

Association of allergic contact dermatitis with a promoter polymorphism in the *IL16* gene

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Background: There is evidence that IL-16, a cytokine that induces chemotactic responses in CD4⁺ T cells, eosinophils, and dendritic cells, plays an important role during different types of cutaneous inflammatory responses, including allergic contact dermatitis (ACD) and atopic dermatitis (AD).

Objectives: We sought to test for association between a promoter polymorphism in the *IL16* gene (T to C transition at position -295) and ACD and AD, respectively.

Methods: *IL16* -295 genotypes were determined in samples from 2 separate case-control studies with white individuals. The first study included healthy individuals (n = 310) and patients with ACD (n = 86). These patients were polysensitized as defined by a contact sensitization to para-substituted aryl compounds and at least one other structurally unrelated allergen. The second study comprised healthy subjects (n = 214) and patients with AD (n = 94).

Results: *IL16* -295 genotypes were differently distributed among polysensitized and healthy control subjects (*P* = .0021). In particular, the *IL16* -295*/C/C genotype was overrepresented among polysensitized individuals (7.0% vs 1.0% in the control group; odds ratio, 7.68; 95% CI, 1.59-48.12). In contrast, there was no evidence for an association between the *IL16* -295 polymorphism and AD.

Conclusion: The *IL16* -295 promoter polymorphism might influence susceptibility to contact allergy. (*J Allergy Clin Immunol* 2003;112:1191-4.)

Key words: *IL-16*, promoter, polymorphism, contact allergy, atopic dermatitis, white

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 one of the major aller-

Abbreviations used

ACD: Allergic contact dermatitis
AD: Atopic dermatitis
DC: Dendritic cell
DTH: Delayed-type hypersensitivity
OR: Odds ratio

gens, and between 5% and 10% are affected by ACD at least once per year.^{1,2} Although regarded as a skin condition typically triggered by environmental factors, ACD might affect certain individuals more easily than others.² For example, the concentrations of certain allergens needed to elicit contact allergic responses might differ between individuals by a factor of greater than 10,000.^{3,4} The individual susceptibility has been attributed to genetic factors, such as carriage of certain HLA alleles.⁵ However, the results of HLA allotype studies have remained largely inconclusive.^{6,7} Polysensitized individuals (ie, individuals sensitized to more than one structurally unrelated hapten) are more easily sensitized to the experimental contact allergen *DNCEB* and show lower elicitation thresholds than monosensitized individuals.⁸ Therefore polysensitization is considered as a phenotypic marker of increased susceptibility to contact sensitization.⁸ It might be speculated that genetic factors are more prominent in this disease subgroup.

IL-16 is a natural soluble ligand to the CD4 molecule that induces chemotactic responses in CD4⁺ cells, such as CD4⁺ T cells, monocytes, and eosinophils.⁹ It is synthesized by CD4⁺ and CD8⁺ T cells and released in response to antigen, mitogen, histamine, and serotonin.⁹ More recently, dendritic cells (DCs) have been identified as another source of IL-16, and DC-derived IL-16 was found to induce strong migratory responses in DCs themselves and in T cells.¹⁰ A role for IL-16 during delayed-type hypersensitivity (DTH) reactions is suggested by the finding that IL-16 is strongly upregulated at DTH reaction sites and that DTH responses in animal models can be suppressed by treatment with anti-IL-16 neutralizing antibodies.¹¹ Moreover, migration of epidermal DCs and contact sensitization are impaired in mice deficient for caspase-1,¹² an enzyme assumed to mediate the release of bioactive IL-16 from DCs.¹³

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Langerhans cells in atopic dermatitis (AD) were found to express high-affinity IgE receptors and to use these structures for the highly efficient uptake of allergens and subsequent induction of allergen-specific T-cell responses, indicating that AD might share some pathogenetic elements with classical DTH responses. Interestingly, increased storage of IL-16 has been observed in epidermal DCs in AD lesions and positive atopy patch test reactions, and stimulation of DCs derived from patients with AD through the high-affinity IgE receptor was shown to enhance the production of IL-16 in these cells.^{13,14}

Functionally relevant polymorphisms (ie, polymorphisms that affect cytokine production) have been identified in a number of human cytokine genes, and the results of epidemiologic studies indicate the presence of associations between such polymorphisms and chronic inflammatory and autoimmune disorders.¹⁵ There is also evidence that cytokine gene polymorphisms contribute to the genetic basis of inflammatory skin conditions.¹⁶⁻¹⁸ Because IL-16 has been implicated in the pathogenesis of ACD and AD, we set out to investigate the possible role of a newly described single nucleotide polymorphism at position -295 of the *IL16* promoter¹⁹ as a genetic component in these 2 types of eczema. To this end, *IL16* -295 genotypes were studied in samples from 2 case-control studies, including polysensitized patients with ACD and patients with AD, respectively.

METHODS

Study participants

The study was approved by the institutional review board of the Medical Faculty of the Georg-August-University, Göttingen. Written informed consent was obtained from all participants. We included 125 unrelated white subjects (85 female and 40 male subjects) with positive patch test reactions toward para-substituted aryl compounds (at least one 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). They were recruited from various departments of dermatology in Germany and Austria collaborating in the multicenter Information Network of Departments of Dermatology.²⁰ The *N*-acetyltransferase phenotypes and genotypes of a subgroup of these patients, as well as other cytokine gene polymorphisms, have been reported previously.^{18,21} All individuals had skin lesions and a medical history suspicious of ACD and were patch tested with the standard series according to the recommendations of the International Contact Dermatitis Research Group.²² Individuals were classified as *monosensitized* if they had a positive patch test reaction to para-compounds alone (mean age, 46.5 ± 15.7 years; 13 male and 26 female subjects) and as *polysensitized* if they had a positive patch test reaction to para-compounds and at least one other chemically unrelated agent of the standard series (mean age, 47.6 ± 18.3 years; 27 male and 58 female subjects).

The control group consisted of otherwise healthy unrelated white individuals (n = 367; mean age, 37.3 ± 13.8 years; 169 female and 198 male subjects) recruited from the blood donor registries of the University Hospitals in Göttingen (n = 175) and Munich (n = 192). Control subjects were divided into those with (n = 57) and without (n = 310) a history of eczema on the basis of a physician-administered questionnaire.

IL16 -295 genotypes were also analyzed in an independent case-control study comprising patients with AD and healthy control subjects.¹⁷ Briefly, this study included white patients with active AD (n = 94) on the basis of the modified Hanifin and Rajka diagnostic criteria for AD defined by the UK Working Party. The disease severity (as determined by the SCORAD index), total serum IgE levels, and levels of specific IgE against 8 typical aeroallergens (SX1 test, CAP system; Pharmacia, Freiburg, Germany) were determined at study entry. The control group (n = 214) consisted of healthy unrelated white individuals without a personal or family history of atopic disease (AD, allergic rhinoconjunctivitis, and allergic asthma), as determined by means of questionnaire. Patients with AD and control subjects were recruited locally at the University Hospital Göttingen.

IL16 promoter polymorphism

Genomic DNA was prepared from PBMCs as previously described.²³ The T-to-C transition at position -295 of the *IL16* promoter was investigated by using PCR-RFLP analysis with *AhdI* as a restriction enzyme according to Nakayama et al.¹⁹ PCR products of heterozygous and homozygous carriers of -295°C were also analyzed by means of PCR-RFLP with *Eam*1105I as a restriction enzyme to validate the results obtained with *AhdI*. DNA products were visualized on 2.5% agarose gels.

Statistical analysis

Individuals with confirmed polysensitization were compared with healthy control subjects without a history of eczema according to a strategy proposed by Morton and Collins to ensure a high statistical power of the study with patients with ACD.²⁴

Deviation from the Hardy-Weinberg equilibrium in the control groups was evaluated by comparing observed and expected genotype frequencies by using an exact goodness-of-fit test. Difference in genotype frequencies were investigated by using an exact χ^2 test of independence. In addition, differences in grouped genotypes were described by using odds ratios and exact 95% CIs,²⁵ with exploratory *P* values from the exact test by Fisher.

RESULTS

The absolute and relative genotype frequencies of the *IL16* -295 polymorphism in the investigated groups are given in Tables I and II. No significant deviation from Hardy-Weinberg equilibrium was detected at the -295 locus in the control group without eczema included in the first case-control study (nominal *P* > .05, Table I) or among healthy control subjects of the second case-control study (Table II), and the distribution of *IL16* -295 genotypes was almost identical in these groups.

The distribution of *IL16* -295 genotypes was significantly different in healthy individuals without a history of eczema compared with that in polysensitized patients with ACD (exact *P* = .0021, Table I). In particular, the -295°C/C genotype was more common in the polysensitized group (7.0%) than in the healthy control group with no history of eczema (1.0%; odds ratio, 7.68; 95% CI, 1.59-48.12). The *IL16* -295°C/C genotype also appeared to be overrepresented in the control group with a history of eczema (Table I).

In contrast, analysis of the second independent case-control study revealed a similar distribution of *IL16* -295 genotypes in patients with AD and healthy control subjects (Table II). The majority of patients with AD had

TABLE I. *IL16* –295 genotypes in patients with ACD

Genotype	Control subjects, n (%)		Patients with ACD, n (%)		Control subjects without eczema vs polysensitized subjects	
	Without eczema, n = 310	With eczema, n = 57	Monosensitized, n = 39	Polysensitized, n = 86	χ^2	Exact <i>P</i> value
TT	218 (70.3)	41 (71.9)	28 (71.8)	63 (73.3)		
TC	89 (28.7)	13 (22.8)	11 (28.2)	17 (19.8)		
CC	3 (1.0)	3 (5.3)	0 (0.0)	6 (7.0)	12.79	.0021

early onset (64% ≤ 5 y), familial (71%), or moderate-to-severe (93%) disease of the extrinsic type (87% sensitized to typical aeroallergens).¹⁷

DISCUSSION

Our study suggests a possible association between polysensitization to contact allergens and a promoter polymorphism in the gene encoding *IL-16*, a cytokine that induces chemotactic responses in CD4⁺ T cells and DCs. Migration of DCs from the epidermis to the regional lymph nodes and chemokine-mediated recruitment of activated T cells from the peripheral blood into the skin represent critical steps during contact allergic responses.²⁶ Therefore a genetically determined alteration in the production of *IL-16* could be of pathophysiologic relevance in the manifestation of contact allergy.

The functional consequence of the *IL16* –295 promoter polymorphism remains to be explored. A pro-inflammatory effect might result from an increased production of *IL-16* in response to activating signals, an impaired responsiveness to anti-inflammatory signals, or both. We have performed some preliminary experiments with PBMCs from healthy individuals (n = 44), indicating that carriage of *IL16* –295**C* might influence the control of *IL-16* production by *IL-10* (not shown). Notably, *IL-10* is upregulated in the skin during contact allergic responses and plays a major part in the endogenous control of cutaneous inflammation, including experimental ACD.²⁷

There are several limitations to the findings of the case-control studies. First, it remains to be addressed in future studies whether the association between the *IL16* –295 polymorphism and ACD is a general feature of contact sensitization or restricted to certain groups of allergens, including para-compounds.

Second, because we did not control for allergen exposure, the possibility cannot be excluded that the control group includes individuals with genetic predisposition but low allergen exposure, whereas the polysensitized group might contain individuals characterized by high allergen exposure but lack of prominent genetic risk factors.

Third, it remains to be seen whether carriage of variant *IL16* –295 alleles is also associated with other types of cutaneous inflammation. Because of recent data consistent with a possible contribution of *IL-16* to the recruitment of CD4⁺ T cells and eosinophils not only in allergic asthma but also in AD,^{14,28} we extended our analysis of the *IL16* –295 polymorphism to samples of a

TABLE II. *IL16* –295 genotypes in patients with AD

Genotype	Control subjects, n (%), n = 214*	Patients with AD, n (%), n = 94
TT	152 (71.0)	64 (68.1)
TC	59 (27.6)	29 (30.9)
CC	1 (0.5)	1 (1.1)

*In 2 individuals the *IL16* –295 genotypes could not be determined.

second case-control study, including individuals with an unambiguous phenotype of AD.¹⁷ One major difference between AD and ACD regarding the cutaneous cytokine milieu is that T_H2-type cytokines dominate in AD, at least in acute lesions, whereas ACD is thought to be driven by T_H1-type cytokines. Therefore the observed association between the *IL16* –295 polymorphism and ACD and the lack of association in AD together with previous findings in Crohn's disease,²⁹ another T_H1-type inflammatory process, might point to a more prominent role of *IL-16* in classical T_H1-type diseases.

The recently proposed danger model has introduced the concept that common pathogenic pathways are involved in irritant and ACD. This model presumes that an antigenic signal on its own tends to produce tolerance and requires the presence of a second pro-inflammatory (danger) signal, such as cutaneous irritancy, to activate the immune system.³⁰ Similarities between these 2 types of dermatitis might also exist at the genetic level because the *TNFA* –308 polymorphism was found to be associated with both irritant³¹ and ACD.¹⁸ Our finding of an association between the *IL16* –295 polymorphism and polysensitization stimulates further research into the possible role of this polymorphism in the cutaneous threshold toward irritants.

Finally, association results obtained from case-control studies might always be influenced by population stratification. The distribution of *IL16* –295 genotypes in the healthy control group without eczema (n = 310, Table I) was in Hardy-Weinberg equilibrium and could be confirmed in a second independent group of healthy individuals (n = 214, Table II). *IL16* –295 genotype frequencies in this group were different, however, compared with those reported in an earlier German study²⁹ (explorative exact *P* from χ^2 test of independence = .037). This earlier study included patients with inflammatory bowel disease (n = 203) and healthy subjects (n = 120) but did not contain information on sex, ethnical background, and precise inclusion criteria. From our data, there is little evidence for ethnicity among German white subjects

because within the group of healthy individuals without eczema, the distribution of *IL16* –295 genotypes was almost identical among subjects recruited in Göttingen ($n = 175$) and Munich ($n = 192$; not shown). However, the general possibility of ethnic differences is suggested by the observation of a higher frequency of *IL16* –295**C* in a Japanese population¹⁹ compared with the findings in healthy white subjects reported here. Differences between our findings and those of the earlier German study might at least partially be explained by different inclusion criteria. For example, the exclusion of individuals with a history of eczema from our healthy control group might contribute to a lower frequency of the *IL16* –295**C/C* genotype because the latter appeared to be higher among otherwise healthy individuals with a history of eczema (Table I).

In summary, we present preliminary evidence for an association between a polymorphism in the promoter region of a cytokine involved in the migration of DCs and T cells and contact allergy. The homozygous combination of the rare allele *IL16* –295**C* appeared to be more common among polysensitized than among healthy individuals without a history of eczema. Our results encourage further research into the possible role of genetic variations of immunoregulatory cytokines in the development of contact sensitization and might help to identify individuals with increased susceptibility to contact allergic reactions.

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