

Different expression of cytokine and membrane molecules by circulating lymphocytes on acute mental stress in patients with atopic dermatitis in comparison with healthy controls

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Background: Mental stress is believed to induce an exacerbation of atopic dermatitis (AD). Until now, however, only few psychoneuroendocrinologic mechanisms underlying the link between psychological stress and exacerbation or maintenance of AD have been described.

Objective: Our purpose was to conduct an investigation of immunologic parameters in the form of membrane molecules and cytokines with potential relevance for the cutaneous inflammation in an established psychological laboratory stress model. **Methods:** Patients with AD (n = 15) and healthy controls (n = 15) were exposed to mental stress, as described in a previous report. In vitro analyses were completed 1 hour before, immediately after, and 1 hour after mental stress exposure. Lymphocyte subpopulations, the cutaneous lymphocyte-associated antigen (CLA), the membrane molecule CD69⁺ (early activation antigen), and intracellular IL-4, IL-5, and IFN- γ in blood-derived lymphocytes were analyzed by flow cytometry. IL-4 in the supernatant of concanavalin-A-stimulated PBMCs was determined by ELISA.

Results: An increase in heart rate and blood pressure was demonstrated during psychological stress in patients with AD and healthy volunteers. We found significantly higher stress-induced increase of CLA⁺ lymphocytes, T helper cells expressing IL-5, and both CD4⁺ and CD8⁺ lymphocytes expressing IFN- γ on mitogenic stimulation in patients with AD in comparison with healthy controls. In addition, we observed an earlier increase in the secretion of IL-4 in the supernatant of mitogen-stimulated lymphocytes during psychological stress in patients with AD in comparison with healthy volunteers.

Conclusion: A higher stress-induced increase of CLA⁺ cells in the circulation in patients with AD compared to healthy controls might indicate an increased ability of T lymphocytes in AD to migrate to the skin during this psychological condition. In addition, the data of this study suggest a different stress-induced cytokine profile in circulating lymphocytes in patients with AD compared to healthy controls. (*J Allergy Clin Immunol* 2001;108:455-62.)

Key words: Atopic dermatitis, T lymphocytes, IL-4, IL-5, IFN- γ , cutaneous lymphocyte-associated antigen, stress, experimental study

The central role of immunologic abnormalities has been described for atopic dermatitis (AD),¹⁻⁵ a number of studies demonstrating the importance of psychological stress in AD exacerbation in children⁶ and adults.⁷⁻¹¹ Possible psychoneuroendocrinologic mechanisms underlying the link between stress and exacerbation or maintenance of the AD have been described.^{1,12,13} Until now, investigations have focused on endocrinologic factors^{1,12,13} or categorized stress effects on T cells and eosinophils in the circulation by immunophenotype.¹ To date, no studies have examined the functional role of these shifts—eg, the stress-induced alteration of different membrane activation markers relevant in acute and chronic eczema reactions—or of intracellular cytokine profiles.

In particular, membrane molecules (eg, cutaneous lymphocyte-associated antigen [CLA]^{2,14} and early activation antigen [CD69⁺]¹⁵) are relevant parameters characterizing the activation of T lymphocytes. These molecules have not been examined in laboratory stress models for patients with AD. Moreover, until now, stress-induced alterations of cytokines have been studied in healthy persons¹⁶⁻¹⁸ but not in patients with AD.

This study therefore focused on the effects of acute psychological stress on these functionally important immunologic parameters in patients with subacute or chronic AD lesions. First, to characterize immune function, we measured the membrane expression of CLA and CD69 by flow cytometry. Second, intracellular IL-4, IL-5, and IFN- γ of mitogen-stimulated blood lymphocytes were characterized by flow cytometry. Finally, IL-4 in the supernatant of concanavalin-A (Con-A)-stimulated PBMCs was determined by ELISA.

METHODS

Subjects and procedure

Fifteen untreated patients (9 female and 6 male; aged 18-51 years) with AD and 15 healthy control subjects, matched by sex, age, and social status, participated in this study after having given their informed consent. All patients with AD fulfilled the criteria of Hanifin and Rajka.¹⁹ None of the patients or controls had any other chronic disease or received medication such as oral corticosteroids, retinoids, methotrexate, and cyclosporine A. Moreover, controls and

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Supported in part by grants from the Deutsche Forschungsgemeinschaft (SFB 244/C13 and 1756/A6).

Received for publication April 5, 2001; revised June 4, 2001; accepted for publication June 5, 2001.

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0091-6749/2001 \$35.00 + 0 1/85/117800

doi:10.1067/mai.2001.117800

TABLE I. Blood pressure and heart rate values 1 hour before (*Baseline*), during (*Stress*), and 1 hour after (*Follow-up*) stress exposure in patients with AD (n = 15) and healthy controls (n = 15)

Parameter/group	Baseline	Stress	Follow-up	2*3 ANOVA		
				P_G dF = 1, 28	P_T dF = 2, 56	P_I dF = 2, 56
RR systolic (mmHg)				—	<.01	—
Patients with AD	129.8 (± 10.5)	147.5 (± 12.7)	131.2 (± 11.2)			
Healthy controls	127.3 (± 9.6)	144.0 (± 13.2)	126.1 (± 9.0)			
RR diastolic (mmHg)				—	<.01	—
Patients with AD	76.6 (± 13.1)	84.9 (± 12.1)	75.3 (± 11.8)			
Healthy controls	76.2 (± 10.2)	84.1 (± 10.0)	72.1 (± 11.6)			
Heart rate (beats/min)				—	<.01	—
Patients with AD	74.9 (± 10.9)	96.6 (± 18.1)	73.4 (± 10.0)			
Healthy controls	75.3 (± 10.1)	94.3 (± 15.1)	72.1 (± 8.6)			

All values are means (± SDs).

Abbreviations used

- AD: Atopic dermatitis
- Con-A: Concanavalin-A
- CLA: Cutaneous lymphocyte-associated antigen
- NK: Natural killer

patients with alcohol or drug abuse were excluded from the study. All patients had subacute or chronic AD lesions when the stress experiment was performed. Patients with acute flares of AD were excluded from this study.

We used a stress model previously established in our laboratory.¹ Experiments were always conducted between 11:30 AM and 1:30 PM. On arrival in the laboratory, each volunteer was seated and rested for 20 minutes. A butterfly needle was then inserted into an antecubital vein, and a blood pressure cuff was placed on the other arm. Twenty minutes later, a first blood sample (Baseline [B]) was drawn to analyze lymphocyte subpopulations (CD4⁺ T helper cells, CD8⁺ T cells, and natural killer [NK] cells). In patients and healthy controls, acute psychological stress was then induced by public speaking and mental arithmetic. Each subject was asked to prepare a lecture (topic: "Children's position in today's society"). After 10 minutes of preparation, the volunteer gave his or her speech in front of an audience of 8 to 12 people. The speech was interrupted after 5 minutes, and the volunteer was instructed to subtract the number 13 from the number 1022 and to continue subtracting 13 from each subsequent number as fast as possible during the next 5 minutes. Immediately after this induction of an acute psychological stress, a second blood sample (Stress [S]) was drawn. A third blood sample (Follow-up [F]) was drawn 60 minutes after the stress exposure.

Cardiovascular parameters

Blood pressure and heart rate were continuously recorded (Criticon Dinamap, Johnson & Johnson, Norderstedt, Germany) and analyzed parallel to the time points of blood sampling.

Preparation and cultivation of PBMCs

PBMCs were isolated from heparinized blood samples by density centrifugation on Ficoll Hypaque (Nycomed Pharma AS, Oslo, Norway; d = 1.077 g/mL), as described previously.¹

Immunofluorescence staining of PBMCs and flow cytometry

PBMCs were stained with a combination of labeled mAbs and measured by flow cytometry through use of standard techniques.²⁰ Expression of surface antigens on gated lymphocytes was assessed

by flow cytometry through use of CD4 (Immunotech, Hamburg, Germany) and CD8 (DAKO, Hamburg, Germany) antibodies; CD8⁺ lymphocytes were further distinguished by the density of CD8 expression on the cell membranes—ie, CD3⁺CD16⁺CD8^{high} T lymphocytes and CD3⁺CD16⁺CD8^{low} NK cells.²¹ The density of CLA (HECA 452, Pharmingen, Hamburg, Germany) and CD69 (Pharmingen) was detected by conventional flow cytometry. The calculation of the absolute numbers of circulating lymphocyte subpopulations was carried out according to the results of standard white blood counts and differential analysis from blood smears.

Intracellular cytokine staining

PBMCs were stimulated with Con-A for 24 h. Thereafter, the cells were harvested, washed twice in phosphate-buffered saline (PBS), fixed with 4% ice-cold phosphate-buffered paraformaldehyde for 15 minutes at 4°C and washed in PBS. The cells were then permeabilised in PBS with 0.1% saponin for 15 min. Thereafter pretitrated cytokine-specific mAbs (anti-IL-4, anti-IL-5, anti-IFN- γ , all from Pharmingen, Hamburg, Germany) diluted in the permeabilisation buffer (PBS-saponin) were added and incubated for 45 minutes at 4°C. After subsequent washings in the permeabilisation buffer, cells were resuspended in PBS for flow cytometric analysis. The calculation of the absolute numbers of circulating lymphocyte subpopulations was carried out considering the results of standard white blood counts and differential analysis from blood smears.

Determination of IL-4 in the supernatant of stimulated PBMC

PBMC were cultured with 10g/mL Con-A. Supernatants were collected at 48 hours for assessment of IL-4. IL-4 was determined by ELISA (R and D Systems, Wiesbaden, Germany).

Statistical analysis

Group (patients with AD or healthy controls) $\times$ time analyses were calculated by 2-factor ANOVAs. For some analyses, the sample sizes were reduced by 1 case. A *P* value of <.05 was considered statistically significant.

RESULTS

Public speaking and mental arithmetic induced significant elevations of blood pressure and heart rate both in patients with AD and in healthy volunteers (P_T < .01 for time effect; Table I). These results indicate that the chosen laboratory stress model induced significant psychological arousal.^{1,22,23}

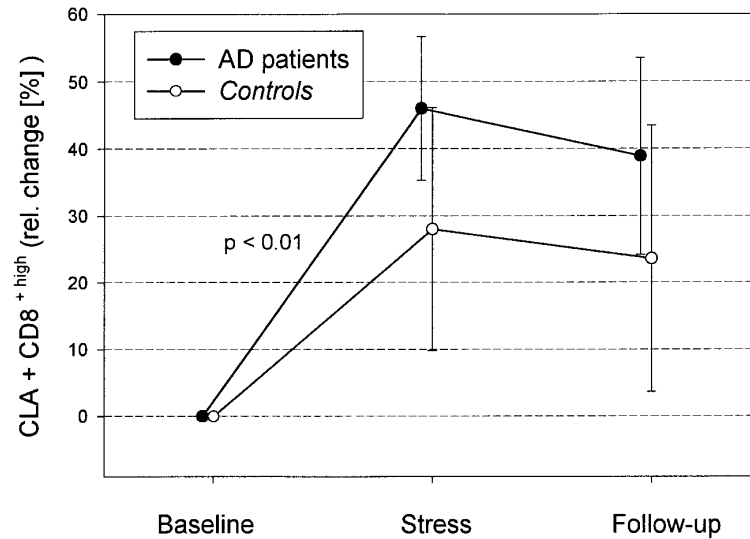


FIG 1. Changes relative to baseline of CLA⁺CD8⁺ lymphocytes (expressed as percent $\pm$ SEs) during (Stress) and 1 hour after (Follow-up) stress exposure in patients with AD (solid circles) and healthy controls (open circles). Group changes were calculated as means of individual changes in percent.

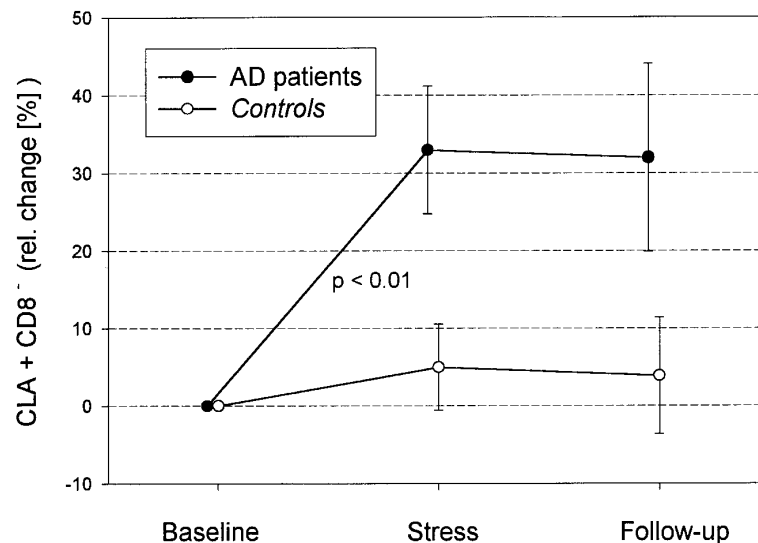


FIG 2. Changes relative to baseline of CLA+CD8⁻ lymphocytes (expressed as percent $\pm$ SEs) during (Stress) and 1 hour after (Follow-up) stress exposure in patients with AD (solid circles) and healthy controls (open circles). Group changes were calculated as means of individual changes in percent.

Phenotypic changes

Confirming our previous findings,¹ the stress situation induced a significant increase of CD8⁺ T lymphocytes in patients with AD ($P[T]_{B-S} < .05$; Table II) but not in healthy volunteers; CD 16⁺ (ie, CD8^{low}) NK cells increased significantly on psychological stress in both groups (data not shown).

A significant stress-induced increase was observable for the CLA+CD8⁺ (T and NK) lymphocytes (Table II). A further differentiation revealed that the increase was significant in the CLA+CD8⁺ T-lymphocyte subpopulation in patients with AD (Fig 1 and Table II) but not in healthy volunteers. In addition, a significant increase of CLA+CD8⁻

lymphocytes (Fig 2 and Table II) was observed for patients with AD ($P[T]_{B-S} < .01$) but not for healthy controls. Furthermore, a significant interaction effect for CLA+CD8⁻ cells could be shown ($P_I < .05$), indicating a significantly different course of the cell counts over time between patients with AD and healthy volunteers.

The following lymphocyte subpopulations bearing the membrane molecule CD69, which is an early activation marker, showed significant alterations over the 3 measurements for patients with AD and healthy controls induced by the experimental design: CD8⁺CD69⁺ cells ($P_T < .01$ for time effect); CD8⁺CD69⁺ T lymphocytes ($P_T < .05$); and CD8⁻CD69⁺ cells ($P_T < .05$; Table II). However, this

TABLE II. Absolute numbers of CD8^{high} cells and different CLA⁺ lymphocyte subgroups (cells/ μ L) 1 hour before (*Baseline*), immediately after (*Stress*), and 1 hour after (*Follow-up*) stress exposure in patients with AD (n = 15) and controls (n = 15)

Membrane activation marker	Lymphocyte subset/group	Baseline (B)	Stress (S)	Contrasts B-S: P(T) _{B-S}	Follow-up (FU)	Contrasts S-FU: P(T) _{S-FU}	1*3 ANOVA	2*3 ANOVA		
							P(T) _{B-S-FU} df = 2	P _G dF = 1, 28	P _T dF = 2, 56	P _I dF = 2, 56
CLA	CD8 ^{high}									
	Patients with AD	370.6 ($\pm$ 167.3)	504.3 ($\pm$ 274.6)	0	422.1 ($\pm$ 132.9)	NS	NS	—	<.05	—
	Healthy controls	339.2 ($\pm$ 168.2)	366.4 ($\pm$ 192.1)	NS	338.5 ($\pm$ 181.6)	NS	NS			
	CD8 ⁺									
	Patients with AD	158.5 ($\pm$ 90.0)	226.3 ($\pm$ 111.8)	<.01	184.5 ($\pm$ 84.8)	<.01	<.01	—	<.01	—
	Healthy controls	164.5 ($\pm$ 71.9)	192.5 ($\pm$ 86.1)	NS	164.7 ($\pm$ 95.8)	NS	NS			
CD69 ⁺	CD8 ^{high}									
	Patients with AD	49.0 ($\pm$ 46.4)	60.7 ($\pm$ 42.7)	<.01	58.4 ($\pm$ 48.7)	NS	<.05	—	<.05	—
	Healthy controls	50.3 ($\pm$ 38.5)	55.2 ($\pm$ 45.9)	NS	53.8 ($\pm$ 41.6)	NS	NS			
	CD8 ⁻									
	Patients with AD	161.7 ($\pm$ 102.4)	203.3 ($\pm$ 116.5)	<.01	191.6 ($\pm$ 109.5)	NS	<.01	—	—	<.05
	Healthy controls	210.1 ($\pm$ 116.5)	210.2 ($\pm$ 93.1)	NS	199.0 ($\pm$ 73.8)	NS	NS			
CD69 ⁺	CD8 ⁺									
	Patients with AD	21.4 ($\pm$ 12.5)	32.6 ($\pm$ 26.7)	NS	18.9 ($\pm$ 13.1)	<.01	<.01	—	<.01	—
	Healthy controls	24.9 ($\pm$ 15.1)	31.5 ($\pm$ 21.0)	NS	18.2 ($\pm$ 11.7)	<.01	<.01			
	CD8 ^{high}									
	Patients with AD	10.7 ($\pm$ 7.2)	12.8 ($\pm$ 8.1)	NS	10.2 ($\pm$ 6.0)	<.05	NS	—	<.05	—
	Healthy controls	8.9 ($\pm$ 5.0)	9.0 ($\pm$ 4.6)	NS	7.4 ($\pm$ 4.1)	NS	NS			
	CD8 ⁻									
	Patients with AD	13.0 ($\pm$ 7.1)	16.8 ($\pm$ 12.1)	NS	11.8 ($\pm$ 6.6)	<.05	NS	—	<.05	—
	Healthy controls	18.5 ($\pm$ 15.4)	19.3 ($\pm$ 12.3)	NS	15.1 ($\pm$ 11.4)	NS	NS			

All values are means ($\pm$ SDs).

NS, Not significant.

was primarily due not to a stress-induced increase but rather to a decrease after stress exposition (Table II).

Changes in cytokine expression and secretion

No significant stress-induced increases of lymphocytes expressing intracellular IL-4 were observed (Table III). Nevertheless, examination of IL-4 levels in the supernatant of Con-A–stimulated PBMCs showed significant stress-induced changes over the 3 measurements for patients with AD and healthy controls ($P_T < .01$ for time effect; Table III). However, the PBMCs of atopic individuals produced a stress-induced course of IL-4 somewhat different from that of controls: the increase of

IL-4 in patients with AD occurred immediately after stress (remaining high for 1 hour after the stress), whereas this level was attained in the healthy controls only in the follow-up (Table III).

The stress situation induced an increase of IL-5–positive CD4⁺ T-helper cells in patients with AD only ($P[T]_{B-S} < .05$; Fig 3 and Table III). No significant increases were detectable in IL-5–positive CD8⁺ and CD8^{high} lymphocytes on psychological stress in either group (Table III).

An increase in the numbers of CD4⁺ and CD8⁺ cells but not in the numbers of CD8^{high} cells positive for IFN- γ after stress was revealed for patients with AD ($P[T]_{B-S} < .05$; Table III) but not for healthy volunteers.

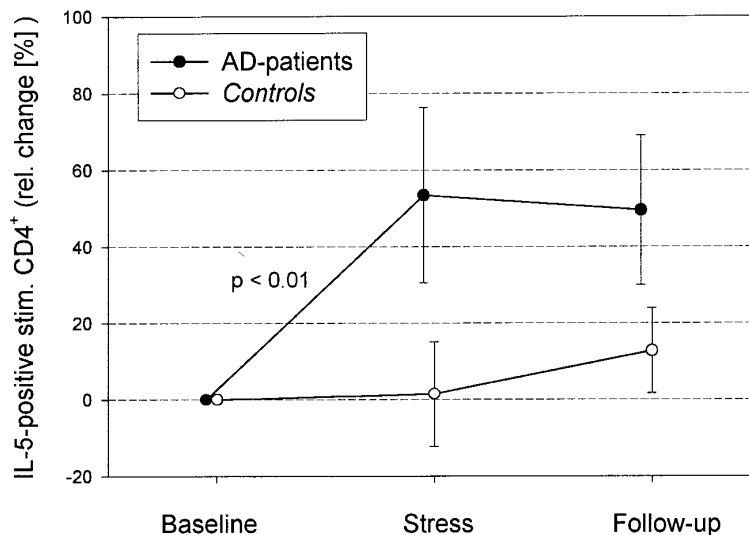


FIG 3. Changes relative to baseline of IL-5-positive stimulated CD4⁺ cells (expressed as percents $\pm$ SEs) during (*Stress*) and 1 hour after (*Follow-up*) stress exposure in patients with AD (*solid circles*) and healthy controls (*open circles*). Group changes were calculated as means of individual changes in percent.

DISCUSSION

In this investigation, we extended previous data showing that immunologically, patients with AD and healthy controls react differently after acute psychological stress. In the present study, we investigated lymphocyte-associated immune parameters, focusing on T lymphocytes, which account for the majority of skin-infiltrating cells that play a prominent role in this skin disease.^{5,24,25}

A number of studies indicate that AD might be a systemic disease reflected by some abnormalities of T lymphocyte numbers and functions not only in the inflamed skin but also in the circulation. It is well known that the number of CD4⁺ cells is increased and the number of CD8⁺ suppressor/cytotoxic lymphocytes decreased in peripheral blood of patients with AD.^{5,24,25} However, we recently showed that psychological stress leads to significantly higher increases in the number of circulating CD8⁺ T lymphocytes in patients with AD in comparison with healthy controls.¹ This observation was confirmed by the current data.

In addition, this study demonstrates for the first time significant stress-induced increases in circulating CLA⁺ lymphocytes in patients with AD but not in healthy controls. The CLA molecule is thought to be involved in selective homing of T cells into inflamed skin, because it mediates binding to E-selectin (CD62E), which is expressed on activated endothelial cells, preferentially in cutaneous vessels. More than 80% of skin-infiltrating T lymphocytes express the CLA molecule.²⁶

In recent years, a number of studies have addressed the characterization of CLA⁺ lymphocytes and the regulation of this membrane molecule. Intracellular cytokine staining revealed that CLA⁺ cells contain high amounts of IL-13 and IL-5 in patients with AD.²⁷ The expression of CLA has been found to be upregulated through super-

antigen induction of IL-12.² IL-12 and/or staphylococcal superantigen stimulation upregulates CLA expression even on T_H2 cells.²⁷ Membrane expression of CLA, which is a carbohydrate molecule, is dependent on the activity of α -fucosyltransferase (FucT-VII).²⁸

It is unknown whether psychological stress leads to a rapid increase of enzyme activity of FucT-VII, leading to higher CLA expression on circulating lymphocytes, or whether CLA⁺ lymphocytes are simply redistributed during stress, leading to higher numbers of these cells in peripheral blood. There is, however, a high probability that the increase of CLA⁺ lymphocytes in the blood is associated with a greater number of cells that can subsequently adhere to CD62E⁺ endothelial cells in cutaneous vessels.

We found an increase of CLA⁺ lymphocytes not only for the CD8⁺ lymphocyte population (which may contain subgroups of T helper cells, B cells, and NK cells) but also for CD8⁺ T lymphocytes on psychological stress in patients with subacute or chronic AD lesions. Interestingly, CLA⁺CD8⁺ T cells isolated from the circulation were recently shown to be as potent as CLA⁺CD4⁺ T cells in the induction of IgE and enhancement of eosinophil survival, which suggests that these cells have more than bystander functions in AD.²⁹

In addition, we studied the CD69 antigen, which is rapidly induced on the membranes of cytokine or mitogen-activated lymphocytes.³⁰⁻³² It is also induced after stimulation of the T-cell receptor/CD3 complex in T cells and by other stimuli.^{33,34} In T cells, CD69 transcripts are already detectable 30 to 60 minutes after T-cell receptor stimulation and decline rapidly after 4 to 6 hours.³⁵ Surface expression can be detected by 2 to 3 hours and is maximal on stimulation with lectins after 24 hours.³⁶ We previously found a fast increase of the CD69 antigen on antigen- or allergen-activated T lymphocytes and on NK cells in cell cultures stimulated with antigens in patients

TABLE III. Absolute numbers of cytokine-positive cells (in cells/ μ L) and IL-4 concentrations (pg/mL) 1 hour before (*Baseline*), immediately after (*Stress*), and 1 hour after (*Follow-up*) stress exposure in patients with AD (n = 15) and controls (n = 15)

Membrane activation marker	Lymphocyte subset/group	Baseline (B)	Stress (S)	Contrasts B-S: $P(T)_{B-S}$	Follow-up (FU)	Contrasts S-FU: $P(T)_{S-FU}$	1*3 ANOVA	2*3 ANOVA		
							$P(T)_{B-S-FU}$ df = 2	P_G dF = 1, 28	P_T dF = 2, 56	P_I dF = 2, 56
IL-4										
	CD4+ Patients with AD	41.2	44.1 ($\pm$ 28.8)	NS ($\pm$ 20.5)	43.2	NS ($\pm$ 16.1)	NS	—	—	—
	Healthy controls	40.7	33.2 ($\pm$ 30.8)	NS ($\pm$ 17.5)	34.2	NS ($\pm$ 16.9)	NS			
	CD8+ Patients with AD	12.9	21.7 ($\pm$ 7.4)	NS ($\pm$ 35.1)	12.2	NS ($\pm$ 4.7)	NS	—	—	—
	Healthy controls	14.6	13.1 ($\pm$ 10.9)	NS ($\pm$ 8.3)	10.2	NS ($\pm$ 7.3)	NS			
	CD8+high Patients with AD	5.9	6.5 ($\pm$ 4.2)	NS ($\pm$ 3.6)	6.6	NS ($\pm$ 3.0)	NS	—	—	—
	Healthy controls	7.0	6.4 ($\pm$ 8.8)	NS ($\pm$ 6.0)	5.4	NS ($\pm$ 5.3)	NS			
IL-4* (ELISA)										
	Patients with AD	— 17.0	24.1 ($\pm$ 10.3)	<.01 ($\pm$ 18.4)	26.2	NS ($\pm$ 15.7)	<.01	—	<.01	—
	Healthy controls	13.2	15.4 ($\pm$ 10.5)	<.01 ($\pm$ 12.5)	20.0	<.01 ($\pm$ 14.8)	<.01			
IL-5										
	CD4+ Patients with AD	13.8	20.2 ($\pm$ 10.8)	<.05 ($\pm$ 18.7)	19.3	NS ($\pm$ 17.2)	NS	—	—	—
	Healthy controls	10.6	8.6 ($\pm$ 6.6)	NS ($\pm$ 3.7)	9.8	NS ($\pm$ 3.5)	NS			
	CD8+ Patients with AD	3.8	6.0 ($\pm$ 4.0)	NS ($\pm$ 7.0)	5.5	NS ($\pm$ 4.8)	NS	—	—	—
	Healthy controls	4.0	3.8 ($\pm$ 3.0)	NS ($\pm$ 2.8)	2.4	NS ($\pm$ 1.9)	NS			
	CD8+high Patients with AD	2.4	2.8 ($\pm$ 2.8)	NS ($\pm$ 2.9)	3.1	NS ($\pm$ 3.6)	NS	—	—	—
	Healthy controls	1.7	1.2 ($\pm$ 1.3)	NS ($\pm$ 1.4)	0.9	NS ($\pm$ 1.2)	NS			
IFN- γ										
	CD4+ Patients with AD	127.5	166.4 ($\pm$ 61.7)	<.05 ($\pm$ 96.1)	166.9	NS ($\pm$ 74.0)	NS	—	—	—
	Healthy controls	131.0	134.0 ($\pm$ 78.0)	NS ($\pm$ 114.5)	134.6	NS ($\pm$ 88.5)	NS			
	CD8+ Patients with AD	210.6	350.1 ($\pm$ 114.9)	<.05 ($\pm$ 196.7)	212.4	<.01 ($\pm$ 104.7)	<.01	—	<.01	—
	Healthy controls	259.9	348.9 ($\pm$ 156.5)	NS ($\pm$ 212.3)	186.6	<.01 ($\pm$ 127.4)	<.01			
	CD8+high Patients with AD	110.0	163.9 ($\pm$ 70.4)	NS ($\pm$ 87.7)	125.3	<.01 ($\pm$ 57.2)	<.01	—	<.01	—
	Healthy controls	112.3	148.3 ($\pm$ 90.6)	NS ($\pm$ 153.0)	107.5	NS ($\pm$ 99.0)	NS			

All values are means ($\pm$ SDs).

NS, Not significant.

*Concentration in the supernants of Con-A-stimulated lymphocytes (pg/mL).

with AD and other patients.³⁰ We presently found a significant alteration of CD69 expression both for patients with AD and for healthy controls during the time course in our stress experiments. However, this was primarily due not to a stress-induced increase but rather to a decrease after stress exposition. One might speculate that some CD69⁺ lymphocytes might have left the circulation 1 hour after acute psychological stress.

Activated T lymphocytes secrete a variety of cytokines that modulate the inflammatory reaction in lesional skin. We found an increase of IL-4 secretion (but not of intracellular IL-4 expression in stimulated CD4⁺ cells or stimulated CD8⁺ T lymphocytes) from mitogen-stimulated T cells isolated from the circulation after psychological stress. These increases were observable both in controls and in patients; however, the increases of IL-4 were revealed earlier in patients with AD than in healthy volunteers. IL-4 functions as an IgE isotype switching factor³⁷ and upregulates important membrane molecules such as CD40, IL-4R, and CD23 on B cells.^{5,25} Importantly, IL-4 inhibits the expression of the IL-12 β 2 subunit on T cells, which is the binding and signal transducing component of the IL-12 receptor, thus strengthening a T_H2 response.³⁸ Furthermore, IL-4 might stimulate the production of eotaxin,³⁹ which is involved in the chemoattraction of eosinophils and other cells. Analysis of mitogen- and allergen-stimulated T cells from peripheral blood demonstrated a dysregulation of cytokine secretion, with an overproduction of IL-4 in patients with AD.^{40,41} Thus, a faster increase in the secretion of IL-4 on stress in AD might affect both lymphatic organs (containing IgE expressing B lymphocytes) and the cutaneous site.

In contrast to IL-4, which is found mainly in acute eczema,⁴² the number of IL-5 expressing cells increase during chronification of AD.⁴³ The major effect of a higher IL-5 expression during the transition from acute to chronic AD is probably related to eosinophil function and survival.^{44,45} We presently observed specific increases of circulating T lymphocytes that are able to express IL-5 on psychological stress in patients with subacute or chronic lesions of AD. From our data, we cannot conclude that stress leads to higher expression of this cytokine or determine whether a lymphocyte subpopulation able to express IL-5 on activation is distributed from other sites of the body into the circulation on mental stress in patients. Moreover, the increase in lymphocytes expressing IL-5 in blood does not necessarily point to similar mechanisms in the skin of the same patients. There is, however, a functional link between observation of a higher IL-5 expression in blood-derived lymphocytes from patients with AD on stress and the rise in eosinophils on psychological stress in patients with AD.¹

In this study we also investigated cytokine IFN- γ , which is overexpressed in chronic lesions of AD.^{46,47} The stress-induced increases of CD4⁺ and CD8⁺ cells expressing IFN- γ were statistically significant in patients with AD but not in healthy controls. We observed a comparable but nonsignificant trend in the subgroup of CD8^{high} T lymphocytes expressing IFN- γ . We can there-

fore conclude that in addition to IFN- γ ⁺ T helper cells and possibly cytotoxic T cells, IFN- γ ⁺CD8^{low} NK cells increase in number during acute psychological stress in patients with AD. IFN- γ might have a variety of profound effects on the perpetuation and enhancement of the inflammatory reaction in the skin in AD (eg, induction of MHC class I and class II molecules, induction of adhesion molecules such as ICAM-1, and induction of cytotoxic responses and apoptosis²⁵). Further studies must examine whether there is a significant increase of IFN- γ expressing CD8⁺ T lymphocytes after a poststress period clearly longer than 1 hour. In addition, the relevance to AD pathophysiology of short-term stress-induced upregulation for IFN- γ in intravascular cell populations, especially in CD4⁺ cells, should be investigated.

In summary, our data show that laboratory stress induces comparable increases in cardiovascular parameters and levels of the stress hormone noradrenaline in patients with AD and healthy controls. In contrast, patients with AD show a higher stress-induced increase in the number of circulating CD8⁺ T lymphocytes and a different cytokine profile of circulating lymphocytes in comparison with healthy controls. Moreover, the finding of a higher percentage of CLA⁺ cells in the circulation induced by psychological stress in patients with AD compared to healthy controls provides first evidence of a possible functional link between changes in lymphocytes in the circulation on psychological stress and the cutaneous processes in the context of inflammation in AD. Further studies should examine whether mental stress produces comparable alterations in lesional skin of patients with AD.

We thank Mrs Petra Kienlin for her excellent technical assistance, and we dedicate this article to Professor Friedhelm Lamprecht on his 60th birthday.

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