

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

Edyta Machura · Bogdan Mazur · Jarosław Kwiecień · Krystyna Karczewska

Received: 5 June 2006 / Accepted: 21 September 2006
© Springer-Verlag 2006

Abstract The role of the type-2 T helper (Th2) cell-mediated immune response in the immunopathogenesis of atopic dermatitis (AD) is well documented. Whether polarized immunoresponse is confined to antigen-specific T cells or is distributed among all T cell subsets is still controversial. We investigated frequencies of interleukin-2 (IL-2), IL-4, interferon-gamma (IFN- γ), and tumor necrosis factor-alpha (TNF- α) producing CD3⁺ and CD4⁺ T cells in peripheral blood from children with atopic dermatitis and healthy subjects with and without in vitro stimulation. Children with severe AD had a significantly lower percentage of CD4⁺ T cells spontaneously expressing IL-4 compared with healthy controls ($p < 0.01$). Polyclonal stimulation significantly increased cytokine production in both AD patients and healthy individuals. Frequencies of CD3⁺ and CD4⁺ producing IL-2, IL-4, IFN- γ , and TNF- α after in vitro stimulation with phorbol-12-myristate 13-acetate (PMA)+ionomycin were comparable in the AD and control groups. In response to PMA/ionomycin, children with AD and asthma symptoms had a significantly lower percentage of CD3⁺ T cells producing TNF- α . We failed to demonstrate evidence of an imbalance with respect to type-

2 cytokine productions in children with AD. Comparable induction of Th1 and Th2 cytokines in polyclonally stimulated peripheral CD3⁺ and CD4⁺ T cells from AD patients and controls puts into question the polarized Th2 immune response as a general characteristic of T cells in children with atopic dermatitis.

Keywords Atopic dermatitis · Flow cytometry · Intracellular cytokines · Th1/Th2

Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disorder, with an increasing prevalence over the past three decades. Numerous immunological abnormalities are implicated in the pathogenesis of AD. The most prominent feature of AD is elevated serum immunoglobulin E (IgE) and eosinophilia, which reflect an increased expression of T helper 2 (Th2) cytokines [24, 32, 34]. There have also been a number of studies demonstrating increased frequency of allergen-specific T cells producing interleukin (IL)-4, IL-5, and IL-13 but little interferon-gamma (IFN- γ) in the peripheral blood of patients with AD [38]. On the other hand, data from skin biopsies revealed that Th1 cytokines also contribute to the pathogenesis of AD. Studies have shown that acute skin lesions contain significantly more cells producing IL-4, IL-5, and IL-13. By contrast, in chronic lesions in AD, IFN- γ predominates over IL-4 [13, 15]. Moreover, peripheral blood mononuclear cells (PBMC) obtained from children with atopic disease revealed increased Th1 and Th2 allergen-induced cytokine response [33]. Whether enhanced Th2 cytokine production in atopic individuals is allergen specific or is a general characteristic of T cells is still not clear.

E. Machura · J. Kwiecień · K. Karczewska
Chair and Department of Pediatric Gastroenterology,
Allergology and Developmental Disorders,
Medical University of Silesia,
Zabrze, Poland

B. Mazur (✉)
NICU, II Department of Pediatrics,
Medical University of Silesia, Laboratory of Immunology,
3 Maja 13/15,
41-800 Zabrze, Poland
e-mail: bmazur@slam.katowice.pl

We therefore investigated cytokine expression in peripheral blood from AD children before and after polyclonal stimuli, which were compared with healthy controls. Allergen-induced T-cell response reflects cytokine production by allergen reactive T cells. On the contrary, polyclonal cytokine responses following T-cell stimulation with mitogen or superantigen allow examination of underlying predisposition of cytokine production.

Materials and methods

Study population

Fifteen children (age range 3–16, mean age 10.9 ± 1.3 years) suffering from moderate to severe AD were enrolled in this study. AD was diagnosed according to the criteria described by Hanifin and Rajka [16]. AD severity was estimated according to the grading criteria of Rajka and Langelanda [31]. The scores of extent, course, and intensity were summed up. Based on total score, seven children were classified as moderate (score 5–7) and eight as severe (score 8–9) AD. All children attended the outpatient clinic of the Department of Pediatric Gastroenterology, Allergology and Developmental Disorders, Medical University of Silesia, Zabrze, for at least 1 year before the study was conducted, and all had positive skin-prick tests (SPT) to at least three allergens (SPT was regarded as positive when mean diameter was at least 3 mm). All patients were SPT positive to mixed pollen grass, 11 to *Dermatophagoides pteronyssinus*, nine to tree pollen and weed, seven to cat hair, three to dog hair, and two to food allergens (eggs, milk). All children had significantly elevated total and relevant allergen-specific IgE levels. The control group consisted of seven healthy children (age range 4–16, mean age 11.0 ± 1.0) years who had no previous clinical history of allergic disease and had negative skin response to common allergen (*D. pteronyssinus*, mixed grass or tree pollen, or cat and dog hair; Allergopharma, Reinbek, Germany). The control group consisted of voluntary children recruited from staff members of the outpatient clinic. None of the AD patient had used oral corticosteroids. Nine patients with AD used low-dose inhalant budesonide (200 µg) because of concomitant asthma symptoms (asthma symptoms were recognized in four children with moderate and five with severe AD). The study was approved by the Ethics Committee of the Medical University of Silesia, Katowice. Written informed consent was obtained from the parents of all patients. Peripheral blood was obtained from all children. Total serum IgE was quantified by fluoroenzymeimmunoassay (UniCap, Pharmacia Diagnostic AB, Uppsala, Sweden) according to the manufacturer's instructions.

Intracellular cytokine staining

Heparinized whole blood was diluted with Roswell Park Memorial Institute (RPMI) medium (BioWhittaker) and calf serum, and after addition of 25 µl (25 ng/ml) of phorbol-12-myristate 13-acetate (PMA; Sigma), 20 µl (1 µg/ml) of ionomycin (Sigma), and 10 µl (2.5 µM) of monensin (Sigma) incubated in a water bath for 4 h at 37°C in tightly capped tubes. Stimulated whole blood was distributed in 100-µl aliquots into tubes containing 20 µl antihumans CD3-FITC and CD4-PerCp (Becton Dickinson), vortexed, and incubated for 20 min at room temperature in the dark. To fix cells, 100 µl of IntraPrep Reagent 1 (DAKO) was added to the samples, followed by vortexing and incubation for 15 min at room temperature in the dark and afterwards washing with 2 ml of Cell Wash solution. Simultaneous permeabilization of the white cells and lysis of the red cells was then carried out by adding of 100 µl of IntraPrep reagent 2 (DAKO) and incubation. Intracellular staining procedure included the addition of 20 µl of anti-IL-2, IL-4, IFN-γ, IFN-α monoclonal antibodies or isotype control mouse antibodies conjugated with phycoerythrin (PE) (Coulter Immunotech). The flow cytometric assessment with FACScan flow cytometry (Becton Dickinson) was performed on the same day. Acquisition was gated on CD3⁺ and CD3⁺ CD4⁺ cells, and then the parentage of CD3⁺ and CD3⁺ CD4⁺ cells producing IL-2, IL-4, IFN-γ, and TNF-α was determined on dot plots. Quadrants were set based upon isotype control. Acquisition was performed both on samples stimulated with PMA and ionomycin and on nonstimulated samples in order to estimate the residual intracellular expression of the cytokines. Activation of the cells was confirmed by estimation of CD69 expression, which was over 95% in all samples. Acquisition and analysis were performed with Cell Quest software package (Becton Dickinson).

Statistical analysis

Statistical analyses were performed using a software package (Statistica, version 3.0), and data were presented as mean value $\pm$ standard error (SE). Comparisons between groups were made with the Kruskal–Wallis test, followed by the Mann–Whitney U test. Correlations were calculated using the Spearman rank test. Values of $p < 0.05$ were considered as statistically significant.

Results

Eosinophil levels were significantly increased in AD patients (mean: 370 ± 40.4) compared with controls (mean: 120 ± 15.8); $p < 0.001$. There was also a significant increase

Table 1 Percentage of CD3⁺ T cells and CD4⁺ T cells producing IL-2, IL-4, IFN- γ , and TNF- α before and after in vitro activation with phorbol 12-myristate 13-acetate (PMA) and ionomycin

Cytokines	T-cell subsets	AD patients (n=15) mean $\pm$ SE	Healthy controls (n=7) mean $\pm$ SE
IL-2	CD3 ⁺	12.0 $\pm$ 1.5 (2.4 $\pm$ 0.4)	11.4 $\pm$ 2.5 (3.4 $\pm$ 1.2)
	CD4 ⁺	7.0 $\pm$ 0.7 (1.7 $\pm$ 0.3)	7.8 $\pm$ 1.3 (2.1 $\pm$ 0.5)
IL-4	CD3 ⁺	7.3 $\pm$ 0.9 (2.2 $\pm$ 0.5)	6.5 $\pm$ 0.3 (3.0 $\pm$ 1.0)
	CD4 ⁺	6.1 $\pm$ 0.7 (1.8 $\pm$ 0.4)	5.0 $\pm$ 0.4 (2.4 $\pm$ 0.6)
IFN- γ	CD3 ⁺	12.5 $\pm$ 0.5 (2.5 $\pm$ 0.5)	11.7 $\pm$ 3.0 (2.5 $\pm$ 0.8)
	CD4 ⁺	7.6 $\pm$ 1.0 (2.0 $\pm$ 0.4)	7.5 $\pm$ 1.4 (2.0 $\pm$ 0.5)
TNF- α	CD3 ⁺	14.5 $\pm$ 1.2 (2.2 $\pm$ 0.6)	12.0 $\pm$ 1.8 (3.4 $\pm$ 0.6)
	CD4 ⁺	9.3 $\pm$ 0.8 (1.7 $\pm$ 0.42)	8.8 $\pm$ 1.2 (2.4 $\pm$ 0.4)

Unseparated whole blood from AD patients and healthy controls was stimulated for 4 h with PMA and ionomycin, and production of interleukin (IL)-2, IL-4, interferon-gamma (IFN- γ), tumor necrosis factor-alpha (TNF- α) of CD3⁺ and CD4⁺ T cells was determined at the single cell levels by intracellular cytokine staining and flow cytometry, as described in [Materials and methods](#). Frequencies of spontaneous cytokine staining (in the presence of monensin alone) are presented in brackets.

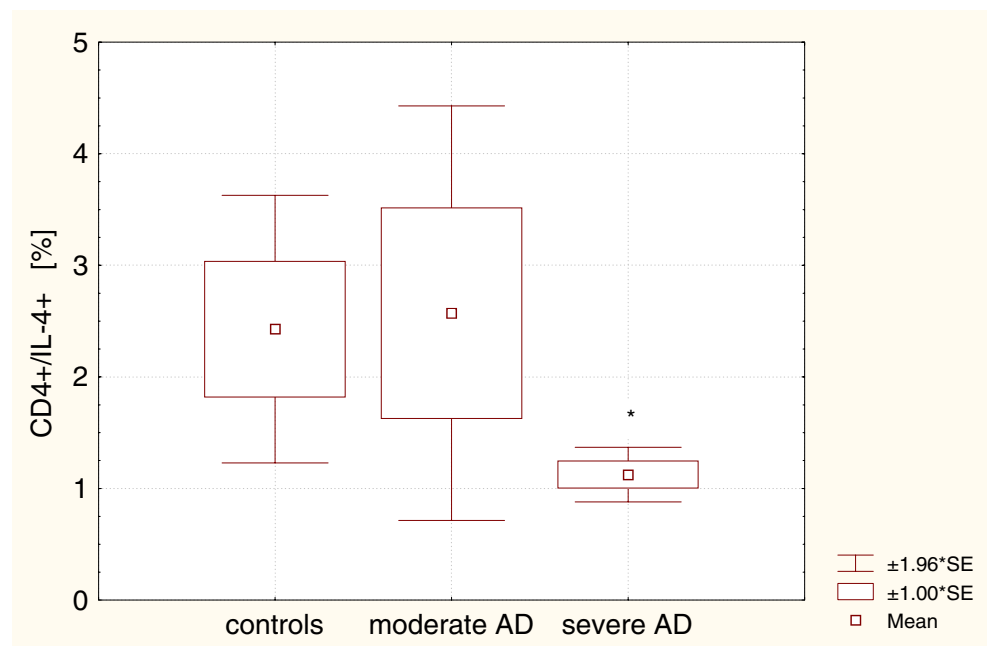
No statistically significant differences between children with atopic dermatitis (AD) and the control subjects were found (p =NS, in all cases).

in total IgE levels in AD patients (mean: 1395.5 $\pm$ 573) compared with controls (mean: 37.2 $\pm$ 13.3); p <0.008. Frequencies of CD3⁺ and CD4⁺ T cells staining positively for IL-2, IL-4, IFN- γ , and TNF- α in unstimulated whole blood were similar in atopic children and in healthy controls (Table 1).

However, after separating patients according to the severity of AD and then comparing them with healthy controls, there was a significant decrease in frequencies of CD4⁺ T cells spontaneously producing IL-4 in children with severe AD; p <0.01 (Fig. 1). Other cytokines were similar in all subgroups.

Although polyclonal stimulation increased cytokine production in AD patients much more intensely than in controls (AD: p =0.001 for CD4⁺/IL-4⁺, in the remaining cases p =0.0006, and controls: p =0.02 in all cases, Fig. 2), frequencies of CD3⁺ and CD3⁺ CD4⁺ T cells producing IL-2, IL-4, IFN- γ , and TNF- α after in vitro stimulation with PMA + ionomycin did not differ between AD patients and the control group (p >0.05) (Table 1). No significant correlations between IgE level or eosinophil number and cytokine production were found. The frequencies of CD3⁺ and CD4⁺ T cells staining positively for IFN- γ after stimulation with PMA and ionomycin from patients with AD, but not from healthy controls, were inversely correlated with age (R : -0.63, p <0.01, R : -0.57, p <0.02, respectively). In response to PMA/ionomycin, children with AD and asthma symptoms had a significantly lower percentage of CD3⁺ T cells producing TNF- α compared with healthy controls and of CD4⁺ T cells producing TNF- α than children with “pure” AD (p <0.01), as shown in Fig. 3. Other cytokines were similar in all subgroups. There were no significant

Fig. 1 Percentage of CD4⁺ interleukin (IL)-4⁺ cells in unstimulated peripheral blood from children with moderate (n =7) and severe (n =8) atopic dermatitis (AD) and control subjects (n =7). The percentage of CD4⁺ IL-4⁺ cells in severe AD was significantly lower than in controls ($*p$ <0.01). Other cytokines [IL-2, interferon-gamma (IFN- γ), tumor necrosis factor-alpha (TNF- α)] were similar in the subgroups. The Mann-Whitney U test was used to determine significant differences between subgroups



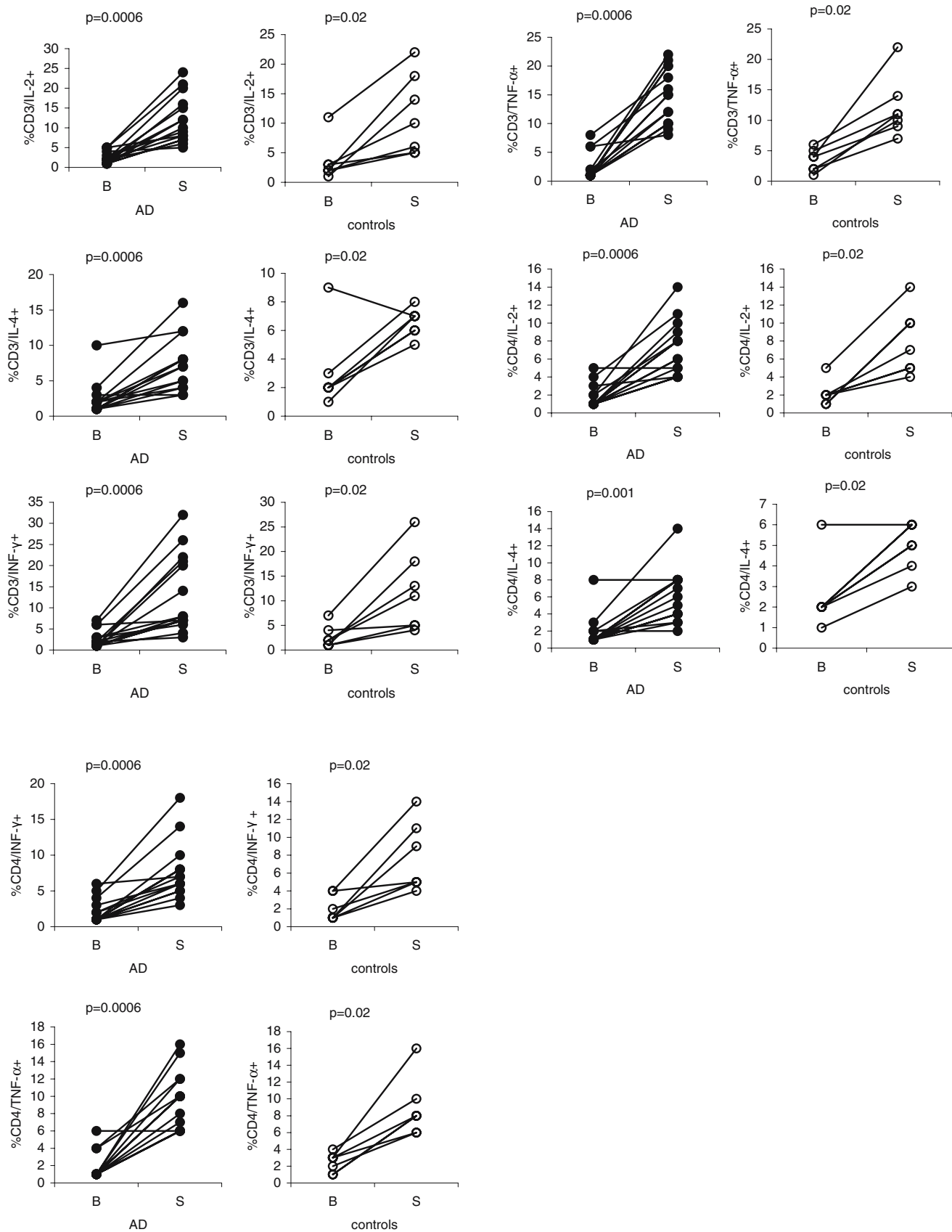


Fig. 2 Frequencies of CD3⁺ and CD4⁺ T cells producing interleukin (IL)-2, IL-4, interferon-gamma (IFN- γ), or tumor necrosis factor-alpha (TNF- α) before and after in vitro stimulation with phorbol-12-myristate 13-acetate (PMA) and ionomycin in healthy and atopic dermatitis (AD) children. Polyclonal stimulation increased production of all cytokines in AD patients much more intensely (for most cases, $p=0.0006$; for CD4⁺/IL-4⁺, $p=0.001$) than in controls ($p=0.02$ in all cases). **B** baseline: spontaneous cytokine staining without stimulation, **S**: cytokine staining with stimulation. The Mann–Whitney U test was used to determine significant differences between spontaneous cytokine staining and cytokine staining with stimulation

differences between the two patient groups in IgE level or eosinophils.

Discussion

There have been conflicting data with respect to cytokine production in AD patients. Therefore, in the present study, we assessed the frequency of intracellular-cytokine-producing T cells in children with AD and healthy, nonatopic controls. We measured the cytokine profile (IL-2, IL-4, IFN- γ , TNF- α) by flow cytometry in unseparated whole blood because this method seems to reflect the situation in circulating cells in vivo in the best way. Frequencies of spontaneous cytokine staining in PBMC were very low and comparable between atopic and healthy controls, as shown in several previous studies [8]. Surprisingly, we found that children with severe AD had a significantly lower percentage of CD4⁺ T cells spontaneously expressing IL-4 compared with healthy children, which may indicate that these cells were recruited from peripheral blood to the site

of inflammation. In contrast to our results, Lonati et al. [25] using reverse transcriptase polymerase chain reaction showed spontaneous expression messenger ribonucleic acid (mRNA) of IL-4 and lower mRNA of IFN- γ in PBMC from AD patients compared with nonatopic subjects. In another study, the level of mRNA for IL-13 and IL-5 but not for IL-4 in PBMC from AD patients was significantly higher compared with controls [1]. In addition, decreased spontaneous IL-4 production by cord blood lymphocytes was reported in children who developed AD during the first year of life [14].

We have shown that both Th1 and Th2 cytokines are equally induced following in vitro stimulation with PMA and ionomycin stimulation in AD patient and controls. Our findings are in line with the results reported by Bullens [6], who demonstrated comparable cytokine production after polyclonal stimulation in allergic patients and controls. Also, Imada et al. [17] demonstrated that IL-2, IL-4, IL-10, and IFN- γ production elicited from PBMC using polyclonal activators did not differ between allergic and nonallergic subjects. In another study, it was shown that despite different levels of memory T cell subsets in peripheral blood in patients with chronic diseases such as leprosy and atopic diseases, cytokine profiles of T-cell subsets were unchanged compared with normal subjects [26]. The results presented in our report differ from most studies, which demonstrate increased IL-4 production and decreased IFN- γ by PBMC after in vitro polyclonal stimulation in AD patients compared with nonatopic subjects [9, 28, 29, 36]. In contrast, Källström et al. [18] showed decreased frequency of intracellular IFN- γ - and IL-4-producing T cells in whole blood preparations from adult patients with AD. Moreover,

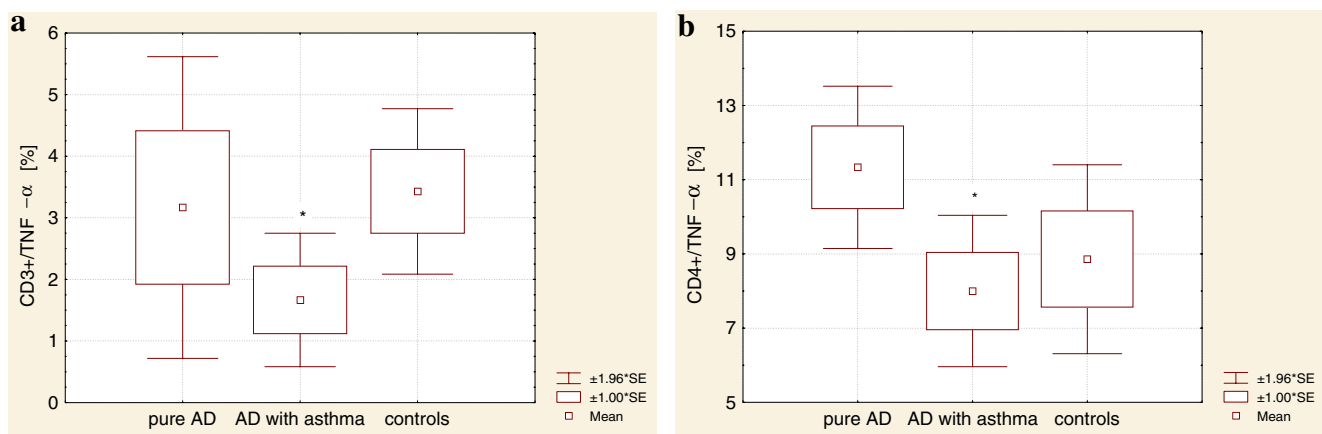


Fig. 3a, b Percentage of CD3⁺ tumor necrosis factor-alpha (TNF- α)⁺ and CD4⁺ TNF- α ⁺ in phorbol-12-myristate 13-acetate (PMA)-and-ionomycin-stimulated peripheral blood from children with “pure” atopic dermatitis (AD) ($n=6$) and children with AD and concomitant bronchial asthma ($n=9$) and healthy children ($n=7$). **a** The percentage of CD3⁺ TNF- α ⁺ in AD children with asthma was significantly lower than in

healthy children ($*p<0.01$). **b** The percentage of CD4⁺ TNF- α ⁺ in “pure” AD was significantly higher than in children with concomitant bronchial asthma ($*p<0.01$). Other cytokines [interleukin (IL)-2, IL-4, interferon-gamma (IFN- γ)] were similar in the subgroups. The Mann–Whitney U test was used to determine significant differences between all subgroups

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 was demonstrated [35]. Also, differential cytokine production of circulating cutaneous-lymphocyte-associated antigen CD4⁺ T cells (CLA⁺ CD4⁺) from AD patients with acute or chronic skin lesions compared with healthy controls was reported [4]. A recent report demonstrated that the Th2 predominance in atopic individuals is partly due to the preferential apoptosis of circulating memory/effector Th1 cells [2]. The discrepancy between our findings and other reports may be the result of the heterogeneous character of the investigated patient group. In a previous study, a different cytokine pattern of stimulated T cells was demonstrated among children with AD and bronchial asthma [21]. In the present study, children with concomitant bronchial asthma had a significantly lower production of TNF- α by stimulated CD4⁺ T cells than did children with “pure” AD and by stimulated CD3⁺ T cells compared with healthy children. The lower percentage of T cells producing TNF- α in children with asthma is conflicting, with results obtained in other studies suggesting that TNF- α , due to its ability to regulate inflammatory-cell infiltration and induction of airway hyperresponsiveness, plays an important role in the pathogenesis of asthma. TNF- α mRNA is more frequently expressed in the airway of asthmatic subjects compared with normal subjects [5]. The increased cytokine release from the bronchoalveolar lavage (BAL) cells and allergen-stimulated PBMC of asthmatic subjects was also evidenced [7, 27]. It may be possible that the part of the observations made in AD children with asthma were influenced by the fact that the children used inhaled corticosteroids (ICS). ICS reduce the expression of effector cytokines in airway T cells, in particular, cytokines IL-4, IL-5, IL-13 and to a lower extent TNF- α . [12, 39]. One study documented the modulating effect of ICS also on PBMC cytokine expression [11]. In addition, corticosteroids increase IL-10 production by T cells and macrophages, which may lead to a decrease in TNF- α synthesis. [19, 30].

It has also been documented that cytokine production seems to be related to age [22]. In our study, we observed that IFN- γ was decreased along with AD patient's age, which was not the case for the control group. Whether this can influence subsequent clinical course of AD remains to be elucidated. Although we used flow cytometry to detect intracellular cytokine expression at the single-cell level, as with most authors of the above-cited reports, some differences in technique, including time stimulation, permeabilization, stimulation agents, Golgi apparatus blockers, antibody, and analyzed T cell subsets, could have an impact on our results [10]. The small number of subjects may also be a drawback of this study; nevertheless, some differences among the particular subgroups were observed. In our

study, we failed to demonstrate a role of IL-4 in patients with AD, but we showed significantly increased total IgE levels and numbers of circulating eosinophils in AD patients compared with the control group. IL-13, another representative Th2 cytokine, was shown to be an important cytokine leading to IgE production, and it is also implicated in the pathogenesis of AD. Indeed, the recently published study supports the role of IL-13 in AD [3, 23]. Unfortunately we were not able to measure IL-13 production in this study. On the other hand, 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—termed regulatory T cells (Tregs)—might play a critical role in controlling the expression these diseases. Current evidence shows that both naturally occurring CD4⁺, CD25⁺, and IL-10-secreting Tregs can suppress Th2 responses to allergen; however, this process may be defective in atopic subjects. [20, 37]

On the basis of these results, e.g., lack of increased intracellular IL-4 expression after in vitro polyclonal stimulation of peripheral CD3⁺ and CD4⁺ T cells in children with AD, we conclude that Th2 predominance is not a general characteristic of T cells among our children with AD.

Acknowledgement This study was supported by grant no NN-2-314/03 from the State Committee for Research in Poland

References

1. Akdis CA, Akdis M, Simon HU, Blaser K (1999) Regulation of allergic inflammation by skin-homing T cells in allergic eczema. *Int Arch Allergy Immunol* 118:140–144
2. Akdis M, Trautmann A, Klunker S, Daigle I, Kucuksezer UC, Deglmann W, Disch R, Blaser K, Akdis CA. T helper (Th) 2 predominance in atopic diseases is due to preferential apoptosis of circulating memory/effector Th1 cells. *FASEB J* 17:1026–1035
3. Aleksza M, Irinyi B, Lukács A, Antal-Szalmás P, Hunyadi J, Szegedi A (2002) 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. *B J Dermatol* 147:1135–1141
4. Antúnez C, Torres MJ, Mayorga C, Cornejo-García JA, Santamaria-Babi LF, Blanca M (2004) 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* 34:559–566
5. Bradding P, Roberts JA, Britten KM, Montefort S, Djukanovic R, Mueller R, Heusser CH, Howarth PH, Holgate ST (1994) Interleukin-4, -5, and -6 and tumor necrosis factor- α in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. *Am J Respir Cell Mol Biol* 10:471–480
6. Bullens DM, van den Keybus C, Dilissen E, Kasran A, Ceuppens JL (2004) Allergen-specific T cells from birch-pollen-allergic patients and healthy controls differ in T helper 2 cytokine and in interleukin-10 production. *Clin Exp Allergy* 34:879–887
7. Cembrzyńska-Nowak M, Szklarczyk E, Ingłot AD, Teodorczyk-Injeyan JA. (1993) Elevated release of tumor necrosis factor- α and interferon- γ by bronchoalveolar leukocytes from patients with bronchial asthma. *Am Rev Respir Dis* 146:291–295

8. Cho SH, Stanciu LA, Begishvili T, Bates PJ, Holgate ST, Johnston SL (2002) Peripheral blood CD4⁺ and CD8⁺ T cell type 1 and type 2 cytokine production in atopic asthmatic and normal subject. *Clin Exp Allergy* 32:427–433
9. Farrell AM, Antrobus P, Simpson D, Powell S, Chapel HM, Ferry BL (2001) A rapid flow cytometric assay to detect CD4⁺ and CD8⁺ T-helper (Th) 0, Th1 and Th2 cells in whole blood and its application to study cytokine levels in atopic dermatitis before cyclosporin therapy. *J Dermatology* 144:24–33
10. Ferry B, Antrobus P, Huzicka I, Farrell A, Lane A, Chapel H (1997) Intracellular cytokine expression in whole blood preparations from normals and patients with atopic dermatitis. *Clin Exp Immunol* 110:410–417
11. Gemou-Engesaeth V, Fagerhol MK, Toda M, Hamid Q, Halvorsen S, Groegaard JB, Corrigan CJ (2002) Expression of activation markers and cytokine mRNA by peripheral blood CD4 and CD8 T cells in atopic and nonatopic childhood asthma: effect of inhaled glucocorticoid therapy. *Pediatrics* 109:1–9
12. Georas SN (2004) Inhaled glucocorticoids, lymphocytes, and dendritic cells in asthma and obstructive lung diseases. *Proc Am Thorac Soc* 1:215–221
13. Grewe M, Bruijnzeel-Koomen CA, Schöpf E, Thepen T, Langeveld-Wildschut AG, Ruzicka T, Krutmann J (1998) A role for Th1 and Th2 cells in the immunopathogenesis of atopic dermatitis. *Immunol Today* 19:359–361
14. Hagendorens MM, Ebo DG, Bridts CH, van de Water L, de Clerck LS, Stevens WJ (2004) Prenatal exposure to house dust mite allergen (Der p1), cord blood T cell phenotype and cytokine production and atopic dermatitis during the first year of life. *Pediatr Allergy Immunol* 15:308–315
15. Hamid Q, Boguniewicz M, Leung DY (1994) Differential in situ cytokine gene expression in acute versus chronic atopic dermatitis. *J Clin Invest* 94:870–876
16. Hanifin JM, Rajka G (1980) Diagnostic features of atopic dermatitis. *Acta Derm Venereol Suppl (Stockh)* 92:44–47
17. Imada M, Simons FE, Jay FT, Hayglass KT (1995) Antigen mediated and polyclonal stimulation of human cytokine production elicit qualitatively different patterns of cytokine gene expression. *Int Immunol* 7:229–237
18. Källström E, Roscher I, Andreasson A, Bäck O, van Hage-Hamsten M (2002) Decreased frequency of intracellular IFN- γ producing T cells in whole blood preparations from patients with atopic dermatitis. *Exp Dermatol* 11:556–563
19. Karagiannidis C, Akdis M, Holopainen P, Woolley NJ, Hense G, Rückert B, Mantel PY, Menz G, Akdis CA, Blaser K, Schmidt-Weber CB (2004) Glucocorticosteroids upregulate FOXP3 expression and regulatory T cells in asthma. *J Allergy Clin Immunol* 114:1425–1433
20. Kearley J, Barker JE, Robinson DS, Lloyd CM (2005) Resolution of airway inflammation and hyperreactivity after in vivo transfer of CD4⁺CD25⁺ regulatory T cells is interleukin 10 dependent. *J Exp Med* 202:1539–1547
21. Kimura M, Tsuruta S, Yoshida T (1999) Differences in cytokine production by peripheral blood mononuclear cells (PBMC) between patients with atopic dermatitis and bronchial asthma. *Clin Exp Immunol* 118:192–196
22. Kimura M, Yamaide A, Tsuruta S, Okafuji I, Yoshida T (2002) Development of capacity 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* 127:191–197
23. La Grutta S, Richiusa P, Pizzolanti G, Mattina A, Pajno GB, Citarrella R, Passalacqua G, Giordano C (2005) CD4⁺ IL-13⁺ cells in peripheral blood well correlates with the severity of atopic dermatitis in children. *Allergy* 60:391–395
24. Leung DY, Bieber T (2003) Atopic dermatitis. *Lancet* 361:151–160
25. Lonati A, Licenziati S, Canaris AD, Fiorentini, Pasolini G, Marcelli M, Seidenari S, Caruso A, de Panfilis G (1999) Reduced of both Th1 and Tc1 lymphocyte subsets in atopic dermatitis (AD). *Clin Exp Immunol* 115:1–5
26. Mitra DK, De Rosa SC, Luke A, Balamurugan A, Khaitan BK, Tung J, Mehra NK, Terr AI, O'Garra A, Herzenberg LA, Roederer M (1999) Differential representations of memory T cell subsets are characteristic of polarized immunity in leprosy and atopic diseases. *Int Immunol* 11:1801–1810
27. Mitsuta K, Matsuse H, Fukushima C, Kawano T, Tomari S, Obase Y, Goto S, Urata Y, Shimoda T, Kondo S, Kohno S (2003) Production of TNF- α by peripheral blood mononuclear cells through activation of nuclear factor kappa B by specific allergen stimulation in patients with atopic asthma. *Allergy Asthma Proc* 24:19–26
28. Nakazawa M, Sugi N, Kawaguchi H, Ishii N, Nakajima H, Minami M (1997) Predominance of type 2 cytokine-producing CD⁺ and CD8⁺ cells in patients with atopic dermatitis. *J Allergy Clin Immunol* 99:673–682
29. Okazaki H, Kakurai M, Hirata D, Sato H, Kamimura T, Onai N, Matsushima K, Nakagawa H, Kano S, Minota S (2002) Characterization of chemokine receptor expression and cytokine production in circulating CD4⁺ T cells from patients with atopic dermatitis: up-regulation of C-C chemokine receptor 4 in atopic dermatitis. *Clin Exp Allergy* 32:1236–1242
30. Peek EJ, Richards DF, Faith A, Lavender P, Lee TH, Corrigan CJ, Hawrylowicz CM (2005) Interleukin-10-secreting “regulatory” T cells induced by glucocorticoids and beta2-agonists. *Am J Respir Cell Mol Biol* 33:105–111
31. Rajka G, Langeland T (1989) Grading of the severity of atopic dermatitis. *Acta Derm Venereol Suppl (Stockh)* 144:13–14
32. Romagnani S (2000) The role of lymphocytes in allergic diseases. *J Allergy Clin Immunol* 105:339–408
33. Smart JM, Kemp AS (2002) Increased Th1 and Th2 allergen-induced cytokine response in children with atopic disease. *Clin Exp Allergy* 32:796–802
34. Spergel JM, Paller AS (2003) Atopic dermatitis and the atopic march. *J Allergy Clin Immunol* 112:118–127
35. Takahashi T, Sasaki Y, Hama K, Furue M, Ishibashi Y (1992) Production of IL-4, IL-2, IFN- γ and TNF- α by peripheral blood mononuclear cells of patients with atopic dermatitis. *J Derm Sci* 3:172–180
36. Teraki Y, Hotta T, Shiohara T (2000) Increased circulating skin-homing cutaneous lymphocyte-associated antigen (CLA)⁺ type 2 cytokine producing cells, and decreased CLA⁺ type1 cytokine-producing cells in atopic dermatitis. *Br J Dermatol* 143:373–378
37. van Oosterhout AJM, Bloksma N (2005) Regulatory T-lymphocytes in asthma. *Eur Respir J* 26:918–932
38. van Reijssen FC, Bruijnzeel-Koomen CA, Kalthoff FS, Maggi E, Romagnani S, Westland JK, Mudde GC (1992) Skin derived aeroallergen-specific T-cells clones of Th2 phenotype in patients with atopic dermatitis. *J Allergy Clin Immunol* 90:184–193
39. Wilson SJ, Wallin A, Della-Cioppa G, Sandstrom T, Holgate ST (2001) Effects of budesonide and formoterol on NF- κ B, adhesion molecules, and cytokines in asthma. *Am J Respir Crit Care Med* 164:1047–1052