



Acute stress affects the physiology and behavior of allergic mice

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ABSTRACT

Physical and psychological stressors have been implicated in acute asthma exacerbation. The objective of the current study was to determine the effects of forced swimming stress (FST) on allergic pulmonary inflammation in BALB/c mice. Eighty female mice were allocated to one of four treatments arranged in a 2 × 2 factorial consisting of two levels of allergy and two levels of stress. The effects of stress and allergy were assessed by examination of cytokines and leukocyte differentials in the bronchoalveolar lavage fluid, corticosterone and immunoglobulin (Ig) E in the plasma, leukocyte differentials in the peripheral blood, natural killer cytotoxicity, and histopathology of the lungs. Behavior was recorded during the FST. Stress and allergy increased plasma corticosterone in mice. Allergy increased IgE concentrations and pulmonary inflammation. Interleukin-4 was greater among allergic stressed and non-stressed mice and stressed, non-allergic mice compared with non-stressed, non-allergic mice. Interleukin-5 (IL-5) and 6 (IL-6) were greater among allergic stressed and non-stressed mice compared with non-allergic mice. Interleukin-5 and 6 were reduced among stressed-allergic mice compared with non-stressed, allergic mice. Stress and allergy shifted mice towards a T-helper 2 response as shown by increased interleukin-4. Stress reduced IL-5 and IL-6 in allergic mice but not non-allergic mice. Pulmonary inflammation was not reduced among allergic stressed mice in spite of elevated glucocorticoids. Mice induced to be allergic responded to FST differently than non-allergic mice. Our findings suggest that stress induces a differential response among allergic and non-allergic mice.

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1. Introduction

Allergic bronchial asthma is a common chronic airway disease affecting 8–10% of children, 3–5% of the adult population, and 100 to 150 million people worldwide [1,2]. Genetic background, environmental factors, viral and bacterial infections, air quality and pollution, all influence allergic pulmonary inflammation and asthma, but stress remains an important factor reported clinically in association with acute exacerbation of asthma.

Asthma is a chronic airway disease characterized by inflammation, bronchial hyperresponsiveness, and remodeling. More specifically, asthma is characterized by a dominant T-helper 2 (Th2) response caused by an over production of the Th2 cytokines (e.g. IL-4, IL-5, IL-13), a shift towards IgE production, and over production of histamines [3,4]. Dysregulation of the T-helper 1 (Th1)/Th2 cytokine balance is considered central in the pathogenesis of asthma and atopic diseases. The over production of Th2 cytokines results in the recruitment and activation of inflammatory mediators, including mast cells, basophils,

and eosinophils further resulting in airway obstructions and hyperresponsiveness [5].

Glucocorticoids and catecholamines are known to cause a shift in the Th1/Th2 balance towards Th2 by suppressing antigen-presenting cells, the Th1 response, and by up-regulating Th2 cytokine production [4]. Multiple studies have demonstrated that glucocorticoids inhibit production of the Th1 cytokines IL-12, interferon- γ (IFN- γ), IFN- α , and tumor necrosis factor- α (TNF- α) by antigen-presenting cells and Th1 cells, and consequently cause up-regulation of the Th2 cytokines IL-4, IL-10, and IL-13 by Th2 cells [6–8].

Stress is often associated with exacerbation of asthma. Perception of stress by an individual usually causes activation of the hypothalamic-pituitary-adrenal (HPA) axis and a subsequent release of glucocorticoids and catecholamines. Therefore, it seems paradoxical that treatment for asthma symptoms usually involves administering glucocorticoids or beta2-agonists as these compounds are potent bronchodilators. However the effect of glucocorticoids and catecholamines on asthma is complex and stress has been shown to enhance and attenuate asthma symptoms [4,9–11]. The fact that different types of stressors (physical versus psychological) can elicit different biochemical and physiological responses may partially account for some of these differing actions of glucocorticoids and catecholamines on atopic disease. Furthermore, the duration of a stressor can differentially affect

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the immune response. An acute stress can enhance the antigen-specific, cell-mediated immune response, but in contrast the same stressor applied chronically will suppress the cell-mediated immune response [12].

The forced swimming test (FST) is a potent activator of the stress response, causing significant increases in corticosterone concentrations in rodents [13–15]. The FST is classified primarily as a physical [16] or primarily as a psychological [17] stressor in the literature, and some researchers consider it a mixture of both [18]. The objectives of this study were to determine the effect of an acute physical and psychological stressor (FST) on the physiology, behavior, and pulmonary inflammatory response in an allergic mouse model.

2. Materials and methods

2.1. Animals and housing

Six to eight week old female BALB/c mice were obtained from Charles River (Portage, MI). All animals were maintained at 21–23 °C with a 12:12 h light–dark cycle. Two mice were housed per bedded shoebox cage with a filter top. Mice were provided an ovalbumin (OVA) free diet and water *ad libitum*. All animal procedures were approved by the Texas Tech University Animal Care and Use Committee.

2.2. Experimental design

Eighty BALB/c mice were randomly allocated into one of four treatments arranged in a 2 × 2 factorial design consisting of two levels of allergy and two levels of stress; 1) non-allergic, non-stressed (CON); 2) non-allergic, stressed (FST); 3) allergic, non-stressed (OVA); and 4) allergic, stressed (OVA + FST). Ovalbumin challenged mice were sensitized on day 7, 14, and 21 with 10 µg of OVA (crude grade IV; Sigma, St. Louis, MO) emulsified in 100 µL of aluminum hydroxide gel (Sigma) and suspended in 100 µL of phosphate buffered saline (PBS) given *i.p.* (Blyth et al., 2000). Non-challenged mice received 200 µL of vehicle *i.p.* on day 7, 14, and 21. On day 28, 29, and 30, OVA challenged mice were exposed for 20 min to aerosolized OVA in PBS (1% w/v) generated with a nebulizer (Minimate Compressor PM6, Precision Medical, Inc., North Hampton, PA). On day 30, stressed mice experienced a forced swim test (FST) for 20 min. Stressed mice were placed in a glass cylinder (27 cm × 12 cm) containing water maintained at 35 (± 2 °C) with a depth of 15 cm. After 20 min of FST, mice were dried and placed on a heating pad until dry then returned to their home cages. Mice were euthanized 50 min after the start of the FST. Allergic mice (OVA and OVA + FST) were euthanized 2.5 h after the last nebulizer exposure. All mice were euthanized on day 30, bronchoalveolar lavage (BAL) fluid and blood were collected and the lungs harvested. Sixteen mice (5 per treatment) were euthanized each day.

2.3. Bronchoalveolar lavage

Mouse tracheas were exposed surgically and cannulated using an 18G needle. Lungs were lavaged with 2 × 0.5 mL aliquots of ice cold PBS/2% fetal bovine serum (FBS). Bronchoalveolar lavage (BAL) fluid was centrifuged at 1200 rpm for 10 min and the supernatant collected and immediately frozen in a –70 °C freezer for later analysis of cytokines. Bronchoalveolar lavage cells were resuspended in 0.5 mL 2% FBS/PBS. One hundred microliters of resuspended BAL cells were spun on a cytofuuge at 8500 rpm (Cytofuuge 2, model M801-22, StatSpin, Inc., Norwood, MA) for 4 min. Slides were fixed and stained using Hematoxylin stain I and II (Fisher Scientific, Houston, TX). Bronchoalveolar lavage leukocyte differential counts were determined under a light microscope at 1000× magnification (Hansen et al., 1999).

Bronchoalveolar lavage fluid supernatant was assayed for IL-4, IL-5, IL-6, interleukin-10 (IL-10), and INF-γ using commercially available kits (Assay Designs, Ann Arbor MI) following manufacturer's instruction. The minimum sensitivity of the assays were 4.34, <5, 1.01, 5.5 and <10 pg/ml, respectively.

2.4. Blood differentials and plasma collection

Blood was collected over heparin from the right ventricle of the heart. Blood samples were centrifuged for 20 min at 8500 rpm, plasma collected, and stored at –70 °C. Whole blood was evaluated to determine total white blood cell (WBC) numbers and leukocyte differential (DIFF) percentages. Total WBC counts were obtained using a WBC dilution kit and hemocytometer under light microscope at 400× magnification (BD Unopette System, Fisher Scientific, Itasca, IL). Whole blood smears were fixed in methanol and stained using Hematoxylin stain I and II (Fisher Scientific) to determine DIFF percentages.

Plasma was assayed for corticosterone using a commercially available radioimmunoassay kit (MP Biomedicals, Orangeburg, NY) following the manufacturer's instructions. The minimum sensitivity of the assay was 7.7 ng/mL.

Plasma was assayed for immunoglobulin E using a commercially available ELISA kit (Bethyl Laboratories, Montgomery, TX) following the manufacturer's instructions. The minimum sensitivity of the assay was 3.9 ng/mL.

2.5. Lung histopathology

Lungs were harvested and placed into 10% formalin for at least 5 days prior to paraffin embedding. Parasagittal sections were sliced (8 µm), stained with hematoxylin–eosin (H/E), and analyzed by bright-field microscopy. Totally embedded lungs of mice were evaluated for seven changes: 1) inflammation of bronchoarterial spaces; 2) peribronchiolar inflammation; 3) perivenular inflammation; 4) inflammation about amuscular blood vessels/lymphatics; 5) inflammation in inter-alveolar spaces, not surrounding blood vessels/lymphatics; 6) subpleural inflammation; 7) eosinophils within the inflammatory aggregates. When all seven conditions were observed, mice lungs were considered to have a full inflammatory response (Fig. 1).

2.6. Natural killer cytotoxicity activity

Spleens were aseptically harvested from mice and placed in a sterile petri dish containing 5 mL Roswell Park Memorial Institute (RPMI). Splenocytes were gently teased out of the spleen capsule using curved forceps. Splenocytes were washed twice in RPMI, resuspended, and counted.

Natural killer cytotoxicity (NK) was measured using a commercially available non-radioactive cytotoxicity detection kit, (Cytotox 96®; Promega Corporation, Madison, WI) and a radioactive NK assay protocol as originally described [19], with modifications. Briefly, splenocytes were used as effector cells and YAC-1 cells (American Tissue Type Culture Collection, Manassas, VA) as target cells. Splenocytes were adjusted to 1 × 10⁴ cells/mL and YAC-1 cells were adjusted to a constant 10,000 cells per well. Samples were run in triplicate at effector-to-target ratios of 12.5:1, 25:1, 50:1, and 100:1, respectively. Percentage cytotoxicity was calculated as previously described [19].

2.7. Behavioral data collection

Behaviors were recorded during the forced swim test using time-lapse video recording in 2-h mode (30 frames/s). The duration of each behavior was analyzed continuously using the

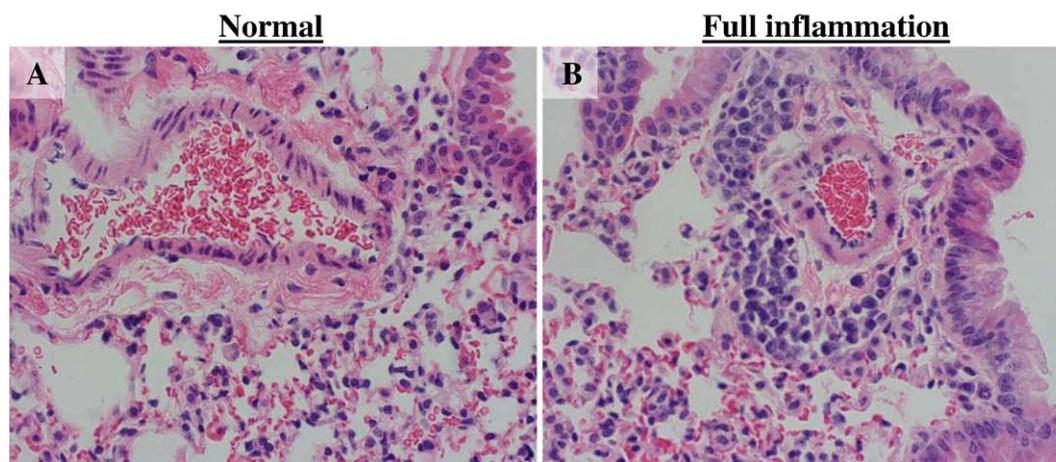


Fig. 1. Pulmonary histopathology of BALB/c mice, normal (left) and inflamed (right). 400× magnification. Note markedly increased pulmonary inflammation characterized by perivascular mononuclear cells and leukocyte infiltrate (B) when compared to normal (A).

Observer 5.0 program (Noldus, Leesburg, VA). Behavior is presented as the percentage of observations of a given behavior over each sampling period and analyzed using analysis of variance using the mixed model procedure of SAS. The 20 min behavior observation period was divided into five, 4 min periods. Behaviors measured included horizontal locomotion, vertical locomotion, horizontal immobility, vertical immobility, sinking locomotion, sinking immobility, total locomotion, total immobility, total vertical behavior, and total horizontal behavior. Horizontal locomotion: body and tail are horizontally aligned with the surface of the water and limbs are moving; the middle of the back is above the water. Vertical locomotion: body and tail are aligned under the surface of the water and only the head is seen above the water surface and limbs are moving. Horizontal immobility: body and tail were horizontally aligned with the surface of the water and limbs are not moving. Vertical immobility: body and tail are aligned under the surface (vertical), the head is above the water surface and limbs are not moving. Sink locomotion: eyes fall below water surface, limbs moving. Sink immobility: eyes fall below water surface, limbs not moving. Total locomotion: horizontal locomotion + vertical locomotion + sinking locomotion. Total immobility: horizontal immobility + vertical immobility + sinking immobility. Total vertical behavior: vertical locomotion + vertical immobility. Total horizontal behavior: horizontal locomotion + horizontal immobility. Seven allergic and two non-allergic mice had to be “rescued” by the observer to prevent drowning. These animals were removed from the behavioral analysis.

2.8. Statistical analysis

All physiology and behavioral data were tested for homogeneity of variance and departures from normal distribution. Data lacking normality were transformed logarithmically using $\log_{10}$. Data were subjected to analysis of variance using the general model procedure of SAS version 9.1 (SAS Inst., Inc., Cary, NC). The main fixed effects were day (five levels), allergy (two levels), and stress (two levels). Random effects of group were also included. The Kruskal–Wallis test (SAS Inst., Inc., Cary, NC) was used to analyze non-parametric data.

In addition, for behavior data, the 20 min behavior observation period was divided into five, 4 min periods. The main fixed effects were day (five levels), period (five levels), and allergy (two levels). Random effects of group were also included. Nine mice were removed from the behavioral analysis due to early removal from the FST to prevent drowning.

3. Results

3.1. Plasma corticosterone and immunoglobulin E

Plasma corticosterone concentrations were greater ($F_{(3,75)} = 83.48$, $p < 0.001$) among mice that experienced FST compared with non-stressed control mice (Fig. 2A). Corticosterone concentrations were greater ($p < 0.001$) among non-stressed-allergic mice compared with control mice. Corticosterone concentrations did not differ ($p > 0.05$) between FST non-allergic and FST allergic mice.

Immunoglobulin E concentrations were greater ($F_{(3,75)} = 56.81$, $p < 0.001$) among non-stressed and stressed-allergic mice compared with non-stressed and stressed non-allergic mice (Fig. 2B). Immunoglobulin E concentrations did not differ between non-stressed-allergic and stressed-allergic mice.

3.2. Bronchoalveolar lavage cytokines

Interleukin-4 concentrations were greater ($F_{(3,73)} = 6.08$, $p < 0.005$) among stressed and non-stressed, allergic mice compared with CON mice. Mice experiencing FST had elevated BAL IL-4 concentrations compared to CON mice (Fig. 3A).

Interleukin-5 concentrations were greater ($F_{(3,73)} = 18.70$, $p < 0.005$) among allergic compared with non-allergic mice regardless of stress (Fig. 3B). Interleukin-5 concentrations were lower ($p < 0.05$) among stressed-allergic mice compared with non-stressed, allergic mice.

Interleukin-6 concentrations were increased ($F_{(3,73)} = 13.09$, $p < 0.001$) among FST mice compared with CON mice (Fig. 3C). Interleukin-6 concentrations were greater ($p < 0.005$) in allergic, non-stressed mice compared with CON and FST mice. Among allergic mice, FST reduced ($p = 0.068$) IL-6 concentrations.

Interleukin-10 concentrations were greater ($F_{(3,73)} = 2.19$, $p < 0.05$) among stressed, non-allergic (432.9 ± 69.1 pg/mL) mice compared with non-stressed, non-allergic mice (175.2 ± 66.2 pg/mL). No other differences ($p > 0.05$) in IL-10 concentrations were observed. Interferon- γ concentrations did not differ ($p > 0.05$) among any of the treatments.

3.3. Splenocyte natural killer cytotoxicity

Natural killer (NK) cytotoxicity did not differ between CON and FST mice. However, among OVA sensitized mice, FST increased ($F_{(3,46)} =$

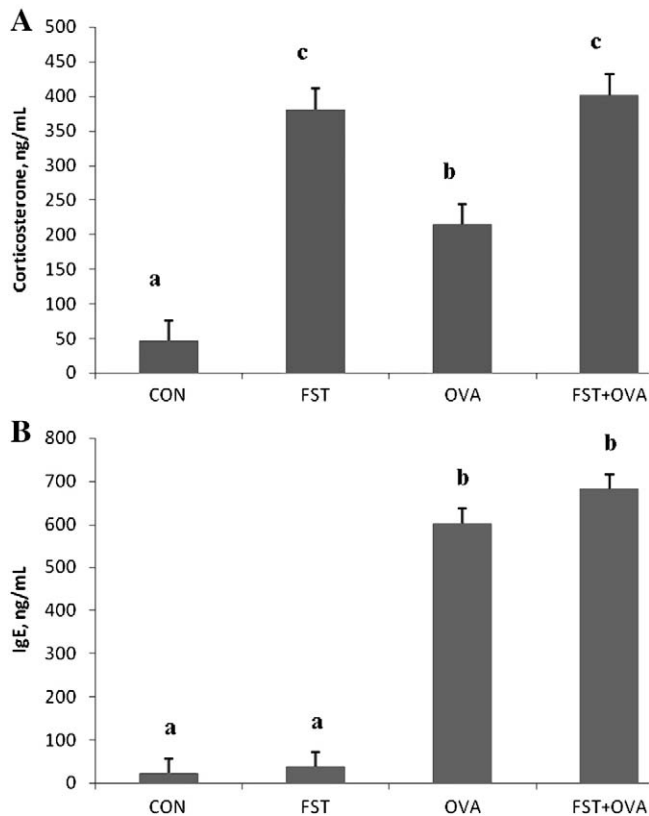


Fig. 2. A. Plasma corticosterone concentrations (ng/mL) of non-stressed, non-allergic (CON, $n=20$), stressed, non-allergic (FST, $n=20$), non-stressed, allergic (OVA, $n=20$), or stressed-allergic (FST + OVA, $n=19$) mice. Least square means accompanied by different subscript are different at $p<0.05$. [allergy $\times$ stress: $F_{(3,75)}=83.48$, $p<0.001$]. B. Plasma immunoglobulin E concentrations (ng/mL) of non-stressed, non-allergic (CON, $n=19$), stressed, non-allergic (FST, $n=20$), non-stressed, allergic (OVA, $n=20$), or stressed-allergic (FST + OVA, $n=18$) mice. Least square means accompanied by different subscript are different at $p<0.05$. [allergy $\times$ stress: $F_{(3,75)}=56.81$, $p<0.001$].

3.26, $p<0.05$) NK cytotoxicity (CON: $37.1 \pm 4.93\%$; FST: $46.0 \pm 3.99\%$; OVA: $42.7 \pm 4.77\%$; FST + OVA: $56.5 \pm 5.28\%$).

3.4. Peripheral and bronchoalveolar lavage differentials

Total white blood cell (WBC) counts were not changed by FST or OVA immunization alone. However, among mice with both FST and OVA, the WBC numbers were reduced ($F_{(3,75)}=5.72$, $p<0.005$) compared with mice in other treatment groups (Table 1). The percentage of lymphocytes was lower and neutrophil percentage higher ($F_{(3,75)}=15.04$, $p<0.001$) among allergic mice compared with non-allergic mice, regardless of stress treatment (Table 1). Consequently, the neutrophil to lymphocyte ratio was greater ($F_{(3,75)}=15.32$, $p<0.001$) among allergic compared with non-allergic mice regardless of stress treatment (Table 1). The percentage of eosinophils did not differ ($p>0.05$) among treatments (Table 1). The percentage of BAL monocyte/macrophages was lower ($F_{(3,75)}=2.75$, $p=0.058$) among allergic, non-stressed compared with CON mice (Table 1). The percentage of BAL eosinophils was greater ($F_{(3,75)}=5.91$, $p<0.005$) among allergic compared to non-allergic mice regardless of stress treatment (Table 1).

3.5. Lung pathology

Full pulmonary lung inflammation was greater ($F_{(3,70)}=471.94$, $p<0.001$) among non-stressed and stressed-allergic mice compared with CON and FST mice. Full pulmonary lung inflammation did not differ between non-stressed-allergic and stressed-allergic mice (CON: $0.0 \pm 2.5\%$; FST: $0.0 \pm 2.5\%$; OVA: $95.0 \pm 2.5\%$; FST + OVA: $100.0 \pm 2.7\%$).

3.6. Mouse behavior

The percentage of time mice spent performing total horizontal ($F_{(4,29)}=6.79$, $p<0.001$) and total vertical ($F_{(4,29)}=5.19$, $p<0.005$) behaviors changed over time (Fig. 4A, B). Mice spent more time ($p<0.001$) performing horizontal behaviors during the first 4 min of

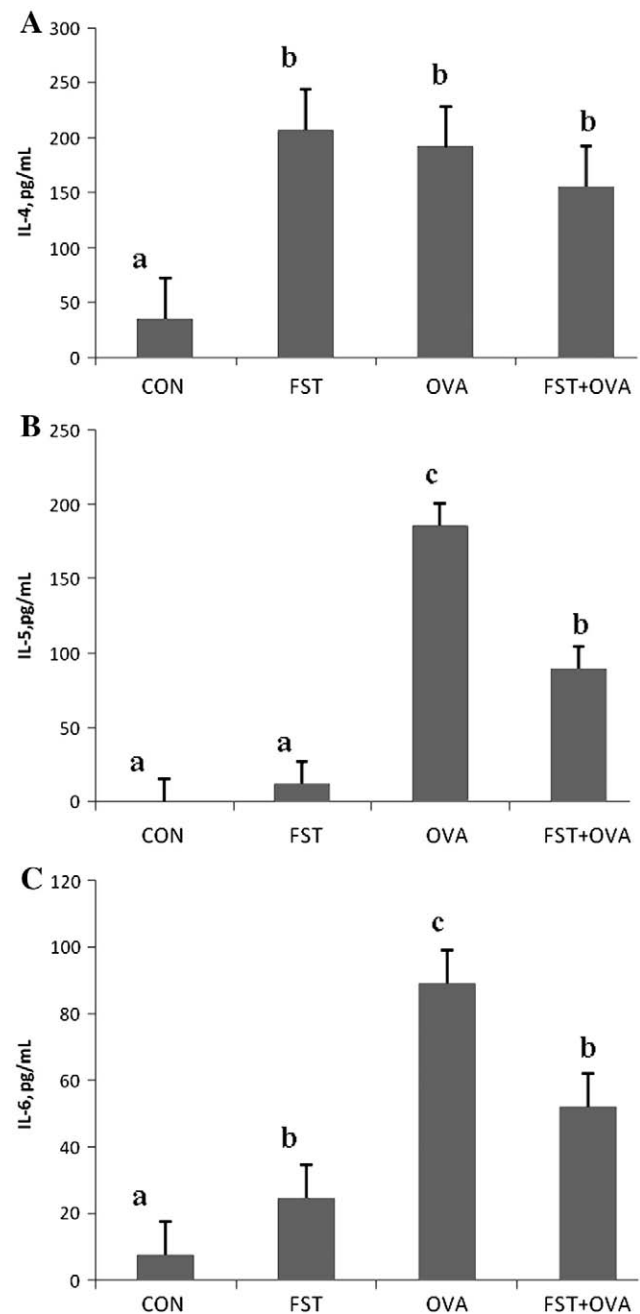


Fig. 3. A. Interleukin-4 concentrations (pg/mL) of non-stressed, non-allergic (CON, $n=20$), stressed, non-allergic (FST, $n=20$), non-stressed, allergic (OVA, $n=18$), or stressed-allergic (FST + OVA, $n=19$) mice. Least square means accompanied by different subscript are different at $p<0.05$. [allergy $\times$ stress: $F_{(3,73)}=6.08$, $p<0.005$]. B. Interleukin-5 concentrations (pg/mL) of non-stressed, non-allergic (CON, $n=20$), stressed, non-allergic (FST, $n=20$), non-stressed, allergic (OVA, $n=19$), or stressed-allergic (FST + OVA, $n=18$) mice. Least square means accompanied by different subscript are different at $p<0.05$. [allergy $\times$ stress: $F_{(3,73)}=18.70$, $p<0.005$]. C. Interleukin-6 concentrations (pg/mL) of non-stressed, non-allergic (CON, $n=20$), stressed, non-allergic (FST, $n=20$), non-stressed, allergic (OVA, $n=19$), or stressed-allergic (FST + OVA, $n=18$) mice. Least square means accompanied by different subscript are different at $p<0.07$. [allergy $\times$ stress: $F_{(3,73)}=13.09$, $p<0.001$].

Table 1

Total white blood cell counts and peripheral and bronchoalveolar lavage (BAL) differentials in non-stressed, non-allergic (CON), stressed, non-allergic (FST), non-stressed, allergic (OVA), or stressed, allergic (FST + OVA) mice.

Measure	Treatment							
	CON	SE	FST	SE	OVA	SE	FST + OVA	SE
N	20		20		20		20	
WBC, no. $\times 10^3$ /mL	93.8 ^b	5.04	89.4 ^b	5.10	82.8 ^b	5.04	65.8 ^a	5.18
<i>Peripheral differentials, %</i>								
Lymphocytes	80.9 ^b	1.90	77.3 ^b	1.92	68.0 ^a	1.90	63.1 ^a	1.90
Neutrophils	16.5 ^a	1.83	19.2 ^a	1.85	27.6 ^b	1.83	31.7 ^b	1.83
Monocytes	2.7 ^a	0.44	3.1 ^a	0.45	4.3 ^{ab}	0.44	4.6 ^b	0.44
Eosinophils	0.0	0.17	0.3	0.17	0.1	0.17	0.4	0.17
Neutrophil:lymphocyte ratio	0.21 ^a	0.044	0.26 ^a	0.045	0.43 ^b	0.044	0.54 ^b	0.044
<i>BAL differentials, %</i>								
Lymphocytes	12.4	3.62	4.9	4.45	11.7	3.56	6.5	3.95
Neutrophils	6.7	1.15	3.7	1.42	6.2	1.14	6.1	1.26
Monocytes	73.0 ^b	7.07	77.3 ^b	8.69	44.9 ^a	6.96	54.0 ^{ab}	7.71
Eosinophils	7.8 ^a	5.72	14.0 ^a	7.03	37.1 ^b	5.64	33.1 ^b	6.24

^{a,b}Least squares means with different superscripts within a row are different ($p < 0.05$).
WBC = total white blood cells.

the FST compared with the following 16 min of the FST. Conversely, mice spent less time ($p < 0.005$) performing vertical behaviors during the first 4 min of the FST compared with the following 16 min of the FST. There was no treatment or treatment $\times$ period effects ($p > 0.05$) for any of the behaviors measured. Allergic mice tended ($p = 0.061$) to need more 'rescuing' than non-allergic mice during the 20 min FST.

4. Discussion

This experiment demonstrated the physiological and behavioral response of allergic and non-allergic mice to a 20 min FST. Corticosterone concentrations were elevated in mice exposed to a

20 min FST compared with non-stressed mice demonstrating that the FST test was effective in significantly increasing corticosterone concentrations and inducing a stress response among mice. Corticosterone concentrations were elevated among non-stressed-allergic compared with CON mice suggesting that allergic mice were also experiencing some level of stress associated with the OVA experience. Non-allergic mice nebulized with PBS had similar corticosterone concentrations as mice nebulized with OVA (unpublished data), suggesting that the increased corticosterone concentrations in allergic non-stressed mice was due to the experience of being nebulized as opposed to a reaction to the OVA. Mice may perceive the experience of being nebulized as stressful due to being isolated in the nebulizer box, repeated handling to and from the nebulizer, or a combination of these stressors. In the present study, CON mice were not nebulized with PBS so as to eliminate the confounding effect that nebulization has on the stress response in mice. This observation has ramifications for studies that use a nebulizer to expose mice to aerosol allergens because the allergic-induction method appears to inadvertently induce stress.

An important feature of asthma is the development of airway inflammation, which is associated with the presence of increased pulmonary eosinophils, neutrophils, macrophage/monocytes, and lymphocytes [20]. In the present study, the percentage of BAL eosinophils was elevated among allergic mice compared with non-allergic mice which is consistent with previously reported observations [21–23]. An increase in IgE concentrations is commonly associated with allergy and asthma in humans and animals [3,24]. In the present study, IgE concentrations were elevated among allergic mice compared with non-allergic mice. Furthermore, full lung pulmonary inflammation was confirmed in histological sections in the lungs of allergic, but not non-allergic mice. Therefore, the combination of the IgE, BAL differentials, and histology data indicates that our model increased pulmonary inflammation and created an asthma-like state in mice.

In the literature, most asthma studies harvest the experimental animals at least 24 h after the last OVA challenge or stressor [22,25]. In the present study, the objective was to determine the immediate effect of stress on the immune response of allergic mice when corticosterone concentrations were maximal. Prior to the commencement of the present study, a time course study was run to determine at what time points cytokines (IL-5 and INF- γ) and leukocytes in the BAL fluid were measurable. Bronchoalveolar cytokine concentrations and leukocyte percentages were similar in mice harvested at 30 min and 24 h after an OVA challenge (unpublished data). In the present study,

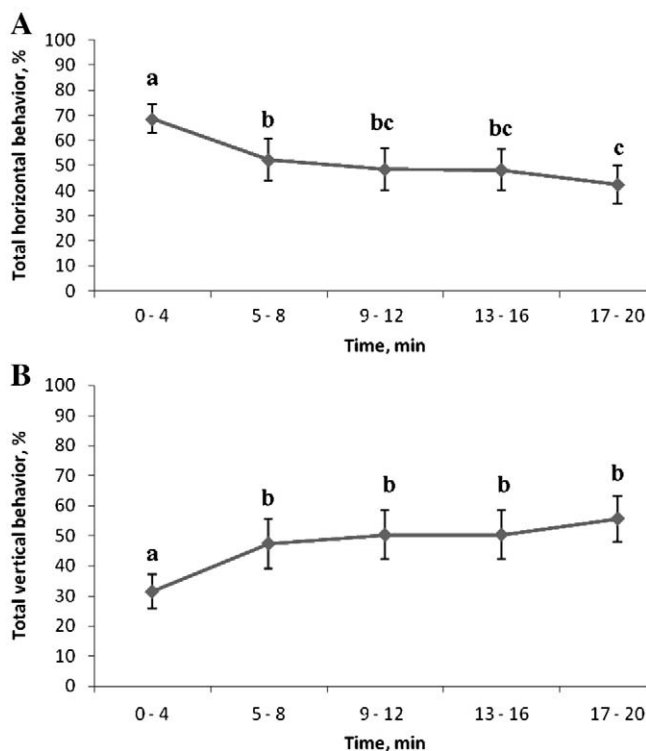


Fig. 4. Total percentage of time spent performing horizontal (A) and vertical (B) behaviors, regardless of treatment ($n = 33$). Least square means accompanied by different subscript are different at $p < 0.05$. Horizontal behavior: [period: $F_{(4,29)} = 6.79$, $p < 0.001$]. Vertical behavior: [period: $F_{(4,29)} = 5.19$, $p < 0.005$].

mice (OVA and OVA + FST) were harvested 3 h after the 3rd OVA challenge and 50 min after the commencement of the FST (FST and OVA + FST). Bronchoalveolar cytokine concentrations and leukocyte percentages were elevated compared with control values, implying that the time of harvest was adequate to observe the effect of acute stress on the immune response to an OVA challenge in mice.

Allergic disease, such as asthma, is thought to be characterized by a dominant Th2 response caused by an over production of the Th2 cytokines (e.g. IL-4, IL-5, and IL-13) and a shift to IgE production [3,5]. In the present study, IL-4 was elevated among non-allergic, stressed mice compared with CON mice. Glucocorticoids mediate a shift towards Th2 by suppressing antigen-presenting cells, the Th1 response, and up-regulating Th2 cytokine production [4]. Stress and allergy did not have an additive effect on the concentrations of IL-4 in BAL fluid; in fact IL-4 was numerically lower among allergic stressed mice compared with allergic, non-stressed mice. Interleukin-4 concentrations were decreased among allergic mice that experienced aerobic exercise over a 4 week period compared with non-exercised, allergic mice [25]. The difference in the IL-4 response between the Pastva et al. [25] study and ours may be due to the fact that these studies used very different stressors. In the present study a 20 min acute physical/psychological stressor was used compared to the 4 week chronic physical stressor [25]. A chronic physical stressor may actually have positive effects if it simulates an exercise effect rather than a stress effect. A possible endocrine explanation for the observed difference in IL-4 concentrations between these two studies is differences in circulating glucocorticoid concentrations. In the present study, corticosterone levels were approximately eight times higher among FST mice than non-stressed controls, however after exposure to a chronic stressor it is common for animals to become habituated to the stressor resulting in a decreased glucocorticoid response. Glucocorticoids were not reported in the Pastva et al. [25] study, so it is difficult to determine if this was the case. When studying the impact of acute or chronic stress or exercise on the physiological response to allergy it is important to define the stressor.

Interleukin-5 is characterized as a Th2 cytokine [26]. In the present study, IL-5 was elevated among allergic mice compared with non-allergic mice, but was reduced among allergic, stressed mice compared to allergic, non-stressed mice. Interleukin-5 concentrations were also decreased among allergic mice that experienced aerobic exercise over a 4 week period compared with non-exercised, allergic mice [25]. The FST seems to have a differential effect on Th2 cytokines dependent on the allergy status of the animal. The reduction in IL-5 response to stress in allergic mice may indicate a “protective” effect of stress among allergic subjects. Even though, IL-5 was reduced in stressed-allergic mice, this reduction in Th2 cytokines was not reflected by a reduction in pulmonary inflammation, so maybe IL-4 plays a larger role in pulmonary inflammation than IL-5. It is also possible that resolution of pulmonary inflammation takes longer than that required for cytokine levels to change. There may also be other factor/s responsible for the acute stress associated allergic pulmonary inflammation. Studies have shown that neurokinins and endogenous opioids are also involved in pulmonary inflammation [21,27]. Further studies are required to determine the exact roles that IL-4 and 5 play in pulmonary inflammation and the interaction between stress and inflammation.

Interleukin-6 is produced by a wide range of cell types including immune, neuroendocrine, and endocrine tissues. Production of IL-6 has been found in response to stress, inflammation, and trauma. In the present study, IL-6 concentrations were elevated among allergic, stressed and non-stressed mice compared with non-allergic mice. Histamine is one of the major mediators of acute inflammation and allergic reactions and can potentiate the production of IL-6 by human monocytes and dendritic cells. Histamine is produced in response to allergic reactions, therefore it follows that IL-6 would be elevated in allergic mice. Interleukin-6 concentrations were elevated in stressed, non-allergic mice compared with CON mice. Elevated IL-6 concentra-

tions have also been recorded in response to physical [28] and several different psychological stressors [28,29]. Interleukin-6 has also been shown to activate the HPA axis in humans [30]. Therefore, elevated IL-6 concentrations in non-allergic, stressed and allergic, stressed and non-stressed mice could have influenced the corticosterone concentrations in these animals, however the corticosterone concentrations do not follow the same pattern as the IL-6 concentration making this unlikely. Interestingly, IL-6 was reduced among allergic, stressed mice compared to allergic, non-stressed mice. More research is needed to fully understand the interaction between IL-6, allergy, and the HPA axis.

The FST is a commonly used acute stressor in rodent studies. The FST is classified as a physical and/or psychological stressor in the literature [16–18]. In pharmacological studies, where the FST is classified as a psychological stressor, immobility during the FST is usually considered to be associated with depressive-like behavior. During the FST rodents can either be immobile in the water or actively swim. Some researchers define immobility during the FST as “despair” behavior as the animal is no longer trying to escape the situation giving the appearance of “learned helplessness” [13]. In the present study, there was no difference in the time spent performing immobile or locomotion behaviors between non-allergic and allergic mice, signifying that allergy did not elicit depressive-like behavior in these mice. However, mice, regardless of their allergic status spent less time swimming horizontally and more time swimming vertically overtime. The change from horizontal to vertical swimming may suggest that these mice became fatigued over time and no longer had the energy to maintain a horizontal posture. During the FST, 22.5% of all experimental mice needed rescuing, further suggesting that these mice were experiencing fatigue. Of the 9 mice needing rescuing, 7 were allergic mice. Allergic mice may have had more trouble breathing due to increased pulmonary inflammation (as shown by histology) resulting in increased fatigue and therefore increased ‘drowning’. Even though the FST can be used as a measure of learned helplessness or depression, in this study it is more likely that the performance of increased ‘drowning’ behavior by allergic mice was a consequence of pulmonary inflammation rather than learned helplessness.

Acute stress appeared to have differential effects on the physiological and behavioral response of mice dependent on their allergy status. Interleukin-5 and 6 were decreased among stressed-allergic mice, but not among non-allergic, stressed mice. Furthermore, these differences in Th2 cytokines were not reflected by changes in pulmonary inflammation between stressed-allergic mice and non-allergic, stressed mice. The interaction between stress, cytokine profiles and pulmonary inflammation among allergic and non-allergic mice is more complex than can be explained entirely by a shift in the balance between Th1 and Th2 systems.

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