Atopic dermatitis patients carrying G allele in –1082 G/A IL-10 polymorphism are predisposed to higher serum concentration of IL-10

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Abstract

Introduction: Atopic dermatitis (AD) is a chronic skin inflammatory disease in which Th2-derived cytokines play an essential role. Aim of the study was to assess interleukin 4, 10 and 13 (IL-4, IL-10 and IL-13) serum concentrations in AD patients and to correlate the values with the occurrence of genotypes of selected polymorphisms in genes encoding these cytokines.
Material and methods: Seventy-six AD patients (mean age 11.4 years) and 60 healthy controls were enrolled in the study. Blood samples were analyzed for IL-4, IL-10 and IL-13 concentrations with ELISA assay and genotyping for –590C/T *IL-4*, –1082A/G *IL-10* and –1055C/T *IL-13* polymorphisms with PCR-RFLP.
Results: The obtained results revealed statistically higher serum concentration of IL-10 and IL-13 in AD patients when compared to healthy controls (10.30 pg/ml vs. 8.51 pg/ml for IL-10 and 5.67 pg/ml vs. 4.98 pg/ml for IL-13). There were no significant differences between AD patients and controls in regard to IL-4 serum level (5.10 pg/ml vs. 7.1 pg/ml). Analyzing the association between level of the examined cytokines and genotype polymorphisms –590 C/T for the *IL-4* gene, –1082 A/G for the *IL-10* gene and –1055 C/T for the *IL-13* gene, we found a statistically higher IL-10 serum level among carriers of the G allele in the –1082 G/A *IL-10* polymorphism both in AD and control groups. We did not find any significant differences between serum level of IL-4 and IL-13 in regard to genotype occurrence in examined polymorphisms: –590 C/T for the *IL-4* gene and –1055 C/T for the *IL-13* gene.
Conclusions: The obtained results confirm the genetic background of IL-10 synthesis in the Polish population.

Key words: atopic dermatitis, interleukin 4, 10 and 13, gene polymorphisms.

Introduction

Atopic dermatitis (AD) is a chronic skin disease of not completely known pathogenesis. Familiar occurrence of AD partially proves the role of genetic factors in its development [1–3]. One set of candidate genes includes genes encoding proteins involved in acquired immunity such as Th2 cy-

tokines. They are located on chromosome 5q31-33 [4, 5]. The role of polymorphisms in these genes in AD pathogenesis may be partially explained by their ability to enhance IgE antibody synthesis [6]. Discrepant results obtained in different populations and low statistical power are the reason why Th2 cytokine gene polymorphisms might not be regarded as crucial factors in AD pathogenesis.

A predominant systemic Th2 imbalance with increased IgE levels and eosinophilia is widely accepted in the pathogenesis of atopic diseases [7]. The enhanced expression of Th2-mediated cytokines, notably interleukin 4, 5 and 13 (IL-4, IL-5, and IL-13), is observed in lesional and non-lesional skin in the acute phase of disease. The IL-4 and IL-13 are involved in the initial phase of skin inflammation and upregulate the expression of adhesion molecules on endothelial cells [7]. In chronic AD skin lesions an increase in Th1-mediated cytokines such as interferon-γ (IFN-γ) and IL-12, as well as IL-5 and GM-CSF, was found [8]. In AD IL-4 knock-out mice models, no eosinophil mobilization to the inflammation site was observed. On the other hand, an increased IL-4 level was found both in serum and skin lesions of AD patients [9]. *IL-4* gene polymorphism was analyzed both in European and non-European AD populations, but a correlation between *IL-4* gene polymorphism in AD patients was found only in some of them [2, 10].

Interleukin-10 is closely associated with immunosuppression of the acquired immune response. It was found that decreased IL-10 serum concentration in AD patients may be correlated with higher activity of the disease [11]. This hypothesis was confirmed by Seneviratne *et al.* [12], who observed *in vivo* that decreased synthesis of IL-10 is associated with a severe course of AD.

Data on *IL-10* gene polymorphisms and AD development are scarce and discrepant [13, 14]. Sohn *et al.* [13] revealed an association between polymorphism in the promoter region of *IL-10* and the clinical picture of AD in children. Lacy *et al.* [14] found higher frequency of TGAC haplotype of the *IL-10* gene in AD patients in whom IgE level was above 1000 IU/ml.

Another protein which is involved in AD pathogenesis is IL-13 [15]. Interleukin 13 is an important regulator of inflammatory immune responses, with key roles in atopy and immunity to parasites. Interleukin 13 is involved in "class switch" of immunoglobulins by B lymphocytes and increases the synthesis of IgG and IgE [16]. Literature data indicate that *IL-13* gene polymorphism correlates with enhanced gene transcriptional activity and in consequence enhanced IL-13 synthesis and inflammatory and allergic response [17].

The essential role of IL-4, IL-10, and IL-13 in AD development and scarce and discrepant data on the subject are the reason why we aimed to assess serum concentration of these proteins in AD patients and to correlate the values with the occurrence of genotypes of selected polymorphisms in genes encoding IL-4, IL-10 and IL-13.

Material and methods

Patients

Seventy-six patients (mean age: 11.4 years; 46 female, 30 male) with AD and 60 healthy controls, age and sex matched, were enrolled in the study. Atopic dermatitis was diagnosed according to criteria proposed by Hanifin and Rajka. The severity of the disease was determined by the modified scoring atopic dermatitis (SCORAD) system [18, 19]. The patients enrolled in the study had moderate AD (mean SCORAD index 23, range: 16–39).

Each patient or his/her parents gave written informed consent before entering the study and all the experiments were approved by the local Ethics Committee. The investigations were carried out in accordance with the Declaration of Helsinki. Serum samples were analysed for IL-4, IL-10 and IL-13 concentration with an ELISA assay (Diaclon, Besanson, France) according to the manufacturer's instructions. Sensitivity of examined methods was 0.7 pg/ml for IL-4, 1.3 pg/ml for IL-10 and 1.5 pg/ml for IL-13.

Genotyping for –590C/T *IL-4*, –1082A/G *IL-10*, –1055C/T *IL-13* polymorphisms

Genomic DNA was prepared from peripheral blood leukocytes using a genomic DNA isolation kit (DNA-Gdansk, II SC, Poland). Analysis of polymorphic variants in the promoter regions of the *IL-4*, *IL-10* and *IL-13* genes was performed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) using the appropriate restriction enzyme (digestion for 2 h). The sequences of oligonucleotides and restriction enzymes used for identification of IL-4 –590 C/T, IL-10 –1082 A/G, and IL-13 –1055 C/T polymorphisms are presented in Table I [20, 21].

Statistical analysis

Data were analysed using the Mann-Whitney *U* test and correlation coefficients were determined by using the Spearman rank correlation test. Chi-squared tests were used to detect a significant deviation in genotype frequency from the Hardy-Weinberg equilibrium. The associations between different dichotomous variables and independent variables were assessed by logistic regression analysis. The odds ratio (OR) and 95% confidence intervals (CI) were calculated using a logistic regression model. A p value of < 0.05

Table I. Sequences of oligonucleotides and restriction enzymes used for identification of *IL-4* –590 C/T, *IL-10* –1082 A/G, *IL-13* –-1055 C/T polymorphisms

Polymorphism	Oligonucleotide sequences	PCR products	Restriction enzyme	Restriction fragments
IL-4 –590 C/T	5'-TGGGGAAAGATAGAGTAATA-3' 5'-TAAACTTGGGAGAACATGGT-3'	195 bp	*Ava* II	Allele C (177 bp and 18 bp), allele T (195 bp)
IL-10 –1082 A/G	5'-TCTTACCTATCCCTACTTCC-3' 5'-CTCGCTGCAACCCAACTGGC-3'	139 bp	*Mn1*I	Allele A (139 bp), allele G (106 bp and 33 bp)
IL-13 –1055 C/T	5'-ACTTCTGGGAGTCAGAGCCA-3' 5'- TACAGCCATGTCGCCTTTT CCTGCTCTTCCGTC-3'	377 bp	*Hpy99* I	Allele C (340 bp and 37 bp), allele T (377 bp)

was considered statistically significant. Statistica 6.0 software (StatSoft, Tulsa, OK, USA) was employed to perform analyses. Values of p lower than 0.05 were considered statistically significant.

Results

The obtained results revealed statistically higher serum concentration of IL-10 and IL-13 in AD patients when compared to healthy controls (10.30 pg/ml vs. 8.51 pg/ml for IL-10 and 5.67 pg/ml vs. 4.98 pg/ml for IL-13). There were no significant differences between AD patients and controls in regard to IL-4 serum level (5.10 pg/ml vs. 7.1 pg/ml). The distribution of genotypes of analyzed *IL-4*, *IL-10* and *IL-13* polymorphisms is consistent with the results presented in our papers published previously [20, 21] (Table II).

Analyzing the association between level of the examined cytokines and genotype polymorphisms –590 C/T for the *IL-4* gene, –1082 A/G for the *IL-10* gene and –1055 C/T for the *IL-13* gene, we found a statistically higher IL-10 serum level among carriers of the G allele (G/A heterozygotes or G/G homozygotes vs. A/A homozygotes) in the –1082 G/A *IL-10* polymorphism both in AD and control groups. We did not find any significant differences between serum level of IL-4 and IL-13 in regard to genotype occurrence in examined polymorphisms: –590 C/T for the *IL-4* gene and –1055 C/T for the *IL-13* gene. The detailed results are presented in Table III. There was no correlation between SCORAD index and IL-10, IL-4 or IL-13 serum level in the examined group of patients ($p > 0.05$ for all comparisons).

Discussion

The prevalence of AD has increased significantly over the past decades [22]. As atopy is a complex disease, multiple experimental studies have focused on the interaction between genetic and environmental factors. Genetic factors have a high impact on the risk of developing AD, and several studies have provided evidence of an association between atopy and multiple genes [23, 24].

According to current knowledge, AD is partially considered as a Th2-derived disease, and IL-4, IL-10 and IL-13 are important in development of allergic inflammation. In certain cohorts of AD patients, a high serum level of these cytokines is observed, which probably depends on the genetic background [2].

Hamid *et al.* [25] observed increased expression of IL-4 mRNA in skin lesions of AD patients,

Table II. The association between *IL-4*, *IL-10* and *IL-13* polymorphisms and atopic dermatitis development

	Genotype	Control		AD patients		Value of p	OR	–95% CI	+95% CI
		n	%	*n*	%				
IL-4	TT	31	51.5	43	56.4	0.383	1	Reference	
	CT	26	43.6	31	41.1	0.482	0.859	0.563	1.311
	CC	3	4.9	2	2.5	0.198	0.457	0.138	1.505
IL-10	GG	14	23.5	16	20.9	0.816	1	Reference	
	GA	26	43.6	35	46.0	0.525	1.190	0.696	2.034
	AA	20	32.8	25	33.1	0.655	1.138	0.645	2.006
IL-13	CC	43	71.1	46	60.1	0.076	1	Reference	
	CT	16	27.5	29	36.8	0.058	1.585	1.016	2.475
	TT	1	1.5	1	3.1	0.224	2.466	0.576	10.556

Table III. IL-4, IL-10 and IL-13 serum levels in AD patients and control group including associations between their concentrations and presence of examined genotypes in –590 C/T for *IL-4*, –1082 A/*IL-10* and –1055 C/T *IL-13* polymorphisms

Genotype	Median level of IL-4, IL-10 and IL-13 levels [pg/ml] (25%; 75%)			
	AD patients (n = 76)		Control group (n = 60)	
–590 C/T *IL-4*:				
C/C	5.30 (4.00, 6.87)	p = 0.938	6.74 (2.94, 8.75)	p = 0.641
C/T	4.95 (3.85, 9.55)		7.71 (3.16, 10.19)	
T/T	4.88 (3.21, 8.75)		7.21 (3.61, 9.87)	
Median total serum level of IL-4	5.10 (3.98, 7.25)		7.31 (5.31, 8.64)	p = 0.328
–1082 A/G *IL-10*:				
A/A	7.65 (7.00, 8.95)	p < 0.001*	7.82 (6.85, 9.76)	p = 0.045**
G/A	10.75 (9.25, 5.40)		8.89 (8.07, 10.94)	
G/G	31.5 (15.82, 40.0)		11.18 (7.26, 12.52)	
Median total serum level of IL-10	10.30 (8.80, 17.80)		8.51 (8.05, 9.23)	p = 0.029
–1055 C/T *IL-13:*				
C/C	5.65 (5.52, 6.03)	p = 0.904	5.01 (3.56, 5.85)	p = 0.438
C/T	5.60 (5.26, 6.29)		4.79 (4.54, 6.07)	
T/T	5.70 (5.17, 6.23)		5.47 (only 1 subject)	
Median total serum level of IL-13	5.67 (5.31, 6.11)		4.98 (4.56, 5.16)	p = 0.008

**G/G vs. A/A p < 0.05; G/G vs. G/A p > 0.05; G/A vs. A/A p < 0.05. **G/G vs. A/A p < 0.05; G/G vs. G/A p > 0.05; G/A vs. A/A p < 0.05*

which is further proof of the role of this cytokine in development of AD. In our previous studies we did not find a statistically significant association between –590 C/T *IL-4* gene polymorphisms and occurrence or severity of the disease [18, 19], which is in line with the results obtained by Tanaka *et al.* [10] and Elliot *et al.* [26].

Although a higher serum IL-4 concentration in AD patients has been observed by many authors [11–13], in our study we did not note this phenomenon. Lack of a positive correlation between –590C/T *IL-4* polymorphisms and AD development as well as IL-4 serum concentration in our patients testifies to the genetic distinction of the Polish population and variety of genetic and immune factors involved in AD pathogenesis, partially dependent on population origin.

In various published studies, different polymorphisms of the promoter region of the *IL-10* gene (–1082A/G; –819 T/C; –571 C/A; –854 C/T; –1117 G/A and 592 A/C) were analysed in AD patients, and in most of them no association with disease development was found [13, 14], which is consistent with our previous study [2, 20, 21]. However, on analysing IL-10 serum concentration in AD patients we observed a significantly higher level in the patients than in the control group. Interestingly, in both examined groups (patients and controls) higher IL-10 serum levels were found in the carriers of the G allele in –1082 G/A polymorphisms. This observation suggests the role of the analysed polymorphism in synthesis of IL-10, but the presence in the study patients only with moderate AD is its limitation and requires further investigations.

The performed studies showed that polymorphic variants in the promoter region of the *IL-13* gene are linked to increased transcriptional activity and enhanced synthesis of IL-13, and development of inflammatory and allergic diseases [27]. In our study we found an association between –1055 C/T IL-13 polymorphism and AD development and higher IL-13 serum level in AD patients. Statistical analysis did not show a link between certain genotypes in –1055 C/T polymorphism and serum concentration of IL-13.

Discrepant results on the genetic background and Th2-derived cytokines in AD patients obtained in different populations confirm the complicated and multifactor pathogenesis of the disease. The role of alteration of adaptive immunity in AD has already been widely discussed. However, recent data clearly point to participation of the innate immune response, and these tightly linked arms of human immunity are the challenge for further research of AD [2, 28].

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