF-α production in vitro and susceptibility to inflammatory diseases. Given the importance of TNF-α in the pathogenesis of CHF, we studied the prevalence of TNF gene polymorphisms in CHF patients and the correlation of genotypes to in vivo TNF-α levels.

Methods and Results—TNFA and TNFB genotypes were determined by the polymerase chain reaction–restriction fragment length polymorphism technique. There were no differences in the TNF allele frequencies between CHF (n=229; TNFA1/2=0.84/0.16, TNFB1/2=0.33/0.67) and control subjects (n=139; TNFA1/2=0.84/0.16, TNFB1/2=0.32/0.68). In 211 patients with CHF, circulating levels of TNF-α and the soluble receptors type I and type II were measured by ELISA: 6.18±3.59 pg/mL, 1768±761 pg/mL, and 4484±1750 pg/mL, respectively. There were no correlations between TNFA or TNFB genotypes and circulating levels of TNF-α or its soluble receptors in the CHF patients.

Conclusions—Despite their association with other inflammatory diseases, neither TNFA nor TNFB polymorphisms are related to the presence of CHF or the elevation of circulating TNF-α. Thus, other factors may be more important in determining the circulating levels of TNF-α in CHF. (Circulation. 1998;97:2499-2501.)

Key Words: genetics ■ heart failure ■ immunology

Tumor necrosis factor-α (TNF-α) is a proinflammatory cytokine with pleiotropic biological effects. Numerous studies have demonstrated that circulating levels of TNF-α are elevated in patients with congestive heart failure (CHF).1–4 There is a significant correlation between the plasma levels of TNF-α and the severity of CHF.5 Furthermore, TNF-α is present in the failing but not the nonfailing human heart.6 In experimental animals, TNF-α depresses cardiac contractility, induces cardiomyocyte apoptosis, and produces dilated cardiomyopathy.6 Therefore, TNF-α might be a key mediator in the development of CHF.

The genes for TNF-α and TNF-β are located in tandem on the short arm of chromosome 6. Genetic polymorphisms in the TNF locus are known to be related to several autoimmune, infectious, and neoplastic diseases.7 Two biallelic polymorphisms have been studied extensively: a G-to-A transition at position −308 in the promoter region of the TNF-α gene (G=TNFα1, A=TNFα2) and a G-to-A transition at position +252 in the first intron of the TNF-β gene (G=TNFβ1, A=TNFβ2).8 Both TNFα2 and TNFβ2 alleles are associated with high TNF-α production.10,11 although contradictory reports exist.9,12 The frequency of the TNFα2 allele increases in rheumatoid arthritis and systemic lupus erythematosus,11 and homozygotes for the TNFα2 allele have a higher risk for death due to cerebral malaria.14 In contrast, the TNFβ2 allele decreases in systemic lupus erythematosus,15 and homozygotes for the TNFβ2 allele in severe sepsis have a higher mortality rate with higher circulating levels of TNF-α.16 Given the importance of TNF-α in the pathogenesis of CHF, we hypothesized that these genetic variations might affect susceptibility to and severity of CHF.

Methods

Two patient populations were studied. To examine the effects of TNF polymorphisms on susceptibility to CHF, we studied a series of 229 patients referred to the University of Pittsburgh Heart Failure Service from April 1996 to April 1997 and 139 age-matched control subjects without a history of coronary disease or heart failure. Controls were recruited from spouses of patients in the study. Given the female predominance of patient spouses, additional healthy male volunteers were recruited through a university advertisement. The population profiles are summarized in the Table.
To examine the effects of TNF polymorphisms on circulating levels of TNF-α, we studied 211 patients in the VEST for TNF Genotype Analysis, which is a subsudy of the Vesnarinone Survival Trial (VEST, directed by Jay N. Cohn, MD). All patients in the VEST study had New York Heart Association class III to IV CHF and a left ventricular ejection fraction ≤35%. The study was approved by the review board at each institution, and subjects gave written informed consent for genetic analysis.

Genotyping of TNF Polymorphisms

Genomic DNA was extracted from peripheral blood with a Puregene kit (Gentra Systems Inc). Primers 5'-AGGCAATAGGTTT-GAGGGCCAT-3' and 5'-TCTCTCCCTGCTCGATTTCCG-3' were used to amplify a DNA fragment of 107 bp containing the variable region of the human TNF-α promoter. The sense primer was modified to incorporate the polymorphic site into an Nci I restriction site. Genomic DNA (100 ng) was amplified with 1.25 U Thermus aquaticus DNA polymerase (Gibco BRL) in 50 μL of reaction mixture containing 20 mmol/L Tris-HCl, 1.5 mmol/L MgCl2, 200 μmol/L of each dNTP, and 0.2 μmol/L of each primer. Polymerase chain reactions were run for 35 cycles: 1 minute at 94°C, 30 seconds at 60°C, and 1 minute at 72°C. The product (10 pg) was electrophoresed on a 5% polyacrylamide gel (29:1, acrylamide:bisacrylamide), subjected to electrophoresis in 1% agarose gel (SeaKem LE, FMC BioProducts). TNF A1 allele gives 2 fragments of 586 and 196 bp, whereas a homozygote for the TNF A2 allele gives 1 fragment of 782 bp. Therefore, a homozygote for the TNF A1 allele (TNFA11) gives 2 bands, whereas a homozygote for the TNFA2 allele (TNFA22) gives 1 band and the heterozygote (TNFA12) 3 bands.

Measurements of Circulating TNF-α and Its Soluble Receptors

Plasma samples were collected at the entry of the VEST study and stored frozen at −80°C. Commercially available ELISA kits (Quantikine, R&D Systems) were used to measure plasma levels of human TNF-α, human soluble TNF receptor type I (sTNFRI), and human soluble TNF receptor type II (sTNFRII) as we described previously. 1

Statistical Analysis

The results are presented as mean±SD. The χ2 test was used to compare allele frequencies between groups. ANOVA and Kruskal-Wallis test were used to examine effects of TNF genotypes on circulating levels of TNF-α and its soluble receptors. Differences were considered significant at a value of P<0.05.

Results

TNF genotypes in each population are summarized in the Table. The observed allele frequencies were similar to those reported in the previous studies, 8,11–15 and no differences were found between CHF and controls: TNFA1/TNFA2=0.84/0.16 (CHF) or 0.84/0.16 (control) and TNFB1/TNFB2=0.33/0.67 (CHF) or 0.32/0.68 (control). Furthermore, there were no differences in functional classes, ejection fraction, or cause of CHF by TNFA or TNFB genotypes (data not shown). The TNFB2 allele was significantly linked to the TNF A1 allele (P<0.00001, data not shown), as reported previously. 12

The Figure summarizes the circulating levels of TNF-α, sTNFRI, and sTNFRII in the VEST patients. Plasma levels of TNF-α were elevated in patients with CHF (n=211, 6.18±3.59 pg/mL) compared with the healthy control subjects in our previous study (n=14, 0.8±0.2 pg/mL, P<0.001). 3 Similarly, CHF patients had elevated plasma levels of sTNFRI and sTNFRII (1768±761 and 4484±2049 pg/mL). These values are nearly identical to that previously reported for CHF patients in studies using identical methods and reagents. 2,4 Neither parametric (ANOVA) nor nonparametric (Kruskal-Wallis) statistics could detect significant associations between the TNFA or TNFB genotypes and circulating levels of TNF-α, sTNFRI, or sTNFRII.

Discussion

This is the first report that has studied TNF polymorphisms in patients with CHF. Despite their association with other inflammatory diseases, 7 neither TNFA nor TNFB polymorphisms were...
related to the presence of CHF or the elevation of circulating TNF-α, thus suggesting that elevated levels of TNF-α in CHF patients are not necessarily linked to genetic polymorphisms of TNF-α or TNF-β.

The disparity between the associations of TNF polymorphisms and autoimmune diseases and the lack of association between TNF polymorphisms and CHF points out a major limitation in performing genetic studies in a population of patients referred to a tertiary hospital because of CHF. Unlike autoimmune diseases, CHF is associated with a high mortality, with as many as 50% of patients dying within the first year after referral. Furthermore, the denominator in CHF patients is undefined, because it is generally believed that patients with myocardial infarction are asymptomatic for variable periods of time before presenting with signs and symptoms of CHF. Therefore, we cannot exclude the possibility that patients homozygous for the TNFA2 or TNFB2 alleles had a more malignant clinical course and died before their presentation at a tertiary referral center and that the results were therefore inherently biased. Indeed, this possibility may explain the enormous disparity among the large number of studies that have assessed the relevance of ACE polymorphisms in patients with CHF. One additional limitation of this study was the slightly greater proportion of women and nonwhite individuals in the control group compared with patients. However, genotype analysis stratified by sex and race (data not shown) did not differ from the group as a whole, arguing that these demographic differences did not mask a true underlying association.

This study confirms previous reports of linkage disequilibrium between specific TNF alleles, TNFA1 and TNFB2. This association appears to be inconsistent with the finding of previous studies\(^{10,11}\) that TNFB2 and TNFA2 but not TNFA1 were associated with increased TNF-α production. In fact, our data demonstrate no association of either TNFA2 or TNFB2 with increased levels of proinflammatory cytokines in patients with CHF. In most previous studies, however, an association was demonstrated by assessing the ability of peripheral blood mononuclear cells to respond to lipopolysaccharides or phytohemagglutinin, whereas we examined baseline levels of plasma TNF-α in patients with severe but stable CHF. Furthermore, although TNF-α expression is in the failing heart,\(^2\) TNF-α levels in the myocardium are known to be different from those in the plasma.\(^{5,6}\) Because circulating levels of TNF-α are also affected by other factors, such as clearance and production by nonmyocardial tissues, it may not reflect the true cytokine burden to the failing heart. Finally, all patients with TNF levels measured in this study had at least class III heart failure. This may decrease the variance of TNF-α levels within the group and limit the power of the study to detect a correlation with TNF genotypes.

In conclusion, the present study does not define an association between the genotypes of TNFA or TNFB polymorphisms and the presence of CHF or circulating levels of TNF-α. Although we cannot exclude the possibility that other factors are important in determining the risk for CHF, additional trials will be needed to clarify the prognostic importance of polymorphisms in the TNF genes. Specifically, studies in patients with compromised left ventricular function after a first myocardial infarction might provide a more appropriate population in which to test this hypothesis.

**Appendix**

**VEST Investigators for TNF Genotype Analysis**

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