



RU486 blocks the anti-inflammatory effects of exercise in a murine model of allergen-induced pulmonary inflammation

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Abstract

In an ovalbumin (OVA)-driven murine model of allergic pulmonary inflammation, we have shown previously that moderate-intensity aerobic exercise training attenuates inflammatory responses, disease progression, and NF- κ B activation within the sensitized lung. Glucocorticoids (GCs), potent anti-inflammatory agents, have been shown to alter transcriptional events that are important in asthmatic pathogenesis, such as NF- κ B activation. Notably, exercise training can alter the production and signaling capacity of endogenous GCs. Because GCs exert their anti-inflammatory effects through binding to intracellular glucocorticoid receptors (GRs), we examined the role of the GR in facilitating the anti-inflammatory effects of exercise. Results show that, in exercised OVA-sensitized mice, treatment with the GR antagonist RU486 blocked the exercise-induced reductions in cellular infiltration of the airways ($p < .05$), KC and soluble VCAM-1 protein levels in the bronchoalveolar lavage fluid ($p < .05$), and NF- κ B translocation and DNA binding within the lung to levels similar to those observed in sedentary OVA-sensitized mice. Importantly, RU486 treatment also blocked exercise-induced increases in GR nuclear translocation to the levels seen in sensitized control mice. Together, these results suggest that GR nuclear translocation and NF- κ B activation play roles in mediating the anti-inflammatory effects of exercise in allergen-mediated lung pathology.

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1. Introduction

Recent reports indicate that aerobic exercise training improves the overall health and fitness of individuals with asthma, an allergen-mediated lung pathology (Emtner et al., 1996, 1998; Hallstrand et al., 2000; Henriksen and Nielsen, 1983; Matsumoto et al., 1999); such improvements include reductions in asthma-related symptomatology, hospital admissions, and medication use. Because these reports suggest collectively that there

may exist an immunological basis for the beneficial results of exercise in asthmatics, we began a study that examined the effects of moderate-intensity aerobic exercise in allergen-induced lung pathology. Using a murine model of allergen-induced pulmonary inflammation, we documented recently that moderate-intensity aerobic exercise training reduces lung inflammatory responses, including leukocyte infiltration, cytokine and chemokine production, adhesion molecule expression, and structural airway remodeling (Pastva et al., 2004). We have also shown that, within the ovalbumin (OVA)-sensitized lung, exercise attenuates activation of NF- κ B, the major inflammatory gene transcription factor implicated in asthmatic pathogenesis (Pastva et al., 2004). The purpose of this present study was to examine a possible mecha-

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nism that underlies the anti-inflammatory effects of exercise in allergen-induced lung pathology.

Exercise stimulates the hypothalamic–pituitary–adrenal (HPA) axis to upregulate its release of neuroendocrine-related factors, including the endogenous glucocorticoid (GC) corticosterone (cortisol in humans), into the circulation (Schleimer, 2000). A growing body of evidence suggests that corticosterone is an important regulator of allergic disease progression and inflammatory responses. For example, GCs have been shown to inhibit the synthesis of a wide variety of inflammatory mediators, including cytokines (IL-1 β , TNF- α , IL-6, and GM-CSF), chemokines (IL-8, MCP-1, and RANTES), adhesion molecules (ICAM-1, VCAM-1), and inflammatory enzymes (cyclo-oxygenase 2, inducible nitric oxide synthase) (Schwiebert et al., 1996). Because of their anti-inflammatory properties, exogenous GCs are used commonly in the treatment of asthma; with prolonged usage, however, these agents trigger a variety of deleterious side effects such as skeletal muscle wasting, glaucoma, osteoporosis, and HPA axis dysfunction, which diminish their overall effectiveness.

GCs exert their anti-inflammatory effects via binding to intracellular glucocorticoid receptors (GRs); these receptors are widely expressed in cells and tissues relevant to the pathogenesis of asthma. GRs are considered ligand-activated transcription factors that act, either directly or indirectly, to regulate gene transcription. Activated GRs can either bind directly to DNA (transcriptional transactivation) or to other transcription factors (transcriptional transrepression) to modulate gene transcription. In particular, GRs can bind NF- κ B (p65 subunit) physically to inhibit its transcriptional activation of inflammatory genes (Adcock and Caramori, 2001). Importantly, activated GRs can also inhibit NF- κ B-mediated gene expression through the induced gene expression of the κ B inhibitor, I κ B (Auphan et al., 1995). Emerging data now suggest that activated GRs may also modulate gene expression via regulation of chromatin structure and initiation complex accessibility to genes as well as via inhibition of the MAPKs ERK1/2, JNK, and p38, which have been implicated in the signaling pathways that promote inflammatory gene expression (Adcock and Lane, 2003).

In this study, we hypothesized that moderate-intensity aerobic exercise induces an anti-inflammatory response in allergen-induced pulmonary inflammation via a mechanism that involves GR nuclear translocation and NF- κ B activation. To test this hypothesis, we treated mice with the GR antagonist RU486 and then monitored inflammatory responses and NF- κ B activation within the exercised and OVA-sensitized (EO) lung. Studies described herein indicate that RU486 prevented the exercise-associated attenuation of inflammatory responses and NF- κ B activation within the sensitized lung.

2. Materials and methods

2.1. Animals

Female BALB/cJ mice (3–5 weeks old; The Jackson Laboratory, Bar Harbor, ME), a strain susceptible to OVA-induced IgE responses (Duguet et al., 2000; Herz et al., 1996), were utilized. Mice were housed in a pathogen-free containment facility and maintained in autoclaved Microisolator cages (Laboratory 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 Institutional Animal Care and Use Committee and were in accordance with the National Institutes of Health recommended guidelines.

2.2. OVA-sensitization and exercise protocol/RU486 treatment

At the start of the protocol, mice were assigned randomly to one of the following six experimental groups: sedentary and nonsensitized (S), sedentary and OVA-sensitized with placebo (SO/placebo), sedentary and OVA-sensitized with RU486 treatment (SO/RU486), exercised and nonsensitized (E), exercised and OVA-sensitized with placebo (EO/placebo), and exercised and OVA-sensitized with RU486 treatment (EO/RU486). OVA-sensitization and moderate-intensity aerobic exercise training were performed as described previously (Pastva et al., 2004). Briefly, OVA-sensitized mice were injected i.p. (200 μ l/mouse) on days 0 and 14 with 50 μ g of alum-precipitated chicken egg OVA (Imject Alum; Pierce, Rockford, IL; grade VII OVA, $\geq$ 98% pure, Sigma–Aldrich, St. Louis, MO). Forty-eight hours post the second OVA i.p., mice were anesthetized with isoflurane and drug pellets containing the glucocorticoid receptor antagonist RU486 (mifepristone; 17 β -hydroxy-11 α -(4-dimethylaminophenyl)-17 α -(1-propynyl)-estra-4,9-dien-3-one; Innovative Research of America, Sarasota, FL) were implanted intrascapularly according to the manufacturer's instructions. The 60-day release, 15 mg pellets were designed to release a continuous flow of the drug at a rate of 10 μ g/h for the duration of the 4 week exercise protocol; this concentration of time-released RU486 has been shown previously to inhibit GR activity in mice (Kruszewska et al., 1998). Control-sensitized groups received a placebo pellet (Innovative Research of America). Following the i.p. injections and pellet implantation and beginning on day 21, mice were exposed to aerosolized OVA at a concentration of 5 mg/ml (in 0.85% NaCl solution) for 30 min/day for five consecutive days. Thereafter, aerosolized boosters of OVA (at the same concentration) were administered for 10 min/day, five consecutive days/week for the remainder

of the study. Mice assigned to the nonsensitized S and E groups received injections and aerosolizations of saline only. We have shown previously that the utilization of this OVA-sensitization scheme in Balb/cj mice initiates an airway inflammatory response and immunoglobulin profile that is consistent with those observed in atopic asthma (Pastva et al., 2004).

One to two hours post-aerosolization, exercised mice (E and EO groups) underwent bouts of ‘forced’ moderately intense aerobic exercise on a motorized treadmill (Exer 6M; Columbus Instruments, Columbus, OH) (Pastva et al., 2004). This mode of ‘forced’ exercise was utilized because exercise intensity and duration could be manipulated experimentally unlike voluntary running or swimming. The motorized treadmill was equipped with a shock plate that was set to the lowest possible voltage. After initial exposure to the treadmill and upon learning that a shock device was there, the mice did not approach the shock device again (personal observation). Exercise bouts were performed three times per week for a total time period of 4 week. During the first week of the exercise regimen, treadmill speed and exercise duration were progressively increased from 10.0 m/min for 30 min to 13.5 m/min for 45 min to acclimate mice to and train mice on the treadmill. In the remainder of the protocol (weeks 2–4), mice exercised for 45 min at 13.5 m/min (0% grade). As noted in our earlier study (Pastva et al., 2004), previous reports have defined moderate-intensity aerobic exercise as brief (15–60 min) bouts of treadmill running at 50–75% maximum O₂ consumption or ~15 to 22 m/min (Fernando et al., 1993; MacNeil and Hoffman-Goetz, 1993; Schefer and Talan, 1996; Woods et al., 1993; Woods et al., 1994) (Dr. Jeffrey Woods, personal communication). Because the EO group received an inflammatory-promoting pulmonary treatment, treadmill parameters were set at the lower end of the max O₂ consumption range so as to reduce the chance of exercise-induced bronchospasm. Repetitive aerobic exercise was tailored to mimic the classic parameters of traditional pulmonary rehabilitation programs; such parameters include intermittent weekly sessions of moderately intense physical aerobic training, with emphasis on the lower extremities, for a period of one month (ACCP/AACVPR Pulmonary Guidelines Panel, 1997; Cooper, 2001). All exercise bouts included brief warm-up and cool-down periods so that the total treadmill time was ~60 min. Twenty-four hours after completion of the sensitization/exercise protocol, mice were euthanized via i.p. injection of ketamine (8.7 mg/100 g body weight) mixed with xylazine (1.3 mg/100 g body weight) and prepared for lung extraction, bronchoalveolar lavage, and cardiac puncture as described below.

2.3. Immunohistochemistry

Lungs were excised, fixed in 3% formaldehyde, and prepared for immunohistochemical analyses as

described previously (Pastva et al., 2004). Sections were stained with antibodies specific for GR or the NF- κ B subunit p65 (Santa Cruz Biotech., Santa Cruz, CA). Sections were microwaved with citrate anhydrous buffer (pH 6.0) to facilitate antigen retrieval and then stained with the respective primary antibody followed by the appropriate Alexa Fluor 564 secondary antibody (Molecular Probes) as described previously (Pastva et al., 2004). Changes in GR nuclear translocation were then determined. Specifically, lung sections were coded randomly and the presence of fluorescence within the nuclei of approximately 100 cells in each lung section was recorded.

2.4. Lung lavage and plasma collection, and analysis

Mice were lavaged or cardiac punctured as described previously (Pastva et al., 2004). In bronchoalveolar lavage fluid (BALF) samples, cell viability was determined via trypan blue exclusion and cell types were differentiated on cytospin preps using Diff-Quik stain. Cell differentials were determined from at least 500 leukocytes using standard hematological criteria. BALF samples were also analyzed for KC and soluble VCAM-1 (sVCAM-1) protein content via ELISA (BioSource International, Camarillo, CA).

For measurement of endogenous GC corticosterone levels, mice were cardiac punctured and blood was collected into EDTA-coated tubes (Becton–Dickinson, Franklin Lakes, NJ) as described previously (Pastva et al., 2004). Care was taken to ensure that the same individual handled all the mice in the study in the same manner to minimize the impact of sacrifice on basal GC measurements. From these blood samples, plasma was isolated and then stored at –20 °C. Endogenous GC corticosterone levels were assayed using an ¹²⁵I RIA kit (ICN Biomedicals, Costa Mesa, CA). All plasma sampling was performed between 7:30 and 11:00 a.m.

2.5. Electrophoretic mobility shift analysis of NF- κ B–DNA binding

Lungs were harvested and nuclear extracts were prepared for electrophoretic mobility shift analysis (EMSA) of NF- κ B–DNA binding as described previously (Propst et al., 2002). Briefly, lungs were homogenized in buffer A (10 mM KCl, 20 mM Hepes, 1 mM MgCl₂, 1 mM DTT, 0.4 mM PMSF, 1 mM NaF, and 1 mM Na₃VO₄) and pelleted at 1000g, 4 °C. Pellets were resuspended and lysed in buffer A plus 0.1% Nonidet P-40 and centrifuged at 3000g, 4 °C. The resulting pellet was resuspended in buffer B (10 mM Hepes, 400 mM KCl, 0.1 mM EDTA, 1 mM MgCl₂, 1 mM DTT, 0.4 mM PMSF, 15% glycerol, 1 mM NaF, and 1 mM Na₃VO₄) and set at 4 °C with constant gentle mixing. Nuclei were

then pelleted at 40,000g and nuclear extracts were dialyzed at 4°C against buffer C (20mM Hepes, 200mM KCl, 1mM MgCl₂, 0.1mM EDTA, 1mM DTT, 0.4mM PMSF, 15% glycerol, 1mM NaF, and 1mM Na₃VO₄). Nuclear extracts were cleared by centrifugation at 14,000g, 4°C. EMSA was performed using NF-κB oligonucleotides (consensus: 5'-AGT TGA GGG TTT CCC AGG C-3'; mutant: 5'-AGT TGA GGC TTT CCC AGG C-3'; Santa Cruz, Santa Clarita, CA). The gel shift reaction was then prepared by incubating ³²P-labeled oligonucleotide (250,000 cpm/reaction) with 10 μg of nuclear extract in 50mM NaCl, 1mM EDTA, 1mM DTT, 10mM Tris-Cl (pH 7.5), 5% glycerol, and 1 μg of poly(dI:dC) at room temperature. For competition analysis, molar excess of the respective unlabeled DNA was included in the initial gel shift reaction mix. Bound and free DNA were resolved by electrophoresis through a 4% polyacrylamide gel at 190 V in 1× TGE buffer (50mM Tris-Cl, 380mM glycine, and 2mM EDTA). Dried gels were processed via autoradiography and densitometric analyses of repetitive samples were performed.

2.6. Sample size and statistical analysis

The results presented in this paper were derived from two independent protocols, which each utilized five to six mice per experimental group (S, E, SO, and EO). Samples were paired as much as possible. For example, all mice in both protocols were cardiac punctured to obtain serum for endogenous glucocorticoid analysis ($n=10$ each experimental group). BALF collected from mice was analyzed for both differential cellular infiltration ($n=5-6$ each experimental group) and for inflammatory mediator (i.e., KC, sVCAM-1) content ($n=5-6$ each experimental group); lavaged lungs were harvested and analyzed for in vivo GR nuclear translocation and VCAM-1 protein expression ($n=5-6$ each experimental group). Because of concerns regarding the mechanical effects of lavage upon NF-κB activation, lungs from mice that were not lavaged were harvested for NF-κB activation via EMSA ($n=6$ each experimental group).

Data were analyzed using SPSS Version 11.0 (SPSS, Chicago, IL). Results are reported as group means $\pm$ SEM. ANOVA determined differences among the group means and the Tukey post hoc analysis determined which group means differed significantly (at a level of $p=.05$).

3. Results

3.1. Exercise mediated increases in endogenous corticosterone levels and GR nuclear translocation in OVA-sensitized mice

Several reports indicate that exercise stimulates an increase in the release of endogenous GC into the circu-

lation (reviewed in Pedersen and Hoffman-Goetz, 2000). As noted above, GC exerts its anti-inflammatory effects via binding to intracellular GRs and subsequently inducing their nuclear translocation. To determine the effects of exercise on endogenous GC production and GR activation in sensitized mice, plasma GC levels and GR nuclear localization were measured in mice that were exercised and/or OVA-sensitized. At the conclusion of the sensitization/exercise protocol, plasma was collected and examined for differences in endogenous GC corticosterone content via corticosterone-specific RIA. In addition, lungs were excised and assessed for differences in GR nuclear translocation via immunohistochemical analysis. As shown in Fig. 1, corticosterone production was enhanced approximately 3-fold in EO/placebo mice as compared with SO/placebo controls. Importantly, exercise also enhanced relative GR nuclear translocation 5-fold in EO/placebo mice as compared with SO/placebo control mice (Figs. 2A, B, and E); such increases in GR nuclear translocation were observed primarily within airway epithelial cells (AECs) (Fig. 2B). No immunofluorescent cross-reactivity was observed in the IgG-negative controls (data not shown).

3.2. RU486 blocked the effects of exercise on GR nuclear translocation

To determine directly whether GR played a role in the anti-inflammatory effects of exercise that we observed in the OVA-sensitized lung (Pastva et al., 2004), we utilized the GR antagonist RU486 to block GR activation in exercised OVA-sensitized mice. RU486

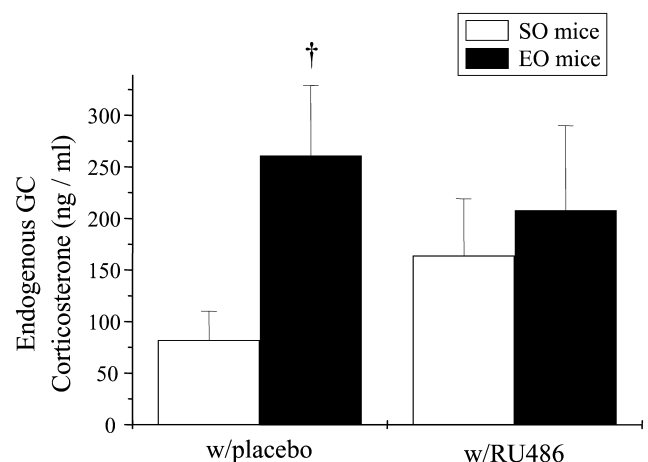


Fig. 1. Exercise induced increases in endogenous GC corticosterone levels in OVA-sensitized mice. Mice were sensitized, exercised, and implanted with RU486 or placebo pellets as described under Section 2. Plasma was isolated from blood collected via cardiac puncture and then analyzed via RIA for changes in endogenous GC corticosterone levels. Results are reported as nanograms per milliliter of endogenous GC corticosterone levels ($n=10$; $p<.05$ as compared with sedentary and OVA-sensitized (SO)/placebo mice).

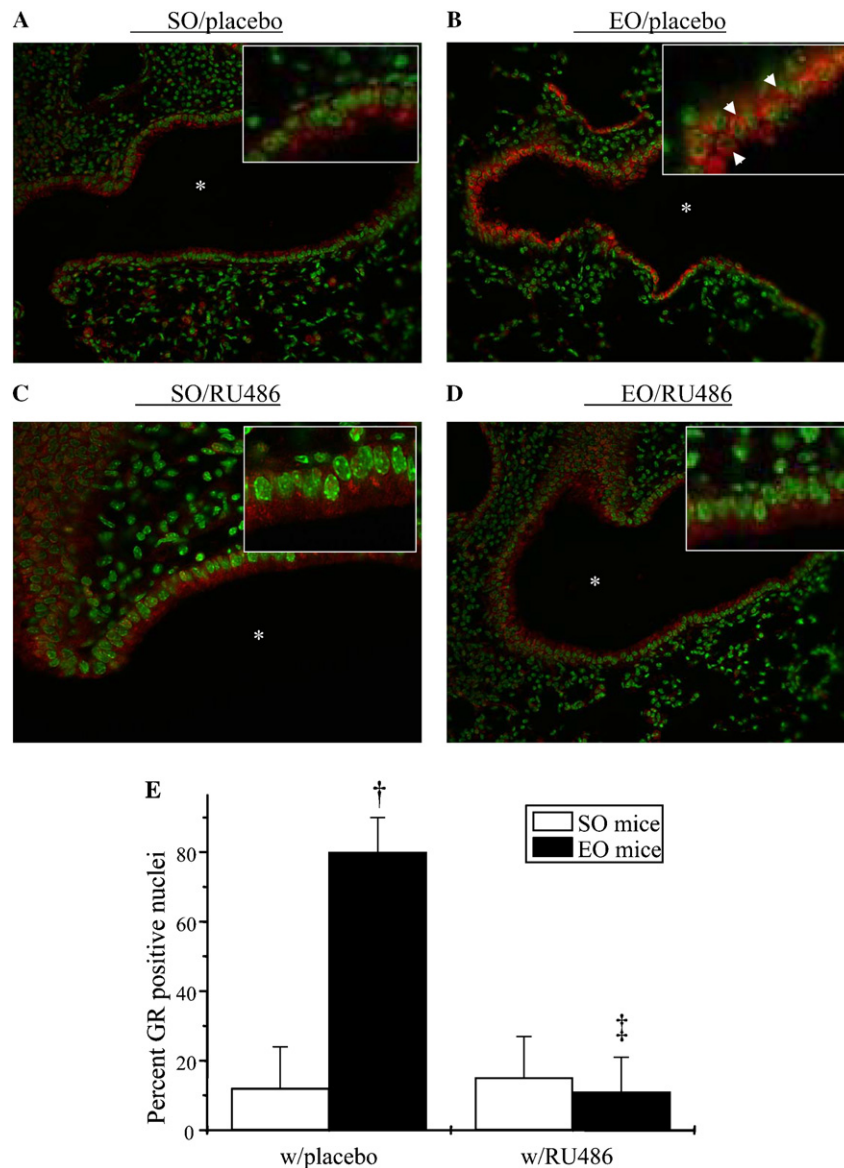


Fig. 2. RU486 blocked exercise-induced increases of GR nuclear translocation within the lungs of OVA-sensitized mice. Nuclear translocation of GR (denoted by arrows) was monitored in (A) SO/placebo, (B) EO/placebo, (C) SO/RU486, and (D) EO/RU486 via immunohistochemical analysis as described under Section 2. Tissue was counterstained with Hoescht to visualize nuclei (green). Representative photomicrographs of six separate experiments are shown (*, airway lumen; magnification, 25 $\times$; inset, 50 $\times$). (E) Relative changes in combined GR nuclear translocation were determined as described under Section 2 ($n = 6$ for each group; $^{\dagger}p < .05$ as compared with SO/placebo control; $^{\ddagger}p < .05$ as compared with EO/placebo control). (For interpretation of the references to colors in this figure legend, the reader is referred to the web version of this paper.)

was developed originally as an anti-GC and was shown to repress GR-mediated transactivation (Agarwal, 1996). RU486 confers antagonistic activity via binding in the 11- β -hydrophobic pocket of the GR and impairing its nuclear translocation and its ability to modify histone structure (Agarwal, 1996). For these analyses, mice were implanted with time-release pellets containing either a placebo control or RU486 and exercised and/or OVA-sensitized as described previously (Pastva et al., 2004).

Upon completion of the exercise/sensitization protocol, blood was collected via cardiac puncture and lungs

were excised; samples were then prepared for corticosterone-specific RIA and immunohistochemical analysis, respectively. Importantly, RU486 treatment did not alter significantly the endogenous corticosterone levels observed in EO/RU486 mice as compared with EO mice that received the placebo control (EO/placebo; Fig. 1). Although not statistically significant, the SO/RU486 appeared to have slightly elevated basal levels of endogenous corticosterone as compared with SO/placebo control mice (Fig. 1). With regard to GR activation, RU486 blocked exercise-mediated increases in GR nuclear translocation (Figs. 2B, D, and E). Notably, the level of

GR nuclear translocation in EO/RU486 mice was not significantly different from that observed in either SO/placebo (Figs. 2A and E) or SO/RU486 mice (Figs. 2C and E).

3.3. RU486 blocked the anti-inflammatory effects of exercise on cellular infiltration within the OVA-sensitized lung

To determine whether RU486 modulated the anti-inflammatory effects of exercise on lung cellular infiltration, mice were implanted with time-release pellets containing placebo or RU486 and then exercised/sensitized as described above. Upon completion of the exercise/sensitization protocol, lungs were lavaged, and lavagates were prepared for cell differential analyses. Data presented in Fig. 3 show that, in EO/RU486 mice, RU486 blocked exercise-mediated reductions in total cell counts (Fig. 3A), including reductions in eosinophil cell counts (Fig. 3B), as compared with EO/placebo mice. It should be noted that total and eosinophil cell count levels in RU486-treated mice were not significantly different from the cell count levels observed in SO/placebo controls.

3.4. RU486 blocked the anti-inflammatory effects of exercise on inflammatory mediator expression in the sensitized lung

Together, chemokines and adhesion molecules play an important role in leukocyte trafficking and transmigration. Specifically, the CXC chemokine KC (human homologue IL-8) induces the chemotaxis and activation of differing cell populations such as neutrophils, T cells, and primed eosinophils. The cell adhesion molecule

VCAM-1, in concert with cytokines, facilitates selective eosinophil accumulation at sites of inflammation. Importantly, VCAM-1 expressed on the cell surface may be cleaved from the plasma membrane of positively expressing cells and released into the local environment as soluble VCAM-1 (sVCAM-1). Increased IL-8 (Folkard et al., 1997) and sVCAM-1 (Zangrilli et al., 1995) levels have been reported in the BALF of asthmatic patients.

In our previous report, we demonstrated that exercise decreased the expression of KC and VCAM-1 in the OVA-sensitized lung (Pastva et al., 2004). To determine whether RU486 modulated the anti-inflammatory effects of exercise on chemokine and adhesion molecule expression, mice were implanted with time-release pellets containing placebo or RU486 and then exercised/sensitized as described above. Upon completion of the exercise/sensitization protocol, lungs were lavaged and lavagates were prepared for ELISA. Results presented in Fig. 4 show that, in EO/RU486 mice, RU486 blocked exercise-mediated decreases in KC and sVCAM-1 protein levels as compared with EO/placebo mice. Notably, the lungs of SO/RU486 and EO/RU486 mice displayed protein levels of both KC (Fig. 4A) and sVCAM-1 (Fig. 4B) that were statistically equivalent to those levels observed in SO/placebo mice.

3.5. RU486 blocked the exercise-associated reductions in NF- κ B nuclear translocation and DNA binding

The activated transcription factor NF- κ B regulates the expression of a variety of genes that encode inflammatory mediators such as KC and VCAM-1 and, thus, has been implicated directly in regulating asthmatic

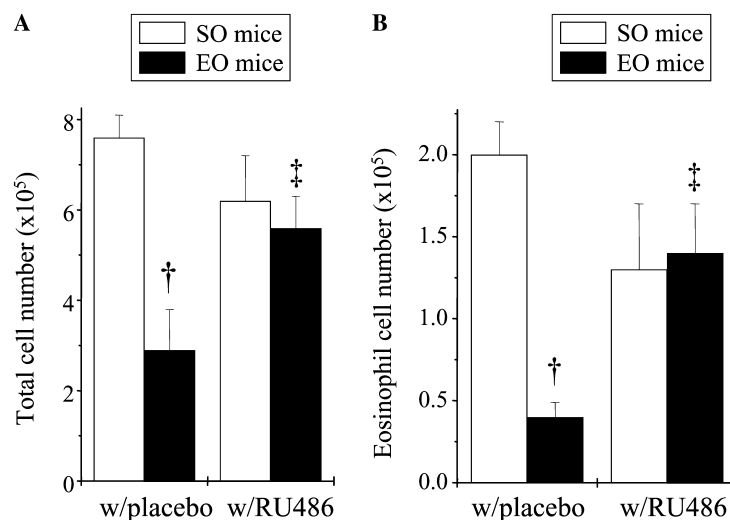


Fig. 3. RU486 blocked the anti-inflammatory effects of exercise on cellular infiltration in OVA-sensitized mice. (A) Cells collected from BALF of mice were analyzed for differences in total cell count as described under Section 2. (B) Differential BALF cell counts were performed using standard hematological criteria; results for the eosinophil cell population are shown ($n = 5-6$ for each group; $^{\dagger}p < .05$ as compared with SO/placebo control; $^{\ddagger}p < .05$ as compared with EO/placebo control).

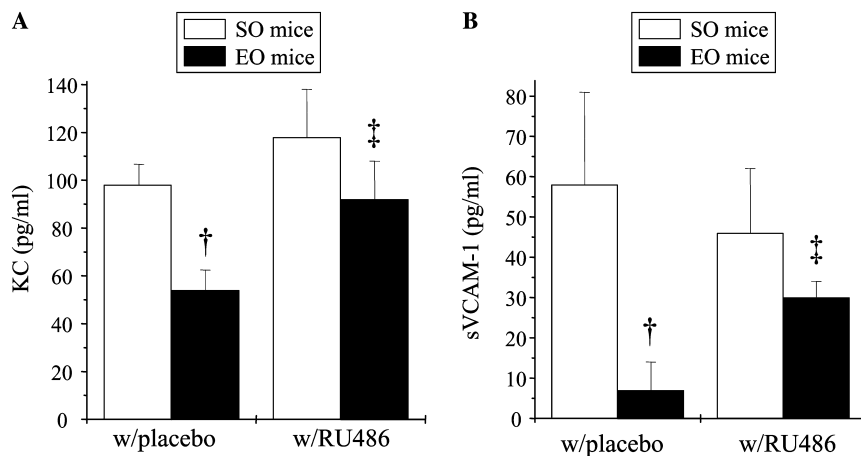


Fig. 4. RU486 blocked the anti-inflammatory effects of exercise on KC and sVCAM-1 levels in the BALF of OVA-sensitized mice. BALF samples were analyzed for (A) KC and (B) sVCAM-1 levels via ELISA. Results are reported as picograms per milliliter of respective protein ($n = 5-6$; * $p < .05$ as compared with sedentary and OVA-sensitized (SO)/placebo control; † $p < .05$ as compared with exercised and OVA-sensitized (EO)/placebo control).

inflammatory responses (Christman et al., 2000). We have demonstrated previously that exercise decreases NF- κ B nuclear translocation and DNA binding in the OVA-sensitized lung (Pastva et al., 2004). To determine whether RU486 modulated the exercise-induced inhibitory effects on NF- κ B nuclear translocation and DNA binding, mice were implanted with time-release pellets containing placebo or RU486 and then exercised/sensitized as described above. Upon completion of the exercise/sensitization protocol, lungs were excised and prepared for immunofluorescence or EMSA. Data presented in Fig. 5 show that, in EO/RU486 mice, RU486 blocked exercise-mediated decreases in NF- κ B (p65) nuclear translocation (Figs. 5A, B, and C) and NF- κ B–DNA binding activity (Figs. 5D and E) as compared with the lungs of EO/placebo mice. Interestingly, the lungs of EO/RU486 mice displayed a pattern and level of NF- κ B nuclear localization (Fig. 5C) and DNA binding activity (Figs. 5D and E) that was equivalent to the pattern observed in SO/placebo control mice (Figs. 5A, D, and E). No cross-reactivity was observed in the IgG-negative controls (data not shown). NF- κ B–DNA binding was confirmed through EMSAs performed with unlabeled (competitor) and mutant NF- κ B oligonucleotide (data not shown).

4. Discussion

The data presented herein demonstrate that the GR antagonist RU486 blocked the effects of exercise in OVA-sensitized mice. Specifically, in the sensitized lung, RU486 blocked the anti-inflammatory effects of exercise on GR nuclear localization, cellular infiltration, chemokine and adhesion molecule production, and NF- κ B

activation. Taken together, these data suggest that moderate aerobic exercise promotes an anti-inflammatory response in allergen-induced lung inflammation via a mechanism that involves GR nuclear translocation and NF- κ B activation.

Treadmill training was utilized as the mode of aerobic exercise in our study because intensity and duration could be manipulated directly unlike voluntary running or swimming. Moreover, treadmill training mimics the use of treadmill exercise, which is often a large component of pulmonary rehabilitation programs. We recognize that treadmill training introduces an unintentional psychological stress on the mice because it comprises a ‘forced exercise’; such a psychological stress may activate the HPA axis and thus cloud our interpretation that aerobic exercise alone lessens airway inflammation. It should be noted, though, that various clinical studies have demonstrated an association between psychological stress and the onset of asthma-like symptoms (Kang and Fox, 2000; Ritz et al., 2000; Sandberg et al., 2000). Repetitive psychological stress has also been shown to enhance airway reactivity and airway inflammation in an OVA-driven murine model of allergic asthma (Joa-chim et al., 2003, 2004). In light of these studies, it appears that psychological stress exacerbates allergen-mediated airway inflammation. Conversely, our results demonstrate that ‘forced exercise’ training attenuated allergen-mediated airway inflammation; nonetheless, the contributions of physical stress versus psychological stress in airway responses require further analysis. In addition, metabolic measurements that quantify exercise intensity and training status are imperative to ascertain the extent of exercise stress and physiological adaptation in the trained mice; the lack of such measurements is a limitation of our current study.

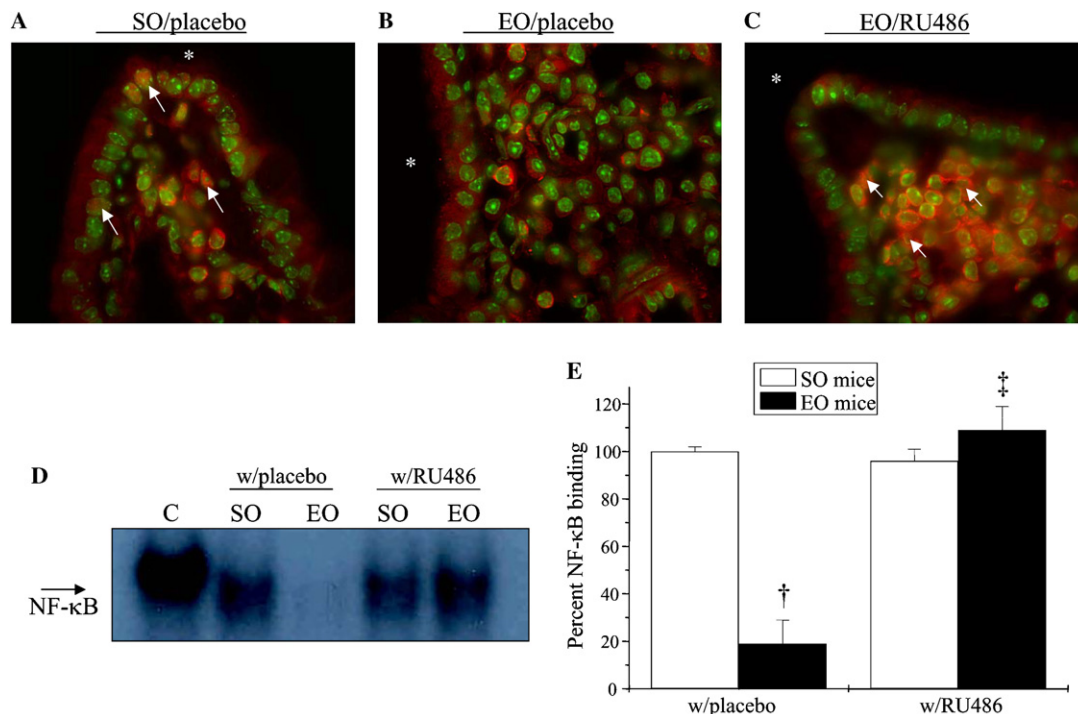


Fig. 5. RU486 blocked exercise-induced decreases of NF-κB nuclear translocation and DNA binding within the lungs of OVA-sensitized mice. Nuclear translocation of p65 (denoted by arrows) was monitored in (A) sedentary and OVA-sensitized (SO)/placebo, (B) exercised and OVA-sensitized (EO)/placebo, and (C) EO/RU486 via immunohistochemical analysis as described under Section 2. (D) NF-κB-specific EMSA was performed as described in Materials and methods. Representative results from six independent experiments are shown (C, positive control) (TNF-α-treated AECs). (E) Densitometric analyses of NF-κB-specific DNA binding as determined via EMSAs were performed ($n = 6$; $^{\dagger}p < 0.05$ as compared with sedentary and OVA-sensitized (SO)/placebo control; $^{\ddagger}p < 0.05$ as compared with exercised and OVA-sensitized (EO)/placebo control).

As a part of the HPA axis, endogenous GCs play important roles in many physiological processes, including the regulation of energy homeostasis, adaptation to stress, modulation of central nervous system function, and modulation of the immune response. Several reports suggest that a single, acute bout of exercise increases circulating levels of GCs (Coleman et al., 1998; Deschenes et al., 1991; Girard and Garland, 2002; Tharp, 1975); however, the effects of prolonged exercise training on endogenous GC levels are much less clear. For example, previous studies that have examined the effects of exercise training on changes in circulating GC levels have reported increases (Sylvester et al., 1989; Tharp and Buuck, 1974), decreases (Virus et al., 1994), or no change (Borer et al., 1992; Dellwo and Beauchene, 1990) in these levels. The conflicting data are most likely the result of differences in exercise parameters (i.e., intensity, frequency, duration, and type), subject fitness level (i.e., trained versus untrained), blood sample timing (i.e., in relation to the diurnal variation of GC production; in rodents, circulating GC levels are lower in the morning hours and higher in the early evening hours), and sacrifice method (Shipp and Woodward, 1998). In our study, we observed that a 4 week protocol of moderate-intensity aerobic training stimulated a significant increase in endogenous GC lev-

els in groups of mice that were exercised, including EO mice. These elevated levels were detected 24 h following the completion of the exercise training protocol, with sampling occurring over the morning hours (7:30–11:00 a.m.). From these observations, we conclude that moderate-intensity aerobic training enhances endogenous GC production.

GCs exert their anti-inflammatory effects through binding to and activating GRs. Once activated, GRs translocate to the nucleus, where they facilitate transcriptional transactivation or transrepression to modulate gene transcription as described above. In our study, we observed that moderate exercise enhanced the nuclear localization of GR, primarily within AECs of OVA-sensitized mice. The GR antagonist RU486 blocked such enhancement and, further, blocked the anti-inflammatory effects of exercise in the sensitized lung. As noted earlier, RU486 did not alter endogenous GC production significantly in EO/RU486 mice as compared with EO/placebo controls. RU486 appeared to elevate slightly the endogenous GC levels of SO/RU486 mice as compared with SO/placebo control mice; however, these differences were not significant statistically. This slight elevation may be the result of an RU486-mediated impairment in the GR-dependent negative-feedback regulation of the HPA axis. Previous studies

with RU486 (Tarcic et al., 1998) and GR knockout (GR $-/-$) mice (Cole et al., 1995) have yielded similar findings.

Activated GR can inhibit the transcriptional activation of genes that encode inflammatory mediators via several independent mechanisms, including modulation of NF- κ B activity. In further support of the mechanistic role of GR, we demonstrate that RU486 blocks exercise-mediated attenuation of NF- κ B (p65) nuclear translocation and DNA binding within the OVA-sensitized lung. These results imply that, within the EO lung, ligated GR facilitates an anti-inflammatory response through the prevention of NF- κ B nuclear translocation and its subsequent DNA binding.

It should be noted that RU486 can also antagonize the actions of the progesterone receptor (PR), which is a member of the steroid receptor family and, therefore, related closely to GR (Cadepond et al., 1997). Despite the similarity of these two receptors, the cognate hormones each display a very distinct set of physiological actions (Wan and Nordeen, 2003). While classic functions of the GR include regulation of metabolism and inhibition of inflammation, the major physiological role of the PR in mammals is to establish and maintain pregnancy (Wan and Nordeen, 2003). In light of these differences between the actions of GR and PR, we believe that the effects of RU486 observed in our study are caused solely by its antagonism of the GR and not of the PR.

As noted above, we believe that the results of our current study demonstrate that exercise mediates its anti-inflammatory effects in the sensitized lung via a mechanism that involves GR nuclear translocation and NF- κ B activation; however, the mechanism that underlies exercise-mediated anti-inflammatory responses may ultimately prove multifactorial. For example, transcription factors other than NF- κ B, including AP-1 and CREB, have been implicated in mediating inflammatory responses and are inhibited by GC-GR complexes (Umland et al., 2002). Despite this possibility, we maintain that the data from our current study show the potential for the use of moderate aerobic exercise as a non-pharmacologic therapeutic in the treatment of allergen-induced airway inflammatory responses. Moreover, we believe that our findings lay the foundation for the development of related clinical studies, which examine the effects of aerobic exercise on atopic asthmatic patients at the molecular and cellular level.

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