

Effect of stress on eotaxin and expression of adhesion molecules in a murine model of allergic airway inflammation

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Abstract

Recently we have shown that sound stress enhances allergic airway inflammation in a combined murine model. In the current study we investigated mediating factors and early kinetics of stress exacerbated allergic airway inflammation. Stress significantly increased allergen induced airway inflammation as identified by leukocyte numbers in BAL fluids. Eotaxin levels from stressed mice were significantly higher 24 h after stress. No differences were found for vascular or cellular adhesion molecule expression or cytokine levels. Our data indicate that the effect of stress on allergic airway inflammation might be mediated by the chemoattractant eotaxin, while Th2 cytokines and expression of adhesion molecules seem not to be differently regulated in stressed and non-stressed mice.

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1. Introduction

Stress has long been hypothesized to be associated with the exacerbation of asthma symptoms (Sandberg et al., 2004, 2000; Smyth et al., 1999; Wright et al., 2002). However, mechanisms linking stress to asthma are not well defined. One possible link might be the effect of stress on inflammatory processes. Liu et al. (2002) demonstrated that in asthmatic students stress increased the number of eosinophilic sputum cells after allergen challenge. In humans, acute stressors can enhance or reduce chemotaxis of peripheral blood mononuclear cells (PBMC) ex vivo, depending on the level of underlying chronic stress (Redwine et al., 2004, 2003). In mice, acute stress increases leukocyte infiltration in vivo and

enhances chemokine- and cytokine-directed infiltration (Viswanathan and Dhabhar, 2005). Various investigators have demonstrated that the expression of adhesion molecules on peripheral leukocytes changes after stress exposure in humans and animals. Psychological stress decreases L-selectin and increases Lymphocyte Function-Associated Antigen-1 (LFA-1) and Mac-1 expression on human lymphocytes (Goebel and Mills, 2000; Mills and Dimsdale, 1996; Redwine et al., 2003). In mice, the expression of LFA-1 and very late antigen 4 (VLA-4) on CD4⁺ cells was altered after a single period of restraint stress. However, the impact of stress on mechanisms of eosinophilic cell trafficking has not been investigated so far.

The accumulation of eosinophilic leukocytes in the airways is a main feature of allergic asthma, which in asthma patients correlates well with asthma severity (Bradley et al., 1991). A variety of molecules and different pathways contribute to eosinophil trafficking to the sites of allergic inflammation. Chemoattractants as well as adhesion molecules regulate eosinophilic inflammation. As shown in a study with eotaxin-deficient mice, eotaxin plays an important role in the early

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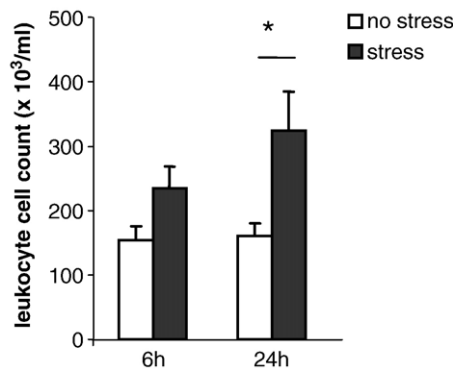


Fig. 1. Total leukocyte numbers in BAL fluid. BAL was performed 6 and 24 h after last allergen aerosol challenge. 24 h after the last challenge stressed mice showed significantly higher total cell numbers than non-stressed mice. * $p < 0.05$.

recruitment of eosinophils after allergen challenge (Rothenberg et al., 1997). Eotaxin is a chemokine binding to the CCR3 receptor, which is present on eosinophils, basophils, mast cells and Th2 cells (Daugherty et al., 1996; Gerber et al., 1997; Kitauro et al., 1996; Ponath et al., 1996; Romagnani et al., 1999; Sallusto et al., 1997; Uguccioni et al., 1997), and was characterized for the first time in the guinea pig in 1994 by Jose et al. (1994).

After being attracted to the target organ inflammatory cells enter the tissue with the assistance of adhesion molecules. Eosinophil cell surface adhesion receptors mediating rolling of eosinophils are P-selectin glycoprotein ligand-1 (PSGL-1) (Broide et al., 1998a; Symon et al., 1996), the $\alpha 4 \beta 1$ integrin and L-selectin (Sriramarao et al., 1994). Vascular P-selectin (Broide et al., 1998a; Symon et al., 1996) and vascular cell adhesion molecule 1 (VCAM-1) (Sriramarao et al., 2000) mediate eosinophil rolling on endothelial cell surface while firm adhesion is mediated by VCAM-1 and intercellular adhesion molecule 1 (ICAM-1) (Broide et al., 1998a,b).

Recently, we demonstrated in a murine model of allergic airway inflammation that stress increases the number of

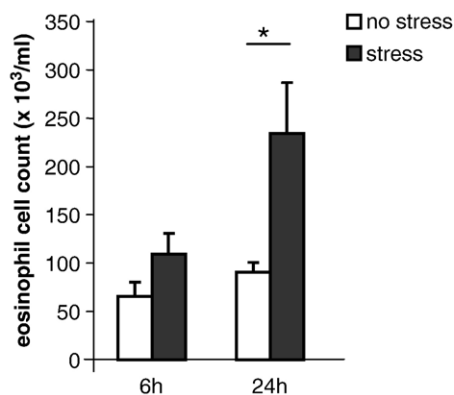


Fig. 2. Eosinophil numbers in BAL fluid. BAL was performed 6 and 24 h after the last allergen aerosol challenge. 24 h after the last challenge stressed mice showed significantly higher eosinophil cell numbers than non-stressed mice. * $p < 0.05$.

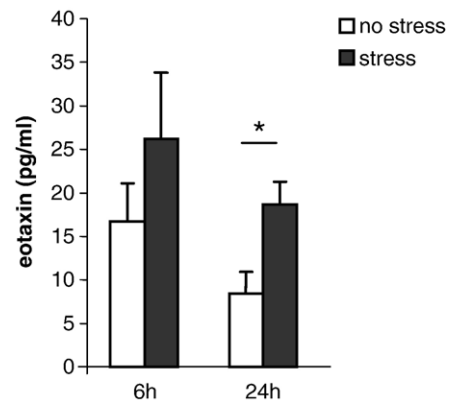


Fig. 3. Eotaxin concentration in BAL fluid. BAL was performed 6 and 24 h after the last allergen aerosol challenge and eotaxin protein concentration was determined by ELISA. 24 h after the last challenge stressed mice showed significantly higher eotaxin protein levels than non-stressed mice. * $p < 0.05$.

eosinophils found in the broncho-alveolar lavage (BAL) fluid and enhances airway reactivity (Joachim et al., 2003, 2004). Given the important role of immune cell recruitment and cell migration in airway inflammation on one hand and the supposed role of stress in mediating leukocyte trafficking on the other hand, in the present study we aimed to investigate the mediating factors and early kinetics of eosinophilic allergic airway inflammation in response to stress.

2. Methods

2.1. Animals

BALB/c mice were purchased from the Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV, Berlin, Germany) and maintained on a 12-h light/dark cycle. 6 mice were housed per cage. Animal care and experimental procedures were followed according to institutional guidelines and conformed to requirement of the state authority for animal research conduct (LaGetSi, Berlin).

2.2. Protocol of sensitization and airway challenge

BALB/c mice were sensitized by intraperitoneal (i.p.) injection of 10 μ g chicken ovalbumin (OVA, Sigma Chemie, Deisenhofen, Germany) emulsified in 1.5 mg $\text{Al}(\text{OH})_3$ (Alum Inject Pierce, Rockford IL, USA) on day 0, 14 and 21. Mice were challenged twice with OVA aerosol (1% OVA diluted in phosphate buffered saline (PBS)) via the airways on day 26 and 27.

2.3. Stress exposure

After i.p. sensitization, mice were randomized in two experimental groups. Coinciding with the first OVA aerosol challenge, one group was exposed to sound stress for a single 24-h period ($n=12$), while the other group remained

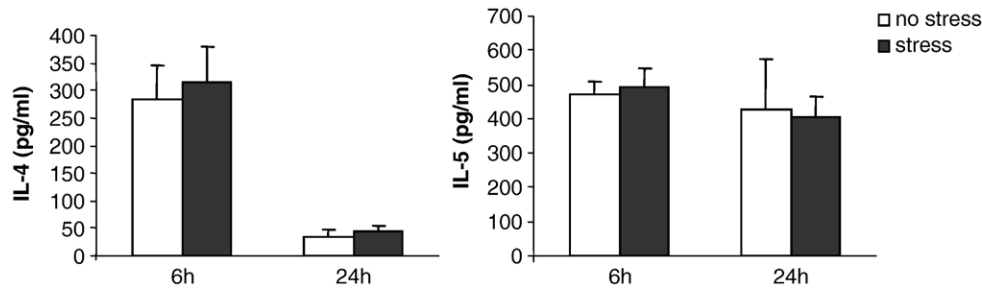


Fig. 4. Cytokine concentrations in BAL fluid. BAL was performed 6 and 24 h after the last allergen aerosol challenge and concentrations of the cytokines IL-4 and IL-5 were determined by ELISA.

undisturbed ($n=12$). Sound stress as an effective inductor of stress reactions has been used in various animal models (Arck et al., 2001, 1995; Clark et al., 1993; Windle et al., 1997). The stress protocol used in this study was adopted from a murine model of stress induced abortion. Here, a 24 h period of sound stress induces maximal abortion rates in pregnant CBA/J mice (Arck et al., 1995). The sound stress was emitted by a rodent repellant device (Conrad Electronics, Germany) at a frequency of 300 Hz (sound pressure level 75–80 dB). The stress device was placed in the mouse cage so that the mice could not escape perception of the sound. During exposure to the stressor in their home cage, stressed animals were kept in a different room separated from control animals. Stressed mice had free access to food and water during the stress period.

2.4. Broncho-alveolar lavage

BAL fluid was obtained 6 h (6h, $n=12$) and 24 h (24 h, $n=12$) after the second airway challenge (day 27 and 28). Animals were sacrificed and tracheas cannulated. Airways were lavaged twice with 0.8 ml ice-cold PBS and the total number of cells in BAL fluid was determined. Cytospins were prepared for each sample. After fixation, cytopins were stained with Diff Quik (Dade Behring, Marburg, Germany) and differential cell counts were performed. Using standard morphological criteria, cells were classified counting at least 100 cells per cytospin.

2.5. Flow cytometry

Cells from blood and lung were obtained for examination of adhesion molecule expression at 6 h and 24 h. Blood was collected in tubes containing heparin. After erythrocyte depletion blood cells were washed twice with PBS. After the broncho-alveolar lavage, lungs were removed, cut into pieces, and incubated in 5 mL PBS containing 175 U/mL collagenase (type IA, Sigma Chemical Co) and 10% FCS for 60 min at 37 °C. The digested lungs were sheared with a sterile 20-gauge needle and filtered through a sterile 45- μ m porous nylon mesh. Mononuclear cells were purified by Lympholyte/M (Cedarlane Laboratories, Canada) gradient centrifugation and washed twice before staining.

BAL cells and cells isolated from blood and lung were incubated with Fc block (BD Pharmingen) for 15 min to inhibit

unspecific binding. Then cells were stained for 30 min on ice with an antibody cocktail containing fluorescein isothiocyanate (FITC)-conjugated anti-CCR3, R-phycoerythrin (PE)-labeled anti-CD49d and allophycocyanin (APC)-conjugated anti-CD162, or their corresponding isotype-matched control antibodies (all antibodies from BD, Pharmingen, Germany). After washing, cells were analyzed on a FACScalibur flow cytometer (BD Immunocytometry Systems). Granulocytes were identified and gated using forward- versus side-scatter characteristics. About 96% of gated cells were CCR3-positive.

2.6. Determination of cytokines and eotaxin in BAL fluids

Interleukin (IL)-4 and IL-5 content of BAL fluids was determined by ELISA as described elsewhere (Herz et al., 1998). Antibodies were obtained from BD Pharmingen, Germany. Detection limits were 16 pg/ml for IL-4 and 27 pg/ml for IL-5. Eotaxin (CCL11) was determined using a commercial kit (R&D Systems, Germany) following the manufacturers' instructions. Detection limit was 3 pg/ml.

2.7. Immunohistochemistry

Immunostaining was carried out on cryostat-cut lung sections from non-stressed and stressed animals sacrificed 6 h and 24 h after the second airway challenge. For VCAM-1 detection, sections were mounted, fixed, washed in Tris-buffered saline (TBS), and incubated with TBS containing 2% normal goat serum. After removing serum block, the tissues were incubated with rat anti-mouse VCAM-1

Table 1
Total and eosinophil cell numbers in blood and lung tissue

	Total leukocyte cell count ($\times 10^6$ /ml)		Eosinophils ($\times 10^5$) per 10^6 /ml	
	No stress	Stress	No stress	Stress
Blood				
6 h	2.6 $\pm$ 0.4	2.6 $\pm$ 0.6	1.3 $\pm$ 0.3	1.2 $\pm$ 0.3
24 h	2.7 $\pm$ 0.4	2.3 $\pm$ 0.3	1.4 $\pm$ 0.4	1.3 $\pm$ 0.3
Lung				
6 h	2.3 $\pm$ 0.5	1.7 $\pm$ 0.1 ^a	1.9 $\pm$ 0.4	1.3 $\pm$ 0.1
24 h	2.1 $\pm$ 0.2	1.5 $\pm$ 0.2 ^a	2.8 $\pm$ 0.4	1.7 $\pm$ 0.2

^a $p \leq 0.05$.

Table 2
Expression of VLA-4 and PSGL-1 on eosinophils from blood, lung and BAL fluid

CCR3/ VLA-4	Blood (% total eosinophils)		Lung (% total eosinophils)		BAL (% total eosinophils)	
	No stress	Stress	No stress	Stress	No stress	Stress
6 h	96.8±0.2	96.9±0.6	95.1±0.5	95.0±1.0	93.9±0.7	91.0±2.6
24 h	92.4±1.2	93.0±0.7	92.4±0.9	86.2±0.8**	97.5±0.2	95.9±1.1
CCR3/PSGL-1						
6 h	97.2±0.9	97.6±0.2	96.0±0.6	96.4±0.8	97.0±0.5	93.4±2.0
24 h	95.3±1.2	96.3±0.5	95.1±0.7	93.1±0.6*	98.6±0.1	97.5±0.7

VLA-4: Very late antigen 4; PSGL-1: P-selectin glycoprotein ligand-1; BAL: broncho-alveolar lavage. ** $p \leq 0.01$, * $p \leq 0.05$.

antibody (BD Pharmingen, Germany), diluted 1:100 in TBS. After overnight incubation and washing steps in TBS, the VCAM-1 signal was detected by means of incubation with a Rhodamin-labeled goat anti-rat secondary antibody (Jackson ImmunoResearch, UK), and counterstaining was performed using DAPI dye (Roche, Germany; 1 µg/ml of methanol) in a subsequent incubation step. Finally, the sections were mounted using Immumount (Shandon, Germany) and viewed by using epifluorescence microscopy.

For P-selectin immunostaining cryostat sections were incubated with avidin-, and biotin-blocking solution (Vector Labs, Germany), followed by another block using protein blocking agent (Immunotech). The rat anti-mouse P-selectin mAb (Pharmingen, Germany) was diluted 1:50 and incubated for 1 h. After washing, biotinylated goat anti-rat secondary antibody (Jackson ImmunoResearch, UK) in TBS containing 2% goat normal serum and 4% mouse normal serum was applied for 30 min. The alkaline phosphatase detection system, Vector ABC-AP (Vector Labs, Germany) was used for development.

VCAM-1 and P-selectin immunoreactivity was quantified by counting the total number of blood vessels and the number of blood vessels stained positive per section under 200× magnification by two blinded independent observers. Then, percentages were calculated.

2.8. Corticosterone determination

To control the corticosterone response to stress an additional group of animals ($n=12$) was sensitized and challenged and half of the mice were stressed as described above. Mice were bled 24 h after the last airway challenge. Blood from non-stressed and stressed mice was collected individually on ice and centrifuged with 10,000 rpm at 4 °C for 10 min. Serum samples were stored at −20 °C until corticosterone determination with a commercially available EIA kit (Diagnostic Systems Laboratories, Texas, USA), following the manufacturer's instructions.

2.9. Statistics

Significance of differences between groups was determined using the non-parametric Mann–Whitney *U*-test.

Significance was set at $P < 0.05$. Results are presented as mean values ± SEM.

3. Results

3.1. Airway inflammation

To determine the number and cell types of leukocytes infiltrating the animals' airways, BAL fluids were analyzed. At 6 h after challenge and/or stress exposure, no difference was found between groups of stressed and non-stressed mice, however, stressed animals showed higher numbers of eosinophils without reaching significance ($p=0.08$; Figs. 1 and 2). A significantly higher number of total leukocytes was found in sensitized and stressed animals at 24 h (mean $324 \pm 61 \times 10^3/\text{ml}$) compared to sensitized, non-stressed mice ($160 \pm 20 \times 10^3/\text{ml}$, $p < 0.05$, Fig. 1). Cell type identification revealed that the difference between the two groups was predominantly based on an increased number of eosinophils ($234 \pm 53 \times 10^3/\text{ml}$ vs. $90 \pm 10 \times 10^3/\text{ml}$, $p < 0.05$, Fig. 2).

3.2. Eotaxin (CCL 11) protein level in BAL fluid

Concentrations of the chemokine eotaxin (CCL 11) decreased with time. 6 h after the last challenge stressed animals had higher levels of eotaxin in BAL fluids without reaching significance (26.2 ± 7.6 pg/ml vs. 16.7 ± 4.4 pg/ml). At 24 h, stressed animals showed significantly higher levels

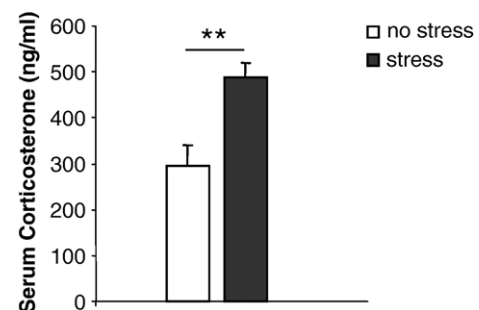


Fig. 5. Corticosterone concentrations in serum. Blood was taken 24 h after the last allergen aerosol challenge and corticosterone concentration was determined in serum by EIA. 24 h after the last challenge stressed mice showed significantly higher corticosterone levels than non-stressed mice. ** $p < 0.01$.

compared to non-stressed mice (18.6 ± 2.7 pg/ml vs. 8.4 ± 2.5 pg/ml, $p < 0.05$, Fig. 3).

3.3. Cytokines IL-4 and IL-5 in BAL fluid

At 6 h, Th2 cytokines IL-4 and IL-5 in BAL fluid were detected in high concentrations in both groups (IL 4: no stress 285 ± 60 pg/ml vs. stress 315 ± 64 pg/ml; IL-5: no stress 470 ± 38 pg/ml vs. stress 492 ± 52 pg/ml). IL-4 was decreased at 24 h in both groups (no stress 35 ± 13 pg/ml vs. stress 44 ± 11 pg/ml) meanwhile IL-5 remained high in non-stressed (426 ± 148 pg/ml) and stressed animals (406 ± 58 pg/ml) at this time point without showing differences (Fig. 4).

3.4. Total and eosinophil cell numbers isolated from blood and lung

Whereas in BAL fluid there was a significant difference in total leukocyte numbers, in blood no difference was detected between total numbers of stressed and non-stressed animals (Table 1). In lung tissue from stressed mice significantly lower total cell numbers were found at both time points compared to non-stressed mice (Table 1).

No significant differences resulted between stressed and non-stressed animals in eosinophil cell counts from blood or lung tissue, however, lungs from stressed mice showed lower numbers 6 h and 24 h after stress.

3.5. Expression of adhesion molecules VLA-4 and PSGL-1 on eosinophils

Next we wondered whether stress would influence the expression of adhesion molecules on eosinophils. Expression of VLA-4 (CD49d) and PSGL-1 (CD162) on eosinophils from BAL fluid, blood and lung tissue was measured by flow cytometry. Expression was high on eosinophils from non-stressed and stressed mice and no percentage difference was found in BAL fluid or blood (Table 2). On eosinophils from lung tissue, the percentage of CD49d and CD162 expression was significantly lower in stressed animals 24 h after allergen/stress exposure compared to non-stressed animals (Table 2).

3.6. Expression of adhesion molecules VCAM-1 and P-selectin in lung tissues

Immunohistochemistry revealed that there was no difference between stressed and non-stressed groups at each time point for VCAM-1 or P-selectin expression on lung vascular endothelium (data not shown).

3.7. Corticosterone response

24 h after the second airway challenge, stressed mice showed significantly higher corticosterone serum levels than non-stressed mice (Fig. 5).

4. Discussion

In the present study we found that stress applied during an ongoing allergic airway inflammation increases total leukocyte numbers and eosinophils in BAL fluid. These increases are mirrored by decreased numbers of total leukocytes and eosinophils in lung tissue. Stress exposure is associated with increased BAL fluid eotaxin levels but does not enhance the expression of adhesion molecules in lung tissue or on eosinophils.

The result of stress induced increase of airway inflammation at 24 h after allergen challenge and stress exposure is in accordance with previous findings from combined models of stress and allergic airway inflammation (Forsythe et al., 2004; Joachim et al., 2003, 2004). At an even earlier time point (at 6 h) a tendency towards higher total leukocyte and eosinophil numbers in BAL of stressed mice is already appreciable. Other studies, that investigated the effect of allergen challenge alone found different kinetics, with increased numbers of eosinophils not earlier than 48 h after challenge (Duez et al., 2004; Tomkinson et al., 2001). In contrast to these studies that used a model of a single challenge with intranasal application, in our model mice were challenged twice on two consecutive days, with the application of stress between aerosol exposures. Here, the time of read out was defined by the second challenge. Taking this into account the time points found for eosinophil influx in BAL fluid of sensitized and challenged mice are comparable to the above mentioned findings.

Eosinophil trafficking in allergic airway inflammation is mediated by a complex multistep process, in which locally expressed cytokines, chemokines and adhesion molecules result in the extravasation of inflammatory and immune cells into the airway mucosa and lumen. IL-5 stimulates the release of eosinophils from the bone marrow into the circulation (Palframan et al., 1998) and prolongs eosinophilic survival (Rothenberg et al., 1989). Earlier studies have shown that the enhancing effect of stress on airway inflammation is not mediated via increased Th2 cytokines IL-4, IL-5 or IL-13 (Forsythe et al., 2004; Joachim et al., 2003). Here, these findings were confirmed and expanded, as no differences for IL-4 or IL-5 were found at time points investigated.

Other crucial factors in the pathway of eosinophilic trafficking are chemoattractants like eotaxin. Eotaxin belongs to the family of CC chemokines, a family of inflammatory and immunoregulatory cytokines. Constitutive expression of eotaxin mRNA was found in tissues like thymus, lymph nodes, skin, lung and intestinal tract (Rothenberg et al., 1995). After allergen challenge eotaxin mRNA is rapidly upregulated in airway epithelium and alveolar macrophages contributing to the early recruitment of eosinophils into inflamed tissue (Li et al., 1997; Lilly et al., 2001). In the current model we found a high expression of eotaxin in BAL fluid 6 h after the last of two airway provocations with decreasing levels at 24 h. Nevertheless, stressed animals showed significantly higher amounts of eotaxin in BAL fluid compared to non-stressed animals. Higher eotaxin levels in

stressed mice suggest that the effect of stress on eosinophils might be at least in part mediated by stress induced eotaxin.

Recent studies in animals indicate that stress not only activates the hypothalamic pituitary adrenal axis (HPA) and the sympathetic nervous system but may further lead to the release of neuropeptides, especially the tachykinin substance P (SP) (for review see [Black, 2002](#)), and thereby induces or exacerbates inflammatory processes. ([Arck et al., 1995](#); [Peters et al., 2005](#); [Singh et al., 1999](#); [Spanos et al., 1997](#); [Zhu et al., 1996](#)) In our model, we found higher concentrations of corticosterone in stressed compared to non-stressed mice. This finding is comparable with other studies investigating stress ([Cao et al., 2002](#); [Bauer et al., 2001](#)) and represents a general adaptation to external threats. The stress effect on airway inflammation seems to be mediated at least in parts by the neuropeptide substance P (SP), rather than corticosterone. Blocking of the main SP receptor inhibited stress enhanced airway inflammation ([Joachim et al., 2004](#)), while the stress effect was not prevented in a comparable model of allergic airway inflammation by the use of a corticosteroid inhibitor ([Forsythe et al., 2004](#)).

The enhanced expression of the chemoattractant eotaxin might be caused by stimulation of airway epithelium or inflammatory cells via neuronal mediators released after stress. In this line SP, which is present in airway sensory neurons and released upon stimulation, stimulates bovine bronchial epithelial cells to express neutrophil chemoattractant mediators thus increasing neutrophil adherence ([DeRose et al., 1994](#); [Von Essen et al., 1992](#)). [Numao and Agrawal \(1992\)](#) demonstrated that neuropeptides like SP potentiated the chemotactic effect of platelet-activating factor (PAF) and leukotriene B₄ (LTB₄) on eosinophils of allergic patients in vitro, suggesting a priming effect of neuropeptides released from sensory nerves in response to neurogenic stimuli. Alternatively, a direct release of eotaxin from neurons might be responsible for increased eotaxin levels in our model, as eotaxin positive airway neurons have been shown recently in a model of allergic airway inflammation in monkeys ([Chou et al., 2005](#)). Eotaxin plays an important role in human asthma ([Lamkhiioued et al., 1997](#)). Therefore, our data might provide further insights into the mechanisms linking stress to asthma.

There is considerable evidence that stress can affect immune functions via neuroendocrine pathways like activation of the hypothalamic pituitary adrenal axis (HPA) and the sympathetic nervous system ([Dhabhar and McEwen, 2001](#); [Madden, 2001](#)). [Dhabhar et al.](#) and others recently demonstrated that acute stress reduces total number and percentages of peripheral white blood cells. Adrenalectomy or blocking of catecholamines reduced the observed effects which were reconstituted by administration of corticosterone ([Dhabhar et al., 1995](#); [Engler et al., 2004](#)). This group also showed that cell numbers recover within 6 h after termination of stress. We similarly report that 6 h and 24 h after stress there is no difference in total numbers of peripheral white blood cells between stressed and non-stressed animals. Furthermore, no

difference was found in circulating eosinophil numbers between non-stressed and stressed mice. In contrast to peripheral leukocyte numbers, total leukocyte numbers isolated from lung tissue were reduced 6 h and 24 h after stress. Little data is available with respect to stress effects on lung cell populations. [Kanemi et al.](#) found decreased lymphocyte numbers in blood and lung immediately after a short period of restraint stress which recovered within 4 h. ([Kanemi et al., 2005](#)) Decreased leukocyte numbers in lung tissue together with increased BAL fluid numbers after stress can be interpreted as stress induced redistribution as proposed by [Dhabhar and McEwen \(2001\)](#). On the other hand, it can not be ruled out completely that the lower cell numbers might reflect an increased cell death rate. In the fast regenerating tissue of the skin's hair follicle apoptosis has been observed as an effect of external applied stress. ([Arck et al., 2001](#)).

Focusing on leukocyte migration into the tissue, the impact of stress on the expression of adhesion molecules in healthy individuals and animals has been studied by various groups, showing a modulating effect of stress on molecules like L-selectin, $\beta 2$ integrins and VLA-4 ([Bauer et al., 2001](#); [Goebel and Mills, 2000](#); [Mills and Dimsdale, 1996](#); [Redwine et al., 2003](#); [Tarcic et al., 1995](#)). To our knowledge it had not yet been investigated how stress effects expression of adhesion molecules related to eosinophilic trafficking in atopic diseases.

We did not find differences in percentage expression of CD49b and CD162 on eosinophils recovered from blood or BAL fluid, as expression was high in stressed and non-stressed groups alike at all investigated time points. In lung tissue total cell counts in stressed animals were significantly lower than in non-stressed mice, while eosinophil numbers were lower without reaching significance. Eosinophils isolated from lung tissue of stressed animals showed lower percentages of CD49b and CD162 at the time point of 24 h after stress and allergen exposure. As lungs tissue was lavaged before the following digestion steps this observation is not due to different cell numbers in airway space. It can be speculated that these results correspond to an increased velocity of eosinophils in traversing the tissue compartment of the lung.

We found no difference for the epithelial expression of VCAM-1 or P-selectin in lung tissues of stressed or non-stressed mice. Therefore there is no indication that stress influences vascular adhesion molecules related to eosinophilic rolling and adhesion in this model. One might suspect from our data that with equal high expression of adhesion molecules in stressed and non-stressed animals the driving force of high numbers of eosinophils and a more rapid influx of cells into the airways might be the enhanced local eotaxin level.

In conclusion we investigated the impact of stress on potential mediators of eosinophil trafficking and found that stress is associated with increased eotaxin levels, which might explain the enhanced number of eosinophils found in BAL fluid. The upregulation of adhesion molecules or Th2 cytokines seem not to be involved in stress enhanced eosinophilic airway inflammation.

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