

Cytokine gene polymorphisms in allergic contact dermatitis

GÖTZ A. WESTPHAL,¹ AXEL SCHNUCH,² ROTRAUT MOESSNER,³ INKE R. KÖNIG,⁴ BIRGER KRÄNKE,⁵
ERNST HALLIER,¹ ANDREAS ZIEGLER⁴ AND KRISTIAN REICH³

Departments of ¹Occupational Health and ³Dermatology, and ²Information Network of Departments of Dermatology (IVDK) at the Department of Dermatology, Georg-August-University, Göttingen; ⁴Institute of Medical Biometry and Statistics, University at Lübeck, Germany; ⁵Department of Occupational Dermatology, University of Graz, Austria

Susceptibility to contact allergy may be influenced by genetically determined alterations in the production of pro- and anti-inflammatory cytokines. This report focuses on functional polymorphisms in the genes encoding for several cytokines involved in the pathogenesis of contact allergic responses, including tumour necrosis factor (TNF)- α (G–238 A, G–308 A), interleukin (IL)-1 β (C–511 G, T+3953 C), its natural antagonist, the IL-1 receptor antagonist (VNTR intron 2), and IL-6 (G–174 C). Polymorphisms were investigated by PCR techniques among polysensitized individuals, defined as individuals with confirmed contact sensitization to *para*-substituted aryl compounds and at least one other structurally unrelated allergen ($n = 86$), and healthy control individuals without a history of eczema ($n = 310$). The distribution of *TNFA*–308 genotypes was significantly different in these groups ($P_{\text{adjusted}} = 0.0378$). Compared with carriers of 2 wild-type alleles (*TNFA*–308*1/1 (*G/G)), carriers of the *TNFA*–308*1/2 (*G/A) and *TNFA*–308*2/2 (*A/A) genotypes tended to be more common among polysensitized individuals [OR = 1.54, 95% CI (0.92–2.55) and OR = 2.36 (0.84–6.51), respectively]. No significantly different distribution of genotypes was detected at any other polymorphic loci among control individuals without eczema and polysensitized subjects. These findings suggest a possible relationship between the *TNFA*–308 polymorphism and contact allergy. The results need to be confirmed in future studies.

Key words: gene polymorphism, interleukin-1 β ; interleukin-6; tumour necrosis factor- α . © Blackwell Munksgaard, 2003.

Accepted for publication 2 December 2002

Allergic contact dermatitis (ACD) and its underlying cause, allergic contact sensitization, is a common phenomenon. Between 15 and 20% of the general population are sensitized to at least 1 of the major allergens, and between 5 and 10% are affected by manifest ACD at least once per year (1). Although regarded as a dermatosis that is typically triggered by environmental factors, ACD may affect certain individuals more easily than others. The individual susceptibility has been attributed to genetic factors, such as the carriage of certain human leukocyte antigens (HLA) alleles (2). However, the results of HLA allotype studies have remained largely inconclusive (3, 4). Polysensitization, i.e. sensitization to more than 1 structurally unrelated hapten, is discussed as a phenotypic marker of increased susceptibility to contact sensitization (5). Hence, it may be speculated that gen-

etic factors are more prominent in this disease subgroup.

According to a simplified model, the pathogenesis of contact allergy can be divided into pre-immunological and immunological phases (6). The former is characterized by the penetration of a substance (pro-hapten) into the skin and its subsequent metabolic transformation into a hapten (7). The immunological phase involves the interaction of immune-competent cells, primarily antigen-presenting cells and T cells, and is regulated by a complex network of cytokines. In particular, the proinflammatory cytokines tumour necrosis factor (TNF)- α , interleukin (IL)-1 β , and IL-6 are likely to play a crucial role during contact allergic reactions (8). IL-1 β is specifically up-regulated in the epidermis after challenge with allergens, but not in response to irritants, and neutralization of IL-1 β

by intradermal injection of neutralizing antibodies (9) or administration of its natural antagonist, the IL-1 receptor antagonist (IL-1Ra) (10), strongly inhibits cutaneous contact hypersensitivity (CHS) reactions. Furthermore, mice deficient in IL-1 β (11) or IL-6 (12) show impaired CHS responses. As genetic polymorphisms have now been identified that affect the activity of xenobiotic metabolizing enzymes and the production of regulatory cytokines, the preimmunological as well as the immunological phase may be influenced by genetic factors.

In line with the concept that genetically determined variations in xenobiotic metabolism influence susceptibility to contact sensitization, we have previously demonstrated associations between polymorphisms of the genes encoding the *N*-acetyltransferases 1 and 2 and the glutathione *S*-transferases M1 and T1 and contact sensitization to *para*-substituted aryl compounds (*para* compounds) and thimerosal, respectively (13–15). In the present study, we used a case-control design to address the possible role of cytokine gene polymorphisms in contact allergy. Polymorphisms in the genes encoding IL-1 β , IL-1Ra, IL-6, and TNF- α were selected on the basis of their functional relevance and the proposed importance of the cytokine during contact allergic reactions. To ensure high statistical power of the study (16), individuals with confirmed polysensitization ($n = 86$) were compared with healthy control individuals without a history of eczema ($n = 310$).

Materials and Methods

Study participants

The study was approved by the Ethics Committee of the Medical Faculty of the Georg-August-University, Göttingen. Written informed consent was obtained from all participants. We included 125 unrelated Caucasian patients (85 females and 40 males) with positive patch test reactions to *para*-substituted aryl compounds (at least 1 of the following: *p*-phenylenediamine, isopropyl-*p*-phenylenediamine, benzocaine, *p*-aminobenzoic acid derivatives, *p*-aminophenol, sulfanilamide, Disperse Orange 3, *p*-aminoazobenzene, 4,4-diaminodiphenylmethane and 2,4-toluenediamine). All individuals had skin lesions and a medical history suggestive of allergic contact dermatitis and were patch tested with the standard series (17). They were recruited from various Departments of Dermatology in Germany collaborating in the multicentre Information Network of Departments of Dermatology (IVDK) (17). The *N*-acetyltransferase phenotypes and genotypes of a subgroup of these patients have been reported previously (13,

14). Patch testing was performed according to the recommendations of the International Contact Dermatitis Research Group (ICDRG) (18). Individuals were further classified as 'monosensitized' if they had a positive patch test reaction to *para* compounds alone (mean age 46.5 ± 15.7 years, 13 males and 26 females), and as 'polysensitized' if they had a positive patch test reaction to *para* compounds and at least 1 other, chemically unrelated component, agent of the standard series (mean age 47.6 ± 18.3 years, 27 males and 58 females). The control group consisted of healthy unrelated Caucasian individuals ($n = 367$; mean age 37.3 ± 13.8 years, 169 females and 198 males) recruited from the blood donor registry of the Department of Transfusion Medicine, University Hospital Göttingen. Control subjects were divided into those with ($n = 57$) and without ($n = 310$) a history of eczema based on a physician-administered questionnaire.

Cytokine gene polymorphisms

Genomic DNA was prepared from peripheral blood mononuclear cells according to Miller and coworkers (19). We investigated functionally relevant polymorphisms of the genes encoding TNF- α (*TNFA*) (20, 21), IL-1 β (*IL1B*) (22, 23), the IL-1 receptor antagonist (*IL1RN*) (24, 25), and IL-6 (*IL6*) (26). Polymorphisms in the *IL1B* gene at positions -511 (23) and +3953 (27) (relative to the transcriptional start), in the *IL1RN* gene in intron 2 (24), and in the *TNFA* gene at positions -238 (28) and -308 (29) were determined by polymerase chain reaction (PCR) in combination with restriction fragment length polymorphism (RFLP) analysis or allele specific PCR as described. A transition from C to G at position -174 of the *IL6* promoter was analysed by PCR-RFLP according to Fishman et al. (26). Alleles are denoted as follows: *IL1B*-511*C=1, *T=2; *IL1B*+3953*T=1, *C=2; *IL6*-174*C=1, *G=2; *TNFA*-238*G=1, *A=2; *TNFA*-308*G=1, *A=2. Alleles 1 (4 repeats, 410 bp), 2 (2 repeats, 240 bp), 3 (5 repeats, 500 bp), 4 (3 repeats, 325 bp), and 5 (6 repeats, 595 bp) of the variable number of tandem repeat (VNTR) polymorphism in intron 2 of the *IL1RN* gene were detected according to their size relative to a 100-bp DNA.

Statistical analysis

To evaluate deviation from the Hardy-Weinberg equilibrium, observed and expected genotype frequencies were compared by an exact goodness of fit test in cases and controls. Odds ratios (ORs) and exact 95% confidence intervals (CIs) (30) were

Table 1. Genotype frequencies in the investigated groups

Locus	Genotype	Controls		Cases		Controls without eczema vs polysensitized cases		
		Without eczema (<i>n</i> = 310) <i>n</i> (%)	With eczema (<i>n</i> = 57) <i>n</i> (%)	Mono-sensitized (<i>n</i> = 39) <i>n</i> (%)	Poly-sensitized (<i>n</i> = 86) <i>n</i> (%)	χ^2	Exact <i>P</i>	adjusted <i>p</i>
<i>IL1B</i> –511	1 1	129 (41.6)	27 (47.4)	15 (38.5)	43 (50.0)	2.061	0.3574	
	1 2	147 (47.4)	22 (38.6)	19 (48.7)	36 (41.9)			
	2 2	34 (11.0)	8 (14.0)	5 (12.8)	7 (8.1)			
<i>IL1B</i> +3953	1 1	182 (58.7)	32 (56.1)	21 (53.8)	51 (59.3)	0.9283	0.6367	
	1 2	109 (35.2)	21 (36.8)	16 (41.0)	32 (37.2)			
	2 2	19 (6.1)	4 (7.0)	2 (5.1)	3 (3.5)			
<i>IL1RN</i>	1 1	158 (51.0)	24 (42.1)	18 (46.2)	47 (54.7)	4.002	0.4038	
	1 2	108 (34.8)	23 (40.4)	18 (46.2)	29 (33.7)			
	1 3,4,5	9 (2.9)	3 (5.3)	3 (7.7)	5 (5.8)			
	2 2	31 (10.0)	7 (12.3)	0 (0.0)	4 (4.7)			
	2 3	4 (1.3)	0 (0.0)	0 (0.0)	1 (1.1)			
<i>IL6</i> –174	1 1	94 (30.3)	27 (47.4)	9 (23.1)	36 (41.9)	4.383	0.1110	
	1 2	156 (50.3)	17 (29.8)	21 (53.8)	34 (39.5)			
	2 2	60 (19.4)	13 (22.8)	9 (23.1)	16 (18.6)			
<i>TNFA</i> –238	1 1	285 (91.9)	54 (94.7)	37 (100.0)	78 (95.1)	1,208	0.5474	
	1 2	23 (7.4)	3 (5.3)	0 (0.0)	4 (4.9)			
	2 2	2 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)			
<i>TNFA</i> –308	1 1	226 (72.9)	43 (75.4)	26 (66.7)	57 (66.3)	11.470	0.0063	0.0378
	1 2	84 (27.1)	14 (24.6)	13 (33.3)	26 (30.2)			
	2 2	0 (0.0)	0 (0.0)	0 (0.0)	3 (3.5)			

calculated to compare genotype frequencies. Difference in genotype frequencies were investigated using an exact χ^2 -test. The overall significance level was set to $\alpha = 0.05$. To correct for multiple testing, testwise *P*-values were adjusted according to Bonferroni–Holm.

Results

The absolute and relative genotype frequencies of the cytokine gene polymorphisms investigated are given in Table 1. In the control group without eczema, the genotype distribution of all investigated polymorphisms was similar to those reported in earlier studies for Caucasian individuals (24, 26, 28, 29, 31, 32). In accordance with previous investigations, genotype frequencies at the *TNFA*–308 locus deviated from Hardy–Weinberg equilibrium in this group (nominal *P* = 0.0044). No significant deviation from Hardy–Weinberg equilibrium was detected at any other locus in the control group without eczema (all nominal *P* > 0.05).

To ensure high statistical power of the study (16), polysensitized individuals were compared with healthy control subjects without a history of eczema. The distribution of *TNFA*–308 genotypes was significantly different in these groups (*P*_{adjusted} = 0.0378). Compared with carriers of 2 wild-type alleles, carriers of the *TNFA*–308*1/2 and *TNFA*–308*2/2 genotypes tended to be more common among polysensitized individuals [OR = 1.54, 95% CI (0.92–2.55) and OR = 2.36 (0.84–6.51), re-

spectively). No significantly differently distributed genotypes were detected among control individuals without eczema and polysensitized subjects at any other polymorphic loci (Table 1).

Discussion

To the best of our knowledge, this is the first study that investigates the possible relationship between polymorphisms in cytokine genes and ACD. We found that the distribution of *TNFA*–308 genotypes was significantly different among polysensitized individuals and healthy control subjects.

The proinflammatory cytokines TNF- α , IL-1 β , and IL-6 are induced during the early phase of experimental contact hypersensitivity responses (8–12, 33). TNF- α is up-regulated in response to experimental contact allergens (33) and regarded as a critical mediator in the pathogenesis of ACD (8). The production of TNF- α is regulated at the transcriptional and post-transcriptional level (34). In humans, a G to A transition has been identified at position –308 within the promoter region of the *TNFA* gene, with the G form (allele 1) being the most common (21). The *TNFA*–308*1 allele contains an activator protein-2 (AP-2) binding element which is disrupted in the *TNFA*–308*2 allele (35). Functional *in vitro* assays suggest that AP-2 can suppress the activity of the *TNFA* promoter in certain cell lines (36). Moreover, there is evidence that *TNFA*–308*2 is associated with increased transcription compared with the *TNFA*–308*1 al-

lele (36, 37), and that individuals carrying at least 1 *TNFA*-308*2 allele are high secretors of TNF- α (21). These findings, together with the observed correlation between carriage of the *TNFA*-308*2/2 homozygous genotype and a fatal outcome of common infectious diseases (38, 39), imply potential biological significance of the -308 polymorphism.

More recent studies suggest that the *TNFA*-308 polymorphisms may also influence cutaneous inflammatory reactions. Allen and colleagues observed an increased frequency of *TNFA*-308*2 and of the *TNFA*-308*2/2 genotype among individuals with low threshold towards irritants (40). In another study, psoriatic patients were treated with the anti-inflammatory cytokine IL-10 and cutaneous mRNA levels of TNF- α were determined by a quantitative PCR method before and after therapy. It was found that the down-regulation of TNF- α in response to IL-10 was significantly lower in individuals carrying a haplotype that includes the *TNFA*-238*2 or -308*2 allele, respectively (41). Although preliminary, these findings indicate a possible association of *TNFA*-308*2 with enhanced susceptibility to skin inflammation, which could be related to an increased sensitivity to inflammatory stimuli or, alternatively, a decreased response towards anti-inflammatory signals.

In line with this hypothesis, we here present evidence that the *TNFA*-308 polymorphism may influence susceptibility to ACD. In particular, carriage of the *TNFA*-308*2/2 genotype may act as a marker of enhanced susceptibility to contact sensitization. However, interpretation of the latter finding has to be cautious since it did not reach statistical significance, possibly due to the limited number of polysensitized individuals included in the study. It should also be noted that the *TNFA*-308 polymorphisms may occur in linkage disequilibrium with other polymorphisms in the *TNFA* gene that affect cytokine production and influence the susceptibility to contact sensitization.

Furthermore, it remains to be addressed in future studies whether the association between the *TNFA*-308 polymorphism and ACD is a general feature of contact sensitization or restricted to certain groups of allergens such as *para* compounds. The proposed role of *TNFA*-308*2 as a genetic component of both irritant and allergic contact dermatitis could reflect common pathogenetic pathways of these types of dermatitis. Moreover, this finding may point to a genetic basis of the recently suggested 'danger' model. This model presumes that an antigenic signal on its own tends to produce tolerance and requires the presence of a second proinflammatory ('danger') signal, such as cutaneous irritancy, to activate the immune system (42).

There are other limitations to the findings in this study. First, we did not control for allergen exposure. Therefore, we cannot exclude the possibility that the control group includes individuals with genetic predisposition but low allergen exposure, while the polysensitized group may contain individuals characterized by high allergen exposure but lack of prominent genetic risk factors. Second, association results obtained from case-control studies might always be influenced by population stratification. The finding of a deviation from Hardy-Weinberg equilibrium at the *TNFA*-308 locus in the control group may be interpreted as evidence that these controls are not a representative population sample. However, similar findings obtained in other different studies (43, 44) and the reported association of carriage of *TNFA*-308*2 with an increased risk for a fatal outcome of severe infectious diseases (38, 39) point to negative selection as another possible explanation for this phenomenon.

The pathogenesis of contact allergy is complex and likely to involve the interaction of a number of genes. Our finding of a possible contribution of a polymorphism in the gene encoding TNF- α , a cytokine involved both in skin irritation and sensitization, encourages future investigations in the field.

Acknowledgements

Contributors to the IVDK were (in alphabetical order): D. Becker, Department of Dermatology, University Mainz; J. Brasch, Department of Dermatology, University Kiel; T. Fuchs, Department of Dermatology, University Göttingen; J. Grabbe, Department of Dermatology, University Lübeck; P. Koch, Department of Dermatology, University Homburg/Saar; H. F. Merk, Department of Dermatology, Technical University Aachen; G. Richter, Department of Dermatology, University Dresden, Germany.

We thank Melanie Walter and Pia Hopmann for excellent technical assistance and Christine Neumann for critically reviewing the manuscript.

This study was supported by the GEPA, Göttingen, Germany, and by a grant from the Deutsche Forschungsgemeinschaft (Re 1475/1-1).

References

1. Schnuch A, Uter W, Geier J, Gefeller O. Epidemiology of contact allergy: an estimation of morbidity employing the clinical epidemiology and drug utilisation research (CEDUR) approach. *Contact Dermatitis* 2002; 47: 32-39.
2. Menné T, Holm N V. Genetic susceptibility in human allergic contact sensitization. *Seminars Dermatol* 1986; 5: 301-306.
3. Emtestam L, Carlsson B, Marcusson J A, Wallin J, Moller E. Specificity of HLA restricting elements for human nickel reactive T cell clones. *Tissue Antigens* 1989; 33: 531-541.

4. Emtestam L, Olerup O. On T-cell recognition of nickel as a hapten. *Acta Derm Venereol* 1996; 76: 344–347.
5. Moss C, Friedmann P S, Shuster S, Simpson J M. Susceptibility and amplification of sensitivity in contact dermatitis. *Clin Exp Immunol* 1985; 61: 232–241.
6. Merk H F, Jugert F K, Frankenberg S. Biotransformations in the skin. In: Marzulli, F N Maibach, H I, eds. *Dermato-toxicology*, 5th edn. pp. 61–73. Washington DC: Taylor & Francis, 1992.
7. Scholes E W, Pendlington R U, Sharma R K, Basketter D A. Skin metabolism of contact allergens. *Toxicol In Vitro* 1994; 8: 551–553.
8. Kimber I, Cumberbatch M, Dearman R J, Bhushan M, Griffiths CEM. Cytokines and chemokines in the initiation and regulation of epidermal Langerhans cell mobilization. *Br J Dermatol* 2000; 142: 401–412.
9. Enk Ah, Angeloni V L, Udey MC, Katz S I. An essential role for Langerhans cell-derived IL-1 beta in the initiation of primary immune response in skin. *J Immunol* 1993; 151: 2390–2398.
10. Kondo S, Pastore S, Fujisawa H, Shivji G M, McKenzie R C, Dinare Sauder D N. Interleukin-1 receptor antagonist suppresses contact hypersensitivity. *J Invest Dermatol* 1995; 105: 334–338.
11. Shornick L P, De Togni P, Mariathasan S et al. Mice deficient in IL-1 beta manifest impaired contact hypersensitivity to trinitrochlorobenzene. *J Exp Med* 1996; 183: 1427–1436.
12. Hope J C, Campbell F, Hopkins S J. Deficiency of IL-2 or IL-6 reduces lymphocyte proliferation, but only IL-6 deficiency decreases the contact hypersensitivity response. *Eur J Immunol* 2000; 30: 197–203.
13. Schnuch A, Westphal G A, Müller M M, et al. Genotype and phenotype of *N*-acetyltransferase 2 (*NAT2*) polymorphism in contact allergy patients. *Contact Dermatitis* 1998; 38: 209–211.
14. Westphal G A, Reich K, Schulz T G, Neumann Ch, Hallier E, Schnuch A. *N*-Acetyltransferases 1 and 2 polymorphisms in para-substituted arylamine-induced contact allergy. *Br J Dermatol* 2000; 142: 1121–1127.
15. Westphal G A, Schnuch A, Schulz T G, et al. Homozygous gene deletions of the glutathione *S*-transferases M1 and T1 are associated with thimerosal sensitization. *Intern Arch Occup Environ Health* 2000; 73: 384–388.
16. Morton N E, Collins A. Tests and estimates of allelic association in complex inheritance. *Proc Natl Acad Sci USA* 1998; 95: 11389–11393.
17. Uter W, Schnuch A, Geier J, Frosch P J. The information network of departments of dermatology (IVDK) in Germany. *Eur J Dermatol* 1998; 8: 36–40.
18. Fregert S. *Manual of Contact Dermatitis*, 2nd edn. Copenhagen: Munksgaard, 1981.
19. Miller S A, Dykes D D, Polesky H. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; 16: 1215–1217.
20. D'Alfonso S, Richiardi P M. A polymorphic variation in a putative regulation box of the *TNFA* promoter region. *Immunogenetics* 1994; 39: 150–154.
21. Wilson A G, de Vries N, Pociot F, di Giovine F S, van der Putte L B A, Duff G W. An allelic polymorphism within the human tumour necrosis factor α promoter region is strongly associated with *HLA A1*, *B8* and *DR3* alleles. *J Exp Med* 1993; 177: 557–560.
22. Pociot F, Molvig J, Wogensen L, Worsaae H, Nerup J. A *TaqI* polymorphism in the human interleukin-1 β (*IL-1 β*) gene correlates with IL-1 β secretion in vitro. *Eur J Clin Invest* 1992; 22: 396–402.
23. di Giovine F S, Takhsh E, Blakemore A I F, Duff G W. Single base polymorphism at -511 in the human interleukin-1 β gene. *Hum Mol Genet* 1992; 1: 450.
24. Tarlow J K, Blakemore A I F, Lennard A, et al. Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86 base pair tandem repeat. *Hum Genet* 1993; 91: 403–404.
25. Santtila S, Savinainen K, Hurme M. Presence of the IL-1Ra allele: 2 (*IL1RN*2*) is associated with enhanced IL-1 β production in vitro. *Scand J Immunol* 1998; 47: 195–198.
26. Fishman D, Faulds G, Jeffery R, et al. The effect of novel polymorphisms in the interleukin-6 (*IL-6*) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J Clin Invest* 1998; 102: 1369–1376.
27. Bioque G, Crusius J B A, Koutroubakis I, Bouma G. Allelic polymorphism in IL-1 β and IL-1 receptor antagonist (*IL-1Ra*) genes in inflammatory bowel disease. *Clin Exp Immunol* 1995; 102: 379–383.
28. Day C P, Grove J, Daly A K, Stewart M W, Avery P J, Walker M. Tumour necrosis factor-alpha gene promoter polymorphism and decreased insulin resistance. *Diabetologia* 1998; 41: 430–434.
29. Chouchane L, Ahmed S B, Baccouche S, Remadi S. Polymorphism in the tumor necrosis factor-alpha promoter region and in the heat shock protein 70 genes associated with malignant tumors. *Cancer* 1997; 80: 1489–1496.
30. Mehta C R, Patel N R, Gray R. On computing an exact confidence interval for the common odds ratio in several 2 $\times$ 2 contingency tables. *J Am Stat Assoc* 1985; 80: 969–973.
31. Mansfield J C, Holden H, Tarlow J K, et al. Novel genetic association between ulcerative colitis and the anti-inflammatory cytokine interleukin-1 receptor antagonist. *Gastroenterology* 1994; 106: 637–642.
32. El-Omar E M, Carrington M, Chow Wh, et al. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature* 2000; 404: 398–402.
33. Cumberbatch M, Dearman R J, Kimber I. Langerhans cells require signals from both tumour necrosis factor alpha and interleukin-1 beta for migration. *Immunology* 1997; 92: 388–395.
34. Rink L, Kirchner H. Recent progress in the tumor necrosis factor- α field. *Int Arch Allergy Immunol* 1996; 111: 199–209.
35. Kroeger K M, Abraham L J. Identification of an AP-2 element in the-323 to-285 region of the TNF-alpha gene. *Biochem Mol Biol Int* 1996; 40: 43–51.
36. Wilson A G, Symons J A, McDowell T L, McDevitt H O, Duff G W. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. *Proc Natl Acad Sci USA* 1997; 94: 3195–3199.
37. Kroeger K M, Carville K S, Abraham L J. The-308 tumor necrosis factor-alpha promoter polymorphism effects transcription. *Mol Immunol* 1997; 34: 391–399.
38. McGuire W, Hill A V, Allsopp C E, Greenwood B M, Kwiatkowski D. Variation in the *TNF*-alpha promoter region associated with susceptibility to cerebral malaria. *Nature* 1994; 371: 508–510.
39. Nadel S, Newport M J, Booy R, Levin M. Variation in the tumor necrosis factor alpha gene promoter region may be associated with death of meningococcal disease. *J Infect Dis* 1996; 174: 63–68.
40. Allen Mh, Wakelin Sh, Holloway D, Baadsgaard O, Barker J N W N, McFadden J. TNFalpha and IL-1 receptor antagonist gene polymorphisms in susceptibility to irritant contact dermatitis. *Br J Dermatol* 1998; 138: 201–205.
41. Reich K, Garbe C, Blaschke V, et al. Response of psoriasis to interleukin-10 is associated with suppression of cutaneous type 1 inflammation, downregulation of the epidermal interleukin-8/CXCR2 pathway and normalization of keratinocyte maturation. *J Invest Dermatol* 2001; 116: 319–329.

42. McFadden J P, Basketter D A. Contact allergy, irritancy and 'danger'. *Contact Dermatitis* 2000; 42: 123–127.
43. Hohler T, Kruger A, Schneider PM, Schorf RE, Knor J, Rittner C, Meyer zum Büschenfelde K, Marker-Hermann E. TNF-alpha promotor polymorphism is associated with juvenile on set psoriasis and psoriatic arthritis. *J Invest Dermatol* 1997; 109: 562–565.
44. Reich K, Mössner R, König I R, Westphal G, Ziegler A, Neumann C. Promoter polymorphisms of the genes encoding tumor necrosis factor- α and interleukin-1 β are associated with different subtypes of psoriasis characterized by early and late disease onset. *J Invest Dermatol* 2002; 118: 155–163.

Address:
Dr G. A. Westphal
Department of Occupational Health
Georg-August-University
Waldweg 37
D-37073 Göttingen
Germany
Tel: + 49 551 396193
Fax: + 49 551 396184
e-mail: gwestph@gwdg.de