

Atopic dermatitis is associated with a low-producer transforming growth factor β_1 cytokine genotype

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Background: Atopic dermatitis (AD) is associated with hyper-responsiveness of lymphocytes to allergens. In acute AD only T_H2-type lymphocytes are activated, whereas in more chronic forms of AD, the activity of both T_H1- and T_H2-type lymphocytes increases. IL-10 and transforming growth factor β_1 (TGF- β_1) are immunosuppressive cytokines that inhibit the activity of both T_H cell types in human subjects.

Objective: The aim of this study was to determine whether children with moderately severe chronic AD had *IL10* or *TGFB1* genotypes known to be associated with low cytokine production.

Methods: Using amplification refractory mutation screening PCR, we examined *TGFB1* and *IL10* gene polymorphisms, which are known to affect cytokine production, in 68 children with moderately severe AD and in 50 nonatopic children.

Results: The odds ratio of children with AD having a low *TGFB1* producer genotype was 4.8 (95% CI, 2.4-9.7) compared with the control subjects ($P < .0001$). There were no differences in the frequency of *IL10* gene polymorphisms between groups.

Conclusion: *TGFB1* genotype may partly explain the strong genetic predisposition to AD. (J Allergy Clin Immunol 2001;108:281-4.)

Key words: Atopic dermatitis, cytokine gene polymorphisms, transforming growth factor β_1 , IL-10

Up to 10% of children in Western communities have atopic dermatitis (AD).¹ The risk of a child having AD is 50% if one parent has atopic disease (AD, asthma, or allergic rhinitis) and 75% if both parents are affected. The concordance in monozygotic twins is 72%, and it is 23% in dizygotic twins.² Genetic factors are therefore thought to play a dominant role in predisposing to this condition. Although a number of candidate genes in various gene clusters (5q, 6p, 11q13, 12q15-24.1, and 14q), especially those associated with determining the production or activity of T_H1 and T_H2 cytokines, have been suggested to predispose to atopic disease, the importance of these factors is still unclear.³⁻⁵

Abbreviations used

AD: Atopic dermatitis

OR: Odds ratio

TGF- β_1 : Transforming growth factor β_1

Atopy is presently thought of as an imbalance between the T_H2- and T_H1-type immune responses, with the cutaneous inflammatory response in AD being at least partly mediated by T_H2 cytokines (IL-4, IL-13, and IL-5).⁶ However, rather than T_H1-type cytokines (IFN- γ) down-regulating the allergic-type immune response, recently they have been found to exacerbate the inflammatory process.^{7,8} A third group of cytokines, which include transforming growth factor β_1 (TGF- β_1) and IL-10, have general immunosuppressive activity, blocking both T_H1- and T_H2-type cytokine activity.^{9,10} In an animal model of asthma, TGF- β_1 reduces airway reactivity and inflammation,^{11,12} and peripheral blood leukocytes of patients with AD express reduced levels of TGF- β_1 mRNA.¹³

Polymorphisms in the *TGFB1* and *IL10* genes have been linked to differences in in vitro cytokine production both in our laboratory^{14,15} and in those of independent groups.^{16,17} Other studies have demonstrated a clinical association between TGF- β_1 polymorphisms and the prevalence of organ fibrosis¹⁴ and hypertension¹⁷ and IL-10 polymorphisms and inflammatory bowel disease,¹⁸ renal transplant outcome,¹⁹ and asthma.²⁰ To date, no significant differences in either IL-10 or TGF- β_1 polymorphisms in AD have been described. The polymorphisms in the *TGFB1* gene affect the amino acid sequence of the signal peptide, so that the +869 alleles encode either leucine or proline at codon 10, and the +915 alleles encode either arginine or proline at codon 25.¹⁴ The -1082 polymorphism in the *IL10* gene lies in the promoter region and affects transcription factor binding.¹⁵ In this study we examined functionally relevant polymorphisms of these 2 immunomodulatory cytokine genes. It was shown that a significantly higher proportion of children with AD had a *TGFB1* gene allele known to be associated with lower production of this cytokine.

METHODS

Patients

All the children enrolled in the study attended outpatient clinics at Booth Hall Children's Hospital in Manchester, United Kingdom. This hospital is a regional referral center for unusually severe or persistent atopic disease, and the study group comprised children with AD

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TABLE I. Demographic data of study groups

	Control subjects	Patients with AD	P value*
No.	50	68	
Age (y)	10 ± 4	8 ± 5	.02
White race	47 (94%)	48 (71%)	.002
Male sex	27 (54%)	37 (54%)	1.0
Family history of atopy	18 (36%)	50 (74%)	.0001

Values are given as mean ± SD where shown.

* χ^2 statistic.

attending the clinic. The mean ± SD percentage of body surface area affected by eczema was 36% ± 33%. The control group consisted of children who did not have AD, asthma, or allergic rhinitis but were attending an adjoining fracture clinic. Permission to conduct the study was obtained from the local research ethics committee (project No. 99060).

Buccal scrapings

Buccal scrapings were done with cytology brushes rubbed gently inside one and then the other cheek for 20 seconds. Cells were shaken into 10 mL of sterile 4% sucrose solution. Cell suspensions were centrifuged at 1200g for 10 minutes. The supernatant was discarded, and the pellet was resuspended in 500 μ L of 10 mmol/L NaCl and 10 mmol/L EDTA (pH 7.5) before being centrifuged at 13,000 rpm in a microfuge for 15 seconds. The supernatant was again discarded, and the pellet was resuspended in 500 μ L of 50 mmol/L NaOH before heating the samples to 100°C for 11 minutes to release the DNA from the cells. After the suspension had cooled, 100 μ L of 1 mol/L TRIS/Cl (pH 7.5) was added, and the mixture was centrifuged at 13,000 rpm for 15 seconds. Five hundred microliters of the supernatant containing the DNA was transferred to a fresh tube, and 5- μ L aliquots were used for each amplification refractory mutation screening PCR analysis, as described below.

Cytokine genotyping

DNA used in this study had been extracted from buccal scrapings as described above or from 5 mL of whole blood by using standard methods. The technique used to genotype individual samples for polymorphisms in the *IL10* (−1082 G > A) and *TGFB1* (+869 C > T [codon 10] and +915 G > C [codon 25]) genes was an amplification refractory mutational system PCR method using primers already described.²¹ Briefly, DNA was amplified in a 10- μ L reaction. Final concentrations of reagents were as follows: 1× AS reaction buffer (ABgene); 200 μ mol/L each of deoxyribonucleoside triphosphates (ABgene); 1.5 mmol/L $MgCl_2$ (ABgene); 8.5% (wt/vol) sucrose; 0.25 units of Thermoprime^{PLUS} DNA Polymerase (ABgene); 5 μ mol/L specific primer mix; 1 μ mol/L internal control primer mix*; and 25 to 100 ng of DNA.

The primer sequences used in the PCR reactions were as follows: TGF- β_1 (+869) generic primer (sense) 5'-tccgtgggatactgagacac-3', primer C (antisense) 5'-gcagcgtagcagcagcg-3', and primer T (antisense) 5'-agcagcgtagcagcagca-3'; TGF- β_1 (+915) generic primer (antisense) 5'-ggctccggttctgcactc-3', primer G (sense) 5'-gtgctgacgctggccg-3', and primer C (sense) 5'-gtgctgacgctggccc-3'; IL-10 (−1082) generic primer (antisense) 5'-cagtccaactga-gaatttg-3', primer G (sense) 5'-ctactaaggctcttgggag-3', primer A (sense) 5'-actactaaggctcttgggaa-3'; and internal control primer 1 (sense) 5'-gccttccaacccttccttc-3' and internal control primer 2

(antisense) 5'-tcacgattctgtgtgttc-3'.

The protocol for the PTC-100 PCR machine (MJ Research) was as follows: 95°C for 1 minute; 95°C for 15 seconds, 65°C for 50 seconds, and 72°C for 40 seconds for 10 cycles; and 95°C for 20 seconds, 59°C for 50 seconds, and 72°C for 50 seconds for 20 cycles. The amplified products were monitored by means of electrophoresis on a 2% agarose gel with ethidium bromide (0.5 μ g/mL).

Statistical analysis

Odds ratios (ORs) and 95% CIs calculated by using logistic regression and χ^2 analysis were used to compare genotype and allele frequencies between groups. Analyses were performed with the SPSS 7.5 software package.

RESULTS

Demographic characteristics

The demographic characteristics of the 118 children aged 1 to 16 years who took part in this study are shown in Table I. Thirty-nine (57%) of 68 children with AD and none of the control group had asthma. Forty-nine (72%) of 68 children with AD compared with 18 (36%) of 50 control subjects had a family history of atopic disease.

TGFB1 and *IL10* cytokine gene polymorphisms

Compared with the control population, in which only 6 of 50 individuals had the +915 C allele of the *TGFB1* gene (codon 25), 27 of 68 children with AD had this allele ($\chi^2 = 8.5$, $P = .004$; Table II). The OR of children with AD having a C allele associated with a low-producer *TGFB* genotype was 4.8 (95% CI, 2.4-9.7; $P = .0001$) when compared with control subjects. There were no statistically significant differences in the frequencies of the +869 alleles of the *TGFB1* gene (codon 10; OR, 0.8; 95% CI, 0.4-1.2) or the −1082 alleles of the *IL10* gene (OR, 1.2; 95% CI, 0.4-3.2) between the 2 study groups. Similarly, significant trends were found when the analysis was performed with only white children (data not shown).

Thirty-nine children in this cohort had asthma, and all of these patients also had AD. Regression analysis with asthma and eczema as covariants showed that although there remained a significant association between TGF- β_1 (codon 25) and AD (OR, 3.6; 95% CI, 1.2-11.0; $P = .02$), there was no significant association between *TGFB1* (codon 25; OR, 1.7; 95% CI, 0.7-4.5; $P = .5$), *TGFB1* (codon 10), or *IL10* polymorphisms and asthma.

Children with AD were subdivided into those with mild-to-moderate disease (affecting <30% of their surface area) and those with severe disease ($\geq 30\%$ of surface area). The group of children with the most severe AD had the highest frequency of the low-producer *TGFB1* (codon 25) genotypes ($P = .002$, Table III). The OR of having a low-producer *TGFB1* (codon 25) genotype compared with the control subjects was 1.7 (95% CI, 0.6-4.5; $P = .3$) in the group with mild-to-moderate disease but 6.2 (95% CI, 2.1-18.2; $P = .001$) in the group with severe disease.

DISCUSSION

These data provide the first evidence that the presence

*Specific primer mix consisted of 10 μ mol/L generic primer and 10 μ mol/L of one of the 2 allele-specific primers, and internal control primers were used to amplify a human growth hormone sequence.

TABLE II. *TGFB1* and *IL10* polymorphism frequencies in children with and without AD

	Control subjects (n = 50)	Patients with AD (n = 68)	P value*
<i>TGFB1</i> polymorphisms, codon 25 (+915)			
Allele frequencies			
G	94 (94%)	109 (80%)	.004
C	6 (6%)	27 (20%)	
Genotype frequencies			
G/G	44 (88%)	41 (60%)	.001
G/C	6 (12%)	27 (40%)	
C/C	0 (0%)	0 (0%)	
<i>TGFB1</i> polymorphisms, codon 10 (+869)			
Allele frequencies			
T	63 (63%)	91 (67%)	.5
C	37 (37%)	45 (33%)	
Genotype frequencies			
T/T	25 (50%)	37 (54%)	.9
T/C	13 (26%)	17 (25%)	
C/C	12 (24%)	14 (21%)	
<i>IL10</i> polymorphisms (−1082)			
Allele frequencies			
G	48 (48%)	62 (46%)	.8
A	52 (52%)	74 (54%)	
Genotype frequencies			
G/G	13 (26%)	16 (24%)	.9
G/A	21 (42%)	30 (44%)	
A/A	16 (32%)	22 (32%)	

Values in bold indicate high-producer genotypes.

*Pearson χ^2 statistic.

TABLE III. *TGFB1* (codon 25) polymorphism frequencies in control subjects, patients with mild-to-moderate AD (<30% of surface area affected), and patients with severe AD (≥30% of surface area affected)

	Control subjects (n = 50)	Patients with mild-to-moderate AD (n = 33)	Patients with severe AD (n = 35)	P value*
<i>TGFB1</i> polymorphisms, codon 25 (+915)				
Allele frequencies				
G	94 (94%)	55 (83%)	54 (77%)	.003
C	6 (6%)	11 (17%)	16 (23%)	
Genotype frequencies				
G/G	44 (88%)	22 (67%)	19 (54%)	.002
G/C	6 (12%)	11 (33%)	16 (46%)	
C/C	0 (0%)	0 (0%)	0 (0%)	

Values in bold are high-producer genotypes.

*Pearson χ^2 statistic.

of a C rather than G residue at position +915 of the *TGFB1* gene—encoding proline at codon 25 is associated with a significantly higher risk of AD. The strongest association was found in children with the most severe disease. The results of our study suggest that *TGFB1* genotype, although important in eczema, may not be as important in the cause of asthma.²² Further studies recruiting children with exclusively AD or asthma are required to examine this question in more detail. The *TGFB1* polymorphism might be relevant itself, or it may be that it is in linkage disequilibrium with another functional polymorphism elsewhere in the *TGFB1* gene. In either case this study suggests that this polymorphism is a significant marker of disease. The study had a power of 80% to achieve significance at the .05 level for this low-producer *TGFB1* genotype, with the obtained OR of 4.8. Hardy-Weinberg equilibrium was obeyed, which is in

keeping with the constant gene frequencies and genotype ratio expected in a randomly breeding population in which the cytokine gene is not associated with excessive mortality before reproductive age.

A possible confounding factor in this study is that there is no absolute guarantee that some children in the control group will not have AD in the future, especially because 36% have a family history of atopic disease (AD, asthma, or allergic rhinitis). However, if some children did have AD, this would be expected to reduce, rather than increase, observed *TGFB1* genotype differences between the groups. Positive skin prick and RAST test results are not specific for AD, and many individuals will have positive laboratory test results of atopy without having overt AD; thus use of these tests would not resolve this issue.

Although we found a significant association between alleles at the +915 position (codon 25) of the *TGFB1*

gene and AD, there was no association between alleles at the +869 position (codon 10) and AD. This disparity may be partly explained by the fact that despite the proximity of these 2 codons, linkage disequilibrium was incomplete. (The C allele of codon 25 was associated with the C allele of codon 10 in 62% of patients, and the G allele of codon 25 was associated with the C allele of codon 10 in 30% of cases.) Polymorphisms in IL-10, although known to determine IL-10 production, were not found to be associated with a predisposition to AD. The study had a power of 80% to achieve significance at the .05 level for *IL10* genotypes, with an OR of 3.0.

How could a lower-producer *TGFB1* genotype predispose children to AD? TGF- β_1 is known to have a number of immunomodulatory actions.²³ On this basis, 2 main hypotheses are proposed. The first involves regulation of the humoral response. This cytokine specifically promotes IgA production by means of immunoglobulin isotype switching in human B lymphocytes.²⁴ In the gut increased levels of IgA may mop up potential food allergens, preventing them from being absorbed and triggering an allergic response. TGF- β_1 can either be produced by the child or be provided in the breast milk of the feeding mother, along with maternal IgA.^{25,26} A decrease in TGF- β_1 activity may result in lower IgA levels and thus increased systemic absorption of potential food allergens. However, the observation that neither breast- nor bottle-fed children with complete IgA deficiency (1:500) have a significant increase in the prevalence of AD or other atopic disease suggests that this mechanism is not likely to be of clinical significance.

The second hypothesis relates to regulation of cellular immunity. TGF- β_1 is produced by a variety of cells. Regulatory T_H3 lymphocytes recently have been found to produce this cytokine after stimulation with IL-4.^{27,28} TGF- β_1 inhibits the activity of antigen-presenting cells, which induce immune responses. Some nonlymphoid cells can also produce TGF- β_1 . Human skin keratinocytes can be stimulated to produce TGF- β_1 but not IL-10.²⁹ It is possible that a relative deficiency of TGF- β_1 in the skin results in the immune response characteristic of AD. The inability of skin keratinocytes to produce IL-10 provides one explanation for the lack of a correlation between polymorphisms in this cytokine gene and atopic eczema.

We suggest that a major genetic predisposition to AD may be the reduced production of local negative feedback circuits controlling the immune response to potential allergens.

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