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CLINICAL REPORT

Tumour Necrosis Factor-α Promoter Polymorphism in Erythema Nodosum

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Erythema nodosum is a common skin disease characterized by erythematous, tender subcutaneous nodules, mostly located on the lower extremities. Little is known about its pathogenesis, although a wide variety of aetiological factors (e.g. bacterial and viral infections, neoplastic diseases and drugs) have been described. Sarcoidosis, a typical granulomatous disease, often occurs in association with erythema nodosum (Loefgren syndrome). Since granulomatous diseases have been closely linked to a deregulated tumour necrosis factor (TNF)-α production, it was tempting to speculate whether TNF-α might play a role in the pathogenesis of erythema nodosum, at least in cases associated with sarcoidosis. A previously described nucleotide exchange, (G→A) at position −308 in the human TNF-α gene promoter, has been shown to be a major cause for enhanced TNF-α production. In the present report, we investigated the genomic TNF-α promoter region in patients suffering from EN with and without underlying sarcoidosis. Our results showed a strong correlation between the uncommon TNF A II allele and sarcoidosis-associated erythema nodosum. Patients with erythema nodosum without underlying sarcoidosis displayed a similar allele frequency compared with controls. Taken together, we provide evidence that erythema nodosum in association with sarcoidosis might be pathogenically linked to altered TNF-α production due to a genetic promoter polymorphism. ***Key words: erythema nodosum; TNF-α promoter polymorphism.***

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Erythema nodosum (EN) is a common skin disease characterized by erythematous, tender subcutaneous nodules, mostly located on the anterior aspects of the lower extremities (1). Lesions usually resolve spontaneously within a few weeks without ulceration or scarring. However, cases without a tendency to self-healing occur and recurrences are a common feature. The onset of lesions is often accompanied by malaise, fever, chills and leukocytosis. Arthropathy and episcleral involvement may be associated with the cutaneous signs. Although association with a variety of diseases has been described, up to now, little is known about the pathogenesis of EN. Diseases that have been linked to EN are sarcoidosis, inflammatory bowel disease, malignancies and bacterial (streptococcal infections, tuberculosis), viral and fungal infections (2). There are also some cases attributed to drugs, e.g. sulfonamides and oral contraceptives (2). Most commonly EN occurs after streptococcal infections and sarcoidosis, a typical granulomatous disease. Sarcoidosis-associated EN had been termed Loefgren syndrome. Loefgren syndrome is characterized by the acute exacerbation of sarcoidosis with bihilar adenopathy, fever, arthropathy and EN.

TNF-α is an important mediator of a wide variety of immunological and inflammatory reactions (3, 4). The TNF-α gene locus is located in the class III region of the major histocompatibility complex (MHC) on the short arm of chromosome 6 (5). A genetic biallelic polymorphism has been described at position −308 of the human TNF-α promoter consisting of the alleles TNF A I (guanine at position −308) and TNF A II (adenine at the position −308) (6). It has been shown that this polymorphic variation within in the promoter region exerts influence on TNF-α production. The TNF A II promoter shows significantly enhanced responsiveness after appropriate stimulation compared with the more common TNF A I promoter (7, 8). Based on these findings, it was tempting to speculate that carriers of the TNF A II allele might be prone to a more severe outcome of inflammatory diseases and might even be susceptible to autoimmune disorders, due to enhanced TNF production. The second hypothesis was fuelled by an association of the TNF promoter polymorphism with specific HLA alleles (9, 10). Indeed, increased allele frequencies of the TNF A II gene were described in patients with severe infectious diseases, such as cerebral malaria (11) and mucocutaneous leishmaniasis (12), and in some autoimmune disorders, such as lupus erythematosus (13) and diabetes mellitus (14) .

TNF-α is mainly produced by activated macrophages (3) and it has also been shown to play an essential role in granuloma formation (15, 16). During granuloma formation TNF-α is required for accumulation of macrophages and differentiation of macrophages into epitheloid cells. The presence of TNF-α is important for both the induction and persistence of well-developed granulomas (16).

In the present report, we addressed the question of whether a TNF-α promoter polymorphism plays a role in the pathogenesis of the acute exacerbation of sarcoidosis presenting as Loefgren syndrome. Our data described a clear association of the uncommon TNF A II allele with this disease while EN of other aetiology showed no association.

MATERIALS AND METHODS

To address the question of whether a −308 TNF-α promoter polymorphism correlates with sarcoidosis-associated EN or EN of other aetiology we compared allele frequencies and genotype distribution defined by NcoI polymorphism in patients suffering from EN and

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pulmonary sarcoidosis (Loefgren syndrome) with a group of patients suffering from not sarcoidosis-associated EN.

Patients

Genomic DNA of 37 age- and sex-matched patients was analysed. Ten patients suffered from EN without underlying sarcoidosis, 10 patients suffered from EN and pulmonary sarcoidosis. Five patients suffering from granuloma annulare and 12 patients suffering from acute drug eruption served as controls. The diagnosis was confirmed by clinical findings and routine histopathology.

Extraction of genomic DNA

Genomic DNA was extracted from paraffin-embedded specimen of skin biopsies taken for diagnostic purposes. After removal of paraffin by extraction with xylene, followed by 2 steps of washing with ethanol, according to a recently described protocol (17), genomic DNA was prepared using a QIAamp Tissue Kit (QIAGEN, Hilden, FRG) according to the manufacturer's specifications; 100 ng of genomic DNA was used for PCR amplification.

PCR amplification

A 107 bp fragment of the TNF promoter region containing the variable −308 nucleotide was amplified by PCR using the primers as described earlier (6):

Sense primer: 5′-AGGCAATAGGTTTTGAGGGCCAT-3′
Antisense primer: 5′-TCCTCCCTGCTCCGATTCCG-3′.

The reaction mixture (total volume of 20 μl) contained 4 μM of each primer and 5 units AmpliTaq-DNA-Polymerase (Perkin Elmer, Weiterstadt, Germany). The cycling conditions were as follows: an initial denaturation step at 94°C for 5 min was followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 64°C for 1 min and primer extension at 72°C for 1.5 min. A final primer extension step of 10 min at 72°C was added.

NcoI restriction enzyme digest

The amplified PCR product was concentrated by ethanol precipitation after phenol chloroform extraction, digested with 1.5 units NcoI restriction enzyme (Boehringer Mannheim, Mannheim, FRG) at 37°C for 1 h and analysed on a 4% agarose gel (FMC NuSieve 3:1 Agarose, Biozym, Hess. Oldendorf, FRG). The DNA was visualized by ethidium bromide staining. With the TNF A I promoter the sense primer resulted in the generation of a NcoI restriction site which was used for analysis of the PCR product by restriction enzyme digest. The PCR fragment of the TNF A II promoter remained unaffected after restriction enzyme digest. After NcoI digest the PCR product of the TNF A I allele generated two fragments of 87 bp and 20 bp, the TNF A II allele only one fragment of 107 bp, and the heterozygous allele TNF A I/II three fragments of 107 bp, 87 bp and 20 bp.

Statistical analysis

The χ^2 test with Yates correction was used to determine the significance of differences in TNF-α genotype distribution between patients with EN and patients suffering from EN and sarcoidosis (Loefgren syndrome) and controls.

RESULTS

Fig. 1 shows NcoI restriction enzyme digests of PCR products of the TNF promoter from patients suffering from Loefgren syndrome and drug eruption, respectively. In patients with drug eruption (Fig. 1a) NcoI digest resulted in a 87 bp band (lane 1 and 3). In Loefgren syndrome two clear-cut bands (107 and 87 bp) were detectable in one patient (Fig. 1b; lane 1), while all others showed one band at 107 bp (Fig. 1b; lane 3). Lanes 2 and 4 served as controls and show PCR products (107 bp band) without addition of NcoI.

The distribution of TNF-α genotypes in patients with EN and controls is shown in Table I. The group of patients suffering from EN and pulmonary sarcoidosis ($n=10$) showed the following distribution of genotypes (Table I): 80% of the individuals were homozygous for the allele TNF A II, 10% were homozygous for TNF A I and 10% were heterozygous for TNF A I/II. This resulted in a TNF A II allele frequency of 0.85.

In EN of other aetiology ($n=10$), the rare homozygous genotype TNF A II was detected in 20%, the heterozygous gene TNF A I/II in 10%, and the homozygous gene TNF A I in 70%. The frequency of the TNF A II allele in EN patients was 0.25. The differences in allele frequencies of the TNF A II allele between Loefgren syndrome and erythema nodosum of other aetiology were statistically highly significant ($p=0.00047$) as were the differences of the allele frequencies between Loefgren syndrome and drug eruption ($p=0.000007$). The differences between the erythema nodosum group and drug eruption were not statistically significant ($p=0.497$).

In the control groups, the distributions were similar to those of healthy individuals (18). In the granuloma annulare group ($n=5$) no TNF A II homozygous was found, 80% were homozygous for the TNF A I and 20% were heterozygous, resulting in an allele frequency of 0.10 for TNF A II. In patients suffering from drug eruption ($n=12$), we observed no homozygous TNF A II allele, 75% were homozygous for TNF A I and 25% were heterozygous. This corresponds to an TNF A II allele frequency of 0.125.

In conclusion, the presented data provided evidence that

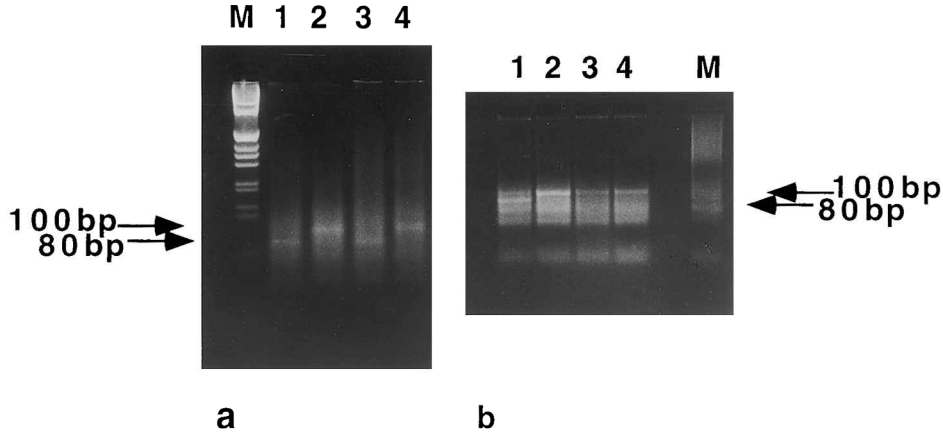

Fig. 1. PCR analysis of TNF promoter polymorphism. (a) NcoI restriction enzyme digest of PCR products from patients suffering from drug eruption (controls). (b) NcoI restriction enzyme digest of PCR products from patients suffering from sarcoidosis-associated erythema nododsum (Loefgren syndrome). Lines 1 and Line 3 with NcoI; lines 2 and 4 without NcoI. M = molecular weight marker.

Table I. *Tumour necrosis factor-α promoter polymorphism (−308): distribution of TNF A alleles*

	n	Homozygous TNF A I	Heterozygous TNF AI/II	Homozygous TNF A II	Allele frequency TNF A I/TNF A II
Erythema nodosum	10	7 (0.7)[a]	1 (0.1)	2 (0.2)	0.75/0.25*
Pulmonary sarcoidosis+erythema nododsum	10	1 (0.1)	1 (0.1)	8 (0.8)	0.15/0.85*/**
Granuloma annulare	5	4 (0.8)	1 (0.2)	0 (0)	0.90/0.10
Drug eruption	12	9 (0.75)	3 (0.25)	0 (0)	0.875/0.125**

[a]Genotype frequencies are shown in parenthesis.
Differences in TNF A II allele frequencies were statistically highly significant (*$p=0.00047$; **$p=0.000007$).

−308 polymorphism is strongly associated with sarcoidosis-associated EN.

DISCUSSION

The present report provides evidence for an association between sarcoidosis and EN (Loefgren syndrome) and the uncommon TNF A II allele. Interestingly, EN of other aetiology showed no correlation with the TNF A II allele. In these patients, the allele frequencies were comparable with those of normal controls. Similarly, granuloma annulare, another granulomatous disease of the skin, and acute drug eruption, which served as controls, showed a TNF allele frequency comparable to that observed in a healthy population.

In a recent study, the allele frequencies of the TNF-α gene were investigated in a series of patients suffering from pulmonary sarcoidosis and a strong, although less pronounced, association of the TNF A II allele with Loefgren syndrome was found (18). The TNF A II allele frequency was 0.41 for Loefgren syndrome compared to the sarcoidosis group, showing an allele frequency of 0.23. Due to these and the findings of our study, it was of particular interest as to whether the TNF A II promoter is a hallmark of the acute exacerbation of sarcoidosis presenting as Loefgren syndrome, or a characteristic feature of EN itself. Although EN shows some clinical, and at late stage also some histopathological, features of a granulomatous diseases (19), the allele frequency of the TNF A II was not increased in EN of other aetiology. This clearly argues against a genetic basis of this disease. However, although not linked to genetic alterations, it is very likely that enhanced TNF-α production might play a role in EN. We have recently found that polymorphonuclear granulocytes are highly activated in EN and show a dramatically enhanced production of reactive oxygen intermediates (20) and it is well known that TNF-α is a potent inducer of reactive oxygen intermediates (21).

A binding motif for a particular transcription factor that could account for enhanced gene expression of TNF-α in TNF A II homozygous patients has not yet been identified. But we recently showed that this region (−308) might play a role in the control of the TNF promoter, at least after stimulation with the activated Raf kinase (22). It had also been shown by other groups that individuals homozygous for the TNF A II allele have higher levels of TNF-α than TNF A I homozygotes (23). These findings had been confirmed by *in vitro* studies showing enhanced TNF-α promoter activity in TNF A II reporter gene assays (7, 8).

Due to the constant finding of TNF A II homozygous gene expression in Loefgren syndrome, the presence of the TNF A II allele might serve as a prognostic parameter to predict sarcoidosis as underlying disease in EN patients, since the allele frequency in EN patients without associated sarcoidosis was similar to normal controls. In the presence of the TNF A II allele careful follow-up of patients suffering from EN should be undertaken for early detection of systemic sarcoidosis.

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REFERENCES

1. Fitzpatrick TB, Eisen AZ, Wolff K, Freedberg IM, Austen KF. Dermatology in general medicine. New York: McGraw-Hill, 1993: 1338–1340.
2. Cribier B, Caille A, Heid E, Grosshans E. Erythema nodosum and associated diseases. A study of 129 cases. Int J Dermatol 1998; 37: 667–672.
3. Beutler B, Cerami A. The biology of cachectin/TNF-α primary mediator of the host response. Annu Rev Immunol 1989; 7: 625–655.
4. Strieter RM, Kunkel SL, Bone RC. Role of tumor necrosis factor-α in disease states and inflammation. Crit Care Med 1993; 21: S447–S463.
5. Carroll MC, Katzman P, Alicot EM, Koller BH, Geraghty DE, Orr HAT, Strominger JL, Spies T. Linkage map of the human major histocompatibility complex including the tumor necrosis factor genes. Proc Natl Acad Sci USA 1987; 784: 8535–8539.
6. Wilson AG, di Govine FS, Blakemore AIF, Duff GW. Single base polymorphism in the human tumour necrosis factor alpha (TNF-α) gene detectable by NcoI restriction of PCR product. Hum Mol Gen 1992; 1: 353.
7. Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor α promoter on transcriptional activation. Proc Natl Acad Sci USA1997; 94: 3195–3199.
8. Krøger KM, Carville KS, Abraham LJ. The −308 tumor necrosis factor-alpha promoter polymorphism effects transcription. Mol Immunol 1997; 34: 391–399.
9. Wilson AG, de Vries N, Pociot F, di Giovine FS, van der Putte LBA, Duff GW. An allelic polymorphism within the tumor necrosis factor α promoter region is strongly associated with HLA A1, B8, and DR3 alleles. J Exp Med 1993; 177: 557–560.
10. Brinkmann BMN, Giphart MJ, Verhoef A, Kaijzel EL, Naipal AMIH, Daha MR, Breedveld FC, Verweij CL. Tumor necrosis factor α-308 gene variants in relation to major histocompatibility complex alleles and Felty's syndrome. Hum Immunol 1994; 41: 259–266.
11. McGuire W, Hill AVS, Allsopp CEM, Greenwood BM, Kwiatkowski D. Variation in the TNF-α promoter region associated with susceptibility to cerebral malaria. Nature 1994; 371: 508–511.

12. Cabrera M, Shaw MA, Sharples C, Williams H, Castes M, Convit J, Blackwell JM. Polymorphism in tumor necrosis factor genes associated with mucocutaneous leishmaniasis. J Exp Med 1995; 182: 1259–1264.
13. Jakob CO, Fronek Z, Lewis GD, Koo M, Hansen JA, McDevitt HO. Heritable major histocompatibility complex class II-associated differences in production of tumor necrosis factor alpha: Relevance to genetic predisposition to systemic lupus erythematosus. Proc Natl Acad Sci USA 1990; 87: 1233–1237.
14. Pociot F, Briant L, Jongeneel CV, Mölvig J, Worsaae H, Abbal M, *et al*. Association of tumor necrosis factor (TNF) and class II major histocompatibility complex alleles with the secretion of TNF-α and TNF-β by human mononuclear cells: a possible link to insulin-dependent diabetes mellitus. Eur J Immunol 1993; 23: 224–231.
15. Kasahara K, Kobayashi K, Shikama Y, Yoneya I, Kaga S, Hashimoto M, *et al*. The role of monokines in granuloma formation in mice: The ability of interleukin 1 and tumor necrosis factor-αto induce lung granulomas. Clin Immunol Immunopathol 1989; 51: 419–425.
16. Kindler V, Sappino AP, Grau GE, Piguet PF, Vassalli P. The inducing role of tumor necrosis factor in the development of bactericidal granulomas during BCG infection. Cell 1989; 56: 731–740.
17. Wright DK, Manos MM. Sample preparation from paraffin-embedded tissues. In: Innis MA, Gelfond DH, Sninsky JJ, White TJ, eds. PCR Protocols: A guide to methods and applications. San Diego: Acad Press, 1990: 153–158.
18. Seitzer U, Swider C, Stuber F, Suchnicki K, Lange A, Richter E, *et al*. Tumour necrosis factor alpha promoter gene polymorphism in sarcoidosis. Cytokine 1997; 9: 787–790.
19. Winkelmann RK, Förström L. New observations in the histopathology of erythema nodosum. J Invest Dermatol 1975; 65: 441–446.
20. Kunz M, Beutel S, Bröcker EB. Leucocyte activation in erythema nodosum. Clin Exp Dermatol 1999; 24: 396–401.
21. Hallet MB, Lloyds D. Neutrophil priming: the cellular signals that say 'amber' but not 'green'. Immunol Today 1995; 145: 3026–3032.
22. Hoffmeyer A, Große-Wilde A, Flory E, Neufeld B, Kunz M, Rapp UR, Ludwig S. Different mitogen-activated protein kinase signaling pathways cooperate to regulate tumor necrosis factor-α gene expression in T lymphocytes. J Biol Chem 1999; 274: 4319–4327.
23. Bouma G, Crusius JBA, Oudkerk Pool M, Kolkmann JJ, von Blomberg BME, *et al*. Secretion of tumour necrosis factor α and lymphotoxin α in relation to polymorphism in the TNF genes and HLA-DR alleles. Relvance for inflammatory bowel disease. Scand J Immunol 1996; 43: 456–463.