Acute Exercise Decreases Airway Inflammation, but Not Responsiveness, in an Allergic Asthma Model

Matt Hewitt¹, Amy Creel², Kim Estell¹, Ian C. Davis³, and Lisa M. Schwiebert¹

¹Department of Physiology and Biophysics, ²Department of Pediatrics, University of Alabama at Birmingham, Birmingham, Alabama; and ³Department of Veterinary Biosciences, The Ohio State University, Columbus, Ohio

Previous studies have suggested that the asthmatic responses of airway inflammation, remodeling, and hyperresponsiveness (AHR) are interrelated; in this study, we used exercise to examine the nature of this interrelationship. Mice were sensitized and challenged with ovalbumin (OVA); mice were then exercised via running on a motorized treadmill at a moderate intensity. Data indicate that, within the lungs of OVA-treated mice, exercise attenuated the production of inflammatory mediators, including chemokines KC, RANTES, and MCP-1 and IL-12p40/p80. Coordinately, OVA-treated and exercised mice displayed decreases in leukocyte infiltration, including eosinophils, as compared with sedentary controls. Results also show that a single bout of exercise significantly decreased phosphorylation of the NF- κ B p65 subunit, which regulates the gene expression of a wide variety of inflammatory mediators. In addition, OVA-treated and exercised mice exhibited decreases in the levels of Th2-derived cytokines IL-5 and IL-13 and the prostaglandin PGE₂, as compared with sedentary controls. In contrast, results show that a single bout of exercise had no effect on AHR in OVA-treated mice challenged with increasing doses of aerosolized methacholine (0–50 mg/ml) as compared with sedentary mice. Exercise also had no effect on epithelial cell hypertrophy, mucus production, or airway wall thickening in OVA-treated mice as compared with sedentary controls. These findings suggest that a single bout of aerobic exercise at a moderate intensity attenuates airway inflammation but not AHR or airway remodeling in OVA-treated mice. The implication of these findings for the interrelationship between airway inflammation, airway remodeling, and AHR is discussed.

Keywords: asthma; aerobic exercise; airway inflammation; remodeling; hyperresponsiveness

Asthma is characterized by the clinical symptoms of wheezing, chest tightness, dyspnea and cough, and by the presence of reversible airway narrowing and/or airway hyperresponsiveness (AHR) to a variety of inhaled bronchoconstrictor stimuli (1). Although multifactorial in origin, asthma is considered an inflammatory process that is the result of an inappropriate and sustained immune response to innocuous antigens. This response involves increased levels of chemokines, cytokines, and prostaglandins that perpetuate tissue damage and airway remodeling through the recruitment and activation of leukocytes, including Th2 cells and eosinophils.

The interplay between the mechanisms that drive AHR, airway remodeling, and airway inflammation has been examined extensively. Numerous studies have suggested a causal

CLINICAL RELEVANCE

Using acute aerobic exercise of ovalbumin-treated mice, we report that airway remodeling and airway hyperresponsiveness (AHR) persist in the absence of airway inflammation. These findings indicate that airway inflammation is not required for development of airway remodeling and AHR.

relationship between increases in cellular inflammation of the airways and AHR (2). Likewise, increases in airway remodeling, including goblet cell hyperplasia and airway wall thickening, have been implicated in the pathogenesis of AHR (2). Because antiinflammatory therapy does not completely resolve AHR in individuals with chronic asthma, the role of inflammation in the initiation and exacerbation of AHR is in question.

Previous reports have demonstrated that acute aerobic exercise at a moderate intensity affects both immune responses and lung function in healthy subjects (3, 4); therefore, we used acute exercise as a tool to further examine the relationship between airway inflammation, airway remodeling, and AHR. Because lung function is compromised in individuals with asthma, we hypothesized that acute aerobic exercise at moderate intensity would attenuate airway inflammatory responses but not airway remodeling or AHR in an ovalbumin (OVA)-driven mouse model of allergic asthma. For this study, mice were sensitized and challenged with OVA or control saline and exercised for one bout at a moderate intensity on a motorized treadmill. At the conclusion of the protocol, changes in leukocyte infiltration, mediator production, airway remodeling, and AHR were monitored. Results presented herein indicate that a single bout of moderate-intensity aerobic exercise attenuated airway inflammation, but not airway remodeling or AHR.

MATERIALS AND METHODS

Animals

Female BALB/cJ mice (3–5 wk old; The Jackson Laboratory, Bar Harbor, ME), a strain susceptible to OVA-induced IgE responses (5, 6), were used. Mice were housed in a pathogen-free containment facility and maintained in autoclaved Microisolator cages (Lab Products, Maywood, NJ). Mice were provided with food (Teklad, Madison, WI) and water *ad libitum*. Mice were allowed to acclimate to housing condition for 1 week before experimental manipulation. All animal treatments were approved by the University of Alabama at Birmingham (UAB) Institutional Animal Care and Use Committee (IACUC) and were in accordance with the National Institutes of Health recommended guidelines.

OVA Treatment and Aerobic Exercise Protocol

At the start of the protocol, mice were assigned randomly into sedentary and exercised groups. Sensitization (Days 0 and 14, intraperitoneally) and challenge (Days 21–25, Day 28 via aerosolization)

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Correspondence and requests for reprints should be addressed to Lisa M. Schwiebert, Ph.D., Department of Physiology and Biophysics, MCLM, Room 955, University of Alabama at Birmingham, 1918 University Boulevard, Birmingham, AL 35294-0005. E-mail: lschwieb@uab.edu

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with OVA (Sigma Chemical, St. Louis, MO) or saline was performed as described previously (7, 8). Exercised mice underwent a single bout of moderately intense aerobic exercise on a motorized treadmill (Exer 6M; Columbus Instruments, Columbus, OH) for 45 minutes at 13.5 m/minute (0% grade) on Day 28 of the protocol. As noted in our earlier study (7), previous reports have defined moderate intensity aerobic exercise as brief (15–60 min) bouts of treadmill running at 50 to 75% maximum O_2 consumption or approximately 15 to 22 m/minute (9–12). The exercise bout included brief warm-up and cool-down periods, so that the total treadmill time was approximately 60 minutes.

Lavage Cell and Protein Analyses

Twenty-four hours after completion of the OVA-treatment/exercise protocol, mice were killed via intraperitoneal injection of ketamine (8.7 mg/kg)/xylazine (1.3 mg/kg) and prepared for bronchoalveolar lavage (BAL) as described previously (7). Cell viability was determined via trypan blue exclusion and cell types were differentiated on cytospin preps using Wright-Giemsa stain. Cell differentials were determined from at least 500 leukocytes using standard hematologic criteria. BAL samples were analyzed for mediator expression via Mouse Multiplex Assay (Bio-Rad Inc., Hercules, CA). PGE_2 was analyzed via enzyme-linked immunosorbent assay (ELISA; Invitrogen, Carlsbad, CA).

NF κ B p65 Phosphorylation Analysis

Twenty-four hours after completion of the OVA-treatment/exercise protocol, lungs were harvested and protein extracts were prepared as described previously (8). Equivalent amounts of protein from each sample were analyzed for differences in total and phosphorylated NF- κ B p65 via ELISA according to the manufacturer's instructions (Millipore, Billerica, MA).

Airway Hyperresponsiveness Analysis

Mice were mechanically ventilated and challenged with increasing concentrations of aerosolized methacholine as described previously 48 hours after the last OVA treatment/exercise bout (13, 14). This time-point was chosen to be consistent with the current literature. Briefly, mice were anesthetized with diazepam (17.5 mg/kg) and ketamine (450 mg/kg) and a tracheotomy tube (18G) was inserted and connected to the inspiratory and expiratory ports of a ventilator (Flexivent; SCIREQ, Montreal, PQ, Canada). Mice were ventilated at a rate of 160 breaths per minute at a tidal volume of 0.2 ml with a positive end-expiratory pressure of 2 to 4 cm H_2O . Increasing concentrations of methacholine (0–50 mg/ml) were administered via aerosolization. From 20 seconds up to 3 minutes after each aerosol challenge, resistance (R) was recorded continuously; an average value of R was taken to express changes in airway function.

Airway Remodeling Analysis

Lungs fixed in 70% alcoholic formalin and paraffin-embedded were stained with Alcian blue–periodic acid-Schiff hematoxylin (PASH) as described previously (7). After random coding, the degree of epithelial cell hypertrophy and mucus production in the PASH analyses was assessed subjectively; a semiquantitative rating scale ranged from 0 (none) to maximal (4). For direct measurements of airway wall thickening (15), tissue sections were visualized under a light field microscope at $\times 200$ magnification. Airways with a longitudinal diameter between 150 and 200 μ m were identified, and differences in airway wall thickness were determined using the imaging program Metamorph. A minimum of six independent airways per tissue section were measured.

Statistical Analysis

Data were analyzed using SPSS Version 11.0. Results are reported as group means $\pm$ SD. A one-way ANOVA determined differences among the group means, and the Bonferroni *post hoc* analysis determined which group means differed significantly (at a level of $P \leq 0.05$).

RESULTS

Acute Aerobic Exercise Attenuates Chemoattractant Production and Leukocyte Infiltration

Production of chemoattractants plays an important role in the recruitment and subsequent activation of immune cells into the bronchial mucosa during an inflammatory response. Elevated levels of cytokines with chemoattractant properties, including KC (CXCL8; murine homolog of human IL-8), RANTES (CCL5), MCP-1 (CCL2), and IL-12p40/p80, have been implicated in airway inflammation (16–19). Data shown in Figure 1 demonstrate that OVA treatment of sedentary mice increased (between 6- and 35-fold) KC, RANTES, MCP-1, and IL-12p40/p80 protein levels significantly as compared with saline-treated controls. A single bout of exercise at moderate intensity attenuated ($\geq 50\%$ decrease) significantly the production of KC, RANTES, and MCP-1 in OVA-treated mice as compared with sedentary controls (Figures 1A–1C). Exercise also decreased IL-12p40/p80 protein content (Figure 1D); however, little or no detectable IL-12p70 protein was observed in either control or experimental mice (data not shown). Sedentary and exercised saline-treated controls expressed equivalent levels of each mediator analyzed (Figure 1).

Concomitant with these results, findings presented in Figure 2 indicate that leukocyte infiltration was increased (~ 30 -fold) significantly in OVA-treated sedentary mice as compared with saline-treated controls. OVA-treated mice that were exercised for a single bout at a moderate intensity exhibited decreases in leukocyte infiltration, including infiltration of macrophages, eosinophils, and neutrophils, when compared with sedentary

Figure 1. One bout of aerobic exercise at a moderate intensity decreased chemokine production in ovalbumin (OVA)-treated mice. Twenty-four hours after protocol completion, bronchoalveolar lavage (BAL) was performed; resulting lavagates were analyzed for changes in (A) KC, (B) RANTES, (C) MCP-1, and (D) IL-12p40/p80 via multiplex assay. Results are reported as pg/ml of respective protein ($^{\dagger}P < 0.006$ as compared with saline-treated, sedentary mice; $^*P < 0.04$ as compared with OVA-treated, sedentary mice; $n = 4$ –8 mice per group). Open bars, sedentary mice; solid bars, exercised mice.

Figure 2. A single bout of moderate intensity aerobic exercise decreased leukocyte infiltration in OVA-treated mice. Twenty-four hours after protocol completion, BAL was performed and resulting lavagates were analyzed for changes in total cells and eosinophils. Results were reported as changes in cell number ($^{\dagger}P < 0.03$ as compared with saline-treated, sedentary mice; $*P < 0.04$ as compared with OVA-treated, sedentary mice; ND, none detected; $n = 4$ mice per group). Open bars, sedentary mice; solid bars, exercised mice.

controls (Figure 2). Sedentary and exercised saline-treated controls exhibited equivalent levels of leukocyte infiltrate.

Acute Aerobic Exercise Decreases NF- κ B p65 Phosphorylation

The activated transcription factor NF- κ B regulates the expression of a wide variety of genes that encode inflammatory mediators, such as KC, MCP-1, and RANTES, and, therefore, has been implicated directly in regulating asthmatic inflammatory responses (20, 21). Phosphorylation of the NF- κ B subunit p65 is a modification associated with enhancement of p65 transactivation potential; as such, it is an indirect measure of NF- κ B activation. Results presented in Figure 3 show that a single bout of exercise at a moderate intensity attenuated p65 phosphorylation in the OVA-treated lung by 50% as compared with sedentary, OVA-treated mice. Saline-treated controls exhibited levels of p65 phosphorylation that were equivalent with exercised, OVA-treated mice (Figure 3).

Acute Aerobic Exercise Attenuates Th2-Derived Cytokine Production

Atopic asthma is considered a Th2-driven disease (22); Th2 cells express the cytokines IL-4, IL-5, and IL-13. Findings presented in Figure 4 demonstrate that OVA treatment of sedentary mice increased IL-5 and IL-13 significantly as compared with saline controls; OVA-treatment had no effect on IL-4 levels (data not shown). A single bout of moderate-intensity exercise attenuated ($\geq 50\%$ decrease) the production of IL-5 and IL-13 protein significantly in OVA-treated mice as compared with sedentary controls (Figure 4). Saline-treated controls expressed equivalent levels of each cytokine analyzed (Figure 4). It should be noted

Figure 3. A single bout of moderate-intensity aerobic exercise p65 phosphorylation in the lungs of OVA-treated mice. 24 h post protocol completion, lungs were harvested, lysed and prepared for analyses of total and phosphorylated p65 levels via p65-specific enzyme-linked immunosorbent assay (ELISA); phosphorylated p65 levels were normalized against total p65 levels. Results are reported as fold difference in phospho-NF- κ B p65 levels as compared with saline-treated, sedentary controls ($^{\dagger}P < 0.05$ as compared with saline-treated, sedentary mice; $*P < 0.05$ as compared with OVA-treated, sedentary mice; $n = 4$ mice per group). Open bars, sedentary mice; solid bars, exercised mice.

that the Th1-derived cytokines IL-2 and IFN- γ were not detected in any experimental or control group (data not shown).

Acute Aerobic Exercise Does Not Affect AHR or Airway Remodeling

AHR to a variety of inhaled bronchoconstrictor stimuli is a hallmark of the asthmatic response. To determine the effect of a single bout of exercise on AHR in OVA-treated mice, mice were mechanically ventilated and challenged with increasing concentrations of methacholine (0–50 mg/ml). Results shown in Figure 5 demonstrate that, at the highest methacholine dose (50 mg/ml), OVA treatment increased lung resistance (R) significantly in sedentary mice as compared with saline-treated controls. A single bout of exercise in OVA-treated mice had no effect on this response (Figure 5).

Previous studies have suggested that airway remodeling is causal to airway hyperresponsiveness (2). To analyze the effect of a single bout of exercise on airway remodeling in OVA-treated mice, lung tissue sections were coded randomly and scored subjectively for assessment of hypertrophy/hyperplasia of the mucosal epithelium, goblet cell, and mucin production; overall airway wall thickening was measured using Metamorph analysis. Representative micrographs shown in Figure 6 demonstrate that OVA treatment in both sedentary and exercised mice increased each of these parameters as compared with saline-treated controls; however, no significant differences

Figure 4. One bout of moderate-intensity aerobic exercise decreased Th2-derived cytokine production in OVA-treated mice. Twenty-four hours after protocol completion, BAL was performed and resulting lavagates were analyzed for changes in (A) IL-5 and (B) IL-13 via multiplex assay. Results are reported as pg/ml of respective protein ($^{\dagger}P < 0.001$ as compared with saline-treated, sedentary mice; $*P < 0.001$ as compared with OVA-treated, sedentary mice; ND, none detected; $n = 4$ –8 mice per group). Open bars, sedentary mice; solid bars, exercised mice.

Figure 5. A single bout of moderate-intensity aerobic exercise had no effect on AHR in OVA-treated mice. Forty-eight hours after protocol completion, mice were assessed for changes in AHR upon methacholine challenge at increasing concentrations via mechanical ventilation. Results are reported as measurements of total lung resistance (R , cm $H_2O/mL/s$; $^*P < 0.04$ as compared with saline-treated, sedentary mice; $n = 4-8$ mice per group). Open squares, sedentary, saline-treated mice; solid circles, exercised, saline-treated mice; open triangles, sedentary, OVA-treated mice; solid triangles, exercised, OVA-treated mice.

between OVA-treated sedentary and OVA-treated exercised mice were observed in scored or measured assessments (data not shown).

Acute Aerobic Exercise Attenuates PGE_2 Levels

Within the asthmatic lung, the prostaglandin PGE_2 has been implicated in the regulation of airway inflammation, Th2 cell differentiation, and AHR (reviewed in Ref. 23). Data presented in Figure 7 show that OVA treatment of sedentary mice increased PGE_2 levels significantly as compared with saline-treated controls. OVA-treated mice that underwent a single bout of moderate exercise, however, displayed significantly decreased ($\geq 70\%$) PGE_2 levels as compared with sedentary controls (Figure 7); these levels were equivalent to those observed in saline-treated controls (Figure 7).

DISCUSSION

In this study, sedentary mice treated acutely with OVA exhibited significant increases in asthma-related responses as compared with saline controls; these responses included production of the inflammation-related mediators KC, RANTES and MCP-1, IL-12p40/p80, and PGE_2 ; recruitment of leukocytes, including eosinophils, into the airways; levels of Th2-derived IL-5 and IL-13; activation of NF- κ B; airway remodeling; and AHR. A single bout of aerobic exercise at moderate intensity decreased significantly each of these responses with the exceptions of airway remodeling and AHR. In exercised mice, the observed increases in AHR were not a consequence of exercise-induced bronchoconstriction; increases in resistance were measured in response to methacholine challenge at 48 hours after OVA treatment and, therefore, reflect the persistent AHR observed in individuals with chronic asthma (2). Collectively, these findings suggest that airway inflammation is not required for the development of airway remodeling and AHR.

Recent work suggests that AHR can be dissociated from cellular inflammation while remaining linked with sustained airway remodeling (24, 25). Specifically, Kariyawasam and coworkers and Crimi and colleagues have each shown that increases in cellular inflammation, including eosinophils, do not positively correlate with increased AHR in patients with asthma (24, 25); however, markers of airway remodeling remain associated with AHR (24). Similarly, Leigh and coworkers have shown that mice that undergo chronic allergen exposure exhibit structural changes that are associated with AHR in the absence of cellular inflammation (26). In contrast, Alcorn and coworkers have indicated that attenuated airway remodeling does not impact airway inflammatory responses or AHR (27). In particular, these authors reported that neutralization of TGF- β 1 responses in the lungs of OVA-treated mice suppressed airway fibrosis while increasing AHR; neutralization had no effect on airway inflammation (27). Collectively, these studies demonstrate that airway inflammation, remodeling, and hyperresponsiveness may not be directly interrelated.

Data in the current study support and extend these previous observations. Because NF- κ B regulates the gene expression of a wide variety of inflammatory mediators, including KC, MCP-1, and RANTES, exercise-mediated attenuation of NF- κ B

Figure 6. A single bout of moderate-intensity aerobic exercise had no effect on airway remodeling in OVA-treated mice. Twenty-four hours after protocol completion, lung tissues were harvested, PASH stained, and prepared for analysis of hypertrophy/hyperplasia of the mucosal epithelium, goblet cell and mucin production, and overall airway wall thickening. Representative micrographs of five to six separate experiments are shown: (A) saline-treated, sedentary; (B) saline-treated, exercised; (C) OVA-treated, sedentary; (D) OVA-treated, exercised (*airway lumen; $\times 10$ magnification [inset, $\times 40$]).

Figure 7. One bout of moderate-intensity aerobic exercise decreased PGE₂ production in OVA-treated mice. Twenty-four hours after protocol completion, BAL was performed and resulting lavagates were analyzed for changes in PGE₂ content via ELISA. Results are reported as ng/ml ([†]*P* < 0.001 as compared with saline-treated, sedentary mice; ^{*}*P* < 0.01, as compared with OVA-treated, sedentary; *n* = 6–8 mice per group). Open bars, sedentary mice; solid bars, exercised mice.

activation likely led to the observed decreases in chemokine production and subsequent leukocyte recruitment into the OVA-treated lung. In addition, decreases in IL-12p40/p80, which acts as a macrophage chemoattractant (19), as well as Th2-derived IL-5 and IL-13, which promote eosinophilic inflammation (28), likely led to decreased cellular inflammation. It should be noted that there is an apparent discordance between the magnitude of exercise-mediated decreases in Th2-derived cytokines and eosinophil infiltration; such discordance suggests compensation by other cytokines and/or temporal differences in the attenuation of each of these targets. In murine asthma models, IL-12 has also been implicated in the regulation of Th1/Th2 responses (28); however, our results indicate that exercise decreases Th2 responses in OVA-treated mice via a manner that is independent of IL-12. Also, the lack of detectable IL-12p70 in both control and experimental samples may explain, in part, the lack of observed Th1 responses in our study; IL-12p70 promotes Th1 responses, while IL-12p40/p80 antagonizes this action (29). Despite the reduction in inflammatory cells, mediators, and Th2-derived responses, increased airway remodeling and AHR persisted in the lungs of exercised, OVA-treated mice.

In addition to their roles in asthma-related airway inflammation, the cytokines IL-12 and IL-13 have been implicated directly in AHR pathogenesis. Gavett and colleagues and Kips and coworkers have both demonstrated that administration of IL-12 during antigen challenge inhibits AHR in mice (30, 31). With regard to IL-13, Grunig and coworkers and Wills-Karp and colleagues have both shown that reconstitution of IL-13-deficient mice with recombinant IL-13 triggered mucus hypersecretion and AHR (32, 33); these studies also suggest that IL-13 can induce AHR in the absence of inflammatory cell recruitment (32, 33). Our results indicate decreases in IL-12 and IL-13 within the lung do not correlate with reduced airway remodeling or AHR. The discrepancy between our results and previous findings may be explained by the differences in mouse model systems used. The previous studies cited above used antigen-treated A/J or C57BL/6 mice, whereas the current study used the BALB/cj strain. It is well accepted that mouse strains differ in their inflammatory and pulmonary responses upon exposure to antigen (5, 34). For example, repeated antigen instillation results in increased eosinophilic inflammation, collagen deposition, and airway wall thickening in A/J and BALB/cj mice as compared with C57BL/6 (34). Likewise, A/J and BALB/cj display a greater degree of AHR as compared with C57BL/6 mice (5). Karp and colleagues demonstrated that the complement factor 5 (C5) gene is expressed differentially between mice that differed in their susceptibility to the development of allergen-induced AHR (35). In light of these strain differences, the interrelationship between airway inflammation, remodeling, and hyperresponsiveness may be regulated via

differential mechanisms. Another possibility is that inflammation primes the airway epithelium and ASM for a bronchoconstrictive response; such priming may involve desensitization of β_2 -adrenergic receptors, which facilitate bronchodilation, via inflammatory-mediated induction of GRK-2 (36). The temporal nature of the inflammation allows it to resolve ahead of the resultant and persistent effects on airway remodeling and AHR (24).

PGE₂ is considered a potent proinflammatory mediator that plays a role in the pathogenesis of inflammatory diseases, such as rheumatoid arthritis; within the lung and airways, however, it is considered protective against asthma-related lung inflammation and AHR (reviewed in Ref. 23). In particular, PGE₂-mediated protection against allergen-induced AHR appears to be secondary to reduced levels of cellular inflammation. For example, administration of PGE₂ *in vivo* has been shown to decrease the number of IL-4- and IL-5-producing (Th2) cells, eosinophils, and metachromatic cells within the BALF of allergen-sensitized subjects; these cell types have been implicated in the initiation and exacerbation of AHR (reviewed in Ref. 23). In addition, PGE₂ appears to play a role in T-lymphocyte trafficking and differentiation within the allergen-sensitized airway (23). In contrast, Liu and colleagues have reported that PGE₂ induces AHR via a signaling pathway that is dependent upon Rho/ROCK activation and Ca²⁺ release (37). Results presented herein suggest that increased PGE₂ levels in the lungs of sedentary, OVA-treated mice do not protect against either airway inflammation, including Th2 responses, or AHR. Such conflicting results may be explained by the expression and use of E prostanoid receptors (EP1–EP4) that mediate the effects of PGE₂ within the lung and airways. E prostanoid receptors are expressed on multiple cell types, including lymphocytes, airway epithelial cells, and ASM cells; multiple E prostanoid receptor subtypes may be expressed in a single cell. Activation of the EP1 and EP3 receptors elevate intracellular calcium levels and promote effector cell activation (23). Conversely, activation of the EP2 and EP4 receptors increases intracellular cAMP concentrations, which inhibit effector cell functions (23). McGraw and coworkers have reported that activation of the EP1 receptor on ASM uncouples the β_2 -AR from its G-protein complex, resulting in reduced β_2 -adrenergic receptor (β_2 -AR) desensitization (38). Guo and colleagues have also shown that the EP2 receptor regulates cytokine-mediated desensitization of the β_2 -AR on ASM (39). Because multiple E prostanoid receptor subtypes may be expressed in a single cell, it is possible that an ongoing inflammatory response would cause a multiplicity of opposing PGE₂-mediated actions within the lung and airways.

In our previous studies, we observed that repeated bouts of aerobic exercise at a moderate intensity attenuated both airway inflammation and remodeling in mice that had been sensitized and challenged with OVA for a total of 7 weeks (7, 8). In addition, OVA-treated mice from our previous studies exhibited a lesser eosinophilic response as compared with OVA-treated mice in the current study. We believe that these seemingly contrasting results between our collective studies are explained by the differences in the length of the OVA treatment and exercise protocols. In particular, the disparity in exercise-mediated effects on airway remodeling is likely due to the difference in the number of bouts of exercise. The effects of exercise on physiologic responses are dependent upon several variables, including the frequency of each bout and the total duration of the exercise protocol (40–45); the mechanism that underlies this disparity may involve changes in the levels of circulating hormones (e.g., glucocorticoids, catecholamines) that are released from the hypothalamic–pituitary axis during exercise. With regard to differences in the extent of airway eosinophilia between these studies, we hypothesize that the extended OVA exposure may

have had a tolerizing effect on the eosinophilic response reported in our previous papers (7, 8). In support of this hypothesis, several studies have demonstrated that prolonged allergen exposure results in decreased inflammatory responses, including airway eosinophilia (46, 34, 47–49).

Using aerobic exercise as tool to investigate the interrelationship between airway inflammation, remodeling, and hyperresponsiveness, we have shown that airway remodeling and AHR persist in the absence of airway inflammation; this is the first report to use exercise as a tool to study this interrelationship. We believe that, with this novel approach, future studies will elucidate the mechanisms that underlie this interrelationship and its role in asthma pathogenesis.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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References

- O'Byrne P. Airway inflammation and asthma. *Aliment Pharmacol Ther* 1996;10:18–24.
- Cockcroft D, Davis B. Mechanisms of airway hyperresponsiveness. *J Allergy Clin Immunol* 2006;118:551–559.
- Gleeson M. Immune function in sport and exercise. *J Appl Physiol* 2007;103:693–699.
- Reeves JT, Linehan JH, Stenmark KR. Distensibility of the normal human lung circulation during exercise. *Am J Physiol* 2005;288:L419–L425.
- Duguet A, Biyah K, Minshall E, Gomes R, Wang C, Taoudi-benchekroun M, Bates JHT, Eidelman DH. Bronchial responsiveness among inbred mouse strains. *Am J Respir Crit Care Med* 2000;161:839–848.
- Herz U, Lupp U, Daser A, Gelfand EW, Renz H. Murine animal models to study the central role of T cells in immediate-type hypersensitivity responses. *Adv Exp Med Biol* 1996;409:25–32.
- Pastva A, Estell K, Schoeb TR, Atkinson TP, Schieberr LM. Aerobic exercise attenuates airway inflammation in a mouse model of atopic asthma. *J Immunol* 2004;172:4520–4526.
- Pastva A, Estell K, Schoeb TR, Schieberr L. RU486 blocks the anti-inflammatory effects of exercise in a murine model of allergen-induced pulmonary inflammation. *Brain Behav Immun* 2005;19:415–422.
- Woods JA, Davis JM, Mayer EP, Ghaffar A, Pate RR. Exercise increases inflammatory macrophage antitumor cytotoxicity. *J Appl Physiol* 1993;75:879–886.
- Woods JA, Davis JM, Mayer EP, Ghaffar A, Pate RR. Effects of exercise on macrophage activation for antitumor cytotoxicity. *J Appl Physiol* 1994;76:2177–2185.
- Schefer V, Talan MI. Oxygen consumption in adult and aged C57BL/6J mice during acute treadmill exercise of different intensity. *Exp Gerontol* 1996;31:387–392.
- MacNeil B, Hoffman-Goetz L. Effect of exercise on natural cytotoxicity and pulmonary tumor metastases in mice. *Med Sci Sports Exerc* 1993;25:922–928.
- Irvin CG, Bates JH. Measuring the lung function in the mouse: the challenge of size. *Respir Res* 2003;4:4–12.
- Tomioka S, Bates JHT, Irvin CG. Airway and tissue mechanics in a murine model of asthma: alveolar capsule vs. forced oscillations. *J Appl Physiol* 2002;93:263–270.
- Viera R, Duarte A, Claudino R, Perini A, Santos A, Moriya H, Arantes-Costa F, Martins M, Carvalho C, Dolhnikoff M. Creatine supplementation exacerbates allergic lung inflammation and airway remodeling in mice. *Am J Respir Cell Mol Biol* 2007;37:660–667.
- Lee NA, Gelfand EW, Lee JJ. Pulmonary T cells and eosinophils: coconspirators or independent triggers of allergic respiratory pathology? *J Allergy Clin Immunol* 2001;107:945–957.
- Tomkinson A, Cieslewicz G, Duez C, Larson K, Lee JJ, Gelfand EW. Temporal association between airway hyperresponsiveness and airway eosinophilia in ovalbumin-sensitized mice. *Am J Respir Crit Care Med* 2001;163:721–730.
- Wells TNC, Proudfoot AEI. Chemokine receptors and their antagonists in allergic disease. *Inflamm Res* 1999;48:353–362.
- Cooper A, Khader S. IL-12p40: an inherently agonistic cytokine. *Trends Immunol* 2006;28:33–38.
- Christman JW, Sadikot RT, Blackwell TR. The role of nuclear factor- κ B in pulmonary diseases. *Chest* 2000;117:1482–1487.
- Poynter ME, Irvin CG, Janssen-Heininger YM. Rapid activation of nuclear factor- κ B in airway epithelium in a murine model of allergic airway inflammation. *Am J Pathol* 2002;160:1325–1334.
- Wills-Karp M. Immunologic basis of antigen-induced airway hyperresponsiveness. *Annu Rev Immunol* 1999;17:255–281.
- Vancheri C, Mastroiuzo C, Sortino M, Crimi N. The lung as a privileged site for the beneficial actions of PGE₂. *Trends Immunol* 2004;25:40–46.
- Kariyawasam HH, Aizen M, Barkans J, Robinson DS, Kay AB. Remodeling and airway hyperresponsiveness but not cellular inflammation persist after allergen challenge in asthma. *Am J Respir Crit Care Med* 2007;175:896–904.
- Crimi E, Spanevello A, Neir M, Ind TW, Rossi GA, Brusasco V. Dissociation between airway inflammation and airway hyperresponsiveness in allergic asthma. *Am J Respir Crit Care Med* 1998;157:4–9.
- Leigh R, Wattie J, Southam D, Hoogh M, Gaudie J, O'Bryne P, Inman R. Dysfunction and remodeling of the mouse airway persist after resolution of acute allergen-induced airway inflammation. *Am J Respir Cell Mol Biol* 2002;27:526–535.
- Alcorn JF, Rinaldi L, Jaffe E, van Loon M, Bates JH, Janssen-Heininger YM, Irvin CG. Transforming growth factor- β 1 suppresses airway hyperresponsiveness in allergic airway disease. *Am J Respir Crit Care Med* 2007;176:974–982.
- Nakajima H, Takatsu K. Role of cytokines in allergic airway inflammation. *Int Arch Allergy Immunol* 2007;142:265–273.
- Goriely S, Neurath M, Goldman M. How microorganisms tip the balance between interleukin-12 family members. *Nat Rev Immunol* 2008;8:81–86.
- Gavett SH, O'Hearn D, Li X, Huang S, Finkelman F, Wills-Karp M. Interleukin-12 inhibited antigen induced airway hyperresponsiveness, inflammation and Th2 cytokine expression in mice. *J Exp Med* 1995;182:1527–1536.
- Kips JC, Brusselle G, Joos G, Peleman R, Tavernier J, Devos R, Pauwels R. Interleukin-12 inhibits antigen-induced airway hyperresponsiveness in mice. *Am J Respir Crit Care Med* 1996;153:535–539.
- Grunig G, Warnock M, Wakil A, Venkayya R, Brombacher E, Rennick D, Sheppard D, Mohrs M, Donaldson D, Locksley R, et al. Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 1998;282:2261–2263.
- Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben T, Karp C, Donaldson D. Interleukin-13: central mediator of allergic asthma. *Science* 1998;282:2258–2261.
- Shinagawa K, Kojima M. Mouse model of airway remodeling. *Am J Respir Crit Care Med* 2003;168:959–967.
- Karp CL, Grupe A, Schadt E, Ewart SL, Keane-Moore M, Cuomo PJ, Kohl J, Wahl L, Kuperman D, Germer S, et al. Identification of complement factor 5 as a susceptibility locus for experimental allergic asthma. *Nat Immunol* 2000;1:221–226.
- Pitcher J, Freedman N, Lefkowitz R. G protein-coupled receptor kinases. *Annu Rev Biochem* 1998;67:653–692.
- Liu C, Tazzeo T, Janssen L. Isoprostone-induced airway hyperresponsiveness is dependent on internal Ca²⁺ handling and Rho/ROCK signaling. *Am J Physiol* 2006;291:1177–1184.
- McGraw DW, Muhlacker K, Schwarb M, Rahman F, Small K, Almoosa K, Liggett SB. Airway smooth muscle prostaglandin-EP1 receptors directly modulate beta2-adrenergic receptors within a unique heterodimeric complex. *J Clin Invest* 2006;116:1400–1409.
- Guo M, Pascual R, Wang S, Valencius C, Panettieri RA, Tilley S, Penn R. Cytokines regulate beta-2-adrenergic receptor responsiveness in airway smooth muscle via multiple PKA- and EP2 receptor-dependent mechanisms. *Biochemistry* 2005;44:13771–13782.
- Ceddia MA, Voss EW, Woods JA. Intracellular mechanisms are responsible for the exhaustive exercise-induced suppression of macrophage antigen presentation. *J Appl Physiol* 2000;88:804–810.
- Nieman DC, Nehlsen-Cannarella SL. The immune response to exercise. *Semin Hematol* 1994;31:166–179.
- Pedersen BK, Hoffman-Goetz L. Exercise and the immune system: regulation, integration, and adaptation. *Physiol Rev* 2000;80:1055–1081.
- Smith LL, Anwar A, Fragen M, Rananto C, Johnson R, Holbert D. Cytokines and cell adhesion molecules associated with high intensity eccentric exercise. *Eur J Appl Physiol* 2000;82:61–67.
- Su SH, Chen H, Jen CJ. Severe exercise enhances phagocytosis by murine bronchoalveolar macrophages. *J Leukoc Biol* 2001;69:75–80.
- Woods JA, Lu Q, Ceddia MA, Lowder T. Exercise-induced modulation of macrophage function. *Immunol Cell Biol* 2000;78:545–553.

46. Sakai K, Yokoyama A, Kohno N, Hamada H, Hiwada K. Prolonged antigen exposure ameliorates airway inflammation but not remodeling in a mouse model of bronchial asthma. *Int Arch Allergy Immunol* 2001;126:126–134.
47. Swirski FK, Sajic D, Robbins CS, Gajewska BU, Jordana M, Stampfli MR. Chronic exposure to innocuous antigen in sensitized mice leads to suppressed airway eosinophilia that is reversed by granulocyte macrophage colony-stimulating factor. *J Immunol* 2002;169:3499–3506.
48. Schramm CM, Puddington L, Wu C, Guernsey L, Gharaee-Kermani M, Phan SH, Thrall RS. Chronic inhaled ovalbumin exposure induces antigen-dependent but not antigen-specific inhalational tolerance in a murine model of allergic airway disease. *Am J Pathol* 2004;164:295–304.
49. van Hove C, Maes T, Joos G, Tournoy K. Prolonged inhaled allergen exposure can induce persistent tolerance. *Am J Respir Cell Mol Biol* 2007;36:573–584.