

TUMOUR NECROSIS FACTOR ALPHA PROMOTER GENE POLYMORPHISM IN SARCOIDOSIS



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Biallelic polymorphisms in the promoter region of the TNF- α gene (*TNFA*) and in the first intron of the TNF- β gene (*TNFB*) have been associated with variation in TNF- α production and with susceptibility to severe diseases. Among other functions, TNF- α plays a pivotal role in regulatory aspects of granuloma formation and sustenance. In sarcoidosis, a systemic granulomatous disorder of unknown aetiology, the clinical course of the disease has been associated with the patient's individual capacity of spontaneous TNF- α production by alveolar macrophages. We determined the *TNFA* and *TNFB* polymorphisms in 101 patients with pulmonary sarcoidosis and 216 healthy blood donors. A highly significant shift to the more uncommon *TNFA2* allele was found in the Löfgren syndrome patient group, which represents the acute form of the disease with frequent spontaneous remission. The results show that gene frequencies of the *TNFA* gene variation are significantly different within the clinical forms of sarcoidosis, indicating that genetic predisposition for TNF- α production may play a role in the pathogenesis of the disease.

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Granuloma formation reflects the host's defence against a foreign pathogen that has resisted destruction by an acute inflammatory response. Granulomatous reactions have been shown to be regulated by numerous endogenous mediators of which the immunoregulatory cytokine tumour necrosis factor α (TNF- α) is thought to play a pivotal role.¹ In addition to the dominant role of TNF- α as an immunomodulator and an essential mediator of the inflammatory response, the location of the TNF gene within the major histocompatibility complex (MHC) has prompted speculation about the role of the TNF gene in the aetiology of MHC-linked diseases. Genetic analysis revealed biallelic polymorphisms at position -308 in the promoter region of the TNF- α gene (*TNFA*)² and in the first intron of the TNF- β gene

(*TNFB*),³ both of which have been associated with variation in TNF- α production.^{4,5,6,7} Pociot *et al.*⁷ showed that individuals carrying the *TNFB2* haplotype had a higher TNF- α -secretory capacity than those carrying the *TNFB1* haplotype, and Bouma and coworkers⁵ provided evidence that carriers of the *TNFA2* allele also secrete higher levels of TNF- α . Recently, these biallelic polymorphisms have been correlated with susceptibility to severe diseases such as cerebral malaria,⁸ diabetes mellitus,⁷ mucocutaneous leishmaniasis⁹ and to poor prognosis in severe sepsis.¹⁰ Thus, evidence is accumulating that there is a genetic predisposition for an individual's capacity of TNF- α production which in turn may affect the outcome of severe infectious disease or the predisposition to autoimmune disease.

In sarcoidosis, a systemic granulomatous disease of unknown aetiology, both an infectious agent and an autoimmune response are discussed as the cause of the disease.¹¹ Considering the fact that the clinical course of sarcoidosis has been correlated with the patient's individual capacity of spontaneous TNF- α production by alveolar macrophages^{12,13} and that TNF- α plays a pivotal role in regulatory aspects of granuloma formation and sustenance, we determined the *TNFA* and *TNFB* polymorphisms in 101 patients with pulmonary sarcoidosis and 216 healthy blood donors to reveal a possible role of these polymorphisms in the pathology of the disease.

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TABLE 1. Distribution of tumour necrosis factor alleles in patients with sarcoidosis and healthy controls

	TNF- α promotor polymorphism (-308)				TNF- β intron 1 polymorphism					
	<i>n</i>	Homozygous <i>TNFA1/1</i>	Heterozygous <i>TNFA1/2</i>	Homozygous <i>TNFA2/2</i>	allele frequency <i>TNFA1/TNFA2</i>	<i>n</i>	Homozygous <i>TNFB1/1</i>	Heterozygous <i>TNFB1/2</i>	Homozygous <i>TNFB2/2</i>	allele frequency <i>TNFB1/TNFB2</i>
Sarcoidosis (all)	101	62* (0.61)	31 (0.31)	8 (0.08)	0.77/0.23	99	14 (0.14)	45 (0.45)	40 (0.40)	0.37/0.63
Löfgren syndrome	16	6 (0.38)	7 (0.43)	3 (0.19)	0.59/0.41†,‡	16	5 (0.31)	5 (0.31)	6 (0.38)	0.47/0.53
Non-Löfgren	85	56 (0.66)	24 (0.28)	5 (0.06)	0.8/0.2	83	10 (0.12)	39 (0.47)	34 (0.41)	0.36/0.64
Controls	216	143 (0.66)	63 (0.29)	10 (0.05)	0.81/0.19	192	23 (0.12)	90 (0.47)	79 (0.41)	0.35/0.65

*Number of individuals (frequencies shown in parenthesis), † $P = 0.0078$, ‡ $P = 0.0212$.

RESULTS

The results of a total of 101 sarcoidosis patients and 216 healthy controls analyzed with respect to the *TNFA* and *TNFB* biallelic polymorphism distribution are summarized in Table 1. The sarcoidosis group as a whole was further subdivided into patients exhibiting Löfgren syndrome (acute symptomatology with fever, arthralgia and erythema nodosum)¹⁴ and non-Löfgren syndrome patients for analysis. Statistical analysis of the *TNFB* data revealed no significant differences in allelic distribution between sarcoidosis patient groups and the control group. However, there was a tendency in the Löfgren syndrome patient group towards a higher prevalence of the *TNFB1* allele. On the other hand, statistical analysis of the *TNFA1/TNFA2* gene frequencies indicated some significant variations. Again, there was no significant difference in allelic distribution between the sarcoidosis patient group as a whole and the control group with, however, a trend to a higher frequency of the *TNFA2* allele. In contrast, a highly significant shift to the more uncommon *TNFA2* allele was found in the Löfgren syndrome patient group compared to the control group ($P = 0.0078$) as well as to the non-Löfgren sarcoidosis patient group ($P = 0.0212$). Apparently, it is the Löfgren patient group that causes the shift to the *TNFA2* allele in the sarcoidosis patient group as a whole, since the allelic distributions of both polymorphisms are identical in the controls and the non-Löfgren sarcoidosis patients.

DISCUSSION

The significant differences found in *TNFA* allele distribution between the Löfgren patient group and the non-Löfgren group as well as the control group suggest that genetic predisposition for TNF- α production may play a role in the clinical course of sarcoidosis. The fact that this polymorphic variation lies within the promoter region of the TNF- α locus makes it a likely candidate for playing a regulatory role in the production of TNF- α . Wilson and coworkers⁴ presented preliminary evidence of a nuclear protein that binds in the region of the polymorphic site and have suggested that the *TNFA2* allele may inhibit a repressor of transcription. More recently, evidence is presented by Bouma et al.⁵ that individuals homozygous for the *TNFA1* allele produce significantly less TNF- α upon stimulation than individuals homozygous for the *TNFA2* allele, whereas heterozygous individuals produce intermediate amounts. Although the direct functionality of this *TNFA* polymorphism remains a matter of some dispute,^{15,16} this last finding nonetheless makes it likely that it may be linked to a

variation in TNF- α production and thus to a variation in the host response to disease. Our results reflect this assumption, since a significantly higher prevalence of the less common *TNFA2* allele in sarcoidosis patients exhibiting Löfgren syndrome is demonstrated. This implies that a genetic predisposition for higher TNF- α production may be an important factor influencing the clinical manifestation of sarcoidosis. Higher levels of the inflammatory cytokine may be one component of several responsible for the acute form of the disease presenting with fever, arthralgia and erythema nodosum. Taking into consideration that the Löfgren syndrome in sarcoidosis represents a favourable course of the disease, one could speculate that a genetic propensity of the affected individual for higher TNF- α production may be beneficial in the elimination of the disease.

In conclusion, the data presented here demonstrate a higher frequency of the *TNFA2* haplotype in patients with Löfgren syndrome when compared to other forms of pulmonary sarcoidosis and a control group. This can be rated as an indication that genetic predisposition for TNF- α production may play a role in the pathogenesis of sarcoidosis, since the *TNFA2* allele is associated with higher levels of TNF- α production. In view of these findings and the fact that prognostic parameters to predict an individual course of the disease do not exist,^{18,19} a follow-up study of prospective design could evaluate the prognostic value of the *TNFA* polymorphism in sarcoidosis.

MATERIALS AND METHODS

Study design

Genomic DNA from blood or tissue samples taken for diagnostic purposes and no longer needed for establishing diagnosis was isolated by standard phenol-chloroform extraction procedures. Analysis of DNA of 101 unrelated patients suffering from pulmonary sarcoidosis and from the blood of 216 unrelated healthy blood donors as a control group was performed. Disease was diagnosed and assessed by chest radiography, bronchoalveolar lavage, and transbronchial biopsy. Patients suffering from acute symptomatology with fever, arthralgia, and erythema nodosum were ascribed as Löfgren syndrome patients.¹⁴ This group consisted of 16 patients with a mean age of 44.3 ± 9.0 and a female:male ratio of 12:4; 15 of these 16 patients showed remission of Löfgren symptomatology in follow-up diagnosis.

Typing of TNF- α and TNF- β genes

For PCR amplification of the *TNFA* and *TNFB* regions, 100 ng of genomic DNA were added to 100 μ l of reaction mixture containing 200 ng of each primer (*TNFA*: 5'-AGG-C AATAGGTTTTGAGGGCCAT-3', 5'-TCCTCCCTGC-TCCGATTCCG-3'; *TNFB*: 5'-CCGTGCTTCGTGCTTT-GGACTA-3', 5'-AGAGGGGTGGATGCTTGGGTTTC-3'),

200 μ M each of dNTPs, 5 U Taq-Polymerase (Perkin Elmer), and PCR reaction buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.5 mM MgCl₂ and 0.1% gelatine). Cycling conditions for *TNFA* were as follows: 38 cycles of 1 min 94°C, 1 min 60°C, 1.5 min (+2 s per cycle) 72°C. The conditions for *TNFB* were analogous, using an annealing temperature of 70°C. Products of 107 and 782 bp were generated for *TNFA* and *TNFB*, respectively. The amplified PCR products were digested with *Nco*I and analysed on an agarose gel visualized by ethidium bromide staining. The restriction digests of the *TNFA* PCR product generated fragments of 87 and 20 bp (*TNFA1*) and 107 bp (*TNFA2*) and of the *TNFB* PCR product fragments of 586 and 196 bp (*TNFB1*) and 782 bp (*TNFB2*).^{2,10}

Statistical analysis

The χ^2 test with Yates correction was used to determine the significance of differences in TNF- α or TNF- β genotype distribution between sarcoidosis patient groups and controls.

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