Stress-induced immunomodulation is altered in patients with atopic dermatitis

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Abstract

Atopic dermatitis (AD) is a chronically relapsing inflammatory skin disease with main symptoms such as eczematous skin lesions and severe pruritus. Although the relevance of stress in the pathology of AD is widely accepted, the underlying biological mechanisms of stress-induced exacerbation of AD symptoms are not fully understood. The specific goal of the present study was to investigate the impact of acute psychosocial stress on atopy-relevant immune functions in AD sufferers. AD patients (n = 36) and nonatopic controls (n = 37) were exposed to a laboratory stressor including a free speech and mental arithmetic tasks in front of an audience (“Trier Social Stress Test,” TSST). Blood samples were collected 10 min before and 1, 10 and 60 min after the stress test as well as 24 h after the experiment at identical time points under resting conditions. Analyses of leukocyte subsets indicated significantly elevated lymphocyte, monocyte, neutrophil and basophil numbers 10 min after the TSST (all p’s < 0.001) with no significant differences between the two groups. In contrast, eosinophil number was found to be significantly elevated only in AD sufferers, but not in control subjects (F(3,213) = 4.8; p < 0.01). Moreover, AD patients but not the control group showed increased IgE levels (F(1,71) = 4.4; p < 0.05) 24 h after the stress test. Exposure to the TSST resulted in elevation of interferon-γ (IFN-γ; F(3,207) = 19.55; p < 0.001) and, further, in attenuation of interleukin-4 (IL-4; F(3,207) = 187.46; p < 0.001) concentrations with no significant differences between both groups (all p’s > 0.05). The present findings suggest that stress may be associated with atopy-relevant immunological changes in AD sufferers, which may be one explanation of the common observation of stress-induced aggravation of symptomatology in this patient group.

Keywords: Stress; Immunomodulation; Atopic dermatitis

1. Introduction

Atopic dermatitis (AD) is a chronic, relapsing inflammatory skin disease characterized by eczematous skin lesions, erythema and intense pruritus. AD presents in early childhood, with a disease onset before the age of 5 in approximately 90% of patients. Epidemiological data indicate that the prevalence of AD has been steadily increased during World War II, affecting now more than 12% of the children in the western world (Boguniewicz, 1997; Nimmagadda and Evans, 1999). Endogenous and environmental factors such as genetic disposition, climate, allergens or microbial organisms (i.e., Staphylococcus aureus) are critical in determining the disease (Werfel and Kapp, 1998). However, research of the last decade strongly suggests an underlying immunoregulatory abnormality in AD.

In AD patients, initial exposure to environmental allergens leads to B cell activation and hypersecretion of allergen-specific immunoglobulin E (IgE). Subsequent cross-binding of IgE molecules located on basophils and mast cells by the allergen leads to the release of vasoactive and proinflammatory mediators inducing hypersensitivity reaction of the skin and initiating an IgE-dependent late-phase (inflammatory) allergic response (Leung and Soter, 2001; Eedy, 2001). It was found that surface-bound IgE enables Langerhans cells and monocytes to process and present allergens in the skin, resulting in recruitment of T helper (TH) cells. It was found that allergen-specific TH cells that are cloned from AD skin lesions secrete high amounts of interleukin-4 (IL-4), interleukin-5 (IL-5) and interleukin-13 (IL-13), but not interferon-γ (IFN-γ),...
reflecting a predominantly TH2 cytokine-secreting profile. The dominance of TH2-like cytokines plays a major role in the pathogenesis of AD. IL-4 and IL-13 stimulate IgE synthesis and induce B cells to switch from other Ig isotypes to IgE. They further stimulate the expression of VCAM-1, an adhesion molecule involved in the recruitment of eosinophils into sites of allergic tissue inflammation (Leung, 1999; Leung and Soter, 2001). Eosinophils are inflammatory cells that are found to be commonly associated with tissue damage in atopy. IL-5 further augments eosinophil production and stimulates eosinophils to secrete toxic proteins (i.e., major basic protein, eosinophilic cationic protein), which are known to contribute to tissue injury through its cytotoxic properties and by its ability to stimulate basophil and mast cell degranulation (Martin et al., 1996).

Besides profound immunoregulatory abnormalities, an increasing number of studies underscore the importance of psychological factors such as stress in the maintenance and chronification of AD symptoms (Buske-Kirschbaum et al., 2001; Tausk and Nousari, 2001). Early studies suggest that in 50–70% of AD patients, severe shock, worry or emotional upset precedes onset or aggravation of the disease (Wittkower and Russel, 1953; Brown, 1972). These observations are in line with more recent findings demonstrating a significant positive relationship between interpersonal stress on a given day and skin condition 24 h later (King and Wilson, 1991). The significance of psychosocial stress in the maintenance and exacerbation of AD is further emphasized by the effectiveness of psychotherapeutic interventions including stress management or relaxation techniques, which have been shown to lead to significant improvement of AD symptomatology (Melin et al., 1986; Ehlers et al., 1995).

Although there is general agreement on the existence of stress influences on AD, the underlying (psycho)biological mechanisms of how stress may affect AD pathology remain to be defined. Accumulating data from psychoneuroimmunology strongly suggest that life stressors, such as bereavement, divorce, overburden by job demands or caregiving for significant others, are linked to significant changes in immune function (Kiecolt-Glaser, 1999). Interestingly, stress also appears to condition atopy-relevant immune responses such as IgE production, degranulation of cutaneous mast cells, leukocyte adhesion, secretion of TH1/TH2-derived cytokines or eosinophil activation (Persoons et al., 1995; Singh et al., 1999; Goebel and Mills, 2000; Paik et al., 2000; Schmid-Ott et al., 2001).

The specific goal of the present study was to investigate the impact of psychosocial stress on atopy-relevant immune parameters and disease severity in patients suffering from AD. It was assumed that in response to stress, AD sufferers may exhibit pathologically relevant immune changes, which may promote allergic inflammation and exacerbation of the disease.

2. Materials and methods

2.1. Subjects

Thirty-six patients (18 men, 18 women; age range: 20–33 years; mean age: 25.0 ± 3.8 years) with AD were recruited through advertisements in a local newspaper and by local dermatologists. All patients were clinically diagnosed with AD and fulfilled the diagnostic criteria established by Hanifin and Rajka (1980). Only patients with a minimum history of AD for 5 years were included. All patients were using topical emollients but no corticosteroids. AD patients suffering from other chronic diseases than AD were excluded from the study. Severity of AD symptoms was determined using the Costa score (Costa et al., 1989). Patients’ mean score was 28.9 ± 16.5, indicating a moderate clinical activity of skin lesions in our AD subjects. For a control group, age- and sex-matched nonatopic subjects (n = 37; 19 men, 18 women; age range: 20–33 years; mean age: 24.5 ± 3.4 years) participated in the study. None of the control subjects had ever suffered from atopy or had a family history of atopy. All control subjects were medication-free and did not suffer from an acute or chronic illness. To control for a potential effect of sex hormones on immunological measures, female AD patients and female control subjects were matched for menstrual cycle phase.

The subjects were told that the study was designed to investigate the effect of acute emotional stress on disease-relevant endocrine and immunological parameters. Participants received a compensation of DM 200 upon completion of the experiment.

2.2. Procedure

All subjects were studied on two consecutive days; experimental sessions were run between 10:00 a.m. and 12:00 a.m.

On experimental day 1, a catheter (Vasofix®; Braun-Melsungen, Germany) was inserted in an antecubital vein 40 min before the experimental treatment. After a rest period of 30 min, a first blood sample was obtained at −10 min. Ten minutes thereafter, all subjects were exposed to the ‘Trier Social Stress Test’ (TSST), which has been described and evaluated elsewhere (Kirschbaum et al., 1993). Briefly, the TSST is a standardized laboratory stressor that mainly consists of a free speech (job interview) and mental arithmetic tasks (serial subtraction) in a role-playing approach in front of an audience. Before the stress test, the subjects received a short introduction to the forthcoming tasks followed by a rest period (2 min) to prepare for their speech. Subjects were then exposed for 4 min to the public-speaking task and 4 min to the mental arithmetic tasks, respectively. In previous studies, the TSST has been repeatedly shown to induce significant activation of the HPA axis, with two- to threefold increases of free cortisol levels (Kirschbaum et al., 1995, 1996). Additional blood samples were drawn 1, 10,
20, 30 and 60 min after the stress test. Finally, all subjects completed a 10-item, five-point visual analog scale of how stressful to them were the free speech and the mental arithmetic tasks in front of the audience.

In addition to the immunological data, cortisol, ACTH and catecholamines were determined before and after the TSST as additional endocrine stress markers. Heart rates were monitored continuously at 1-min intervals during the experiment. The effect of the TSST on these parameters is summarized elsewhere (Buske-Kirschbaum et al., in press).

Experimental day 2 served as a control day. All subjects were treated as described for day 1, except that the subjects were not exposed to the TSST on this day. The experimental protocol was approved by the local ethics committee and written informed consent was obtained from all subjects before participating in the experiment.

2.3. Symptom severity (Costa score)

The Costa score represents a simple and reproducible scoring system of AD (Costa et al., 1989) and consists of scoring 10 criteria reflecting the severity of AD (erythema, oedema, vesicles, crusts, excoriations, scales, lichenifications, pigmentation, pruritus, loss of sleep) and 10 topographic sites (feet, knees, legs, hands, arms, face, scalp, buttock, anterior and posterior aspects of the trunk). The severity criteria were scored from 0 (no lesion) to 7 (extremely severe). For the topography item, each of the severity criteria were scored from 0 to 7. For the 10 criteria reflecting the severity of AD, erythema, oedema, vesicles, crusts, excoriations, scales, lichenifications, pigmentation, pruritis, loss of sleep). The topography item in involvement. The total of the severity criteria (score I) reflects AD severity; the total of the topography item reflects the area of the involved skin (score II). The sum of scores I and II represents the total score (score III) reflecting general disease severity.

2.4. Leukocyte subsets

Venous blood was collected in EDTA tubes 10 min before \( t_1 \) and 1 \( t_2 \), 10 \( t_3 \) and 60 min \( t_4 \) after the TSST on day 1 and at identical time points on day 2, respectively. Blood cell counts were determined using a hematology analyser (Technikon H3; Bayer Diagnostics, Germany). The variation coefficients of the cell populations are <14%.

2.5. IgE

A blood sample was obtained 10 min before the TSST and at an identical time point on day 2. Total serum IgE concentrations were determined using an enzyme-linked immunosorbent assay (ELISA; IBL, Hamburg, Germany) according to the manufacturer’s instructions.

2.6. Cell cultures

PBMCs were isolated from heparinized blood samples by Ficoll (Pharmacia, Freiburg, Germany) gradient centrifugation, washed three times in RPMI 1640 (Biochrom, Berlin, Germany) and resuspended at 1 \( \times 10^6 \) cells/ml in RPMI 1640 supplemented with 10% fetal calf serum (FCS; Biochrom). A total of 1.5 \( \times 10^6 \) cells were stimulated with 5 \( \mu \)g/ml PHA (Murex Diagnostica, Burgwedel, Germany) in 24-well microtiter plates at 37 °C in a 5% CO₂ humidified atmosphere. After 48 h, supernatants were harvested and stored at −80 °C until processed for cytokine measurement.

2.7. Cytokines (IL-4, IFN-γ)

Blood samples were obtained 10 min before and 10, 30 and 60 min after the TSST and at identical time points on day 2. IL-4 and IFN-γ concentrations were determined by use of enzyme-linked immunosassays (IL-4: OptEIA, Pharmingen, Hamburg, Germany; IFN-γ: Quantigen™, Pharmingen) with a detection limit of 4 pg/ml for IL-4 and 0.04 4 U/ml for IFN-γ, respectively. The 96-well microtiter plates were coated with a purified monoclonal mouse antihuman IL-4 or IFN-γ diluted in carbonate buffer. After overnight incubation at room temperature, plates were washed three times with phosphate-buffered saline (PBS) plus 0.05% Tween 20. Wells were blocked with PBS plus 10% FCS for 1 h. After washing, supernatants were incubated in duplicates for 2 h at room temperature with parallel IL-4 or IFN-γ standards, respectively. For IL-4 determination, plates were washed and incubated for 1 h with a horseradish peroxide-conjugated monoclonal rat antihuman IL-4 (Pharmingen), diluted 1:250 in PBS plus 10% FCS. After washing, the plates were developed with substrate solution. To determine IFN-γ, the detection antibody was prepared 15 min before use by mixing a monoclonal biotinylated mouse antihuman IFN-γ, diluted in PBS plus 10% FCS at a concentration of 0.36 μg/ml with an avidin–horseradish peroxidase enzyme reagent.

After a 30-min incubation, 1 N H₂SO₄ was added to each well to stop reaction. Plates were read on a spectrophotometer at wavelength 450 nm. Levels of IL-4 and IFN-γ were determined from the optical density readings at 450 nm using Dynatech-MRX (Denkendorf). The intraassay and interassay variations for IL-4 and IFN-γ were <10%, as indicated by the manufacturer.

2.8. Statistical analysis

For all immune parameters with repeated measures (leucocyte subpopulations, IL-4, IFN-γ), ANOVAs were computed on the absolute cytokine levels or cell counts to test for stress-induced changes (‘Time effect’), overall differences between AD patients and controls (‘Group effect’) or different response profiles between the two groups (‘Group-by-Time effects’).

In case of significant interaction effects, Newman–Keuls post-hoc tests were computed. IgE levels on days 1 and 2 were compared by Student’s t-tests for dependent and
independent variables. Likewise, the statistical significance of the change in the total area of lesional skin (in mm) from experimental days 1 to 2 was computed by Student’s *t*-tests for dependent variables.

### 3. Results

As summarized in Table 1, exposure to the TSST led to significant changes in leukocyte subsets, i.e., lymphocyte, monocyte, neutrophil and basophil number, while no changes were found under resting conditions on day 2. As for changes in leukocyte subsets, no significant differences were observed between AD patients and controls on either day (data not shown; all Group effects, all Group × Time effects: *p* > 0.05).

In contrast, analysis of eosinophil number yielded a significant Group (*F*(1,71) = 12.62; *p* < 0.001) and a significant Group × Time (*F*(3,213) = 4.8; *p* < 0.01) effect, indicating significantly elevated eosinophil number in AD patients and, further, a pronounced increase of this cell type in response to stress when compared to the control group (see Fig. 1A). On day 2, again, a significant Group (*F*(1,67) = 11.7; *p* < 0.005) but no Time or Group × Time effect (all *p*'s > 0.05) could be observed (see Fig. 1B).

Mean IFN-γ levels increased significantly from 6404 ± 574.9 to 8860 ± 727.7 pg/ml (*t* = 5.97; *p* < 0.001) 20 min after stress for the total group with no significant differences between AD patients and controls. Similarly, no significant differences were observed between the groups with regard to IL-4 levels. For the total group, IL-4 levels significantly dropped following the TSST from 83.1 ± 4.9 to 69.5 ± 4.5

### Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Day 1 (TSST)</th>
<th>Day 2 (Rest)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>t</em>1</td>
<td><em>t</em>2</td>
</tr>
<tr>
<td>Lym</td>
<td>AD</td>
<td>1.8 ± 0.08 &amp; 2.4 ± 0.08 &amp; 2.1 ± 0.12 &amp; 1.7 ± 0.08 &amp; 87.0 &amp; &lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Con</td>
<td>1.7 ± 0.08</td>
</tr>
<tr>
<td>Mon</td>
<td>AD</td>
<td>0.49 ± 0.02 &amp; 0.60 ± 0.02 &amp; 0.48 ± 0.02 &amp; 0.45 ± 0.01 &amp; 27.5 &amp; &lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Con</td>
<td>0.45 ± 0.02</td>
</tr>
<tr>
<td>Neu</td>
<td>AD</td>
<td>3.7 ± 0.18 &amp; 4.4 ± 0.22 &amp; 3.9 ± 0.20 &amp; 4.0 ± 0.19 &amp; 50.0 &amp; &lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Con</td>
<td>3.4 ± 0.27</td>
</tr>
<tr>
<td>Baso</td>
<td>AD</td>
<td>0.51 ± 0.05 &amp; 0.71 ± 0.07 &amp; 0.58 ± 0.06 &amp; 0.46 ± 0.04 &amp; 6.73 &amp; &lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Con</td>
<td>0.43 ± 0.05</td>
</tr>
<tr>
<td>Eos</td>
<td>AD</td>
<td>0.25 ± 0.04 &amp; 0.30 ± 0.04 &amp; 0.22 ± 0.03 &amp; 0.23 ± 0.03 &amp; 8.42 &amp; &lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Con</td>
<td>0.12 ± 0.01</td>
</tr>
</tbody>
</table>

Lym = lymphocytes (× 10^6 cells/ml); Mon = monocytes (× 10^6 cells/ml); Neu = neutrophils (× 10^6 cells/ml); Baso = basophils (× 10^5 cells/ml); Eos = eosinophils (× 10^6 cells/ml).

Values are total number of cells ± standard error of the mean (S.E.M.). *F* and *p* values indicate the respective Time effect.

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**Fig. 1.** Changes of eosinophil number (A) in response to the TSST and (B) under resting conditions in patients with AD and nonatopic controls.
pg/ml ($t = 5.89; p < 0.001$). In addition, no significant changes of cytokine levels were observed on day 2 (all $p$’s $> 0.1$).

As expected, significantly elevated IgE levels were observed in AD patients (299.9 $\pm$ 59.7 IU/l) when compared to controls (107 $\pm$ 34.2 IU/ml), as indicated by a significant Group effect ($F(1,71) = 9.97; p < 0.005$). However, while no significant alteration of IgE concentrations was found from days 1 to 2 in the control group (IgE level day 2: 102 $\pm$ 33.2 IU/ml), a significant increase of IgE levels could be observed in AD patients (IgE level day 2: 361.4 $\pm$ 71.2 IU/ml) 24 h after the TSST (Time $\times$ Group effect: $F(1,71) = 4.40; p < 0.05$).

To establish the effect of the experimental stressor in inducing exacerbation of AD symptoms, AD symptomatology on days 1 and 2 in the patient group was evaluated. Analysis of the Costa score indicated a worsening of skin condition (day 1: 28.9 $\pm$ 3.08, day 2: 30.03 $\pm$ 3.32; $t = -2.00; p = 0.05$) in that a significant increase of the percentage of the involved skin could be determined 24 h after stress exposure (day 1: 6.97 $\pm$ 0.83, day 2: 7.55 $\pm$ 0.81; $t = 2.48; p < 0.02$). No change was found with respect to symptom severity (day 1: 21.93 $\pm$ 2.43, day 2: 22.48 $\pm$ 2.66; $t = 1.03; p > 0.05$). Aggravation of AD symptomatology was not related to the immunological responses to the TSST (all $p$’s $> 0.05$) or the subjective stress rating (all $p$’s $> 0.05$). Analysis of the subjective stress rating indicated that the subjects experienced the TSST as being stressful (mean “stress score”$: 5.59 \pm 3.08$) with no significant difference between the two groups ($t = 0.75; p = 0.67$). Besides the psychological stress experience, the TSST yielded insignificant biological stress responses as indicated by significant elevation of heart rates and significantly increased cortisol, ACTH and catecholamine levels after the stress test (Buske-Kirschbaum et al., submitted).

4. Discussion

There is increasing acceptance that stress may be a relevant factor in the maintenance and aggravation of AD. To better understand how stress may affect exacerbation of AD, the present study investigated the effect of psychosocial stress on immune responses known to be pathologically significant.

After exposure to the TSST, an acute psychosocial stressor, a significant increase of eosinophil number, was found in AD sufferers, but not in healthy controls. This observation is in line with a previous report (Schmid-Ott et al., 2001) and may be of clinical relevance. Eosinophils are considered to play a pivotal role in chronic allergic inflammation and eosinophilia has often been described in patients with chronic AD (Uehara et al., 1990). There is growing evidence that recruitment and activation of eosinophils are characteristic features of the late-phase reaction of allergic inflammation and are assumed to precede chronification of AD. Eosinophils are assumed to contribute to tissue injury in AD through their capability to release cytotoxic mediators, which are found to be elevated in sera of patients with AD and are positively correlated with disease severity (Kägi et al., 1992; Martin et al., 1996). Our observation of increased eosinophil number after the TSST in AD sufferers may suggest increased sensitivity of this inflammatory cell to stress influences and may represent one potential mechanism in stress-induced worsening of skin condition in AD. The mechanisms of increased recruitment of eosinophils in the peripheral blood after stress in AD patients are not clear. It is well accepted that mobilization of leukocyte subsets into the periphery under stressful conditions is mainly under the control of the adrenergic system (Benschop et al., 1996; Carlson, 2001). However, distinct aberrations of the adrenergic responsiveness, i.e., an altered receptor density or receptor sensitivity of leukocytes, has been reported in AD patients (Sawai et al., 1995; Niemeier et al., 1996).

Analysis of IgE levels yielded a similar pattern of results allowing a comparable line of reasoning. AD sufferers showed elevated basal IgE levels, which were significantly increased 24 h after exposure to the stressor. There is general agreement that IgE represents a key molecule in AD pathology. IgE stimulates basophils and mast cells and is of major importance in the propagation of immediate hypersensitivity reaction. IgE further enables Langerhans cells and monocytes to present allergen-initiating activation of atopy-relevant TH2 cells (Mudde et al., 1990; Leung and Soter, 2001). The finding of elevated IgE concentrations after psychosocial stress is intriguing since it could be an additional piece in the puzzle of how stress may contribute to AD pathology. While our knowledge of how stress may be involved in IgE synthesis is still very limited, there are some data linking the HPA axis to IgE regulation. For instance, Ray et al. (1987) found a stimulating effect of glucocorticoids on IgE production by mononuclear cells in vitro. Most interestingly, only in AD subjects did treatment of mononuclear cells lead to increased IgE concentrations, suggesting an altered sensitivity of IgE-producing B cells for glucocorticoids in AD patients.

Finally, elevated concentrations of IFN-γ and reduced levels of IL-4 were found in response to the TSST in our patient group. This observation points to a TH1- rather than to a TH2-like cytokine response profile under stressful conditions, which is in contrast to our initial expectations. In the present study, it was hypothesized that stress may influence the activity of TH1 and TH2 cell subsets in polarizing the immunological balance toward a TH2 response. This idea was supported by previous data in rats showing a shift towards a TH2 cell dominance in response to psychological stress (Elenkov and Chrousos, 1999). In addition, it was found recently that exam stress resulted in decreased IFN-γ and increased IL-10 levels, suggesting a stress-induced polarization towards a TH2-like response (Maes et al., 1998; Paik et al., 2000). Thus, at a first glance, the present data may be confusing. Most recently, however, a striking result was that a biphasic response of the TH1/TH2
cell subsets can be observed in AD. Sequential analyses of skin biopsies obtained from atopy patch test sites in AD sufferers suggested that, initially, a TH2 response with elevated levels of IL-4 can be found, followed by a second shift toward a TH1 secretion pattern with high amounts of IFN-γ 24 h later (Thepen et al., 1996). The model of sequential TH cell activation is increasingly accepted. It has been proposed that during the acute allergic response, local inflammation is initiated by IL-4 and IL-5 (TH2 response), whereas the consolidation and aggravation of AD are dominated by a secretion of IFN-γ (TH1 response) (Grewe et al., 1998). On the basis of this pathogenic concept, a stress-induced shift towards a TH1 rather than a TH2 cell response pattern could be expected especially in patients in remission, which would be in agreement with our findings. It should be emphasized, however, that the model of a biphasic pattern of T cell activation has been exclusively demonstrated in skin biopsies. It remains to be determined whether, also systemically, a disease state-dependent activation of TH cell subtypes may occur.

To summarize, when stressed, AD patients show elevated eosinophil number, increased IgE levels and further distinct alterations in cytokine concentrations. These immunological changes are known to be characteristic features of allergic inflammation. The present findings suggest a “shift” towards an atopy-relevant immune response pattern to psychosocial stress in AD sufferers and could be one potential explanation of the often stated stress-induced exacerbation of skin atopy.

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References


