

ORIGINAL ARTICLE

Investigation of cytokine levels and their association with SCORAD index in adults with acute atopic dermatitis

E Vakirlis,^{†,*} E Lazaridou,[†] TG Tzellos,[†] S Gerou,[†] D Chatzidimitriou,[‡] D Ioannides[†]

Departments of [†]Dermatology and Venereology and [‡]Microbiology, School of Medicine, Aristotle University of Thessaloniki, Greece

*Correspondence: E Vakirlis. E-mail: svakirlis@hotmail.com

Abstract

Background Atopic dermatitis (AD) is a chronic inflammatory skin disease with increasing frequency over the last decades, especially in adults. Cytokines orchestrate atopic skin inflammation.

Objectives The aim of this study was to compare serum levels of cytokines in adult patients with acute AD (AD1) with other groups of AD patients and controls and investigate the possible association between such cytokines and disease severity.

Methods We measured cytokine levels using flow cytometry in 21 adult patients with acute AD, 12 adults with chronic AD, 10 children with acute AD and 10 healthy adults.

Results Flow cytometry analysis of cytokines revealed that interleukin 10 (IL-10), IL-6, interferon γ (IFN- γ) and IL-4 levels were significantly decreased in AD1 group compared with controls, whereas IL-2 and tumour necrosis factor (TNF) did not differ. Comparison of AD1 group with adults chronic phase group showed that IgE, eosinophil and IL-2 levels remained unaltered, whereas IL-10, IL-6, IFN- γ , IL-4 and TNF were significantly decreased. SCORAD and IgE levels were significantly increased, IL-10, IL-6 and IFN- γ were decreased and TNF, IL-2, IL-4 and eosinophil levels remained unchanged in AD1 group compared with children acute phase group. Within AD1 group correlation analysis revealed that IgE and TNF levels were significantly associated with AD severity. Coefficient of determination analysis revealed that TNF and IgE levels could explain 49.14% and 35.28% of the variance of SCORAD.

Conclusions These data indicate that serum IgE and TNF levels correlate with AD severity and that serum cytokines are downregulated in AD1 group. Further studies are clearly needed to elucidate cytokines' role in adults with AD.

Received: 6 February 2010; Accepted: 22 June 2010

Keywords

atopic dermatitis, cytokines, SCORAD index, TNF

Conflict of interest

The authors state no conflict of interest and no funding.

Introduction

Atopic dermatitis (AD) is a chronic, relapsing, pruritic, inflammatory skin disease with complex genetic and immunological background.¹ It is a superficial skin inflammation which can seriously affect health status of infants, children and adults.² Frequency of AD has been increasing gradually over the last decades, especially in older people.^{3–5} Adult AD has been relatively ignored in epidemiological research.⁶ However, in developed countries adults make up approximately 80% of the population, meaning that up to a third of all subjects with the disease are adults. Lifetime prevalence of AD has been reported to be 10–20% in children and 1–3% in adults worldwide. In developed

countries it has increased two- to three-fold over the past three decades and there is evidence suggesting that it continues to increase.^{7,8}

Pathogenesis of AD remains to be elucidated and includes disturbed skin barrier function, frequent allergic responses against allergens, defects in the antimicrobial immune defence and genetic predisposition.^{2,8} Recent studies provide insights into the underlying immunological mechanisms, suggesting that chemokines and cytokines in peripheral blood and skin orchestrate atopic skin inflammation.⁹

In this study, we have tried to elucidate the role of the complex interdependent network of the rapidly growing protein

superfamilies of cytokines in the peripheral blood of Greek AD population, especially adults with acute AD, using flow cytometry.

Materials and methods

Forty-three consecutive patients (25 males, 18 females) with AD, diagnosed according to the criteria of Hanifin and Rajka, visiting our Department between January 2009 and June 2009, were recruited for this study and distributed into four (4) groups, according to their age and disease activity. Our target group was adults with acute AD (group 1). Patients' details are shown in Table 1.

Our control group consisted of 10 healthy, age-matched individuals, who did not have any signs or symptoms of AD nor a personal history of other atopic conditions (asthma, rhinitis and conjunctivitis) and did not receive any antihistamines, or systemic and topical corticosteroids.

Individuals were excluded from the study, if they suffered from other inflammatory skin diseases, such as psoriasis, seborrhoeic dermatitis and ichthyosis, from systemic infections, or overt asthmatic symptoms such as cough, wheeze and shortness of breath or exercise-induced bronchospasm. Furthermore, patients were not treated with systemic corticosteroids, ultraviolet radiation (UVR), immunomodulating drugs (cyclosporine and/or topical pimecrolimus or tacrolimus) or oral antihistamines with or without topical corticosteroids, for a period of 4 weeks before entering this study. They were allowed to use a topical corticosteroid or oral antihistamine after the blood samples were collected. Some patients with AD had a personal history of respiratory allergy, which was inactive.

Specimens' collection

Blood samples were collected from all patients during exacerbation of AD, except for the 10 patients who were in the chronic phase. They were kept at room temperature for 30 min till they coagulated, and were centrifuged at 550 g for 10 min. The obtained serums were preserved at -70 °C and dissolved just before the analysis.

Informed consent was obtained from each patient or their parents. This study was approved by the Institutional Ethical Committee and Declaration of Helsinki protocols were followed.

Laboratory examinations

Full blood and eosinophil counts were measured using a routine haematology analyser. Total Serum IgE levels (ROCHE) were measured according to the manufacturer's protocol, using the analyser ELECSYS 2010 (ROCHE, Tokyo, Japan). Total IgE levels lower than 100 IU/mL for adults, <15 IU/mL for neonates 1–12 months, <60 IU/mL for 1- to 5-year-old children, <90 IU/mL for 6- to 9-year-old children and <200 IU/mL for children between 10 and 15 years old were within normal values.

Determination of specific IgE levels (IMMULITE, DPC-SIE-MENS, Flanders, NJ, USA) was performed for a mix of food allergens (casein, milk, egg yolk, egg white, wheat, fish and tomato) or aeroallergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* and *Parietaria officinalis*). Specific IgE < 0.1 kU/L was considered normal.

Detection of cytokines in the serum of patients and healthy controls was performed according to the manufacturer's instructions. The BD CBA Human Th1/Th2 Cytokine Kit II (Becton Dickinson, San Diego, CA, USA) was used to quantitatively measure interleukin 2 (IL-2), IL-4, IL-6, IL-10, tumour necrosis factor (TNF) and interferon γ (IFN- γ) protein levels, in a single sample.

Assessment of disease severity

Atopic dermatitis severity was assessed by the same dermatologist using the Scoring Atopic Dermatitis (SCORAD) index of the European Task Force on atopic dermatitis.¹⁰ Briefly, it is defined by the following parameters: (i) extent, (ii) intensity of skin lesions and (iii) subjective symptoms such as pruritus and sleep loss. The final score was calculated by using the following equation: $a/5 + 7b/2 + c$. In this study, patients were grouped into low, mild and severe disease, according to SCORAD values, ranging between 0 and 25, 25 and 50, or >50 respectively.¹¹

Table 1 Patients' characteristics

Group number	Disease status	Disease activity	Sample size	Age group (years)	Gender	SCORAD*	IgE†	RAST test
1	AD	Acute	21	≥18	15 males 6 females	2 low 13 moderate 6 severe	13 positive 8 negative	10 positive 11 negative
2	AD	Acute	10	0–2	6 males 4 females	2 low 6 moderate 2 severe	4 positive 6 negative	4 positive 6 negative
3	AD	Chronic	12	≥18	4 males 8 females	10 low 2 moderate	9 positive 3 negative	6 positive 6 negative
4	Control	—	10	≥18	6 males 4 females	—	10 negative	10 negative

*SCORAD classification: low = 0–25, mild = 25–50, severe > 50.

†IgE classification: normal limits: <100 IU/mL for adults, <15 IU/mL for neonates 1–12 months, <60 IU/mL for 1- to 5-year-old child, <90 IU/mL for 6 to 9-year-old children, and <200 IU/mL for children between 10 and 15 years old.
AD, atopic dermatitis.

Statistical analysis

Statistical analyses were performed using SPSS 12.0 (Statistical Package for Social Sciences; SPSS Inc., Chicago, IL, USA) for Windows. Normality tests were performed for all dependent variables. For comparison studies the paired Student's *t*-test was used for variables following normal distribution whereas for others Wilcoxon signed ranks test was performed. The relationship between SCORAD and other variables was investigated using Pearson product-moment correlation coefficient (*r*) for variables following normal distribution whereas for others Spearman's rank order correlation (*ρ*) was performed. Preliminary analyses were performed to ensure no violation of the assumptions of linearity and homoscedasticity. All *P*-values were two-tailed, and differences were considered significant when the *P*-value was less than or equal to 0.05. Summary data are expressed as mean ± SEM.

Results

Patients' characteristics are shown in Table 1. All patients had C-reactive protein, antistreptolysin O and erythrocyte sedimentation rate within normal values.

Comparison of adults acute phase group (group 1) with children acute phase (group 2)

Results are shown in Table 2 and Fig. 1. Comparison between adults acute phase group and children acute phase group revealed that SCORAD and IgE levels were significantly increased in adults acute phase group (*P*-values 0.005 and 0.003 respectively). Eosinophil levels were not altered (*P*-value 0.59). Flow cytometry analysis of cytokines revealed that IL-10, IL-6 and IFN- γ were decreased in adults acute phase group (*P* values 0.01, 0.005 and 0.0001 respectively), whereas TNF, IL-2 and IL-4 remained unaltered (*P*-values 0.098, 0.88 and 0.079 respectively).

Comparison of adults acute phase group with adults chronic phase group

Results are shown in Table 2 and Fig. 2. The two groups did not differ significantly in age (*P*-value 0.123). Comparison between the two groups revealed that SCORAD was significantly increased, whereas IgE and eosinophil levels remained unaltered in adults acute phase group (*P*-values 0.0001, 0.963 and 0.254 respectively). Flow cytometry analysis of cytokines revealed that IL-10, IL-6,

Table 2 Patients' descriptives

Group number	Age* (years)	SCORAD	IgE	Eosinophils	IFN- γ	TNF	IL-10	IL-6	IL-2	IL-4
1	35.6 ± 5.7	47.3 ± 3.5	176.3 ± 48.5	0.51 ± 0.11	2.11 ± 0.1	3.47 ± 0.42	3.77 ± 0.2	2.91 ± 0.2	5.82 ± 1.7	2.47 ± 0.2
2	0.71 ± 0.2	31.1 ± 2.6	30.7 ± 10.37	1.17 ± 0.48	3.98 ± 0.5	3.73 ± 0.35	5.32 ± 0.5	4.65 ± 0.5	2.76 ± 0.2	2.91 ± 0.03
3	30.7 ± 2.2	19.7 ± 1.8	153 ± 15.7	0.22 ± 0.006	13.63 ± 4	11.63 ± 4.9	6.8 ± 0.45	10.61 ± 3	2.65 ± 0.2	3.33 ± 0.1
4	31 ± 1.68	—	80.39 ± 3.29	0.21 ± 0.01	4.86 ± 0.52	3.7 ± 0.42	5.56 ± 0.2	4.8 ± 0.41	3.32 ± 0.5	3.3 ± 0.24

*Mean ± SEM.

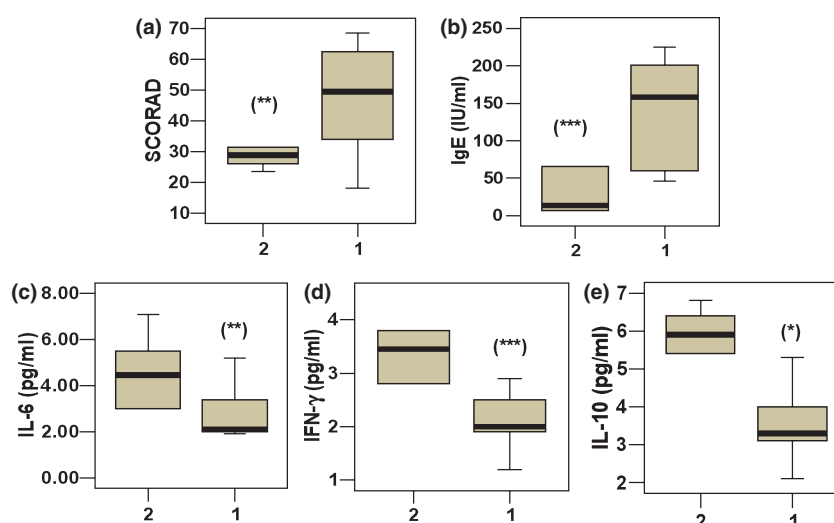


Figure 1 Comparison of adults acute phase group with children acute phase group. Comparison between adults acute phase group and children acute phase group revealed that (a) SCORAD and (b) IgE levels were significantly increased in adults acute phase group. Flow cytometry analysis of cytokines revealed that (c) IL-6, (d) IFN- γ and (e) IL-10 were decreased in adults acute phase group. The length of the box contains 50% of cases. The line across the inside of the box represents the median value. Lines protruding from the box go out to the variable's smallest and largest values. **P*-value between 0.05 and 0.01, ***P*-value between 0.01 and 0.005, ****P*-value >0.005. 1, adults acute phase group; 2, children acute phase group.

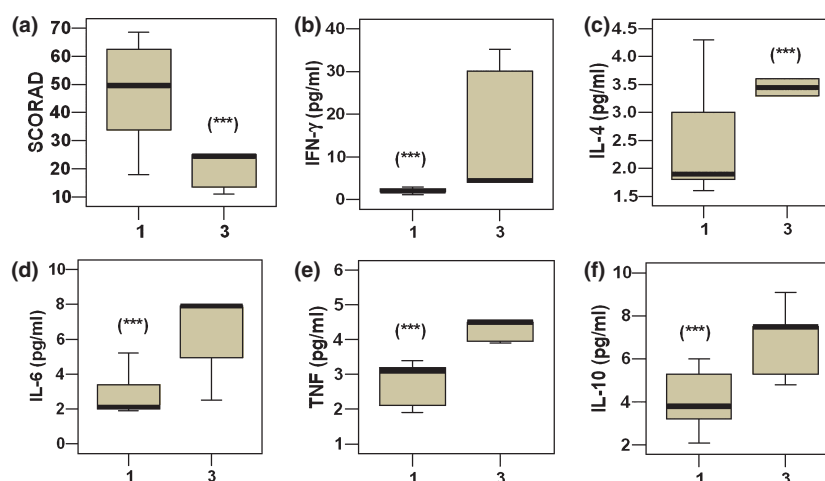


Figure 2 Comparison of adults acute phase group with adults chronic phase group. Comparison between the two groups revealed that (a) SCORAD was significantly increased. Flow cytometry analysis of cytokines revealed that (b) IFN- γ , (c) IL-4, (d) IL-6, (e) TNF and (f) IL-10 were significantly decreased in adults acute phase group. The length of the box contains 50% of cases. The line across the inside of the box represents the median value. Lines protruding from the box go out to the variable's smallest and largest values. (***) = P -value >0.005 . 1, adults acute phase group; 3, adults chronic phase group.

IFN- γ , IL-4 and TNF were significantly decreased in adults acute phase group (all P values <0.0001), whereas IL-2 remained unaltered (P -value 0.816).

Comparison of adults acute phase group with control group

Results are shown in Table 2 and Fig. 3. The two groups did not differ significantly in age (P -value 0.611). Comparison between the two groups revealed that IgE and eosinophils levels, although highly increased, did not differ significantly in adults acute phase group (P -values 0.138 and 0.116 respectively). Flow cytometry analysis of cytokines revealed that IL-10, IL-6, IFN- γ and IL-4 were significantly decreased in adults acute phase group (P -values 0.0001, 0.0001, 0.002 and 0.024 respectively), whereas IL-2 and TNF remained unaltered (P -values 0.966 and 0.252 respectively).

Correlation study revealed an association of TNF and IgE levels with SCORAD within adults acute phase group

Results are shown in Table 3. Correlation study within adults acute phase group revealed that TNF and IgE levels were significantly correlated with SCORAD (correlation coefficient 0.701 and 0.594 respectively). For the other variables a non-significant correlation was found. Coefficient of determination analysis revealed that TNF and IgE levels could explain 49.14% and 35.28% of the variance in SCORAD score.

Comparison between moderate and severe SCORAD severity within adults acute phase group

Results are shown in Table 4 and Fig. 4. The two groups did not differ significantly in age (P -value 0.930). Comparison between

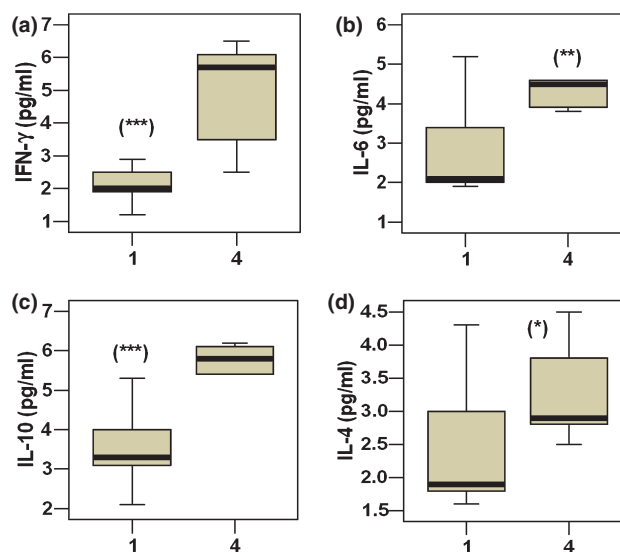


Figure 3 Comparison of adults acute phase group with control group. Flow cytometry analysis of cytokines revealed that (a) IFN- γ , (b) IL-6, (c) IL-10 and (d) IL-4 were significantly decreased in adults acute phase group. The length of the box contains 50% of cases. The line across the inside of the box represents the median value. Lines protruding from the box go out to the variable's smallest and largest values. * P -value between 0.05 and 0.01, ** P -value between 0.01 and 0.005, *** P -value >0.005 . 1, adults acute phase group; 4, control group.

the two groups revealed that IgE and eosinophil levels remained unaltered (P -values 0.331 and 0.093 respectively). Flow cytometry analysis of cytokines revealed that IL-6 and TNF were significantly

Table 3 Correlations and coefficient of determination between SCORAD and age, IgE, eosinophils, IFN- γ , TNF, IL-6, IL-10, IL-2 and IL-4

	Age	IgE	Eosinophils	IFN- γ	TNF	IL-6	IL-10	IL-2	IL-4
SCORAD (<i>n</i> = 21)									
CC	0.269	0.594*	0.394	-0.009	0.701*	0.385	0.039	0.132	0.224
COD (%)	7.23	35.28	15.52	0	49.14	14.82	0.15	1.74	5.01
Sig (2-tailed)	0.238	0.005	0.077	0.970	0.000	0.085	0.865	0.569	0.330

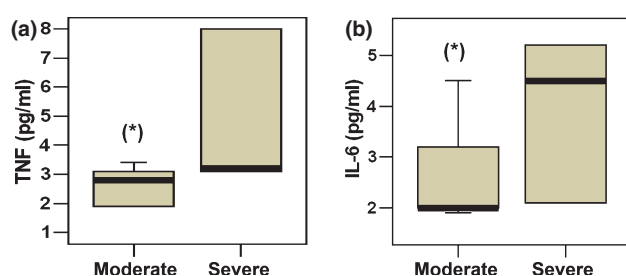
*Strong correlation.

CC, correlation coefficient; COD, coefficient of determination; Sig, significance.

Table 4 Patients' characteristics in moderate and severe SCORAD severity scale within adults acute phase group

SCORAD classification*	Age† (years)	IgE	Eosinophils	IFN- γ	TNF	IL-10	IL-6	IL-2	IL-4
Moderate	38.23 $\pm$ 7.7	203.62 $\pm$ 69.4	0.38 $\pm$ 0.1	2.26 $\pm$ 0.1	3.08 $\pm$ 0.4	6.06 $\pm$ 2.8	2.57 $\pm$ 0.2	4.36 $\pm$ 1.7	2.34 $\pm$ 0.2
Severe	38.66 $\pm$ 10.1	435.66 $\pm$ 179.4	0.93 $\pm$ 0.2	1.86 $\pm$ 0.2	4.76 $\pm$ 1.0	16.16 $\pm$ 7.5	3.93 $\pm$ 0.5	10.26 $\pm$ 4.6	2.93 $\pm$ 0.4

*SCORAD classification: low = 0–25, mild = 25–50, severe > 50.

†Mean $\pm$ SEM.**Figure 4** Comparison between moderate and severe SCORAD severity within adults acute phase group. Flow cytometry analysis of cytokines revealed that (a) TNF and (b) IL-6 were significantly increased in severe SCORAD group compared with moderate SCORAD severity group. The length of the box contains 50% of cases. The line across the inside of the box represents the median value. Lines protruding from the box go out to the variable's smallest and largest values. **P*-value between 0.05 and 0.01.

increased in severe SCORAD group compared with moderate SCORAD severity group (*P*-values 0.04 and 0.017 respectively), whereas IL-2, IL-4, IL-10 and IFN- γ remained unaltered.

Discussion

In this study, we used a method based on unseparated whole blood and investigated serum levels of several cytokines of both Th1 and Th2 type (IL-2, IL-4, IL-6, IL-10, IFN- γ and TNF) in adult patients with AD, to assess the possible relationship between such cytokines and disease severity (SCORAD index) and clarify the discrepancy between results from the previous studies. Cytokine concentrations in the serum are often too low to be measured using enzyme-linked immunosorbent assay (ELISA) and reported values obtained using ELISA vary considerably.^{12–14} The technique of multiparametric flow cytometry enables cytokine production to

be studied at the single-cell level and has the advantage to rapidly measure the cytokine production of thousands of individual cells and detect simultaneously more than one cytokine in the same cell.^{15–17} Moreover, flow cytometry provides several advantages when compared with conventional ELISA methodology, as: (i) the required sample volume is approximately one-sixth the quantity necessary for conventional ELISA assays because of the detection of six analytes in a single sample, (ii) single set of diluted standards is used to generate a standard curve for each analyte and (iii) the experiment takes less time than a single ELISA and provides results that would normally require six conventional ELISAs. A drawback of our study is the relatively small sample size. However, the previous studies demonstrating significant results included smaller sample sizes.^{14,15}

In this study, we investigated the concentration of cytokines in serum of adults with acute AD compared with: (i) children with acute AD, (ii) adults with chronic AD and (iii) healthy non-atopic adults. For the adult groups studied, age did not differ significantly. Most patients of our study suffered from mild or moderate AD. Sixty-five percent of total number of patients presented with pathological value of total IgE and 50% had at least one positive result in certain allergens in RAST test. These results are in agreement with the previous studies.^{18,19}

Our results show that concentration of TNF is increased in patients with chronic disease, in which the Th1 type predominates. The mean concentration of TNF is also slightly higher in healthy controls than in adult patients in acute phase, without reaching statistical significance.

An important finding of our study is that in group 1 patients' concentration of TNF is higher (i) in patients suffering from severe AD when compared with those with moderate and (ii) in patients with increased IgE compared with those with normal values of IgE.

Moreover, in our research it seems that severity index (SCORAD) has strong positive correlation with TNF ($P = 0.0001$) and IgE ($P = 0.005$) in group 1. However, it appears that the severity of disease depends more on TNF (29.92%) than on eosinophils (22%) and IgE (21.25%). This evidence provides a crude indication of a relationship between severity of AD and TNF. TNF is distinguished in TNF- α and in TNF- β . It has been demonstrated that TNF- α and TNF- β are downregulated along with IFN- γ in *in vitro* studies of human peripheral blood mononuclear cells (PBMC).^{20,21} These findings support the hypothesis that Th1 cytokines are downregulated in AD. In other studies, on the contrary, serum concentration of TNF- α in patients with AD is increased and has a positive correlation with the severity of disease.²² Another study indicated that TNF- α was found to be increased in AD and the main inducer of chemokine IP-10 from fibroblasts (and not IFN- γ or the IL-4), which is particularly increased in AD.^{23,24}

It is well known that AD is related to increased production of IgE. Normal serum IgE levels do not necessarily mean that atopy does not exist. The production of IgE from B cells depends on cytokines produced by T cell and is mainly correlated with IL-4. On the contrary, IFN- γ inhibits the synthesis of IgE. Our results show that IgE has a strong correlation with TNF ($P = 0.0001$), IL-4 ($P = 0.003$), IL-6 ($P = 0.004$), and is also correlated with eosinophils ($P = 0.005$) and SCORAD index ($P = 0.005$). We found increase in eosinophils and total IgE levels in peripheral blood from patients with acute lesions, indicating that this stage probably reflects a Th2 response.¹⁵ These results are in line with almost all of the literature.^{25,26} Källström *et al.*, Laske with Niggemann and other researchers lead to the conclusion that IgE is related to the severity of eczema.^{27,28} One recent study showed that 66% of children with mild to moderate AD did not have measurable allergen specific IgE in their serum, but total serum IgE levels were significantly associated with the severity of dermatitis.¹⁹ By contrast, there are reports which could not demonstrate a significant correlation between SCORAD and IgE levels.²⁹ This correlation depends also on other factors, such as gender, age, environmental exposure to allergens and positive familial background for atopic diseases or the coexistence of other allergic illnesses, e.g. asthma (a reason for which a lot of patients were excluded from our study).

Another important result of our study is that SCORAD index and IgE levels increase with age. The most likely explanation for this result is that babies and small children with mild and moderate AD are cured fast and their illness does not last for a long time, while the symptoms and the SCORAD index of patients with serious AD last more. The increasing levels of IgE can be interpreted from the increasing severity, as the greater damage of the skin can easily lead to an increased danger of transepidermal sensitization from more allergens.

We observed that concentration of IFN- γ was higher in group 2 (infants with acute AD) compared with group 1 (adults with acute AD). IFN- γ was decreased along with AD patients' age. This

conclusion is in line with Machura *et al.* and Kimura *et al.*, who found that although the amount of IFN- γ production in children with AD was lower than the one found in non-atopic children, it was higher in infants with AD than in non-atopic infants.^{14,30} It has also been documented that cytokine production seems to be related to age. It has to be stressed out that there are studies that could not detect IFN- γ with ELISA.^{31,32}

The results presented in our study differ from other studies, which demonstrate increased IL-4 production and decreased IFN- γ by PBMC after *in vitro* polyclonal stimulation in AD patients compared with non-atopic subjects.¹⁵ Lonati *et al.*, using RT-PCR (reverse transcriptase polymerase chain reaction), showed spontaneous mRNA expression of IL-4 and lower mRNA levels of IFN- γ in PBMC from AD patients compared with non-atopic subjects.³³ In another study, mRNA levels of IL-13 and IL-5 but not IL-4 in PBMC from AD patients were significantly higher compared with controls.³⁴ By contrast, Källström *et al.* showed decreased frequency of intracellular IFN- γ - and IL-4-producing T cells in whole blood preparations from adult patients with AD.²⁸ Moreover, no significant differences in IL-4 and IFN- γ production were shown, but lower IL-2 and TNF- α by PBMC between AD and non-AD individuals were demonstrated.²¹ Van der Velden *et al.* and Borres *et al.* also reported increased IL-4 levels at 6 months of age which then subsequently decreased.^{35,36}

The discrepancy between our findings and other studies may be the result of the heterogeneous character of the investigated patient group and differences in technique used. To our knowledge, this is the first report in Greek population. It is well known that environmental factors play a role in the development of AD. Probably, the Mediterranean nutrition plays also an important role in prevention of AD, as in Greece its prevalence is less than 5%. The small number of subjects may also be a drawback of this study; nevertheless, some differences among the particular subgroups were observed.

Atopic dermatitis is a multifactorial disease, and hereditary, environmental and immunological factors are all involved in its pathogenesis. Genetic background has been clearly implicated in AD.³⁷ It is well documented that genetic abnormalities in cytokines play an important role in immune responses and have been implicated in the pathogenesis of AD.^{2,37} Such abnormalities and polymorphisms in the genes of TNF- α , IL-4, IL-4R, IL-12R, IL-6 and IL-10 have been previously published.^{37,38} However, although these polymorphisms may act as specific markers of AD and have been associated with symptom of atopy, no confirmatory evidence exists proving a cause-effect relationship between a specific locus and the occurrence of AD phenotype or AD severity.³⁷⁻³⁹ The findings of our study suggest a significant role for TNF, a possible therapeutic target, in the pathogenetic mechanism of the disease and also stress the need for further investigation of the cytokine profile in AD. We failed to demonstrate a role of IL-4 in patients with AD, but we showed significantly increased total IgE levels and number of circulating eosinophils that may be used along

with TNF as important markers in the assessment of disease severity and follow-up of patients with AD. Another possible marker of AD severity could be IL-31, as it has been correlated with SCORAD index in adult AD patients.⁴⁰ Moreover, IgE and serum IL-12 levels have been correlated with AD severity in children.^{41,42} On the contrary, IL-16 serum levels were significantly higher in children with AD, but no correlation was found between IL-16 and disease severity.⁴³

Over the past few years, it has become clear that Th1/Th2 imbalance is insufficient to explain many experimental observations in allergic diseases and other subset of T cells might play a critical role in controlling the expression of these diseases. On the basis of these results, we conclude that Th2 predominance is not a general characteristic of T cells among our patients with AD. Further studies are clearly needed to give deeper insight into the significant role of cytokines in AD.

References

- Bussmann C, Maintz L, Hart J *et al.* Clinical improvement and immunological changes in atopic dermatitis patients undergoing subcutaneous immunotherapy with a house dust mite allergoid: a pilot study. *Clin Exp Allergy* 2007; **37**: 1277–1285.
- Novak N. New insights into the mechanism and management of allergic diseases: atopic dermatitis. *Allergy* 2009; **64**: 265–275.
- Nishioka K. Atopic eczema of the adult type in Japan. *Australas J Dermatol* 1996; **37**: 7–9.
- Vartiainen E, Petäys T, Haahtela T *et al.* Allergic diseases, skin prick test responses, and IgE levels in North Karelia, Finland, and the Republic of Karelia, Russia. *J Allergy Clin Immunol* 2002; **109**: 643–648.
- Pónyai G, Hidvégi B, Németh I *et al.* Contact and aeroallergens in adulthood atopic dermatitis. *J Eur Acad Dermatol Venereol* 2008; **22**: 1346–1355.
- Herd RM, Tidman MJ, Prescott RJ, Hunter JAA. Prevalence of atopic eczema in the community: the Lothian atopic dermatitis study. *Br J Dermatol* 1996; **135**: 18–19.
- Leung DYM, Boguniewicz M, Howell MD *et al.* New insights into atopic dermatitis. *J Clin Invest* 2004; **113**: 651–657.
- Leung DYM, Bieber T. Atopic dermatitis. *Lancet* 2003; **361**: 151–160.
- Homey B, Steinhoff M, Ruzicka T, Leung DYM. Cytokines and chemokines orchestrate atopic skin inflammation. *J Allergy Clin Immunol* 2006; **118**: 178–189.
- European Task Force on Atopic dermatitis. Severity scoring of atopic dermatitis: the SCORAD index. Consensus Report of the European Task Force on atopic dermatitis. *Dermatology* 1993; **186**: 23–31.
- Bordignon V, Sinagra JL, Trento E *et al.* Antigen specific cytokine response in pediatric patients with atopic dermatitis. *Pediatr Allergy Immunol* 2005; **16**: 113–120.
- Matumoto T, Miike T, Yamaguchi K *et al.* Serum levels of soluble IL-2 receptor, IL-4 and IgE-binding factors in childhood allergic diseases. *Clin Exp Immunol* 1991; **85**: 288–292.
- Hashimoto S, Amemiya E, Tomita Y *et al.* Elevation of soluble IL-2 receptor and IL-4, and non elevation of IFN- γ in sera from patients with allergic asthma. *Ann Allergy* 1993; **71**: 455–458.
- Machura E, Mazur B, Kwiecień J, Karczewska K. Intracellular production of IL-2, IL-4, IFN- γ , and TNF- α by peripheral blood CD3 + and CD4 + T cells in children with atopic dermatitis. *Eur J Pediatr* 2007; **166**: 789–795.
- Farrell AM, Antrobus P, Simpson D *et al.* A rapid flow cytometric assay to detect CD41 and CD81 T-helper (Th) 0, Th1 and Th2 cells in whole blood and its application to study cytokine levels in atopic dermatitis before and after cyclosporin therapy. *Br J Dermatol* 2001; **144**: 24–33.
- Jung T, Schauer U, Heusser C *et al.* Detection of intracellular cytokines by flow cytometry. *J Immunol Methods* 1993; **159**: 197–207.
- Prussin C. Cytokine flow cytometry: understanding cytokine biology at the single-cell level. *J Clin Immunol* 1997; **17**: 195–204.
- Akdis CA, Akdis M, Bieber T *et al.* Diagnosis and treatment of atopic dermatitis in children and adults: European Academy of Allergology and Clinical Immunology/American Academy of Allergy, Asthma and Immunology/PRACTALL Consensus Report. *Allergy* 2006; **61**: 969–987.
- Flohr C, Johansson SG, Wahlgren CF, Williams H. How atopic is atopic dermatitis? *J Allergy Clin Immunol* 2004; **114**: 150–158.
- Jirapongsanuruk O, Donahue H, Trumble A, Leung D. The modulation of cytokine and IgE production by tumor necrosis Factor- β in atopic dermatitis. *J Invest Dermatol* 2000; **114**: 200–203.
- Takahashi T, Sasaki Y, Hama K *et al.* Production of IL-4, IL-2, IFN- γ , and TNF- α by peripheral blood mononuclear cells of patients with atopic dermatitis. *J Dermatol Sci* 1992; **3**: 172–180.
- Sumimoto S, Kawai M, Kasajima Y, Hamamoto T. Increased plasma tumour necrosis factor- α concentration in atopic dermatitis. *Arch Dis Child* 1992; **67**: 277–279.
- Villagomez MT, Bae SJ, Ogawa I *et al.* Tumour necrosis factor- α but not interferon- γ is the main inducer of inducible protein-10 in skin fibroblasts from patients with atopic dermatitis. *Br J Dermatol* 2004; **150**: 910–916.
- Ackermann L, Harvima IT. Mast cells of psoriatic and atopic dermatitis skin are positive for TNF- α and their degranulation is associated with expression of ICAM-1 in the epidermis. *Arch Dermatol Res* 1998; **290**: 353–359.
- Antunez C, Torres MJ, Mayorga C *et al.* Different cytokine production and activation marker profiles in circulating cutaneous-lymphocyte-associated antigen +T cells from patients with acute or chronic atopic dermatitis. *Clin Exp Allergy* 2004; **34**: 559–566.
- Aral M, Arican O, Gul M *et al.* The relationship between serum levels of total IgE, IL-18, IL-12, IFN- γ and disease severity in children with atopic dermatitis. *Mediators Inflamm* 2006; **2006**: 73098.
- Laske N, Niggemann B. Does the severity of atopic dermatitis correlate with serum IgE levels? *Pediatr Allergy Immunol* 2004; **15**: 86–88.
- Källström E, Roscher I, Andreasson A *et al.* Decreased frequency of intracellular IFN- γ producing T cells in whole blood preparations from patients with atopic dermatitis. *Exp Dermatol* 2002; **11**: 556–563.
- Kaminishi K, Soma Y, Kawa Y, Mizoguchi M. Flow cytometric analysis of IL-4, IL-13 and IFN- γ expression in peripheral blood mononuclear cells and detection of circulating IL-13 in patients with atopic dermatitis provide evidence for the involvement of type 2 cytokines in the disease. *J Dermatol Sci* 2002; **29**: 19–25.
- Kimura M, Tsuruta S, Yoshida T. Differences in cytokine production by peripheral blood mononuclear cells (PBMC) between patients with atopic dermatitis and bronchial asthma. *Clin Exp Immunol* 1999; **118**: 192–196.
- Kimura M, Yamaide A, Tsuruta S *et al.* Development of the capacity of peripheral blood mononuclear cells to produce IL-4, IL-5 and IFN- γ upon stimulation with house dust mite in children with atopic dermatitis. *Int Arch Allergy Immunol* 2002; **127**: 191–197.
- Yoshizawa Y, Nomaguchi H, Izaki S, Kitamura K. Serum cytokine levels in atopic dermatitis. *Clin Exp Dermatol* 2002; **27**: 225–229.
- Lonati A, Licenziati S, Canaris AD *et al.* Reduced of both Th1 and Tc1 lymphocyte subsets in atopic dermatitis (AD). *Clin Exp Immunol* 1999; **115**: 1–5.
- Akdis CA, Akdis M, Simon HU, Blaser K. Regulation of allergic inflammation by skin-homing T cells in allergic eczema. *Int Arch Allergy Immunol* 1999; **118**: 140–144.
- van der Velden VH, Laan MP, Baert MR *et al.* Selective development of a strong Th2 cytokine profile in high-risk children who develop atopy:

- risk factors and regulatory role of IFN-gamma, IL-4 and IL-10. *Clin Exp Allergy* 2001; **31**: 997–1006.
- 36 Borres MP, Björkstén B. Peripheral blood eosinophils and IL-4 in infancy in relation to the appearance of allergic disease during the first 6 years of life. *Pediatr Allergy Immunol* 2004; **15**: 216–220.
- 37 Mizutani H. Cytokines in atopic dermatitis (Eczema). In Ring J, Przybilla B, Ruzicka T, eds. *Handbook of Atopic Eczema*, 2nd edn. Springer, Berlin Heidelberg, 2006: 350–354.
- 38 Reich K, Westphal G, König IR *et al.* Cytokine gene polymorphisms in atopic dermatitis. *Br J Dermatol* 2003; **148**: 1237–1241.
- 39 Darsow U, Wollenberg A, Simon D *et al.* ETFAD/EADV eczema task force 2009 position paper on diagnosis and treatment of atopic dermatitis. *J Eur Acad Dermatol Venereol* 2010; **24**: 317–328.
- 40 Raap U, Wichmann K, Bruder M *et al.* Correlation of IL-31 serum levels with severity of atopic dermatitis. *J Allergy Clin Immunol* 2008; **122**: 421–423.
- 41 Hon KL, Lam MC, Leung TF *et al.* Are age-specific high serum IgE levels associated with worse symptomatology in children with atopic dermatitis? *Int J Dermatol* 2007; **46**: 1258–1262.
- 42 Piancatelli D, Bellotta L, Del Beato T *et al.* Total IL-12 levels are increased in paediatric atopic dermatitis: correlations with age and disease severity. *Int J Immunopathol Pharmacol* 2008; **21**: 359–365.
- 43 Belloni Fortina A, Tonin E, Pigozzi B *et al.* IL-16 serum level in children with atopic dermatitis. *Int J Immunopathol Pharmacol* 2006; **19**: 841–845.