

# Timing-Dependent Effects of Restraint Stress on Eosinophilic Airway Inflammation in Mice

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## Key Words

Allergy · Asthma · Airway inflammation · Glucocorticoids · Hypothalamic-pituitary-adrenal axis · Restraint stress

## Abstract

**Background:** Chronic stress has been proposed to aggravate allergic inflammation, whereas acute stress may have functional beneficial effects. The aim of this study was to investigate the influence of timing of single short restraint stress (RST) in a model of eosinophilic airway inflammation.

**Methods:** The airways of ovalbumin (OVA)-sensitized mice were exposed to an intranasal OVA challenge. RST was applied in two different ways; either 2 h before (pre-stress) or after (post-stress) the OVA challenge, respectively, or as a combination of stress before and after (double-stress) the OVA challenge. One group of mice was also treated with metyrapone (ME) prior to a pre-stress challenge. The inflammatory cell response was evaluated in bronchoalveolar lavage fluid (BALF), lung and nasal tissue, as well as bone marrow.

**Result:** RST applied prior to the OVA challenge (pre-stress) inhibited OVA-induced airway inflammation in BALF and lung tissue, and reduced nasal histopathology compared to

unstressed mice. Given as post-stress or double-stress, RST did not affect the inflammation in BALF, lungs or nasal tissue. Pre-treatment with ME prevented the pre-challenge stress evoked decrease in inflammation in BALF and lungs. **Conclusion:** Effects of RST on eosinophilic airway inflammation appear to be strongly dependent on timing and, as could be judged from the ME inhibition pattern, also corticosterone dependent. Hypothalamic-pituitary-adrenal axis activation probably influences eosinophilic inflammation through specific sequences of compartmental activation and thereby timing effects are evident on cellular recruitment pattern during the allergic reaction.

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## Introduction

Allergic asthma is a chronic inflammatory disease of the airways, characterized by eosinophilia, reversible airway obstruction, airway hyperresponsiveness, increased mucus production and remodeling. Studies have indicated that chronic stress can aggravate the allergic inflammation in mild asthmatics [1] and emotional stress in-

creases the number of exacerbations [2], whereas acute stress has been shown to improve allergen-induced airway obstruction [3].

Acute stressors may stimulate the immune system and thus lead to functional beneficial effects, but when a stressful event is repeated or even persists and becomes chronic it may be associated with a maladaptive immune system. This may be at the level of a blunted hypothalamic-pituitary-adrenal (HPA) axis response or a down-regulation of the peripheral glucocorticoid (GC) receptors [4, 5]. The intensity, duration and frequency of the stressor are of vital importance for how individual tissue compartments cope with this event. Stress is associated with activation of the HPA axis, which results in increased levels of GCs [6]. GCs have been shown to have the capacity to modulate immune and inflammatory responses in several ways [6]. Hence, GCs have been shown to shift the immune response from Th1 to Th2, by suppressing Th1 cytokine production [7] and also by a direct effect on Th2 cells by increasing IL-4 and IL-13 production [8]. GCs have also been shown to mediate changes in leukocyte distribution in the body, which have significant consequences for the ability of the immune system to perform its surveillance and effector function [9].

Increased activation of the sympathetic nervous system is also a physiologic response to stress, which leads to elevated levels of catecholamines (CAs), mainly epinephrine and norepinephrine, which in turn influence the immune system [10, 11]. Through inhibition of Th1 and stimulation of Th2 cytokine secretion, CAs can cause a selective suppression of cellular immunity and a shift toward a systemic Th2-mediated immunity. Still, in certain conditions, local response to CAs in specific compartments of the body may actually boost local cellular immune response; for example, it has been found that CAs can actually boost the IL-8 production in the lungs and thus promote recruitment of leukocytes to a local inflammation [reviewed in 11].

The actions of the inflammatory cells in airway allergy are tightly regulated by a network of Th2 cytokines, such as IL-4, IL-5 and IL-13. These cytokines will assist the recruitment and activation of eosinophils [12, 13], which is one of the hallmarks of allergic asthma. As evident from animal models, shortly after allergen challenge of the airways, bone marrow (BM) eosinophils decrease due to their migration to the blood and peripheral tissues such as lungs. Later, an increase in eosinophils, both in BM and in peripheral tissues occurs, which results from eosinopoiesis [14]. Lymphocytes, mainly activated CD4+ T cells, increase after allergen challenge and play an im-

portant role in allergic inflammation by inducing B cells to produce IgE, but also to release cytokines, such as IL-4 and IL-5, which is essential in the eosinophil recruitment [13, 15].

Several cross-sectional studies have shown a clear association between asthma and rhinitis, and this has led to the concept of 'united airways' [reviewed in 16]. A systemic connection between the nose and lungs via soluble mediators and BM response has been suggested as a possible linking mechanism [17]. Most of the studies on allergic airway inflammation examine only lower airways, and, to the best of our knowledge, we could not find any study that analyzed the stress response in both upper and lower airways concurrently.

Acute restraint of mice, which has many physiological similarities to emotional stress in humans, such as HPA axis and sympathetic nervous system activation, is often used to examine the influence of stress on the murine immune system [10, 18]. Several reports have shown that restraint stress (RST) elevates the plasma level of corticosterone and reduces cellular accumulation and inflammation in target organs of mice [10, 19–21]. In animal models of airway allergy, different stressors and duration of the stressor have been shown to evoke varied effects on the modulation of inflammatory response [4]. For example, in a model of airway allergy, bronchoalveolar lavage fluid (BALF) eosinophils decreased after 2 h of RST in Balb/C mice, whereas in C57BL/6 the eosinophil level was unaltered [22], indicating genetic variations of the inflammatory response. In another study by Joachim et al. [23], an increase in the BALF eosinophils was detected in Balb/C mice with allergic airway inflammation exposed to 24 h of sound stress, indicating that a more chronic stress may aggravate the allergic inflammation. Bowers et al. [24] found that different stressors elicit different physiological and behavioral responses and that a RST evokes a significantly higher GC response than other stressors, such as low temperature, forced swimming or isolation. These findings indicate that the response to stress in the immune system is complex. It has also been suggested that the timing of stress exposure could have an effect on the outcome in the immune system [25], but this has not been systematically examined.

The aim of this study was to investigate the effects of alterations in timing of stress exposure on allergic airway inflammation in both upper and lower airways, as well as in BM. We used an ovalbumin (OVA) intraperitoneal sensitization and nasal challenge model for airway allergy in Balb/C mice, since this has been well characterized. Stress was applied as a RST immediately before or after

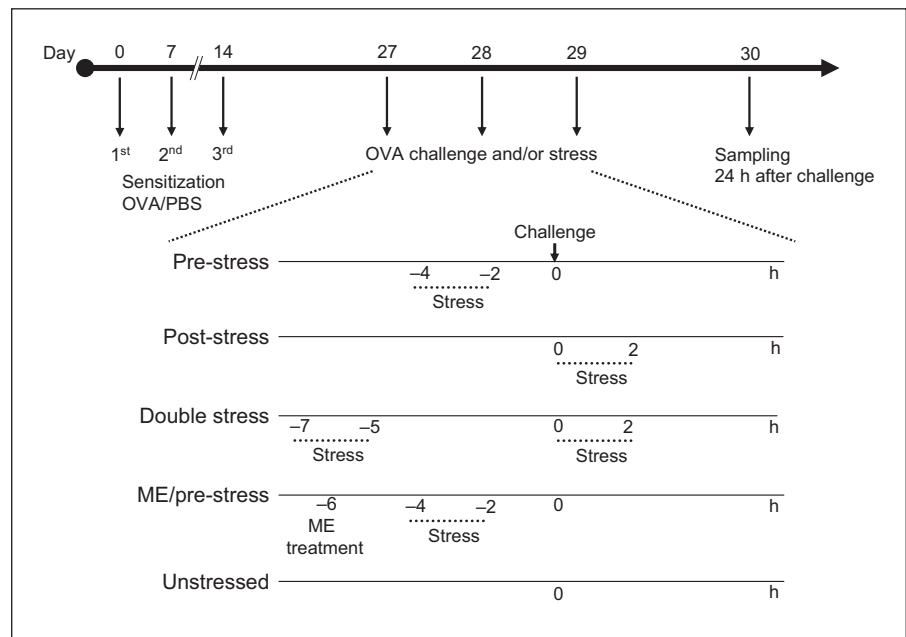


**Fig. 1.** Illustration of OVA sensitization and challenge, as well as stress and ME treatment protocols in mice. RST was exerted on 3 consecutive days as a 2-hour single or repeated restraint. Metyrapone was administered as 100 mg/kg i.p. All sampling was made 24 h after last challenge.

the allergen challenge procedure. In addition, stress exposure both before and after the allergen challenge was used to analyze whether double-stress would further influence the response pattern.

## Materials and Methods

### Animals

Male BALB/c mice were obtained from B&K Universal AB (Sollentuna, Sweden). They were 6–8 weeks old at the initiation of the sensitization protocol. They were allowed food and water ad libitum and kept in a room with a 12-hour light-dark cycle. There were 15 animals in each protocol group. The local committee on animal ethics approved all experiments.

### Sensitization

Mice were sensitized with 10  $\mu$ g OVA grade III (Sigma Chemical, Co., St. Louis, Mo., USA) and 4 mg/ml  $\text{Al}(\text{OH})_3$  (Sigma Chemical) suspended in 0.2 ml PBS, given as an intraperitoneal (i.p.) injection in the mornings on day 0, 7 and 14. A control group was PBS sensitized and received only 4 mg/ml  $\text{Al}(\text{OH})_3$  suspended in 0.2 ml PBS i.p.

### Airway Allergen Challenge

Antigen challenge procedures were performed in all mice repeatedly in the mornings on day 27, 28 and 29, in all groups, by intranasal instillation of 100  $\mu$ g OVA suspended in 50  $\mu$ l PBS during slight  $\text{CO}_2$  anesthesia.

### Stress Procedure

The animals were placed into plastic tubes (27 mm diameter, 88 mm long) fitted with caps and multiple punctures to allow

ventilation. The tubes were placed in horizontal position and the animals were left for a continuous 2-hour period of restraint. Groups were restrained either before, after or before and after the OVA challenges (fig. 1). One group was treated with 100 mg/kg metyrapone (ME; Aldrich Chemical Company Inc., Milwaukee, Wisc., USA) i.p. before a pre-challenge stress.

### Tissue Collection and Preparation

The mice were sacrificed on day 30 (24 h after the last OVA challenge) with a lethal i.p. injection of sodium pentobarbiturate (Apoteksbolaget, Umeå, Sweden).

### Bronchoalveolar Lavage

The trachea was exposed surgically and gently flushed with 400  $\mu$ l PBS three times via a plastic cannula (1.3  $\times$  32 mm, BD Venflon™ Pro, Becton Dickinson infusion therapy, Helsingborg, Sweden) to collect bronchoalveolar cells. The BALF was centrifuged at 1,200 rpm in an Eppendorf centrifuge (4515D) for 10 min. The cell pellet was resuspended in PBS and centrifuged in a cytospin centrifuge (Shandon Cytospin 2; Shandon Southern Products, Ltd., UK) at 450 rpm for 5 min, air-dried and stained with May-Grünwald Giemsa or Luxol fast blue (LFB). The slides for LFB staining were fixed with CAM [a combination of citrate buffer (Sigma Diagnostics, St. Louis, Mo., USA), acetone (Histolab, Göteborg, Sweden), and methanol (Merck, Darmstadt, Germany)] for 5 min, and then rinsed in distilled water. Eosinophils were stained with Solvent blue 38 (Sigma Chemical) in alcohol saturated with urea (Sigma Chemical) for 30 min. This staining shows eosinophils as bright blue-green. Counterstaining with Mayer's hematoxylin was used. A differential cell count was performed on the May-Grünwald Giemsa-stained slides and an eosinophil count on the LFB-stained slides. Evaluation of slides was performed under light microscopy counting 300 leukocytes in a blinded manner.

### *Lung Histology*

The lungs were removed surgically. One lobe was immersed in Stefanini's fixation and the other lobe in 4% buffered formaldehyde. The formaldehyde-fixed lungs were then embedded in paraffin, sectioned and stained with hematoxylin-eosin and the slides were coded. Histological evaluation of inflammatory cells was performed in a blinded manner, and semiquantitatively graded as 0 = no cell infiltration, 1+ = few, 2+ = moderate, 3+ = abundant. The Stefanini-fixed lungs were rinsed in Hank's balanced salt solution after 24 h and snap frozen in liquid nitrogen. Then, they were cut into 10- $\mu$ m-thick sections and stained for eosinophilic peroxidase (EPO) as described previously by Korsgren et al. [26]. The amount of EPO-positive cells in the lungs was semiquantitatively graded as 0 = no EPO-positive cells, 1+ = few, 2+ = moderate, 3+ = abundant in a blinded manner.

### *Nasal Tissue*

The noses were removed, trimmed and fixed in 4% formaldehyde (5 noses from each group). The specimens were decalcified with 24% ethylenediaminetetraacetic acid (Tritriplex III, Merck) at 37°C for approximately 1 month. Following dehydration in ethanol and embedding in paraffin, the whole mounted complexes were sectioned (5  $\mu$ m) and stained with hematoxylin-eosin and Alcian blue-periodic acid Schiff pH 2.5 for morphological studies and mucus-producing cells. A histopathological examination was performed in a blinded manner on the nasal mucosa and a semi-quantified grading system was then employed regarding: (1) ciliary loss, (2) inflammatory cells in the mucosa, and (3) secretion. Each parameter was estimated on a three-graded scale, ranging from 0 'no increase' to 3 'pronounced increase'.

### *Bone Marrow*

The BM cells were collected by carefully flushing 1 ml PBS via a needle (0.6  $\times$  25 mm) from the end-opened right femur of each mouse. The cytocentrifugation and staining were performed in a similar way as for the BALF cells. Eosinophils were exclusively defined as cells containing eosinophilic granules.

### *Statistical Analysis*

Statistical analysis was performed with Kruskal-Wallis analysis of variance, followed by Mann-Whitney U test for between-group comparisons. A *p* value of <0.05 was considered statistically significant. Results are expressed as mean  $\pm$  SEM. All statistical analysis was performed with Statistica 7.0 (StatSoft Inc, Tulsa, Okla., USA).

## **Results**

### *Lower Airway Inflammation*

BALFs were analyzed to determine the levels of leukocytes infiltrating the airways in stressed and unstressed animals. Total leukocytes in BALF increased significantly (*p* < 0.01) in the OVA-sensitized and OVA-challenged (unstressed) group ( $9.53 \times 10^5 \pm 9.6 \times 10^4$ /ml) as compared to the PBS-sensitized group ( $4.75 \times 10^5 \pm 9.1 \times 10^4$ /ml). A significant increase in the proportion and

number of eosinophils in the BALF was seen in response to OVA challenge in OVA-sensitized groups compared to the PBS-sensitized group (OVA, unstressed:  $17.33 \pm 5.2\%$ ,  $1.89 \times 10^5 \pm 6.2 \times 10^4$ /ml; PBS:  $0.8 \pm 0.3\%$ ,  $3.04 \times 10^3 \pm 1.1 \times 10^3$ /ml; *p* < 0.01).

Animals subjected to RST prior to the OVA challenge (pre-stress) displayed a significant decrease in eosinophil proportion and number compared to the unstressed OVA group (fig. 2). RST applied after the OVA challenge (post-stress) or both before and after the OVA challenge (double-stress) did not influence the eosinophil proportion or number as compared to the unstressed OVA group. Also, the pre-stressed group displayed a decreased eosinophilia as compared to both the post-stress and double-stress groups (fig. 2). Lymphocyte levels decreased in proportion and number in the pre-stressed group as compared to the unstressed OVA group (pre-stress:  $0.02 \pm 0.02\%$ ,  $471 \pm 471$ /ml; unstressed:  $0.21 \pm 0.05\%$ ,  $2,130 \pm 501$ /ml; *p* < 0.01) which was not seen in groups subjected to post-stress or double-stress. There were no differences in the proportion of macrophages or neutrophils between any of the groups.

To investigate whether effects of RST applied prior to the OVA challenge on inflammatory cell response were dependent on endogenous corticosterone release, mice were treated with the GC synthesis inhibitor ME before the stress and OVA challenge. Metyrapone treatment prior to the pre-challenge stress prevented the pre-stress-caused decrease (*p* < 0.05) of eosinophil (fig. 2) and lymphocyte proportion and number (ME/pre-stress eosinophils:  $1.61 \times 10^5 \pm 4.1 \times 10^4$ /ml; lymphocytes:  $0.21 \pm 0.05\%$ ,  $2,173 \pm 618$ /ml).

The lungs of OVA-sensitized unstressed mice displayed increased inflammatory scores, reflecting an increase in inflammatory cells around the blood vessels and in the peribronchial area and epithelial shedding, as compared to the lungs of the PBS-sensitized animals (OVA, unstressed:  $1.54 \pm 0.23$ ; PBS:  $0.72 \pm 0.16$ ; *p* < 0.05). An increase in the amount of EPO-positive cells in the lung tissue was seen as well (OVA, unstressed:  $2.15 \pm 0.23$ ; PBS sensitized:  $1.33 \pm 0.14$ ; *p* < 0.01). The pre-stressed group showed a decrease (*p* < 0.05) in inflammatory scores compared to the unstressed group (fig. 3). There were no differences in EPO-positive cells in any of the stressed groups compared to the unstressed groups (pre-stress:  $1.80 \pm 0.14$ , post-stress:  $1.93 \pm 0.22$ , double-stress:  $2.07 \pm 0.21$ ).

The decrease in inflammatory score seen in the pre-stressed group was prevented when mice were treated with ME before the RST (*p* < 0.05), and there was no dif-

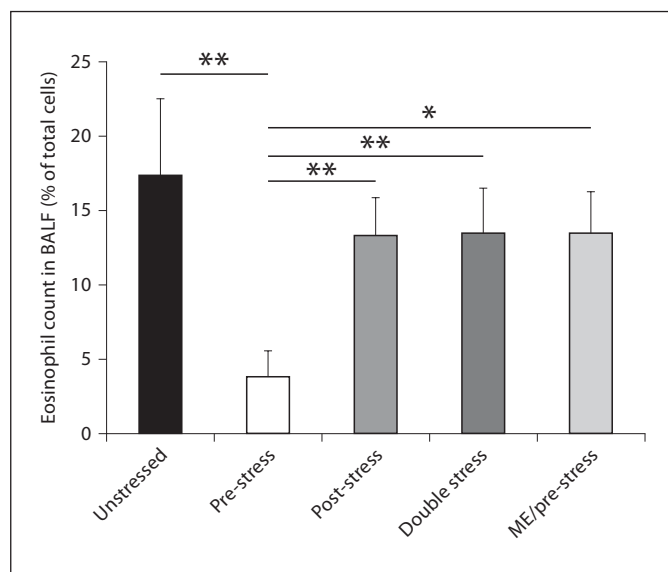


**Fig. 2.** BALF eosinophil count as percent of total cells in OVA-sensitized and -challenged Balb/C mice. The mice were exposed to a single or double 2-hour RST according to the protocol (fig. 1). Mice in the ME/pre-stress group were treated with ME (100 mg/kg) before exposure to a 2-hour RST according to the protocol (fig. 1). \*  $p < 0.05$ , \*\*  $p < 0.01$ . Results are shown as mean  $\pm$  SEM.

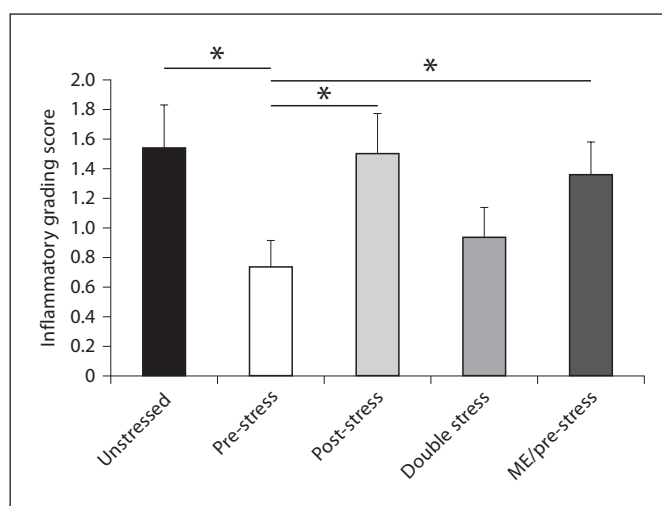
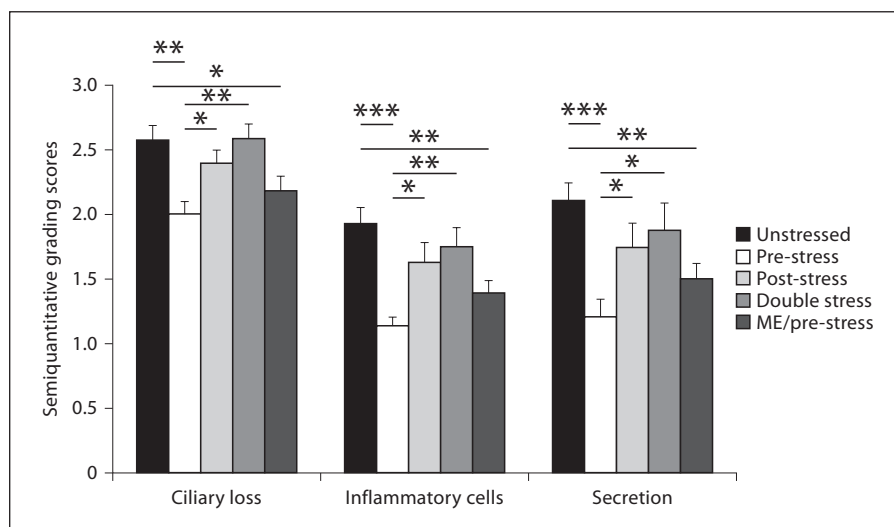


**Fig. 3.** Semiquantitative grading of lung inflammatory grading scores in OVA-sensitized and OVA-challenged mice. Balb/c mice were exposed to a single or double 2-hour RST according to the protocol (fig. 1). Mice in the ME/pre-stress group were treated with ME (100 mg/kg) before exposure to a 2-hour RST according to the protocol (fig. 1). \*  $p < 0.05$ . Results are shown as mean  $\pm$  SEM.

**Fig. 4.** Semiquantitative grading of nasal mucosa pathology scores (ciliary loss, inflammatory cell influx and secretion) in OVA-sensitized and OVA-challenged mice. Balb/c mice were exposed to a single or double 2-hour RST according to the protocol (fig. 1). Mice in the ME/pre-stress group were treated with ME (100 mg/kg) before exposure to a 2-hour RST according to the protocol. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ . Results are shown as mean  $\pm$  SEM.



ference between the ME/pre-stressed group and the unstressed mice in inflammatory score (fig. 3).

#### Upper Airway Inflammation

In the nasal tissue, pathological findings, including inflammatory cell influx, mucus secretion and ciliary

loss, were semiquantitatively graded. An increase in pathological findings were seen in the OVA-sensitized (unstressed) group as compared to the PBS-sensitized (OVA, unstressed: ciliary loss:  $2.57 \pm 0.12$ , inflammatory cells:  $1.93 \pm 0.13$ , secretion:  $2.11 \pm 0.14$ ; PBS: ciliary loss:  $2.05 \pm 0.12$ , inflammatory cells:  $1.14 \pm 0.06$ , secre-

tion:  $0.95 \pm 0.13$ ;  $p < 0.01$ ). In the pre-stressed group, a decrease in inflammatory cell influx, mucus secretion and ciliary loss was seen compared to the unstressed ( $p < 0.01$ ), post-stress ( $p < 0.05$ ), and double-stress ( $p < 0.05$ ) groups (fig. 4).

In the presence of ME, decreased inflammatory cell influx, mucus secretion and ciliary loss were seen in animals subjected to pre-stress as compared to the unstressed group ( $p < 0.05$ ; fig. 4).

#### *Bone Marrow*

The airway eosinophilia is dependent on the eosinophilopoiesis and egression of eosinophils from the BM. We detected an increase ( $p < 0.001$ ) in the proportion and number of eosinophils in the OVA-sensitized unstressed group ( $7.48 \pm 0.78\%$ ,  $8.99 \times 10^5 \pm 9.78 \times 10^4/\text{ml}$ ) as compared to the PBS-sensitized group ( $3.90 \pm 0.33\%$ ,  $3.99 \times 10^5 \pm 6.62 \times 10^4/\text{ml}$ ). There was no effect of stress on the BM eosinophil count and proportion (pre-stress:  $6.40 \pm 0.61\%$ ,  $6.59 \times 10^5 \pm 1.1 \times 10^5/\text{ml}$ ; post-stress:  $6.77 \pm 0.44\%$ ,  $7.64 \times 10^5 \pm 1.07 \times 10^5/\text{ml}$ ; double-stress:  $7.90 \pm 0.66\%$ ,  $6.85 \times 10^5 \pm 1.06 \times 10^5/\text{ml}$ ).

#### **Discussion**

In this study, we demonstrate that the effects of acute RST applied prior to the allergic airway inflammation decreased the inflammatory response in cellular compartments such as lungs, BALF and nasal tissue. In the nasal tissue, we could also demonstrate decreased mucosal histopathology in response to the pre-challenge RST. In contrast to stress applied prior to the OVA challenge, stress applied after did not affect the allergic airway inflammation. Also, when an acute stress prior to the OVA challenge was combined with a stress period after the OVA challenge, no alterations of the inflammation in BALF, lungs or nasal tissue were demonstrated. The inhibitory effect of the airway inflammation in the BALF and lungs due to the pre-stress seemed to be corticosterone dependent, since it was markedly reversed by ME pre-treatment.

When a short stress was applied before each OVA challenge, we detected a protective effect of the inflammation, demonstrated as decreased eosinophils and lymphocytes in BALF, reduced inflammatory cell influx in the nasal mucosa and inflammatory scores in the lungs. The inhibition of the inflammatory response is suggested to be the result of increased endogenous GC levels, since the inflammation was re-established with a pre-stress

treatment of the GC synthesis inhibitor ME. In line with this, almost 10-fold increases of endogenous GCs have been shown after 2 h of RST in mice, and this indicates that the HPA axis is activated [20]. Forsythe et al. [21] have shown that the use of a specific corticosteroid receptor inhibitor, RU 486, reversed the decrease in BALF inflammatory cells which acute stress caused in airway allergy in mice. Also in other stress models without allergic inflammation it has been shown that acute stress inhibits the influx of inflammatory cells to the site of inflammation and the main suppressive effect was GCs dependent [18, 27]. These findings are in agreement with our findings that an inhibition of endogenous GCs reversed the decrease in allergic airway inflammation caused by a pre-challenge stress, indicating that the stress effects on the eosinophils and lymphocytes in BALF are GC dependent.

Eosinophils are prominent in airway allergy and they are sensitive to suppressive function of GCs. Specific cell types respond differently depending on sensitivity for GCs, and eosinophils are considered to be one of the more sensitive cell types in an asthmatic inflammation [28]. Decreased airway inflammation following GC treatment is associated with a prevention of BM eosinophil progenitor expansion and terminal differentiation [29]. GCs may also prevent eosinophil migration into the airways and the appearance of an increased number of eosinophil precursors in the circulation [30]. Apoptosis of airway tissue eosinophils is often associated with the effects of a GC treatment, although this has not been compellingly shown in vivo [28, 31, 32]. It has been shown that apoptotic eosinophils are often found in the airway lumen [33].

A reduction in recruitment and activation of eosinophils could also be due to the decreased levels of lymphocyte causing reduced levels of IL-4 and IL-5. A decrease in lymphocytes in BALF caused by an acute stress in an allergic model, as described in our study, has also been demonstrated by Okuyama et al. [22] and was probably due to the demonstrated ability of GCs to redistribute the lymphocyte population from peripheral tissues to the lymphoid and BM compartments [30]. Also in our study, pre-stress-induced decrease in lymphocytes appeared to be GC dependent due to ME inhibition.

Acute stress leads to elevated levels of corticosterone and probably affects an inactivated cellular compartment more than the ones that already have an ongoing inflammation. In the pre-stressed group, the stress procedure was performed in time so that increased corticosterone levels would be achieved, according to previous reports

[9, 34], at the time of the allergen challenge. When we applied a double-stress, the incentive was to mimic the establishment of a more chronic repetitive stress response. The timing was made so that corticosterone levels, according to previous studies, returned to baseline levels [9, 34] before the allergen challenge. The post-stress was made to investigate how the effects from pre-challenge stress change; due to that, the inflammation process was already initiated. In contrast to the pre-stressed group, the post-stressed and double-stressed animals displayed no differences in BALF inflammatory cells, nasal inflammation or lung pathology as compared to the unstressed group. This may be due to the already manifested inflammation in the corresponding compartments due to earlier allergen challenge, and thus corticosterone production and release in response to the stress will not have protective effects on the already recruited and activated inflammatory cells. Another mechanism explaining the relative lack of reductive outcome of the double-stress is the inherent cellular autoregulatory effect of the first stress episode, giving a regulation of the GC receptors [5] resulting in less anti-inflammatory effect of the endogenous GCs at that time point.

OVA sensitization and challenge are associated with increased numbers of IL-5-responsive eosinophil progenitor cells in BM in both humans and mice [36]. In the BM, we detected an increase in eosinophils in OVA-sensitized and -challenged mice, and this has also been shown previously by us and other groups [35–37]. Acute stress did not have any significant effect on the OVA-induced BM eosinophilia in our study. One explanation for this could be that any eventual effects in the BM due to stress might have occurred earlier than the time of BM evaluation, and that an alteration in BM eosinophil numbers may have been restored by eosinophil progenitor expansion [38].

Few studies have been performed to evaluate the effects of short-time stress on nasal pathology in airway allergy, despite the fact that many of the symptoms in airway allergy arise from the nasal tissue and that the lung symptoms are correlated with the nasal symptoms [16]. The findings of increased inflammatory cell influx, mucus secretion and ciliary loss in response to an allergen challenge in the unstressed group indicate that we have an ongoing inflammation and remodeling process in the nasal tissue. We also show that a pre-stress may prevent or reduce this process. We found that the nasal pathology followed the same pattern as in lung tissue and BALF, where stress applied before each challenge decreased the pathology scores almost to the same levels as

the PBS-sensitized mice. Nishioka et al. [39] presented a study with relatively short (8-hour) repeated RST on mice with rhinitis, where a small but not significant decrease in nasal eosinophils was seen, indicating that this type of stress can be protective against nasal inflammation. ME treatment did not totally inhibit the pre-stress-provoked decrease in inflammatory score in nasal tissue; thus, the mechanisms for the pre-stressed inhibition of inflammatory score in the nose is suggested to be less dependent on GCs. Instead, it may be regulated by CAs and corticotrophin-releasing factor, where the proinflammatory effects are mediated, at least in part, through mast cell activation [40]. Similar to our study, it has been shown that stress can affect physiological changes in the airways that contribute to asthma [41]. Akiyama et al. demonstrate that acute stress, mediated in part through the action of corticotrophin-releasing factor, has a stimulatory effect on the ion secretion by rat tracheal epithelium, and is accompanied by evidence for mast cell activation and degranulation.

In summary, the present study shows that stress effects on inflammatory cells in BALF, as well as lung and nose pathology are strongly dependent on timing, i.e. when the stress is applied during the inflammatory process in an airway allergy model. The different stress effects may also be explained by variations in sensitivity for stress-released mediators and hormones in participating cellular compartments, such as lungs, nose or BM. To analyze long-term effects on allergy, stress hormone release pattern and effects on specific peripheral participating compartments need to be concomitantly analyzed.

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