A TNF-α Promoter Polymorphism Is Associated with Juvenile Onset Psoriasis and Psoriatic Arthritis

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Tumor necrosis factor-α is considered to be one of the important mediators in the pathogenesis of psoriasis. A strong association of juvenile onset psoriasis with the major histocompatibility complex encoded HLA-Cw6 antigen has been reported but it is unclear whether Cw6 itself or a closely linked gene is involved in the pathogenesis. This study has focused on the association of promoter polymorphisms of the major histocompatibility complex encoded tumor necrosis factor-α gene with psoriasis and psoriatic arthritis. Tumor necrosis factor-α promoter polymorphisms were sought by sequence-specific oligonucleotide hybridization and by direct sequencing in Caucasian patients with juvenile onset psoriasis and with psoriatic arthritis and in healthy controls. A mutation at position –238 of the tumor necrosis factor-α promoter was present in 23 of 60 patients (38%; $p < 0.0001$; $p_{corr} < 0.008$) with juvenile onset psoriasis and in 20 of 62 patients (32%; $p < 0.0003$; $p_{corr} < 0.03$) with psoriatic arthritis, compared with seven of 99 (7%) Caucasian controls. There was a marked increase of homozygotes for this mutation in the psoriasis group. Another mutation at position –308 was found in similar proportions of patients and controls. Our study shows a strong association of the tumor necrosis factor-α promoter polymorphism at position –238 with psoriasis and psoriatic arthritis. Our findings suggest that this promoter polymorphism itself or a gene in linkage disequilibrium with tumor necrosis factor-α predispose to the development of psoriasis. *Key words: cytokines/HLA antigens/linkage disequilibrium/major histocompatibility complex. J Invest Dermatol 109:562–565, 1997*

Recent studies have found a strong genetic background for psoriasis and psoriatic arthritis, which is emphasized by a high concordance rate among monozygotic twins of 55–70% (Elder *et al*, 1994a). Although genome-wide searches for linked genes in affected families have come up with several chromosome regions co-segregating with the disease, the strongest association has been established for genes of the major histocompatibility complex (MHC). For psoriasis the highest relative risk (RR) has been found for HLA-Cw6, particularly in patients with early onset of the disease (< 40 years) and a positive family history (Elder *et al*, 1994b). Patients with psoriatic arthritis (PsA) have a higher frequency of HLA-B27 and the association of PsA with HLA-Cw6 is weaker (Gladman *et al*, 1987). The HLA association of psoriasis has been shown to include the B locus (B17, B37, B57) and to extend to the class II genes HLA-DRB1*0701, -DQA1-*0201, and -DQB1*0303 (Schmitt-Egenolf *et al*, 1993). Almost all psoriasis-associated HLA antigens are in linkage disequilibrium and one possible explanation could be that in addition to HLA class I and class II genes other genes encoded within the MHC are involved in the disease pathogenesis. One such candidate is tumor necrosis factor-α (TNF-α). The gene for TNF-α is located within the class III region of the MHC between HLA-B and HLA-DR (Campbell and Trowsdale, 1993).

Several reports have implicated TNF-α as an important cytokine in the pathogenesis of psoriasis. TNF-α concentrations have been shown to be increased in early psoriatic lesions (Ettehadi *et al*, 1994). Monocytes of psoriatic patients produce higher levels of TNF-α than controls (Bonifati *et al*, 1994). TNF-α and interferon-γ (IFN-γ) have been shown to stimulate the release of interleukin-8 (IL-8) by keratinocytes and fibroblasts (Larsen *et al*, 1989; Barker *et al*, 1990; Nickoloff *et al*, 1991). IL-8 is a signal for T-cell and neutrophil migration into the epidermis. In addition TNF-α and IFN-γ can induce the production of transforming growth factor-α (TGF-α) by keratinocytes which is believed to drive hyperproliferation of these cells (Nickoloff *et al*, 1991; Barker *et al*, 1991).

TNF-α expression is controlled at the transcriptional and post-transcriptional level. Two G *versus* A transitions in the promoter region at positions –308 (Wilson *et al*, 1993) and –238 (D'Alfonso and Richiardi, 1994) have been shown to influence TNF-α expression. At position –308 allele 308.2 (A at –308) is associated with higher constitutive and inducible levels of TNF-α (Wilson *et al*, 1994), whereas for the TNF-238.2 (A at –238) allele functional consequences are not yet clear (Pociot *et al*, 1995).

In this investigation we show that the TNF-238.2 promoter polymorphism is associated with a higher RR to develop psoriasis than HLA class I antigens. This promoter variant is part of the juvenile onset psoriasis-associated haplotype Cw6-B57-TNF-238.2-DR7.

MATERIALS AND METHODS

Patients Sixty Caucasian patients with psoriasis vulgaris fulfilling the criteria of juvenile onset psoriasis (onset not later than at the age of 40 years and a positive family history) and 62 Caucasian patients with PsA were recruited from

Manuscript received March 21, 1997; revised June 5, 1997; accepted for publication June 24, 1997.

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Abbreviations: MHC, major histocompatibility complex; PsA, psoriatic arthritis; RR, relative risk; TNF-α, tumor necrocis factor-α.

the Department of Dermatology, University Clinic of Mainz, for this study. The psoriasis group included 21 female (35%) and 39 male (65%) subjects. The median age of the patients was 43.7 y (range 22–79 y). PsA was defined as inflammatory arthritis, seronegative for rheumatoid factor and associated with psoriasis (Gladman, 1994). All PsA patients were seen by a rheumatologist (E.M.-H. or P.H.) and joint involvement confirmed by radiologic or scintigraphic investigations. These patients were negative for rheumatoid factor and all but two also had juvenile onset psoriasis. All patients had psoriasis vulgaris. Twenty-three were female (37.1%) and 39 (62.9%) were male; the median age of the patients was 52.7 y (range 34–82 y) with a medium disease duration of 10.7 y (range 2–39 y). Twenty-nine patients had peripheral arthritis and 34 spondyloarthropathy (18 with peripheral involvement). The normal control population consisted of 99 unrelated, healthy, white Caucasoid persons from routine consecutive paternity cases studied at the Institute of Legal Medicine. Patients came from the Mainz area. There were 47 female and 52 male individuals. Health status was determined by a questionaire.

Data concerning HLA class I and class II associations of these study populations have been published previously (Höhler *et al*, 1996).

TNF-α promoter polymorphisms A 328-bp fragment spanning position −396 to −69 of the 5′ untranslated region of the TNF-α gene was amplified using primers TNF-396 (5′-TTCCTGCATCCTGTCTGGAA-3′) and TNF-69 (5′- CAGCGGAAAACTTCCTTGGT-3′). A panel of previously HLA-DR-typed samples was initially typed for the polymorphisms by sequencing the entire 328-bp fragment. This led to the identification of a number of heterozygous and homozyous individuals for each of the four polymorphisms. TNF-promoter alleles in the study subjects were detected by dot blot analysis of amplified DNA incorporating in every analysis a panel of sequenced reference samples. Four digoxigenin-labeled oligonucleotide probes were used for the identification of the promoter alleles (D'Alfonso and Richiardi, 1994). Filters were hybridized at 40°C and washed with tetramethylammonium chloride at the temperatures previously described (D'Alfonso and Richiardi, 1994). Hybridization and color detection with antidigoxigenin antibody coupled alkaline phosphatase were carried out according to the manufacturer's instructions (Boehringer, Mannheim, Germany).

Statistical analysis The frequencies of TNF-α promoter alleles were compared between patients with juvenile onset psoriasis, PsA, and controls by chi square tests with Yates correction for small numbers. When multiple comparisons are made significant associations may arise by chance. To avoid such errors p values were corrected with the number of alleles tested in the entire association study (18 for HLA-A, 38 for HLA-B, 10 for HLA-C, 13 for DRB1, and four for TNF-α; 83 alleles tested). RRs associated with a particular allele were calculated using a chi-square distribution: RR = (n1 × n4)/(n2 × n3), where n1 is the number of patients with allele x, n2 is the number of controls with allele x, and n3 and n4 are the corresponding proportions of individuals in patient and control groups not carrying allele x (Dyer and Warrens, 1994). In each group the distribution of TNF-α promoter genotypes was checked for deviations from Hardy–Weinberg equilibrium using an exact test (Guo and Thompson, 1992). Linkage disequilibrium between HLA-C, HLA-B, DRB1*, and TNF-α was tested by chi-square test and by an exact test (Zaykin *et al*, 1995). The p values were corrected for the number of alleles tested (38 for HLA-B, 10 for HLA-C, 13 for DRB, and four for TNF-α; 65 alleles tested); p values < 0.05 were regarded as significant.

RESULTS

TNF-238.2 is increased in psoriasis and in PsA We found a significant increase of subjects carrying A at position −238 in the psoriasis (38% *vs* 7%; $p < 0.0001$) and the PsA group (32% *vs* 7%; $p < 0.0003$) (**Table I**). Gene frequencies for the TNF-238.2 allele in controls were similar to those reported in the literature for west European caucasians (D'Alfonso and Richiardi, 1994; Pociot *et al*, 1995). TNF-promoter allele frequencies were similar among male and female patients.

The observed differences were further underlined by the presence of five TNF-238.2 homozygous persons in the psoriasis group. Homozygosity for TNF-238.2 is expected in only 1.2 of 1000 investigated subjects according to the gene frequency of TNF-238.2 (3.5%) in the control population. We did not observe any homozygous subject in the PsA group which is probably due to chance as we would have expected 1.6 homozygous subjects in this group according to the Hardy–Weinberg equation. There were no differences in the frequency of the TNF-308.2 polymorphism and variation at this locus was independent of the TNF-238.2 polymorphism. In PsA patients there was no association between any promoter polymorphism and the pattern of joint involvement.

Table I. TNF-α promoter genotype frequencies in the investigated groups

Locus/genotype	Controls (n = 99)[c]		Psoriasis (n = 60)		PsA (n = 62)	
	n	%	n	%	n	%
TNF-α −238						
G/G	92	93	37[a]	62	42[b]	68
G/A	7	7	18	30	20	32
A/A	0	0	5	8	0	0
TNF-α −308						
G/G	73	74	52	87	47	76
G/A	20	20	7	11	11	18
A/A	6	6	1	2	4	6

[a] $p < 0.0001$, $p_{corr} < 0.008$; G/A and A/A *versus* G/G in psoriatic patients compared with controls.
[b] $p < 0.0003$, $p_{corr} < 0.03$; G/A and A/A *versus* G/G in subjects with PsA compared with controls.
[c] n, number of investigated patients.

Table II. Linkage disequilibrium between HLA-DR, HLA-B, and HLA-Cw alleles and the TNF-238.2 alelle

		TNF-238. 2 +	TNF-238. 2 −	N	p
Psoriasis					
Cw6	+	21	13	58	$p < 0.0003$; $p_{corr} < 0.02$
	−	2	22		
B57	+	20	1	58	$p < 0.00001$; $p_{corr} < 0.0006$
	−	3	34		
DR7	+	15	13	58	$p < 0.06$; p_{corr} = NS[a]
	−		8	22	
PsA					
Cw6	+	11	10	61	$p < 0.04$; p_{corr} = NS
	−	9	31		
B57	+	9	2	61	$p < 0.0009$; p_{corr} = NS
	−	11	39		
DR7	+	5	8	61	p = NS
	−	15	33		

[a] NS, not significant.

Linkage disequilibrium between TNF-238.2 and HLA-B57 The previously published data on HLA class I and class II antigens in our population (Höhler *et al*, 1996) were used to test TNF-α polymorphisms at positions −238 and −308 for linkage disequilibrium to HLA-B, HLA-C, and HLA-DRB1 (**Table II**). In the psoriasis group there was very strong linkage disequilibrium to HLA-B57 ($p < 0.00001$; $p_{corr} < 0.0006$) and a weaker one to Cw6 ($p < 0.0003$; $p_{corr} = 0.02$) and to DRB1*0701 [p_{corr} = not significant (NS)]. This is reflected by the fact that only eight of 23 (34.8%) observed TNF-238.2 alleles in this group occurred independently from HLA-B57 (five patients were homozygous for TNF-238.2 and carried another B allele in addition to B57) compared with 11 of 20 alleles (55%) in the PsA group. In the PsA patients there was a weaker linkage disequilibrium to B57 ($p < 0.0009$; p_{corr} = NS) and even less to HLA-Cw6 (p_{corr} = NS) and to DRB1*0701 (p_{corr} = NS). In addition, we observed a very strong conservation of the Cw6-B57-TNF-238.2 haplotype in psoriasis subjects which accounted for 15 of 23 (65.2%) TNF-238.2 carrying haplotypes compared with eight of 20 (40%) in the PsA group.

Highest RR conferred by TNF-238.2 The highest RR to develop psoriasis was associated with homozygosity for TNF-238.2 in the psoriasis group and with heterozygosity for this allele in the PsA group (**Table III**). Differences between psoriasis and PsA in the importance of the TNF-238.2 polymorphism are partially explained by differences

Table III. RRs conferred by the TNF-238.2 allele compared with Cw6, B57, and DRB1*0701

Allele	Psoriasis RR	PsA RR
TNF-238.2 homozygous	65	[a]
TNF-238.2 heterozygous and homozygous	8.2	6.3
HLA-Cw6	5.3	2.0
HLA-B57	14	4.6
HLA-DRB1*0701	1.3	0.65

[a]No TNF-238.2 homozygous individuals with PsA were observed.

in HLA class I and class II frequencies. The high RR in TNF-238.2 homozygotes is paralleled by a strong RR for HLA-B57.

DISCUSSION

More than 20 years after the initial description of an HLA association of psoriasis (Russell *et al*, 1972; White *et al*, 1972) there is still little evidence for a direct involvement of HLA-Cw6 as an antigen-presenting molecule in the disease pathogenesis. Recent investigations have shown that the extended haplotype Cw6-B57-C4A6-DR7 rather than only Cw6 is disease associated (Schmitt-Egenolf *et al*, 1993). Therefore other MHC-encoded genes may be attractive candidates to explain the observed HLA association. Our study shows that the TNF-238.2 promoter polymorphism has a stronger association with juvenile onset psoriasis and PsA than Cw6. It is impossible to dissect the strong association of B57 and TNF-238.2 in the psoriasis group any further because of the strong linkage disequilibrium between these two alleles. The high RR for psoriasis conferred by homozygosity for TNF-238.2 and the increased frequency of TNF-238.2 in the PsA group argue for the importance of this polymorphism. In PsA subjects, TNF-238.2 shows the strongest disease predisposition (RR = 6.3) although there is a slightly different HLA class I association compared with the psoriasis group (Cw6: 33.3% *vs* 56.7%, respectively; B57: 15.9 *vs* 36.7%; RRs in **Table III**).

In current models of psoriasis TNF-α is thought to be an important mediator in the initiation phase of cutaneous inflammation in psoriasis (Barker *et al*, 1991). Physical and chemical stimuli (Barker *et al*, 1991) have been shown to induce TNF-α production by keratinocytes. TNF-α release induces the activation of dermal endothelial cells with the expression of adhesion molecules like ICAM-1 (Barker *et al*, 1990), ELAM-1 (Bevilacqua *et al*, 1987), and VCAM-1 (Osborn *et al*, 1989) and the recruitment of inflammatory cells like T-lymphocytes (Valdimarsson *et al*, 1995) and neutrophils. In the subsequent antigen-dependent amplification phase of inflammation, TNF-α and IFN-γ are released by the recruited inflammatory cells. These cytokines are important stimulators for the MHC class II expression of keratinocyte (Barker *et al*, 1991). In addition they stimulate the production of chemotactic substances like IL-8 (Barker *et al*, 1990) with the consequence of further amplification of the inflammatory process. TNF-α and IFN-γ have also been shown to stimulate TGF-α production by keratinocytes (Nickoloff *et al*, 1991), the cytokine thought to be responsible for the hyperproliferation of the keratinocytes.

The TNF-238.2 polymorphism lies in a putative regulatory box (Y-box) of the TNF-α promoter region which binds to regulatory DNA-binding proteins like NF-Y (D'Alfonso and Richiardi, 1994). Nucleotide position −238 is strongly conserved among different animal species, raising the possibility that it is functionally important (Shakov *et al*, 1990). The Y-box is believed to contribute to optimal promoter activity (D'Alfonso and Richiardi, 1994). A single base pair substitution in the Y-box of the HLA-DQA1 promoter causes significantly decreased transcription of the gene (Haas *et al*, 1995; Morzycka-Wroblewska *et al*, 1997). Monocytes of TNF-238 A/G heterozygous subjects show increased TNF-α production after LPS treatment or allogenic stimulation (Pociot *et al*, 1995), although the differences in TNF-α production did not reach statistical significance because of the low number of persons tested.

In our study a much stronger RR to develop psoriasis or PsA is associated with the TNF-238.2 promoter mutation than with HLA-Cw6. As long as there is no definite proof of a direct functional involvement of Cw6 in psoriasis other genes should be considered as possible candidates. Several genes might be involved in the disease pathogenesis as is suggested by the strong haplotype conservation observed in our study, particularly in the psoriasis group. A cytokine imbalance caused by altered TNF-α secretion in patients carrying the TNF-238.2 mutation could be an important factor in the disease pathogenesis. Further studies are required to assess the functional consequences of this polymorphism in affected individuals.

We would like to thank Dr. Peter Hasenclever, Karl-Aschoff-Klinik, Bad Kreuznach, Germany, for referring patients for this study.

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