

Prominent Involvement of Activated Th1-Subset of T-Cells and Increased Expression of Receptor for IFN-Gamma on Keratinocytes in Atopic Dermatitis Acute Skin Lesions

Liborija Lugović^a Jasna Lipozenčić^b Jasminka Jakić-Razumović^c

Departments of ^aDermatology and Venereology, University Hospital 'Sestre milosrdnice', ^bDermatology and Venereology, and ^cPathology, Zagreb University Hospital Center, Zagreb, Croatia

Key Words

Atopic dermatitis · Interferon-gamma · Th1 cells

Abstract

Background: Atopic dermatitis (AD) is an allergic skin disease mediated by antigen-specific IgE and an important role has been ascribed to CD4⁺ cells (Th cells). The objective of the study was to evaluate humoral and cellular immunological factors in the blood and the skin lesions of AD patients, and to analyze the presence of inflammatory cell-surface markers in blood and skin biopsies. **Methods:** The parameters for monitoring of 40 AD patients included results of prick test to inhalant allergens and epicutaneous (patch) test to contact allergens; values of total IgE, serum immunoglobulins (IgG, IgA, IgM) and different cell markers in the sera (CD3, CD4, CD8, CD20, CD21, CD23, HLA-DR). We also analyzed the presence of inflammatory cell-surface markers (CD3, CD4, CD8, CD20, CD20, CD1a, CD23, CD29, CD45Ro, IFN γ + markers) in the biopsies of skin lesions from 10 AD patients and 5 healthy controls (HCs) by immunohistochemical analysis (method of avidin-biotin immunoperoxidase). **Results:** Beside increased total serum IgE and

positive skin tests, a significantly higher percentage of CD23⁺ cells with lower percentage of CD21⁺ cells was revealed in peripheral blood of AD patients in comparison to HCs. A positive epidermal expression of the majority of markers of T cells (CD3⁺, CD4⁺, CD8⁺, CD29⁺, CD45Ro⁺, IFN γ +) and those of Langerhans' cells (LCs) (CD1a, CD23⁺), without those of B cells (CD20⁺) were noted in AD patients, but no in the skin of HCs. Furthermore, significant difference was also found between the two groups for increased expression of CD3, CD4, CD8, CD29, CD45Ro, IFN γ + markers (markers for IFN γ receptor) and higher intraepidermal CD23⁺ LCs and intradermal CD1a⁺ LCs in AD skin lesions. **Conclusions:** The obtained results suggest involvement of various humoral factors with increased production of IgE and cooperation between Th subsets and LCs, with higher production of related cytokines, and disturbed cellular immunity, including epidermal LCs with IgE receptors of high and low affinity in AD. The annotation of activated Th1 cells with increased producing of IFN γ in acute AD skin lesions is notable, and might lead to IFN γ binding to keratinocytes and consequently inflammatory skin changes in the disease.

Copyright © 2005 S. Karger AG, Basel

Introduction

Atopic dermatitis (AD) is an allergic skin disorder that occurs in individuals with atopy, and is characterized by extreme itching, erythema, excoriations, and lichenifications at predilection sites. Although the pathogenesis of AD has not yet been fully elucidated, immune disorders of humoral (type I) and cellular (type IV) immunity have been implicated [1–4]. There is an increasing evidence that the activation of T-helper cells (Th2 cells producing IL-4, IL-5 and IL-6, IL-9, IL-10, IL-13, IL-25, or Th1-cells producing IL-2, interferon- γ (IFN γ), lymphotoxin), repeated release of mediators and cytokines from skin mastocytes, allergen-induced activation of Langerhans' cells (LCs) and lymphocytes may be involved in the pathogenesis of AD and lead to chronic inflammatory alterations in the skin of AD patients [1–4]. Disturbed humoral immunity and relative imbalance between Th1- and Th2-cells with overproduction of IL-4 and disturbed cellular immunity including epidermal LCs with IgE receptors of high-affinity Fc ϵ RI are prominent findings in AD [1–5]. The low-affinity receptor Fc ϵ RII (CD23) is expressed on LCs, human B cells, monocytes, follicular dendritic cells, is important for regulation of IgE synthesis and triggers the release of tumor necrosis factor (TNF), IL-1, IL-6, and granulocyte-macrophage colony-stimulating factor (GM-CSF) from human monocytes. It has been recently revealed that the influx of CD4 $^{+}$ lymphocytes has been related to the upregulation of IL-16 in AD skin lesions, and circulating β -chemokines (Eotaxin and Rantes) and IL-16 correlate with severity of the disease in the children with AD [5].

Besides the history of atopy and characteristic clinical picture, laboratory tests are also useful in setting the diagnosis of AD, although there is no reliable laboratory test or lesion pathognomonic for AD. Many observations suggest that environmental allergens play an important role in the disease onset and exacerbation, which is demonstrated by in vivo (skin tests) and in vitro (e.g. determination of total and specific IgE antibodies) testing [5, 6].

Skin tests and determination of increased IgE values are common diagnostic parameters, but immunophenotyping of cell markers on T-cells (e.g. CD3, CD4, CD8, HLA-DR), B cells (e.g. CD21 and CD23) and other cells may contribute to the recognition of factors involved in the pathogenesis of AD. The use of immunophenotyping has lately allowed the cells in the sera and cellular infiltrate in AD skin lesions to be analyzed in more detail, thus to get a better insight into the pathogenesis of AD.

The objective of this study was to examine different immunological and allergological parameters in the blood and skin of AD patients, and to define the most important immunological factors involved in the pathogenesis of AD.

Materials and Methods

Subjects

Forty patients with AD (23 females and 17 males, aged 1–68 years) and 20 healthy controls (HCs) (12 females and 8 males, aged 5–63 years) participated in clinical examination. AD patients had typical clinical signs of AD and the diagnosis of AD was made using the criteria by Hanifin and Rajka [7]. None of the patients had received any treatment for at least 1 week before the study.

Biopsy specimens obtained from skin lesion of 10 AD patients (3 females and 7 males with AD, aged 25–50 years) were submitted to immunohistochemical analysis (cell immunophenotyping), and the results were compared with those recorded for the skin of 5 healthy control subjects (4 females and 1 male, aged 25–50 years). Biopsy specimens were obtained from acute-stage skin lesions at typical AD localizations. Normal skin biopsies, without selected pathological changes taken from the routine material examined at the Institute of Pathology, served as controls.

Skin Tests

Skin prick tests to standard series of inhalant allergens were performed using commercially prepared solutions (supplied by the Institute of Immunology, Zagreb). Negative (50% mix of glycerin and physiological saline) reaction and positive (histamine) controls were included. The tests were performed on clinically uninvolved skin on the subjects' forearms. The wheal diameter was measured at 20 min and a diameter of >3 mm was taken as positive. The result was positive in at least one allergen of standard test series [1, 2].

Epicutaneous (patch) test was carried out with allergens of the standard series according to the International Contact Dermatitis Research Group (ICDRG) in adult patients with AD and children over 5 years of age. Positive patch tests included reactions to 1 or more plus (+) on the contact allergens according to ICDRG [1, 2].

Analysis of Peripheral Blood

Values of total IgE were determined by the standardized radioimmunosorbent test (Pharmacia, Uppsala, Sweden) and enzyme-linked immunosorbent assay (ELISA) methods. The upper limits for normal IgE values were 150 IU/ml, whereas in children it was evaluated according to their age. Values of IgG, IgA, and IgM were determined by radial immunodiffusion.

Immunophenotyping of cells in the blood was analyzed by flow cytometer (FACSCalibur, San Jose, Calif., USA), and specific antibodies (Becton Dickinson, Heidelberg, Germany) by three-color immunofluorescence analysis. The following antibodies were used: CD3, CD4, CD8, CD20, CD2, CD23 and HLA-DR [8, 9]. Reference values were the following: CD3 $^{+}$ T cells 59–86%, CD4 $^{+}$ cells 29–65%, CD8 $^{+}$ cells 14–54%, CD20 $^{+}$ cells 2–20%, CD21 $^{+}$ cells ($12.6 \pm 3.8\%$), CD23 $^{+}$ cells (0–8%), HLA-DR $^{+}$ cells (10–32%) [8, 9].

Analysis of Skin Lesions

Immunohistochemical analysis of the skin biopsy specimens was performed by standard avidin-biotin immunoperoxidase method. Half of skin sample was snap frozen in liquid-resistant nitrogen and used for determination of IFN γ + cells (cells with positive markers for IFN γ). The rest of the sample was fixed in formalin, paraffin embedded, at 4 μ m histological sections, and used for determination of CD4, CD1a, CD23, CD29, CD45Ro, and IFN γ + cells, deparaffinized in xylol, and rehydrated in graded alcohol, and then incubated with nonimmune horse serum for 20 min to block the nonspecific antibody binding. Upon incubation, the serum was removed from the slides, and the slides were incubated with primary antibodies for 30 min. The following primary antibodies were used: anti-CD3, -CD4, -CD8, -CD23 (Dako, Glostrup, Denmark), and anti-IFN γ + (Serotec). We analyzed only CD23 markers on LCs with characteristic LC morphology in the skin.

Upon incubation with primary antibody, the sections were washed three times in phosphate-buffered saline (PBS) buffer, and a secondary antibody (biotinylated multi-link swine anti-goat-mouse-rabbit immunoglobulins) was applied. After 30 min, incubation slides were washed in PBS, and incubated with biotin-avidin peroxidase conjugate (ABC/HRP kit; Dako, Copenhagen, Denmark) for 30 min. The preparations were washed in PBS, and the staining immunoperoxidase was visualized with diaminobenzidine (DAB; Dako) in PBS with 0.01% H₂O₂. The slides were counterstained with hematoxylin and embedded in the medium [10–12]. Positive controls were used in each staining.

Interpretation of Results

The markers of T-cells (CD3+, CD4+, CD8+, CD29+, CD45Ro+), B cells (CD20+), and those of Langerhans' cells (CD1a, CD23+) were determined in the dermis and epidermis. Cell counting in the dermis and epidermis was done under a light microscope. Total cell count per mm² dermis area, and total cell count per mm epidermis length were determined. Intensity of IFN γ + staining was determined using semiquantitative method and designated as follows: 0 (no staining), + (some staining), or ++ (abundant staining), and according to involved thickness, and the results were classified as 1 (rare), 2 (some), 3 (many), 4 (abundant).

Statistical Analysis

Tables of mean values, minimum and maximum values were determined for all studied parameters. Statistical analysis was done by comparison of the results obtained in the two groups using Mann-Whitney test. The correlations between total IgE and prick test (number of positive allergens), between values of total IgE and expression of CD4, between total IgE and CD23 in the blood were also examined in each AD patient. Differences were considered significant at $p < 0.05$.

Table 2. IgG, IgA, IgM and IgE values in AD patients

Immunoglobulins	IgG (n = 40)	IgA (n = 40)	IgM (n = 40)	IgE (n = 40)
Normal	39 (97.5%)	39 (97.5%)	40 (100%)	11 (27.5%)
Increased	0	0	0	29 (72.5%)
Decreased	1 (2.5%)	1 (2.5%)	0	0

Results

The study results show that most of AD patients had more positive reactions in prick test (in 27 of 40 (67.5%) cases) (table 1). The most common positive reactions were to house dust, followed by mites and feathers. Twenty-six (65%) of 40 AD patients showed positive patch test reactions with the standard series of contact allergens. The most positive reactions were to nickel (14 patients), cobalt-chloride [13], and bichromate [13].

All HCs Had Negative Skin Test Results

Values of total IgE in AD patients were mostly increased (table 2). Patients with AD showed increased values of IgE in 29/40 (72.5%) cases. Other immunoglobulins (IgG, IgA, IgM) were within normal limits in the majority of AD patients (table 2).

Expression of CD3+, CD4+, CD8+ cells in the blood of AD patients were mostly normal (CD3 in 37/40, CD4 in 35/40, CD8 in 40/40 cases), but increased CD23 on B cells were noted in a quarter of AD patients (10/40) (table 3). Values of CD21 were normal in half (20/40) of the AD patients, but decreased in 18/40 AD patients. HLA-DR expression was normal in almost all patients (38/40). Although mostly normal expression of markers was obtained in the results, it was noticed that AD patients had statistically significantly higher percentage of CD23+ cells and lower percentage of CD21+ cells and CD3+ cells than HCs ($p < 0.05$) (table 3).

Table 1. Skin tests results in AD patients (n = 40) and HCs (n = 20)

		Prick test	Patch test
AD (n = 40)	positive	27 (67.5%)	26 (65.0%)
	negative	13 (32.3%)	14 (35.0%)
HCs (n = 20)	positive	0	0
	negative	20 (100.0%)	20 (100.0%)

Table 3. Determination of cell markers in AD patients' and healthy controls' blood

	AD	Healthy controls	z	p
CD3				
Normal	37	20		
Increased	0	0	-2.152	0.031
Decreased	3	0		
CD4				
Normal	35	20		
Increased	4	0	-0.251	0.802
Decreased	1	0		
CD8				
Normal	40	20		
Increased	0	0	-1.115	0.265
Decreased	0	0		
CD20				
Normal	39	20		
Increased	1	0	-0.283	0.777
Decreased	0	0		
CD21				
Normal	20	20		
Increased	2	0	-3.760	0.000
Decreased	18	0		
CD23				
Normal	30	20		
Increased	10	0	-3.201	0.001
Decreased	0	0		
HLA-DR				
Normal	38	20		
Increased	0	0	-0.393	0.694
Decreased	2	0		

Analysis of CD4/CD8 ratio in the blood of AD patients showed a median of 1.68.

All HCs had normal values of CD3+, CD4+, CD8+, CD20+, CD23+, CD21+, and HLA-DR+ cells in the peripheral blood (table 3).

The results also revealed the correlation between total serum IgE and prick test positive results in AD patients ($p = 0.023$, $\rho = 0.359$), but no correlations between total IgE and serum CD4+ cells ($p = 0.413$), and no correlations between total IgE and serum CD23+ cells ($p = 0.925$) were found.

Results of immunophenotyping (CD3, CD4, CD8, CD20, CD1a, CD23, CD29, CD45Ro and IFN γ + cells) of skin biopsies of AD skin, compared with nonatopic healthy skin, are shown in table 4. Analysis of infiltrating cells in AD patients and HCs showed substantial differences in the infiltrating cell count, especially in the large number of T-cells in skin lesions of AD, which was not observed in HCs (fig. 1). Table 4 shows the number of infiltrating cells with specified cell markers in AD patients and control subjects (for CD3: epidermis 4–32 (median 17.5), dermis 65–258 (median 113); for CD4: epidermis 1–25 (median 6), dermis 15–212 (median 67); for CD8: epidermis 1–16 (median 7.5), dermis 12–168 (median 59.5)). Study results show that the predominant cells in AD skin were CD3+, CD4+ and CD8+, and also a higher expression of CD1a, CD29, CD45Ro, and IFN γ + cells in them (fig. 2). The significant difference was recorded in

Table 4. Determination of cell markers in the skin (e = epidermis, d = dermis) of AD patients and HCs

	AD		HCs		z	p
	median	min–max	median	min–max		
CD3 (e)	17.5	4–32	0	0–1	-3.092	0.002
CD3 (d)	113	65–258	3	2–9	-3.067	0.002
CD4 (e)	6	1–25	0	0–0	-3.130	0.002
CD4 (d)	67	15–212	1	1–2	-3.090	0.002
CD8 (e)	7.5	1–16	0	0–1	-3.033	0.002
CD8 (d)	59.5	12–168	2	1–5	-3.065	0.002
CD20 (e)	0	0–2	0	0–0	-1.035	0.301
CD20 (d)	1.5	0–32	8	4–12	-0.299	0.194
CD1a (e)	31.5	5–102	16	12–21	-0.980	0.327
CD1a (d)	17.5	3–42	4	2–8	-2.513	0.012
CD23 (e)	12	3–68	0	0–0	-3.124	0.002
CD23 (d)	10.5	2–19	5	4–6	-0.867	0.386
CD29 (e)	8	1–18	0	0–0	-3.121	0.002
CD29 (d)	61	21–114	3	1–4	-3.065	0.002
CD45Ro (e)	21	6–41	0	0–0	-3.133	0.002
CD45Ro (d)	147	71–272	5	4–6	-3.067	0.002
IFN γ + cells (e)	3	1–4	0	0–0	-3.208	0.001
IFN γ + cells (d)	1	1–1	0	0–0	-3.742	0.000

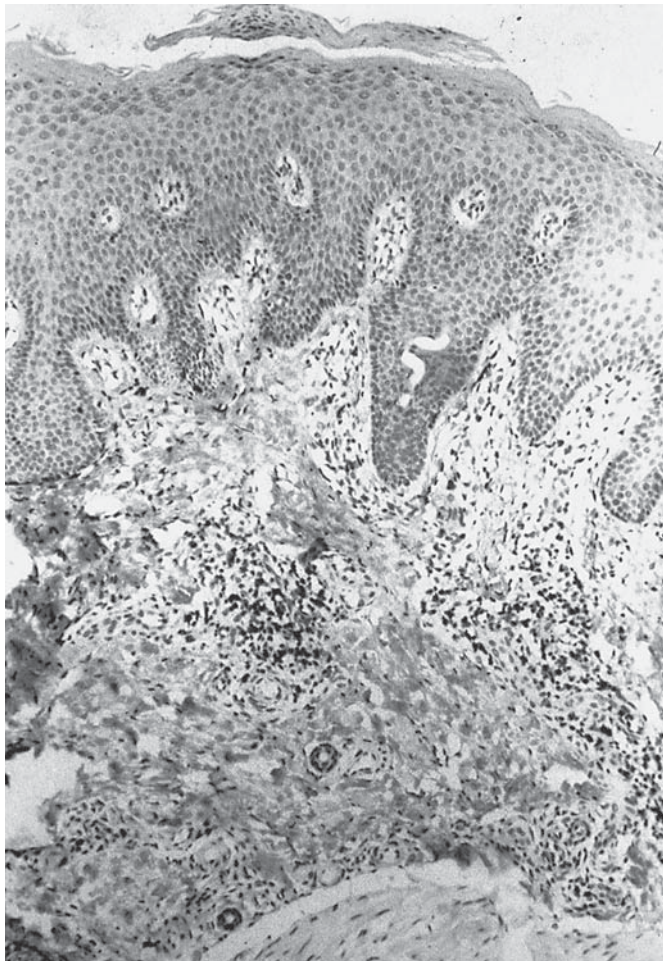


Fig. 1. Skin lesion of AD with numerous T cells in the dermis. Immunohistochemical staining with anti-CD3 antibody (Dako). Counterstaining with hematoxylin. $\times 20$.

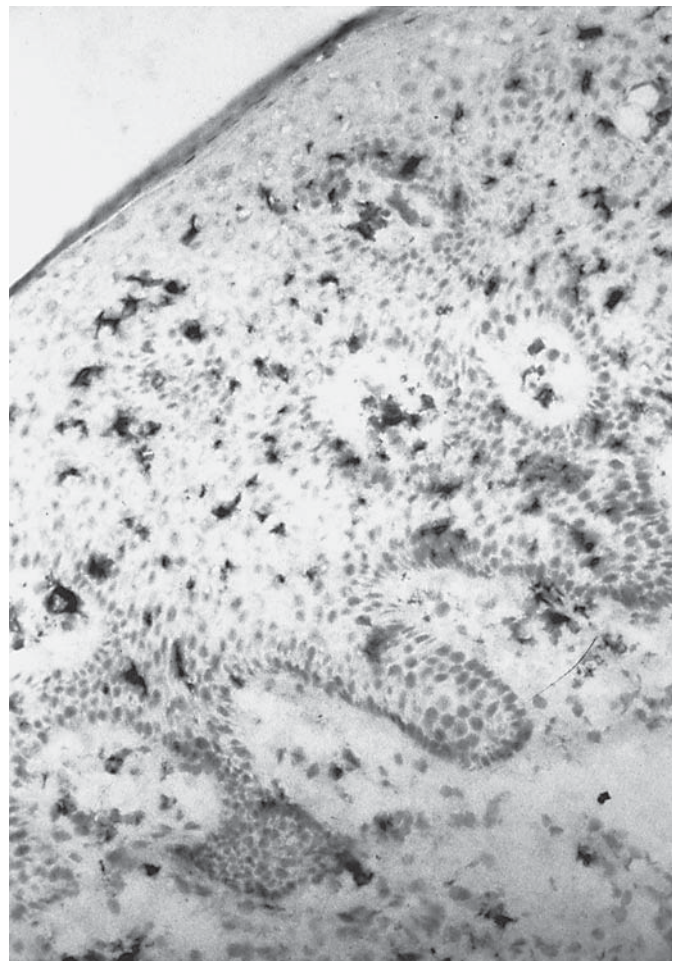


Fig. 2. Skin sample of a patient with AD immunohistochemically stained with anti-CD1a antibody (Dako). There is an increased number of Langerhans' skin cells. Counterstaining with hematoxylin. $\times 40$.

higher skin expression of CD1a+ LCs in the dermis and higher CD23+ LCs count in epidermis of AD patients than in skin of HCs ($p = 0.012$ for dermal CD1, $p = 0.002$ for epidermal CD23).

Comparison of the results obtained in AD patients and HCs yielded a statistically significant difference in a higher number of infiltrating cells expressing CD3, CD4, CD8, CD29, CD45Ro, IFN γ + markers, and increased CD1a+ LCs in the dermis, increased CD23+ LCs in the epidermis in AD patients compared to HCs ($p < 0.05$).

In AD patients, the CD4/CD8 ratio in the epidermis ranged from 0.13 to 4.17 (the mean being 1.33), and CD4/CD8 ratio in the dermis varied from 0.17 to 4.92 (the mean being 1.49), whereas the respective figures in HCs were 0 in the epidermis and ranged from 0.2 to 1 in the

dermis. The AD intralesional infiltrates were observed to contain only a small CD20+ lymphocyte count.

The analysis of AD skin lesions revealed that in epidermis CD1a+, CD45Ro+, CD3+, and CD23+ cells dominated and in dermis CD3+, CD45Ro+, CD4+, and CD8+ cells were mostly found.

On the other hand, in HCs epidermis only CD1a were expressed, and in dermis CD23+, CD45Ro+, and CD1a+ cells were rarely found.

The analysis of AD skin lesions revealed statistically significant correlation between number of CD4+ cells and CD23+ LCs in the epidermis ($p = 0.005$, $\rho = 0.809$) and dermis ($p = 0.036$, $\rho = 0.665$).

A positive epidermal expression of the majority of markers of T-cells (CD3+, CD4+, CD8+, CD29+,

CD45Ro+, IFN γ +) and those of Langerhans' cells (CD1a, CD23+), without those of B-cells (CD20+), was noted in AD patients, but not in the skin of HCs (table 4).

The expression of CD29 and CD45Ro was found in dermis and epidermis of all AD patients, but only in dermis of few HCs. The higher number of CD3+ cells with CD29 and CD45Ro markers was revealed in dermis and epidermis of AD skin lesions, but not in skin of HCs who expressed them only rarely in dermis (table 4).

It was also observed that IFN γ + cells were found in epidermis of all AD patients but only rarely in their dermis, but the expression of IFN γ + cells was not noted in HCs.

The analysis of expression of LCs markers revealed in skin of AD patients statistically significantly higher expression of CD1a in the dermis and significantly higher CD23+ LCs number in the epidermis in comparison with HCs (table 4).

Discussion

There are different attempts to determine the various allergic parameters in AD: by means of skin tests (early hypersensitivity tests), total or specific IgE (determination by RAST or ELISA), specific provocation tests, etc. [1, 2]. The results vary and the percentage of positive skin test reactions ranges from 50 to over 90%. However, the pathogenic role of other environmental inhalant and nutritive allergens is rather difficult to demonstrate because of the ubiquitous occurrence of allergens, and the frequent polyvalent IgE sensitization. In this study, skin tests to standard series of inhalant allergens identified that the most common allergens causing skin allergy were house dust, followed by mites and feather, confirming the role of this environmental allergens in the occurrence of AD. The correlation between total IgE and prick test ($p = 0.023$) in AD patients was found in our study, supporting the role of IgE in AD. However, despite the raised level of serum IgE in the majority of AD patients, it may be within normal limits, even in individual patients with extensive skin lesions, and determination of IgE level is of no pathognomonic significance [2].

Besides early hypersensitivity, delayed hypersensitivity of type IV (cell-mediated immunity) is considered to be involved in the pathogenesis of AD [1, 2]. Therefore, determination of the cell markers on lymphocytes (e.g. CD3, CD4, CD8) could be of importance for the disease. The recent studies have indicated that the receptors for IgE of high affinity (Fc ϵ RI) and low affinity (Fc ϵ RII,

CD23) have an important role in the pathogenesis of AD. In AD patients, the analysis of CD23 receptors on the peripheral blood mononuclear cells (PBMC) showed elevated CD23 expression, and CD21, in a lesser degree, when compared to controls, and positive correlation between CD23 and total serum IgE levels in the tested allergic children, stressing the role of CD21–CD23 ligand-pair interaction in IgE-mediated diseases [2]. Determination of CD3, CD4, CD8, CD21, CD23 markers on T and B lymphocytes of peripheral blood showed normal values in most AD patients, but significantly higher CD23 expression and lower CD3+ and CD21+ cells in AD patients in comparison with HCs, and the cells were HLA-negative. The obtained results are in accordance with those of other authors, as we also confirmed the significantly higher expression of CD23 in the peripheral blood of AD patients in acute phase than in HCs. The obtained results suggest parameters of early and delayed immunity in the blood of AD patients with those of interactions of IgE, T-cells and CD23 receptor for IgE of low affinity (Fc ϵ RII) with consequence of skin inflammation in AD.

Besides skin tests, peripheral blood testings, histologic picture and immunohistochemical analysis of AD skin biopsy have also identified different immunological factors involved in AD. Many authors report on the results of their long-term studies of various markers expressed on the surface of the cells infiltrating AD skin lesions [6, 13].

Analysis of AD skin lesions reveals a mononuclear infiltrate consisting of lymphocytes, predominantly by CD4+ T-cells, monocytes, and an increased intraepidermal LC count, mainly in the perivascular parts of the dermis, and to a lesser extent in the epidermis [14–17]. Perivascular infiltrate in the dermis of AD skin lesions consisted of activated helper CD4+ T lymphocytes expressing HLA-DR+ (an increased number of activated memory T-cells), a large number of APCs such as CD1a+ cells (dendritic cells, DCs) (10–15%), macrophages (10%), and interdigitating reticulum cells (20%) [18]. It has been noted that APCs, IgE bearing LCs and inflammatory dendritic cells (IDECs) express the high-affinity IgE receptor (Fc ϵ RI) and play an important role in allergen presentation of Th2 to Th1 cells. The increased expression of Fc ϵ RI on DCs in AD skin is due to enhanced expression of Fc ϵ RI γ chain and is preserved by increased IgE levels [5]. Recent study analysis shows that keratinocytes, mast cells and IDECs release tumor necrosis factor- α (TNF- α) and IL-1 cytokines, which induce expression of VCAM and facilitate the extravasation of inflammatory cells into the AD skin [5].

In AD skin lesions, mostly CD4+ lymphocytes, with a predominance of CD4+CD29+ lymphocytes (helper/inductor, 87.9%), and some CD4+CD45RO+ lymphocytes (suppressor/inductor, 6%) were found [19]. It was also demonstrated that IL-4+ cells prevail in the incipient stage of AD, while IFN γ + cells prevail over IL-4+ cells in the late and chronic stage of AD [19]. Thus, Th2-cells producing Th2-cytokines probably play a role in the initiation of eczematous reaction, whereas Th1-cytokines are involved in the exacerbation of chronic lesions [13]. In skin lesions of AD patients a significantly higher CD23+ cell count (>3-fold), and a greater number of CD25+ cells, HLA-DR+ cells and CD45RO+ cells (memory T-cells) were also found [20]. The results of our study are generally consistent with the data listed above.

Our study supports increased infiltration of CD3+ and CD4+ cells with expression of CD29 and CD45RO (memory T-cells) in AD skin lesions, suggesting the infiltration of activated Th1-cells in acute skin lesions of AD patients. Although both produce IL-2, IL-10, and IL-3, Th1- and Th2-subsets of CD4+ T-cells produce mainly different cytokines, and expression of their cell markers differs [4, 21–25]. While Th1-subsets express CD45RO and CD29, and produce IFN γ and help in cellular immunity (T-cells), Th2 subsets express CD45RO, but not CD29, and produce IL-4, IL-5, IL-6, and help in humoral immunity (B-cells). It is important to add that Th1- and Th2-cells are not two distinct CD4+ T-cell subsets, but simply represent polarized forms of the highly heterogeneous CD4+ Th-cell-mediated immune response [21–25]. Our results showed that in skin lesions of all AD patients memory Th1 subsets of T-cells (CD45RO and CD29) dominated, supporting participating of cellular immunity mediated by Th1-cells in acute phase of AD.

It has also been noted that immunological abnormality of Th1 immunity in AD patients is induced concomitantly with both the quantitative, qualitative and functional defect of memory type CD4+ T-cells, as supported by significantly reduced number of CD4+ CD45RO+ memory type T-cells found on PBMC of AD patients [26].

The expression receptors for IFN γ (IFN γ + cells) on keratinocytes in acute lesions of all AD patients also establish Th1-cell involvement and their cytokine productions, what slightly occurs in the dermis, where only rarely IFN γ + cells were found. It might be that in acute AD skin lesions, prominent activation of Th1 cells with increased production of IFN γ plays an important role, leading to IFN γ binding to keratinocytes and consequently inflammatory skin changes in AD. It also could be that

increased IFN γ may inhibit AD development by inhibiting Th2 differentiation and cytokine release, but once initiated, the disease may be exacerbated by the Th1-cytokines via inflammatory mechanisms. This notion is also supported by other authors and findings of increased numbers of IFN γ -secreting cells in AD progression, although it is still unclear if switch from Th2 to a Th1 response represents progression of disease or is an attempt to downregulate inflammation [23].

The upregulated IFN γ receptor in AD patients is probably connected with gene for IFN γ receptor, which is a highly expressed gene, as revealed in monocytes from AD patients [27]. This gene is involved in MHC class I antigen presentation and recognition of antigens, and the up-regulation of gene suggests that circulating monocytes in AD patients may be primed to differentiate into effector cells by conditions associated with AD [27]. It is important to mention that in some allergic patients dysfunction of interferon-gamma receptor 1 (IFNGR1) gene (IFNGR) was found, and that L467P in the IFNGR1 gene is one of candidate susceptibility genes for allergic diseases [28]. Recently it was noted that in the IFNGR1 gene Leu 467 Pro was revealed, a single-nucleotide substitution, which is associated with allergic diseases [28].

However, it could be that keratinocytes contribute to the inflammation by expressing IFN γ , the most potent keratinocyte activator, and a variety of proinflammatory molecules [29]. It was also revealed that suppressor of cytokine signaling (SOCS)1 and SOCS3 are negative regulators of IFN γ signaling and are induced in many cells by IFN γ itself or by other cytokines, supporting impaired IFN γ -dependent inflammatory responses in keratinocytes overexpressing the suppressor of cytokine signaling 1 [29]. Our study results indicate prominently higher expression of receptor for IFN γ in AD skin than in non-atopic subjects' skin, which may be due to their difference in their intrinsic ability to respond to proinflammatory stimuli. The other study results have also revealed that keratinocytes cultured from AD patients had much higher IFN γ -induced release of cytokines (IL-1 α , IL-1 receptor antagonist (IL-1ra), GM-CSF, and TNF- α), indicating they are hyperresponsive to IFN γ in terms of cytokine release [30].

It has been revealed that nonallergic subjects' blood exhibits allergen-specific Th1 responses, although they have neither allergic symptoms nor any type of inflammatory response to allergen challenge [24]. Our examination of skin of nonallergic healthy subjects revealed no Th1 response or IFN γ + cells in epidermis and dermis.

Determination of IgE expressed on LCs in skin lesion infiltrate and increased intraepidermal and intradermal deposition of IgE-a confirm interaction between IgE and LCs and connection of early and delayed hypersensitivity reactions in AD. Our study supports increased number of IgE on the LCs and expression of low affinity (FcεRII, CD23) in acute AD skin lesions. Namely, AD skin contains an increased number of IgE-bearing LCs, that via FcεRI or FcεRII, which are thought to bind allergens and are more strongly expressed in lesional skin in AD than in other inflammatory diseases, as supported by our results. It has also been shown that LCs are hyperstimulatory to helper T-cells and can activate helper T-cells to the Th2 phenotype in the initiating phase of AD [5].

Our analysis of AD skin lesions also revealed significant correlation between numbers of CD23+ LCs and CD4+ cells in the epidermis and dermis, indicating in-

creased Th-cells and LCs participation and cooperation between cell markers with involvement of humoral and cellular immunity and increased activation of Th1-subsets. On the other hand, Goto et al. [31] have noted that the numbers of epidermal LCs were negatively correlated with the clinical severity of the AD lesions, indicating that epidermal LCs may decrease in some conditions of AD, probably in lesions with severe inflammation.

Although the study included a relatively small number of subjects, the results obtained point to a conclusion that many immune factors are involved in the pathogenesis of AD. Disturbed humoral immunity with increased production of IgE and disorders of cellular immunity including epidermal LCs with IgE receptors of high and low affinity, relative imbalance between Th1- and Th2-subsets of CD4+ T-cells producing cytokines indicate prominent immune disorders in AD.

References

- Braun-Falco O, Plewig G, Wolff HH, Burgdorf WHC: *Dermatology*, ed 2, completely revised. Berlin, Springer, 2000.
- Lugović L, Lipozenčić J: Are respiratory allergic diseases related to atopic dermatitis? *Coll Antropol* 2000;24:335-345.
- Galli E, Cicconi R, Rossi P, Casati A, Brunetti E, Mancino G: Atopic dermatitis: Molecular mechanisms, clinical aspects and new therapeutic approaches. *Curr Mol Med* 2003;3: 127-138.
- Kuby J: *Immunology*, ed 4. New York, Freeman, 2002.
- Leung DY, Boguniewicz M, Howell MD, Nomura I, Hamid QA: New insights into atopic dermatitis. *J Clin Invest* 2004;113:651-657.
- Lugović L, Lipozenčić J, Jakić-Razumović J: Atopic dermatitis: Immunophenotyping of inflammatory cells in skin lesions. *Int J Dermatol* 2001;40:489-494.
- Hanifin JM, Rajka G: Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Stockh)* 1980;92:44-47.
- Sket-Janković N, Valinger Z, Treščec A: Imunokemija: radioimunotest, enzimoimunotest i srodne tehnike. U: Dekaris D, Čulo F i sur. *Klinička imunologija u nas*. Zagreb, Naprijed, 1990, pp 98-109.
- Rabatić S, Gagro A: The use of flow cytometry in monitoring allergic diseases. *Period Biol* 1996;98:191-194.
- Yoshino T, Mukuzono H, Aoki H, Takahashi K, Takeuchi T, Kubonishi I, Ohtsuki Y, Motoi M, Akagi T: A novel antibody recognizing a helper/inducer T cell subset. *Am J Pathol* 1989; 134:1339-1346.
- Poppema S, Lai R, Visser L: Monoclonal antibody OPD4 is reactive with CD45RO but differs from UCHL1 by the absence of monocyte reactivity. *Am J Pathol* 1991;139:725-729.
- Pirenne H, Aufard Y, Eljaafari A, Bourillon A, Ouri JF, Le Gac S, Blot P, Sterkers G: Comparison of T cell functional changes during childhood with the ontogeny of CDw29 and CD45RA expression on CD4+ T cells. *Pediatr Res* 1992;32:81-86.
- Werfel T, Marita A, Grewe M, Renz H, Wahn U, Krutmann J, Kapp A: Allergen specificity of skin-infiltrating T cells is not restricted to a type-2 cytokine pattern in chronic skin lesions of atopic dermatitis. *J Invest Dermatol* 1996; 107:871-876.
- Mihm MC, Soter NA, Dvorak HN, Austen KF: The structure of normal skin and the morphology of atopic dermatitis. *J Invest Dermatol* 1976;67:305-312.
- Braathén LR, Forre O, Natvig JG, Eeg-Larsen T: Predominance of T lymphocytes in the dermal infiltrate of atopic dermatitis. *Br J Dermatol* 1979;100:511-519.
- Uno H, Hanifin JM: Langerhans cells in acute and chronic epidermal lesions of atopic dermatitis, observed by L-DOPA histofluorescence, glycol methacrylate, thin section and electron microscopy. *J Invest Dermatol* 1980;75:52-60.
- Leung DYM, Bhan AK, Schneeberger EE, Geha RS: Characterization of mononuclear cell infiltrate in atopic dermatitis using monoclonal antibodies. *J Allergy Clin Immunol* 1983;71:47-56.
- Zachary CB, Allen MH, MacDonald DM: In situ quantification of T-lymphocyte subsets and Langerhans cells in the inflammatory infiltrate of atopic eczema. *Br J Dermatol* 1985; 112:149-56.
- Watanabe K, Kondo N, Fukutomi O, Takama T, Agata H, Oril T: Characterization of infiltrating CD4+ cells in atopic dermatitis using CD45R and CD29 monoclonal antibodies. *Ann Allergy* 1994;72:39-44.
- Xu X-J, Banarjee P, Rustin MHA, Poutter LW: Modulation by Chinese herbal therapy of immune mechanisms in the skin of patients with atopic eczema. *Br J Dermatol* 1997;136: 54-59.
- Aleksza M, Lukacs A, Antal-Szalmas P, Hunyadi J, Szegedi A: Increased frequency of intracellular interleukin (IL)-13 and IL-10, but not IL-4, expressing CD4+ and CD8+ peripheral T cells of patients with atopic dermatitis. *Br J Dermatol* 2002;47:1135-1141.
- Biedermann T, Rocken M, Carballido JM: TH1 and TH2 lymphocyte development and regulation of TH cell-mediated immune responses of the skin. *J Invest Dermatol Symp Proc* 2004;9:5-14.
- Weiss E, Mamelak AJ, La Morgia S, Wang B, Feliciani C, Tulli A, Sauder DN: The role of interleukin 10 in the pathogenesis and potential treatment of skin diseases. *J Am Acad Dermatol* 2004;50:657-675.
- Romagnani S: Immunologic influences on allergy and the TH1/TH2 balance. *J Allergy Clin Immunol* 2004;113:395-400.
- Nakatani T, Kaburagi Y, Shimada Y, Inaoki M, Takehara K, Mukaida N, Sato S: CCR4 memory CD4+ T lymphocytes are increased in peripheral blood and lesional skin from patients with atopic dermatitis. *J Allergy Clin Immunol* 2001;107:353-358.

- 26 Matsuyama T, Urano K, Ohkido M, Ozawa H, Ohta A, Kaneko S, Yahata T, Takita C, Nishimura T: The quantitative and qualitative defect of CD4+ CD45RO+ memory-type T cells are involved in the abnormality of TH1 immunity in atopic dermatitis patients. *Clin Exp Allergy* 1999;29:687–694.
- 27 Nagata N, Oshida T, Yoshida NL, Yuyama N, Sugita Y, Tsujimoto G, Katsunuma T, Akasawa A, Saito H: Analysis of highly expressed genes in monocytes from atopic dermatitis patients. *Int Arch Allergy Immunol* 2003;132:156–167.
- 28 Aoki M, Matsui E, Kaneko H, Inoue R, Fukao T, Watanabe M, Teramoto T, Kato Z, Suzuki K, Suzuki Y, Kasahara K, Kondo N: A novel single-nucleotide substitution, Leu 467 Pro, in the interferon-gamma receptor 1 gene associated with allergic diseases. *Int J Mol Med* 2003;12:185–191.
- 29 Federici M, Giustizieri ML, Scarponi C, Girolomoni G, Albanesi C: Impaired IFN-gamma-dependent inflammatory responses in human keratinocytes overexpressing the suppressor of cytokine signaling 1. *J Immunol* 2002;169:434–442.
- 30 Pastore S, Corinti S, La Placa M, Didona B, Girolomoni G: Interferon-gamma promotes exaggerated cytokine production in keratinocytes cultured from patients with atopic dermatitis. *J Allergy Clin Immunol* 1998;101:538–544.
- 31 Goto T, Soma Y, Ra C, Kawa Y, Kubota Y, Mizoguchi M: Enhanced expression of the high-affinity receptor for IgE (Fc(epsilon)RI) associated with decreased numbers of Langerhans cells in the lesional epidermis of atopic dermatitis. *J Dermatol Sci* 2001;27:156–161.