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# Decreased frequency of intracellular IFN- $\gamma$ producing T cells in whole blood preparations from patients with atopic dermatitis

Källström E, Roscher I, Andreasson A, Bäck O, van Hage-Hamsten M. Decreased frequency of intracellular IFN- $\gamma$  producing T cells in whole blood preparations from patients with atopic dermatitis. *Exp Dermatol* 2002; 11: 556–563. © Blackwell Munksgaard, 2002

**Abstract:** There have been contradictory reports on the shift in the T-cell cytokine expression pattern of peripheral blood mononuclear cells from patients with atopic dermatitis (AD); more specifically the interleukin (IL)-4 and interferon (IFN)- $\gamma$  profiles. The aim of this study was to shed further light on this contradiction by measuring the intracellular cytokines IL-4 and IFN- $\gamma$  by flow cytometry on unseparated whole blood to obtain results that, as accurately as possible, reflect the situation in circulating cells *in vivo*. The patient group including 64 patients with AD was compared with 18 nonatopic healthy adults. The results showed that the percentage of CD4<sup>+</sup> T cells expressing IFN- $\gamma$  was significantly decreased ( $P \leq 0.001$ ), as well as the percentage expressing IL-4 ( $P < 0.05$ ) in AD patients compared with healthy controls.

Furthermore, in supernatants from whole blood samples stimulated with phorbol 12-myristate 13-acetate and ionomycin, production of IFN- $\gamma$  was significantly decreased, while IL-4 production remained unchanged in AD patients compared with healthy controls. We also investigated if there was a relationship between serum IgE level and Phadiatop<sup>®</sup>, a screening test for atopy, vs. the levels of IL-4 and IFN- $\gamma$ , but found no correlation with either. However, there was a significant correlation between disease severity and the level of total IgE ( $r = 0.67$ ,  $P < 0.05$ ). In conclusion, our results support the evidence for a decreased ability of peripheral CD4<sup>+</sup> T cells to produce IFN- $\gamma$  among AD patients.

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## Introduction

Atopic dermatitis (AD) is a common chronic inflammatory skin disorder with intense pruritus and typical cutaneous symptoms, and is frequently seen in patients with a family history of atopy (1). Patients with AD are a heterogeneous group of whom about 80% show immediate type skin reactions and elevated serum IgE levels. A previous study stated that the level of total IgE did not necessarily correlate with the activity of the disease (2). Recent studies indicate that the marked elevation of IgE is the result of T-cell dysregulation in AD patients (3). Lately, the term 'Atopic Exzema/Dermatitis Syndrome (AEDS)' has been recommended for AD to reflect the heterogeneity of this

patient group with respect for IgE-associated mechanisms (4).

During the past few years it has become increasingly clear that T-cells contribute to the abnormal regulation of the immune response in atopic diseases. Studies on T-cell clones support the concept that activation of a subpopulation of CD4<sup>+</sup> T-helper (Th) cells leads to the release of various cytokines important for the pathogenesis (5). CD4<sup>+</sup> T-helper cells are subdivided into two subgroups (Th 1 and Th 2) based on their cytokine production profiles. Much interest has been focused on the shifts of T-cell activity towards a T-helper 2-type cytokine response in allergy (6). However, there is controversy about the shift of the T-cell cytokine expression pattern of the peripheral

blood mononuclear cells (PBMC) from patients with AD (7–11) and whether or not the percentage of IL-4-producing cells is increased in AD patients. Both Nakagawa et al. and Jung et al. found a decreased interferon(IFN)- $\gamma$ -producing capacity in CD4<sup>+</sup>T cells in atopic patients but no significant elevation of IL-4-producing cells (12,13). In contrast, Nakazawa et al. and Ferry et al. found that the frequency of IL-4-producing CD4<sup>+</sup> cells was significantly higher in patients with AD than in healthy controls (14,15).

Recently Sander et al. (16) reported a method for detecting intracellular cytokines using fluorescence microscopy, and Jung et al. modified the method for use in analysing cytokine-producing cells by flow cytometry (17). Several groups have since used flow cytometry for detection of intracellular cytokines. So far, most groups have examined PBMC or *in vitro* cloned T cells. However, recent reports showed that the levels of IFN- $\gamma$  in whole blood cultures were higher than in purified PBMC preparations (18,19). We therefore decided to adapt the method for flow cytometric detection of intracellular cytokines (IL-4 and IFN- $\gamma$ ) in unseparated whole blood, to obtain results that, as accurately as possible, reflect the situation in circulating cells *in vivo*.

The aim of this study was to elucidate the characteristics of cytokine-producing cells in AD patients by using a method based on unseparated whole blood, and to clarify the discrepancy between cytokine production results from previous studies. We decided to measure the intracellular expression patterns of IL-4 and IFN- $\gamma$  in AD patients. The frequency of IL-4- and IFN- $\gamma$ -producing T cells was correlated with the severity of the disease (severity score), IgE levels and Phadiatop® (a screening test for atopy).

## Materials and methods

### Patients

During an 18-month period (1997–98) 64 adult patients with atopic dermatitis attended the Dermatology Outpatient Clinic at the Lund University Hospital. All 64 patients with AD (20 male, 44 female, mean age 31 years, range 19–80) fulfilled the diagnostic criteria of Hanifin and Rajka (1). The severity of the dermatitis was estimated according to the grading criteria of Rajka and Langeland (20) and was based on the following criteria: extent ('rule of nine'), course (by history) and intensity (disturbance of night sleep by itching). The total score ranged from 3 to 9, and the disease was classified as mild (3,4), moderate (5–7) or severe (8,9). Patients were asked about their prior diagnosis of allergic rhinitis (AR) and/or allergic asthma (AA). Those without a prior diagnosis were asked about their history of wheezing or whistling in the chest, spontaneously or in combination with exercise or cold weather, sneezing or runny or stuffy nose in the absence of a cold or flu, and of history of itchy-watery eyes.

Peripheral blood was obtained from all 64 patients and from a control group of 18 nonatopic healthy adults (age 23–57 years, mean 42 years) who had no history of AA, AR and AD and were negative in Phadiatop®. None of the patients had undergone treatment with systemic steroids.

### Total IgE and Phadiatop®

Total IgE in serum was determined using the Pharmacia CAP System™ IgE FEIA (Pharmacia Diagnostics AB, Uppsala, Sweden). The cut-off was 2 kU/l. Sera were also screened with Phadiatop®, a mixture of common inhalant allergens (birch, timothy grass, mugwort, olive, cat, dog, horse, *Dermatophagoides pteronyssinus* and *farinae*, *Cladosporium herbarum* and *Parietaria juclacia*) (Pharmacia Diagnostics AB). The Phadiatop® results were reported as positive ( $\geq 0.35$  kU/l) or negative. The analyses were performed according to the instructions of the manufacturer.

### Monoclonal antibodies and reagents

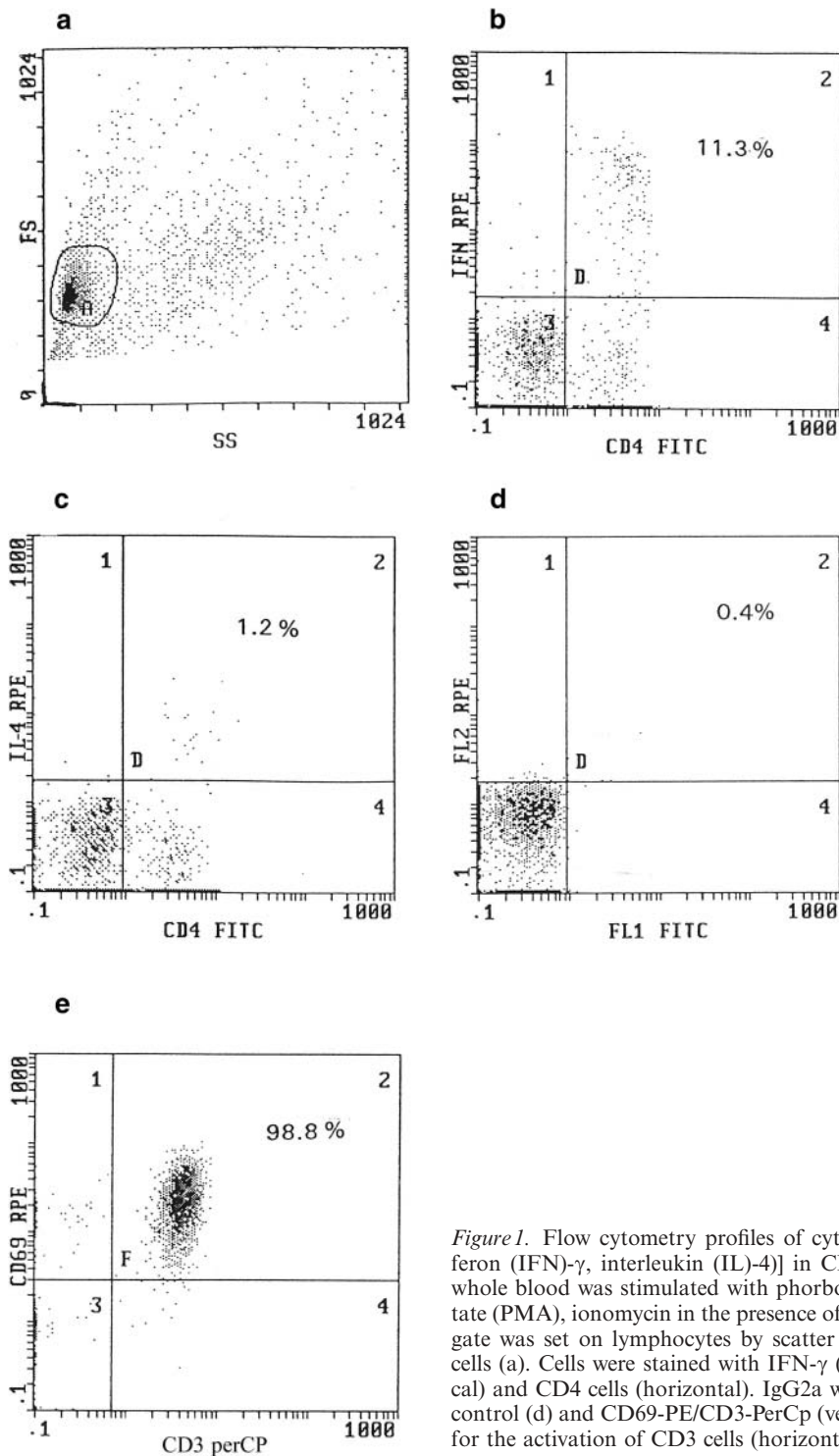
Phycoerythrin (PE)-labelled FastImmune antihuman-IL-4 (BD 340451, 0.0125 mg/ml), PE-FastImmune antihuman-IFN- $\gamma$  (BD 340452, 0.0075 mg/ml), FastImmune antihuman-CD69/CD3-peridinin-chlorophyll (PerCp)(0.00625 mg/ml and 0.003 mg/ml, respectively) and for cell surface staining fluorescein isothiocyanate (FITC)-labelled-anti-CD4 were obtained from Becton Dickinson Immunocytometry Systems (San Jose, CA). As the control antibody, PE-mouse IgG2a (BD 340459, 0.025 mg/ml) from the same company was used. Phorbol 12-myristate 13-acetate (PMA), ionomycin, brefeldin A (BFA) and paraformaldehyde (PFA) were purchased from Sigma (St. Louis, MO). Phorbol 12-myristate 13-acetate 0.1 mg/ml, 0.5 mg/ml ionomycin and 5 mg/ml BFA were used, unless otherwise stated. Kits for fixation and permeabilization (Reagent A and B, number code GAS-001–002) were obtained from Caltag Laboratories (An der Grub, Austria) and RPMI 1640 was from Gibco Brl, Life Technologies Ltd (Paisley, Scotland). Round-bottomed polystyrene tubes were purchased from Sarstedt AB (Landskrona, Sweden).

### *In vitro* stimulation of whole blood

Heparinised whole blood (500  $\mu$ l) was suspended in 500  $\mu$ l RPMI-1640 medium alone (unstimulated), or in RPMI-1640 in the presence of the following activators, 20  $\mu$ l PMA and 2  $\mu$ l ionomycin (stimulated). The samples were then incubated at 37°C in 5% CO<sub>2</sub> in an incubator for 30 min before 2  $\mu$ l BFA was added. Brefeldin A enables the accumulation of cytokines in the Golgi complex by interrupting intracellular transport processes.

### Permeabilization and cell staining

To detect the intracellular expression of cytokines, a modification of the methods of Jung et al. (17) and Picker et al. (21) was used. A panel of anti-CD4-FITC, anti-interferon (IFN)- $\gamma$ -PE, anti-IL-4-PE and anti-CD69-PE/CD3-PerCp was analysed. A total of 50  $\mu$ l of whole blood was incubated with 5  $\mu$ l of Mab FITC-anti-CD4 in the dark for 15 min at room temperature. Thereafter, 50  $\mu$ l of fixation medium (Reagent A) was added, followed by 15 min incubation at room temperature. After incubation the cells were washed once with 2 ml phosphate buffer solution (PBS)/0.5% BSA by centrifugation at 300  $\times$  g for 5 min. Supernatants were removed and 50  $\mu$ l of permeabilization medium (Reagent B) and 10  $\mu$ l of each monoclonal antibody, anti-



*Figure 1.* Flow cytometry profiles of cytokine synthesis [interferon (IFN)- $\gamma$ , interleukin (IL)-4] in CD4 cells. Unseparated whole blood was stimulated with phorbol 12-myristate 13-acetate (PMA), ionomycin in the presence of brefeldin. An analysis gate was set on lymphocytes by scatter properties and CD4<sup>+</sup> cells (a). Cells were stained with IFN- $\gamma$  (b) and IL-4 (c) (vertical) and CD4 cells (horizontal). IgG2a was used as the isotype control (d) and CD69-PE/CD3-PerCp (vertical) was the control for the activation of CD3 cells (horizontal) (e).

IFN- $\gamma$ -PE, anti-IL4-PE and anti-CD69-PE/CD3-PerCp, was added to separate the tubes containing the cell pellets. To determine non-specific binding an isotype-matched IgG2a control antibody was used in parallel. Anti-CD69-PE/CD3-PerCp was used as the control for the activation of CD3 cells. The samples were vortexed and incubated for another 15 min at room temperature. The permeabilized cells were finally washed in 2 ml PBS/0.5% BSA by centrifugation at 300  $\times$  g for 5 min. Supernatants were removed and the cells were resuspended in 500  $\mu$ l

1% PFA, and stored at 2–8°C in the dark. The fixed cells were then analysed within 24 h in a flow cytometric analyser (Epics XL-MCL, Coulter Corporation, Miami, FL). Leukocyte subpopulations were distinguished by their different light-scattering properties; forward scatter reflects the cell size and side scatter reflects the complexity/granularity. A minimum of 3000 cells was analysed within the lymphocyte cluster, and the results are expressed as a percentage of cytokine-producing cells in a population of specified T-helper cells (Fig. 1).

*Kinetics of intracellular cytokine production*

The kinetics of IL-4 and IFN- $\gamma$  induction in CD4<sup>+</sup> T cells were analysed in blood from two AD patients after 4, 6, 8, 24, 30 and 48 h of stimulation. Whole blood samples were stimulated, cell stained and analysed as described earlier.

*Detection of IL-4 and IFN- $\gamma$  in supernatant*

Whole blood samples were stimulated, as previously described, with PMA and ionomycin but without BFA. The supernatants were collected 4 h after stimulation and stored at -70°C until analysis. Concentrations of IFN- $\gamma$  and IL-4 were measured using an ELISA kit (Quantikine human IL-4, Quantikine human IFN- $\gamma$ , R & D Systems Europe, UK). The detection range was 31.2–2000 pg/ml for IL-4, and 15.6–1000 pg/ml for IFN- $\gamma$ . Samples with concentrations above the upper range were further diluted and re-analysed.

*Statistical analysis*

Statistical calculations were performed using Mann-Whitney and Kruskal-Wallis tests for between- and within-group comparisons. Values of *P* less than 0.05 were accepted as being statistically significant. For correlations between cytokine production and IgE levels Spearman's rank correlation test (*r<sub>s</sub>*) was used.

**Results**

*Serological data and severity scores*

The demographic data from 64 investigated AD patients are shown in Table 1. In the AD group, consisting of 20 males and 44 females, 38 patients had a history of allergic asthma and/or allergic rhinitis. The severity of the dermatitis estimated according to the grading criteria of Rajka and Langland ranged from 3 to 8 with a mean of 4.4. Nine patients were not scored.

Forty-three patients had a positive Phadiatop® and 19 were negative. None in the control group had a positive Phadiatop®. In two patients Phadiatop® was not analysed. The level of total serum-IgE in the AD patients ranged between 2.7 and 16690 kU/l, mean 136 kU/l, and in the healthy non-atopic group between 3 and 62 kU/l, mean 29.4 kU/l. There was a significant correlation between the severity score and level of total IgE (*n* = 55, *r* = 0.67, *P* < 0.05).

*Kinetics of intracellular cytokine production*

The percentage of CD3<sup>+</sup>/CD4<sup>+</sup> T cells expressing IL-4 and IFN- $\gamma$  after stimulation with PMA/ionomycin for 4, 6, 8, 24, 30 and 48 h was investigated in whole blood samples from two AD patients. The secretion of both IL-4 and IFN- $\gamma$  peaked at 4 h (Fig. 2).

Table 1. Demographic data from 64 patients with atopic dermatitis

Case	Sex	Disease	Age	IgE kU/l	Phadiatop	Severity score
1	F		19	21	Negative	3
2	F		20	14	Negative	3
3	F	AR	24	179	Positive	4
4	M	AR	25	88	Positive	3
5	F	AA	24	233	Positive	Missing
6	M		27	971	Positive	5
7	M		34	89	Positive	3
8	F	AA, AR	26	91	Positive	Missing
9	M		21	94	Positive	3
10	F	AA, AR	43	1980	Positive	Missing
11	F		24	37	Positive	3
12	F	AA, AR	24	42	Positive	Missing
13	M	AR	27	19	Positive	3
14	F		20	25	Negative	3
15	F		30	140	Positive	4
16	F		30	34	Negative	3
17	F	AA, AR	21	491	Positive	4
18	F	AR	29	371	Positive	Missing
19	F	AA	29	566	Positive	6
20	F		21	32	Positive	3
21	F	AA	30	3232	Positive	5
22	F		24	32	Positive	3
23	M	AA, AR	22	301	Positive	Missing
24	F	AA, AR	30	3262	Positive	5
25	F		19	299	Negative	3
26	F	AA, AR	24	93	Positive	Missing
27	F	AA	60	771	Positive	6
28	M		24	481	Positive	4
29	M		44	49	Negative	3
30	F	AA	40	4863	Positive	5
31	F	AA, AR	28	413	Positive	3
32	F	AA, AR	47	3321	Positive	7
33	F		25	11	Negative	4
34	F		21	129	Negative	3
35	M	AA, AR	32	4130	Positive	4
36	F	AR	36	40	Positive	3
37	F	AA	80	35	Negative	4
38	F	AA, AR	47	4000	Positive	Missing
39	F		24	61	Positive	6
40	F	AA, AR	25	5000	Positive	5
41	F	AR	35	131	Negative	5
42	F		21	53	Negative	3
43	M	AA, AR	27	3479	Positive	5
44	F	AR	25	17	Positive	4
45	M	AA, AR	48	3583		8
46	M	AA	28	9630		8
47	M		23	65	Negative	4
48	M	AA	35	2002	Positive	5
49	M	AA, AR	33	5000	Positive	5
50	F	AA, AR	59	5000	Positive	6
51	F		53	2,7	Negative	3
52	M	AR	43	9950	Positive	6
53	F		24	15	Negative	4
54	F		38	41	Negative	3
55	F	AA, AR	26	8260	Positive	8
56	F	AA, AR	43	16690	Positive	8
57	F		55	13	Negative	3
58	M	AR	36	2635	Positive	7
59	M		19	35	Negative	3
60	M		48	14	Negative	3
61	F	AR	40	4895	Positive	8
62	F	AR	20	14	Negative	4
63	F		32	79	Positive	3
64	M	AR	27	196	Positive	Missing

AA = allergic asthma. AR = allergic rhinitis



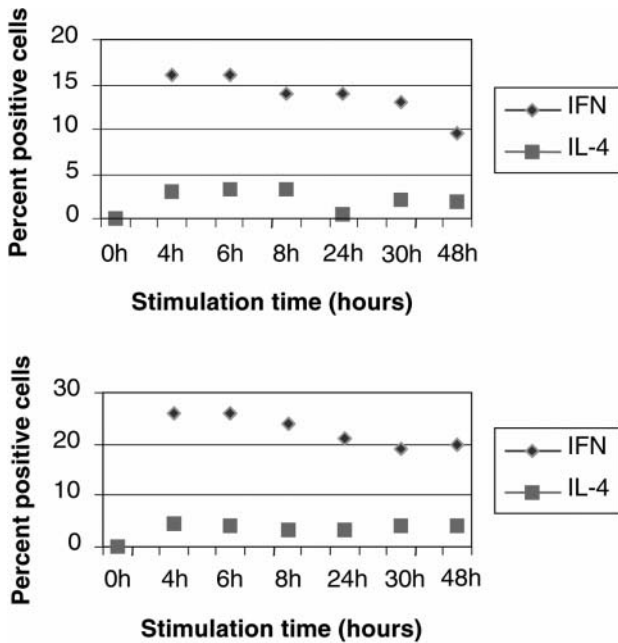


Figure 2. Kinetics of cytokine production. Whole blood samples from two patients with atopic dermatitis (AD) stimulated with phorbol 12-myristate 13-acetate (PMA) ionomycin in the presence of brefeldin.

Flow cytometric detection of IL-4 and IFN-γ in CD4<sup>+</sup> cells

Figure 3 shows the percentage of CD3<sup>+</sup>/CD4<sup>+</sup> IFN-γ<sup>+</sup> and IL-4-producing T cells in the patients with AD and in the healthy control group. The percentage of CD4<sup>+</sup> T cells expressing either IFN-γ or IL-4 were both significantly decreased (IFN-γ  $P < 0.001$ ; IL-4:  $P = 0.0135$ ) in atopic dermatitis patients compared with healthy controls. The proportions of IFN-γ-producing cells among CD4<sup>+</sup> cells ranged from 1.1 to 24% (mean 9.8%) and for CD4<sup>+</sup> IL-4 producing cells from 0.4 to 7.6% (mean 2.5%). Among the 18 subjects in the healthy control group the values for CD4<sup>+</sup> IFN-γ producing cells were between 8.4 and 30% (mean 19.9%) and 1.4–7.6% (mean 3.3%) for CD4<sup>+</sup> IL-4-producing cells.

There was no correlation between either IFN-γ or IL-4 and the severity score of the disease in the patient groups. Furthermore, there was no difference in the percentage of CD3<sup>+</sup>/CD4<sup>+</sup> T cells producing IFN-γ or IL-4 between female and male patients with AD. In addition, there was no relationship between the percentage CD4<sup>+</sup> T cells expressing IFN-γ and IL-4 in PMA-stimulated or ionomycin-stimulated whole blood samples from the patient group and serum IgE or Phadiatop®.

Production of IL-4 and IFN-γ measured by ELISA

In nine patients with AD and in nine healthy controls we measured concentrations of IL-4 and IFN-γ in the supernatant of the whole blood samples stimulated with PMA and ionomycin. We found that the patient group had a significantly decreased production of IFN-γ ( $681.0 \pm 535.7$  pg/ml) compared with that in healthy controls ( $6722.2 \pm 3792.7$  pg/ml) (Table 2). There was no difference in the IL-4 levels.

Discussion

In this study we have analysed the frequency of intracellular-cytokine-producing T cells at the single cell level using a method based on whole blood obtained from 64 patients with AD and compared the results with those from 18 normal control subjects. We found that the AD patients had a significantly lower percentage of CD3<sup>+</sup>/CD4<sup>+</sup> T cells expressing IFN-γ as well as IL-4 compared with the healthy controls. In addition we also measured IFN-γ and IL-4 concentrations in the supernatant after stimulation with PMA and ionomycin but without brefeldin in nine patients and nine healthy controls. The patient group

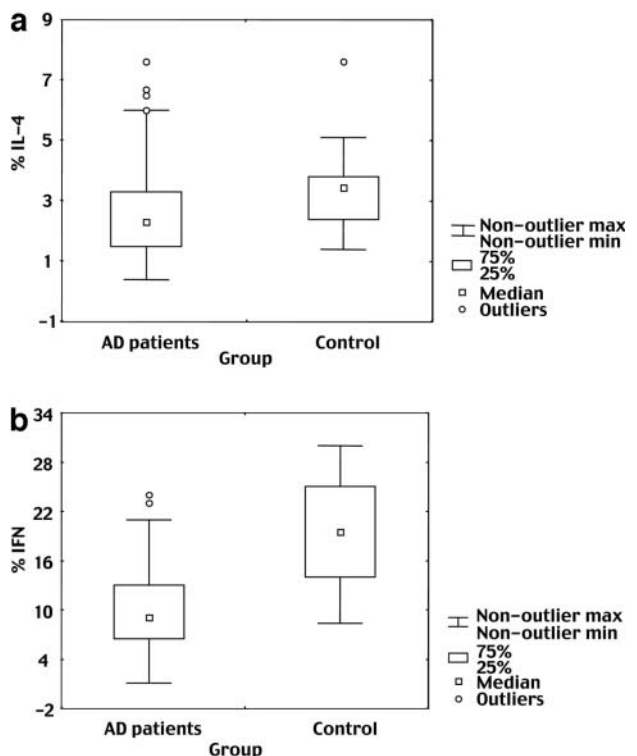


Figure 3. The percentage of CD3<sup>+</sup>/CD4<sup>+</sup> interleukin (IL)-4- (a) and interferon (IFN)-γ- (b) producing T cells in patients with atopic dermatitis (AD) and in healthy controls.

Table 2. Comparison of interleukin-4 and interferon- $\gamma$  production in supernatants from whole blood stimulated with phorbol 12-myristate 13-acetate and ionomycin among atopic dermatitis patients and healthy controls

Subject	AD patients		Healthy controls	
	IL-4 (pg/ml)	IFN- $\gamma$ (pg/ml)	IL-4 (pg/ml)	IFN- $\gamma$ (pg/ml)
1	140	350	42	2400
2	85	420	45	7500
3	69	320	57	4900
4	65	150	430	7000
5	180	720	67	11400
6	46	650	36	1400
7	39	420	290	12900
8	190	1300	94	5100
9	170	1800	97	7900
Mean	109.3	681.1	128.7	6722.2
SD	60.5	535.7	137.4	3792.7

AD = atopic dermatitis

IL = interleukin

IFN = interferon

showed a significantly decreased level of IFN- $\gamma$  compared with the healthy controls. Earlier studies have shown similar results such as reduced IFN- $\gamma$  production after *in vitro* stimulation of PBMCs from patients with AD and from healthy subjects (7,9,10,22). However, all these cited studies are based on measurements of cytokine levels, and although they provide information on the total amount of IL-4 and IFN- $\gamma$  present, they do not take into account the production of cytokines (IL-4 or IFN- $\gamma$ ) by a specific cell type, for example CD4<sup>+</sup> T-cells, CD8<sup>+</sup> T-cells, mast cells or other cell types (23,24). We used flow cytometry to detect intracellular cytokines. With this method we were able to rapidly measure the intracellular cytokine production of thousands of individual cells and to detect different cytokines in the same cell. We were also able to recognise subtypes of T cells.

It has recently been reported that monocytes rather than T cells are the major source of IL-10 in AD, and that monocytes in AD have an excessive production of PGE<sub>2</sub> leading to a decreased IFN- $\gamma$  production (33,34). This indicates that removal of monocytes in T-cell cultures may increase the level of IFN- $\gamma$ . However, higher levels of IFN- $\gamma$  have been demonstrated in whole blood cultures than in conventional cultures of concentrated PBMC (10 × 6/ml) (18,19). We decided to use unseparated whole blood to detect intracellular cytokines, which is in line with the work by Maino et al. (35) and Ferry et al. (15), as this, as accurately as possible, mirrors the conditions *in vivo*. When this technique is used, the T cells do not have to be cloned or exposed to *in vitro* conditions for a long time before examination, and hopefully the results more accurately reflect the situation in circulating cells *in vivo*.

Patients with AD are characterised by enhanced IgE production (25,26). It has been shown that the class switch of IgE and production of IgE in B cells is regulated by cytokines produced by T cells (27,28). In particular, stimulation with IL-4 is necessary. In contrast, IFN- $\gamma$  inhibits IgE synthesis. Therefore enhanced IgE synthesis in patients with AD may be caused by up-regulation of Th2 cytokine-producing cells, especially those that produce IL-4, and IgE synthesis may be suppressed by Th1 cytokine-producing cells, especially IFN- $\gamma$ -producing cells. We could not find any correlation between the total serum IgE level and the frequency of IL-4- or IFN- $\gamma$ -producing CD4<sup>+</sup> T cells in patients with AD. However, it should be noted that even in analysis using T-cell clones from lesional skin of AD patients no correlation was found between the proportion of IL-4-producing cells or IFN- $\gamma$ -producing cells and the total amount of IgE level in serum (24). Our findings provide evidence for a defective ability of peripheral CD4<sup>+</sup> T cells to produce IFN- $\gamma$  in AD patients in contrast to healthy subjects, which is in agreement with previous studies (12–14). The mechanism underlying reduced IFN- $\gamma$  production by CD4<sup>+</sup> T cells in AD patients might be a disease-specific defect in CD4<sup>+</sup> subsets of cells in AD patients, or there might be other inhibitory cytokines that influence the synthesis of IFN- $\gamma$  production. However, these mechanisms need to be further investigated.

We found that CD4<sup>+</sup> T cells in AD patients besides having a reduced IFN- $\gamma$  production have a significantly decreased IL-4 production. Jung et al. (13) and Nakagawa et al. (12) also demonstrated a decreased proportion of IFN- $\gamma$ -producing CD4<sup>+</sup> T cells, but without any significant increase in IL-4 production. The results from these studies are not in line with the results reported by Nakazawa *et al.*, where an increase in frequency of IL-4 but a decrease in IFN- $\gamma$ -producing CD4<sup>+</sup> T cells from AD patients was found (14). Furthermore, Sato et al. showed a decreased ability to produce IFN- $\gamma$  but an enhanced production of type 2 cytokine (IL-4) in AD patients with high levels of IgE (29). The discrepancy between our findings may be the result of differences in the methods used or investigated patient groups. Nine of the patients in this study used medication (terbutalin and salmeterol for inhalation) and only four inhaled low-dose budesonid. However, Jung et al. (13) found that medication could not explain reduced IFN- $\gamma$  production in atopic patients unless they used high-dose steroid therapy (dexamethasone up to 1000 ng/ml), which might contribute to a reduced ability to produce IFN- $\gamma$ .

While our results did not suggest a role for IL-

4 in patients with AD, there are other studies on cytokines such as IL-13 that have been shown to support IgE synthesis (31). Furthermore, in a recently published study by Katagari et al. (32) the levels of mRNA for IL-13 were shown to be significantly greater in PBMC from AD patients than from controls. Therefore it is of interest to monitor other intracellular cytokines in different lymphocyte subsets in order to determine their relationship to IgE synthesis and to AD.

In conclusion, our results obtained at a single cell level support the evidence for a decreased ability of peripheral CD4<sup>+</sup> T cells to produce IFN- $\gamma$  among AD patients. The decreased ability of CD4<sup>+</sup> T cells to produce IL-4 in patients with AD was also demonstrated, as well as poor correlation between IL-4 and IgE. This indicates that other still unknown soluble mediators may be involved in the regulation of IgE production. The mechanism underlying these findings remains to be further elucidated to increase our understanding of the pathogenesis of AD.

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