Whole-Body Periodic Acceleration Modifies Experimental Asthma in Sheep

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Rationale: **Nitric oxide is released from vascular endothelium in response to increased pulsatile shear stress. Nitric oxide inhibits mast cell activation and is antiinflammatory and therefore might be protective in asthma.**

Objectives: **We determined if a noninvasive motion platform that imparts periodic sinusoidal inertial forces to the whole body along the spinal axis (pGz) causing release of endothelial nitric oxide modulates experimental asthma in sheep.**

Methods: **Allergic sheep were untreated (control) or were treated with pGz alone or after receiving intravenously the nitric oxide synthase inhibitor N_w-nitro-L-arginine methyl ester (L-NAME) before aerosol challenge with *Ascaris suum*, and the effect on antigen-induced airway responses was determined. Bronchoalveolar lavage cells obtained 6 h after antigen challenge were analyzed for nuclear factor-κB (NF-κB) activity in the respective groups.**

Results: **pGz treatment for 1 h before antigen challenge reduced the early airway response and blocked the late airway response but did not prevent the antigen-induced airway hyperresponsiveness 24 h after challenge. Administration of L-NAME before pGz completely reversed this protection, whereas L-NAME alone did not affect the antigen-induced responses. NF-κB activity was 1.9- and 1.8-fold higher in the control and L-NAME + pGz groups, respectively, compared with pGz-treated animals. Extending the pGz treatment to twice daily for 3 d and then 1 h before antigen challenge blocked the early and late airway responses, the 24-h airway hyperresponsiveness, and the airway inflammatory cell response.**

Conclusion: **Whole-body pGz modulates allergen-induced airway responses in allergic sheep.**

Keywords: animal models; asthma therapy; nitric oxide; noninvasive periodic acceleration

Nitric oxide (NO) has varied physiologic and pathophysiologic roles in biological systems. NO is formed during the conversion of L-arginine to L-citrulline by the enzyme NO synthase (NOS). There are inducible (i) and constitutive forms of the enzyme. The generation of NO by iNOS (NOS II) occurs after exposure to proinflammatory cytokines, and the levels of NO produced are sustained and in the nanomolar range. In the lung, cell sources of iNOS include airway epithelium, macrophages, monocytes, and eosinophils. The constitutive forms of NOS are found in neurons (nNOS, NOS I) and endothelial cells (eNOS, NOS III). In contrast to iNOS, the release of NO from nNOS and eNOS occurs in bursts, and NO levels are in the picomolar range (1).

Studies evaluating the role of NO on mast cell/basophil activation using NO donor drugs *in vitro* indicate that NO can inhibit degranulation and cytokine production (2–4). These findings are consistent with *in vivo* studies indicating that NO donors can inhibit mast cell degranulation, mast cell–dependent granulocyte adhesion, and microvascular leakage (5). In addition, NO inhibits activation of nuclear factor-κB (NF-κB), a transcription factor that regulates the expression of a number of inflammatory cytokines (6, 7), in tumor necrosis factor (TNF)–stimulated endothelial cells (8), in lipopolysaccharide/TNF-stimulated microglia (9), and in interleukin (IL)-1β/IFN-γ–stimulated astroglial cells (10). Mast cells are also a source of TNF (11). These findings suggest that NO (through inhibition of mast cell activation and cytokine release), mast cell–dependent inflammatory processes, and inhibition of NF-κB, could be beneficial in modulating many of the pathophysiologic processes that contribute to asthma. Despite this suggestive evidence, a previous study in patients with mild asthma indicated that blocking endogenous levels of NO with N_w-nitro-L-arginine methyl ester (L-NAME) did not protect or worsen antigen-induced airway responses (12). In contrast, transgenic mice that overexpressed eNOS were found to be protected against antigen-induced airway hyperresponsiveness (AHR) and showed a reduced inflammatory cell response compared with wild-type control mice (13). The differences in the results of the two studies could be related, in part, to the source of NO being manipulated.

The vascular endothelium releases NO through the action of eNOS in response to blood flow–induced changes in laminar and pulsatile shear stress (14–16). Adams and colleagues (17, 18) described a noninvasive motion platform that oscillates an animal in a foot-to-head direction (z-plane) along the spinal axis. The platform varies the frequency and intensity of acceleration in a controlled fashion, imparting periodic sinusoidal inertial forces (pGz) to the body that add pulses to the circulation as the body accelerates and decelerates. Subsequent studies in healthy human subjects (19, 20), in patients (20, 21), in whole animals *in vivo* (15, 22), and in isolated vessels *in vitro* indicate that application of pGz results in the generation of eNO (15); consistent with these findings is the observation that the effects of pGz can be blocked by L-NAME (15). Furthermore, *in vivo* studies demonstrate that the pGz-induced release of eNO provides protection in different models of hemodynamic inflammation (17, 23, 24). Because pGz activates eNOS by the addition of pulses to the circulation throughout the entire body and the diffusion distance for NO from the microcirculation has a range of 150–300 μm within 4–15 s (25), eNO has the potential to act on mast cells and smooth muscle cells within the airways.

Although the previous study in patients found no effect on airway responses with pharmacologic blockade of NO during antigen provocation (12), that study did not evaluate whether increased concentrations of NO released by activation of eNOS could modulate allergic airway responses. Based on previous work demonstrating that pGz can increase eNO (15, 19–22), this question can now be addressed by using pGz to stimulate eNOS to cause the release of eNO *in vivo* and determining the effects on antigen-induced airway responses.

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The sheep has been used extensively to study the pathophysiology of asthma (26). A primary strength of the model is the ability to repeatedly and accurately assess changes in measures of pulmonary function after provocation with antigen and other irritant stimuli. These functional changes have been characterized extensively and are similar to the respective pathophysiologic changes seen in human subjects in clinical studies (26). Therefore, in this study we tested the hypothesis that pGz could modulate allergen-induced airway responses by examining the effects of pGz treatment on allergen-induced early (EAR) and late (LAR) airway responses, post–antigen-induced AHR, and airway inflammatory cell recruitment in allergic sheep. Studies were performed in the presence and absence of L-NAME. Our results show that pGz can block these antigen-induced airway responses and that this protection is reversed by L-NAME. Mechanistically, the protective effects of pGz seem to be mediated, in part, through suppression of NF-κB. Preliminary results of these studies have been reported in abstract form (27, 28).

METHODS

Detailed methods are contained in the online supplement.

Animal Preparation

Sheep (24–43 kg) with airway hypersensitivity to *Ascaris suum* antigen were used. All sheep had previously been shown to develop EAR, LAR, and AHR to inhaled *A. suum* antigen (26, 29). The Mount Sinai Medical Center Animal Research Committee, which is responsible for ensuring the humane care and use of experimental animals, approved the procedures used in this study.

Whole-body pGz

Whole-body pGz was accomplished with a motion platform device as previously reported (17–20); but the device used in this study was adapted to support a sheep in the cart system. The animals in carts were secured to the platform for the designated treatment times depending on the specific protocol. Acceleration parameters for all studies were f = 2 Hz; Gz = ±0.2 (17, 18).

For control studies, the animals in carts were placed on the platform for the appropriate time without motion.

Airway Mechanics

Mean pulmonary airflow resistance (RL) in cm H_2O/L/s was measured with the esophageal balloon technique (30–32).

Concentration Response Curves to Carbachol Aerosol

Airway responsiveness was determined from cumulative concentration response curves to inhaled carbachol. Data are expressed as PC400, which is the cumulative carbachol concentration in breath units (BU) that increased RL by 400% (30–32).

Bronchoalveolar Lavage

Total and differential cell counts were determined by bronchoalveolar lavage (BAL) (30–32).

NF-κB Analysis of BAL Cell Pellet

NF-κB activity in nuclear extracts from BAL cell pellets recovered from sheep with and without pGz and pGz + L-NAME treatment was assessed using the NF-κB ActivElisa (Imgenex, San Diego, CA). Values are reported as optical density per 10^6 cells × 100 at 405 nm.

Experimental Protocols

Protocol 1: Effect of acute pGz on antigen-induced responses with and without L-NAME. Baseline airway responsiveness was obtained before pGz treatment (f = 2 Hz; Gz = ±0.2) or control. On the challenge day, measurements of RL were obtained before treatment and 30 min after treatment. Then the animals were challenged with *A. suum* antigen. RL was remeasured immediately after and serially from 1 to 8 h after challenge. Post-challenge determinations of airway responsiveness were obtained 1 d after antigen challenge to assess the development of AHR. This study was repeated except that the animals were treated with L-NAME 0.5 h before pGz treatment. L-NAME was dissolved in 20 ml sterile 0.9% NaCl and given intravenously at a dose of 25 mg/kg, based on previous work (33). The same five sheep were used in the different arms of this protocol. Treatments were randomized, and a minimum interval of 2 wk elapsed between antigen challenges.

To determine if pGz treatment induced a generalized stress response, we measured serum cortisol levels before treatment, 15 min after a 1-h pGz treatment, and 6 h after treatment in six sheep. Studies were done in pairs with control sheep (no pGz) positioned next to the treated animals. The same study was performed in six L-NAME + pGz–treated animals and six L-NAME–treated control animals (no pGz). Sampling times were based on previous work showing that sheep responded to a stress response with an immediate and sustained elevation in systemic cortisol (34).

Protocol 2: Effect of L-NAME on antigen-induced responses. To control for the effects of L-NAME alone on antigen-induced responses, four sheep were treated with L-NAME (25 mg/kg intravenously) or intravenous saline (control) 1.5 h before antigen challenge. The time interval between treatment and allergen challenge was chosen to mimic that used in Protocol 1.

Protocol 3: Effect of acute pGz treatment when given after antigen challenge on LAR and AHR. In five sheep, baseline airway responsiveness was determined before antigen challenge. On the antigen challenge day, RL was measured, and the sheep were challenged with antigen. RL was measured immediately after challenge and 1 and 2 h after challenge. In a randomized fashion, the sheep were treated with pGz (f = 2 Hz; Gz = ±0.2) between 2 and 4 h or remained untreated. After treatment, RL was measured as described in Protocol 1. Airway responsiveness was remeasured on the following day.

Protocol 4: Effect of multiple pGz treatments on antigen-induced responses. Protocol 4 was the same as Protocol 1 except that pGz treatments (f = 2 Hz; Gz = ±0.2) were given twice a day for 3 d and then 1 h before antigen challenge on Day 4. This protocol was performed in four sheep. We repeated this study in one control group (n = 9) and in a pGz-treated group (n = 9) to determine if multiple pGz treatments affected antigen-induced airway inflammatory cell recruitment. For these studies, BAL was performed before and 24 h after antigen challenge, and total cells and cell differentials were measured.

Protocol 5: Effect of acute pGz treatments with and without L-NAME on BAL cell NF-κB activity. Protocol 5 was conducted in three separate groups of six sheep each: control (no treatment), pGz-treated (1 h), or L-NAME + pGz. Treatments were given as described in Protocol 1. RL was measured as described up to 6 h. At this time, the animals underwent BAL, and the recovered cell pellet was analyzed for NF-κB activity.

Statistical Analysis

Analyses were conducted with Sigma Stat 3.1 for Windows (Systat Software, Inc., Point Richmond, CA). If data failed the normality test, they were $\log_{10}$ transformed before analysis. Multiple group analysis was performed using a one-way analysis of variance (ANOVA; Protocol 5) or one-way repeated measures ANOVA (Protocol 1). *Post hoc* pairwise comparisons were made with the Student-Neumann-Keuls test. Two group comparisons were made with paired or unpaired *t* test where appropriate (Protocols 2, 3, and 4). Significance was accepted at $p < 0.05$ using a two-tailed analysis. Values in the text, table, and figures are presented as mean ± SEM.

RESULTS

Effects of Acute pGz on Antigen-induced Responses (Protocol 1)

Figure 1A illustrates the time course of the response to antigen with and without pGz treatment in the presence and absence of L-NAME, and Figure 1B illustrates the effects of these treatments on antigen-induced AHR. pGz significantly reduced the EAR and blocked the LAR when compared with control (Figure 1A), but pretreating the animals with L-NAME abolished this protection (Table 1). We assessed the area under the curve (AUC, % change RL/h) for the EAR (0–4 h) and LAR (4–8 h).

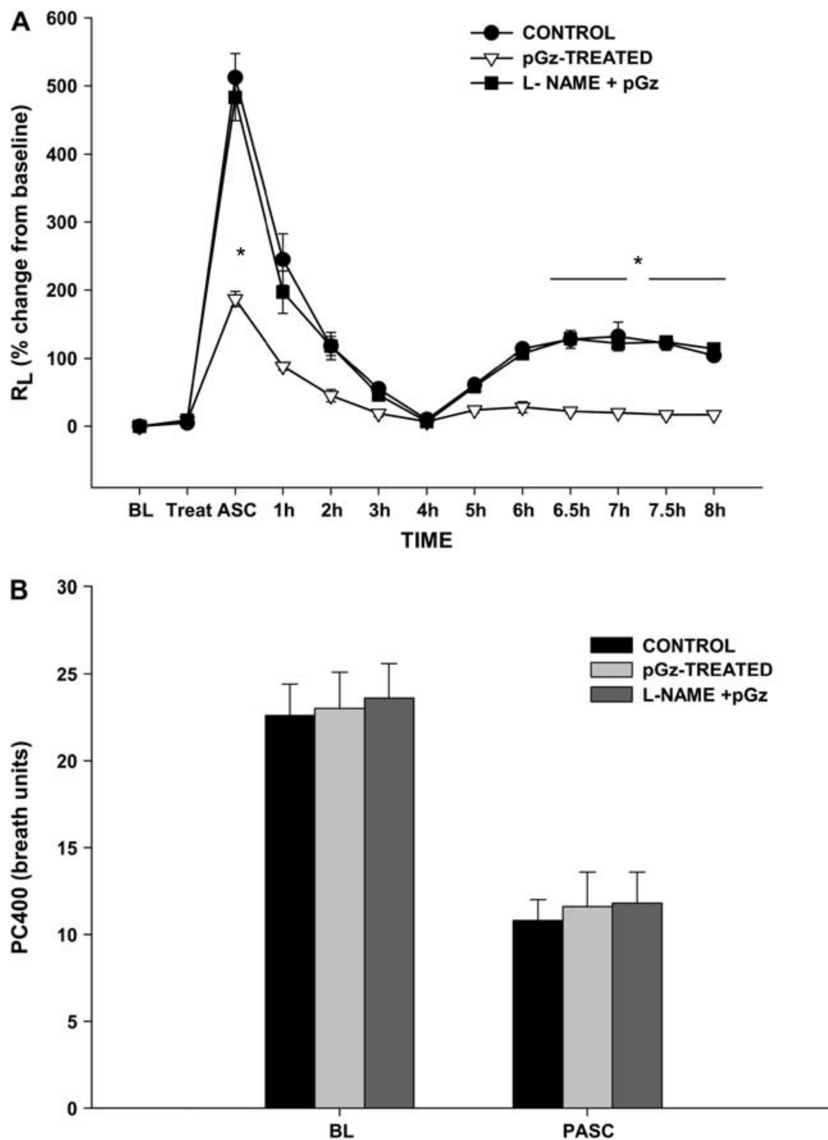

Figure 1. Effect of 1 h pGz treatment with or without N_w–nitro-L–arginine methyl ester (L-NAME) on the time course of antigen-induced changes in lung resistance (RL) in allergic sheep (*A*). pGz was administered for 1 h before Ascaris (ASC) challenge. pGz significantly reduced the early airway response and blocked the late airway response when compared with the control and L-NAME + pGz treatment arms. (*B*) Effects of pGz on airway responsiveness to inhaled carbachol expressed as the PC400 at baseline (BL) and 24 h after antigen challenge (PASC). A fall in the PC400 indicates the development of antigen-induced airway hyperresponsiveness (AHR). pGz had no effect on AHR. Values are mean ± SEM for five sheep. * $p < 0.05$ versus control and L-NAME + pGz treatment arms (*see* Table 1 for statistical analysis of peak responses and text for analysis of area under the curve).

During the control trial, the EAR AUC was 811 ± 67, and the LAR AUC was 368 ± 26. Treatment with pGz reduced the EAR AUC by 61 ± 5% to 302 ± 32 ($p < 0.05$) and reduced the LAR AUC by 77 ± 4% to 82 ± 8% ($p < 0.05$). These effects were reversed by treating the sheep with L-NAME before applying pGz. In the L-NAME + pGz arm, the EAR AUC and LAR AUC were 735 ± 53 and 358 ± 13, respectively. These values did not differ from the control responses but were significantly different ($p < 0.05$) from pGz alone. The protective effects of pGz on these antigen-induced responses were not related to a change in bronchial tone because there was no difference in post-treatment RL among the three trials (Table 1).

Despite the protection against the antigen-induced bronchoconstrictor responses, the single treatment with pGz did not protect against the post-challenge AHR (Figure 1B, Table 1). In the control trial, PC400 at 24 h fell to 10.8 ± 1.2 BU from a prechallenge value of 22.6 ± 1.8 BU ($p < 0.05$), resulting in a decreased post-/prechallenge PC400, which is indicative of AHR. When the animals were treated with pGz, the post-/prechallenge PC400 showed a similar decrease, as the prechallenge PC400 value 23.0 ± 2.1 BU fell to 11.6 ± 2.0 BU after challenge. The degree of AHR in the L-NAME + pGz–treated animals was similar to the other two arms (Figure 1B, Table 1).

Although pGz had no effect on basal airway tone, the protective effects on the antigen-induced EAR and LAR with pGz could have resulted from a decrease in airway smooth muscle responsiveness. To check this, we measured PC400 before and after a 1-h treatment with pGz. PC400 was 30.6 ± 0.4 BU before and 30.6 ± 1.6 BU after a 1-h pGz treatment, indicating that pGz treatment did not affect airway smooth muscle responsiveness (individual responses are presented in Figure E1 in the online supplement).

To ensure that the protective effect of pGz on the antigen-induced EAR and LAR was not related to the induction of a stress response in the animals, we compared serum cortisol levels in pGz-treated and control sheep without and with L-NAME pretreatment. Cortisol levels in pGz-treated and control animals in the absence or presence of L-NAME showed a similar profile with cortisol levels declining throughout the day (Figures 2A and 2B). More importantly, there was no evidence of a cortisol surge in the pGz-treated sheep, suggesting that the protective effect of pGz was not due to a stress-related increase in serum cortisol.

Effect of L-NAME on Antigen-induced Responses (Protocol 2)

Consistent with the previously reported human data, treatment with L-NAME alone had no effect on the antigen-induced EAR,

TABLE 1. SUMMARY OF PULMONARY AIRFLOW RESISTANCE AND AIRWAY RESPONSES BEFORE AND AFTER TREATMENT WITH pGz

Treatment	Baseline RL	Post-treatment RL	EAR (%)	LAR (%)	PC400 Ratio
Protocol 1					
pGz (n = 5)	0.97 ± 0.01	1.04 ± 0.03	187 ± 11*†	32 ± 6*†	0.48 ± 0.03
Control (n = 5)	0.96 ± 0.01	1.01 ± 0.02	512 ± 36	141 ± 11	0.49 ± 0.07
L-NAME + pGz (n = 5)	0.97 ± 0.01	1.06 ± 0.02	483 ± 34	133 ± 6	0.49 ± 0.04
Protocol 2					
L-NAME (n = 4)	0.97 ± 0.01	1.11 ± 0.04	480 ± 40	133 ± 2	0.51 ± 0.02
Control (n = 4)	0.97 ± 0.01	0.97 ± 0.01	467 ± 49	134 ± 5	0.54 ± 0.04
Protocol 3					
pGz postantigen (n = 5)	0.98 ± 0.01	—	434 ± 75	28 ± 6*	0.49 ± 0.05
Control (n = 5)	0.96 ± 0.01	—	469 ± 69	144 ± 6	0.50 ± 0.06
Protocol 4					
Multiple pGz treatments (n = 4)	0.97 ± 0.01	1.11 ± 0.04	193 ± 13*	25 ± 4*	0.99 ± 0.04*
Control (n = 4)	0.98 ± 0.01	1.06 ± 0.04	541 ± 38	144 ± 5	0.50 ± 0.04

Definition of abbreviations: EAR = early airway response; LAR = late airway response; L-NAME = N_w-nitro-L-arginine methyl ester; RL = pulmonary airflow resistance (cm H_2O/L/s).

Values are mean ± SE; n = number of experiments.

* $p < 0.05$ versus control.

† $p < 0.05$ versus L-NAME + pGZ.

LAR, or AHR (Figures 3A and 3B, Table 1). These data, in addition to those from the first series of experiments, indicate that the pGz-induced protection is mediated, at least in part, through an NOS-dependent pathway.

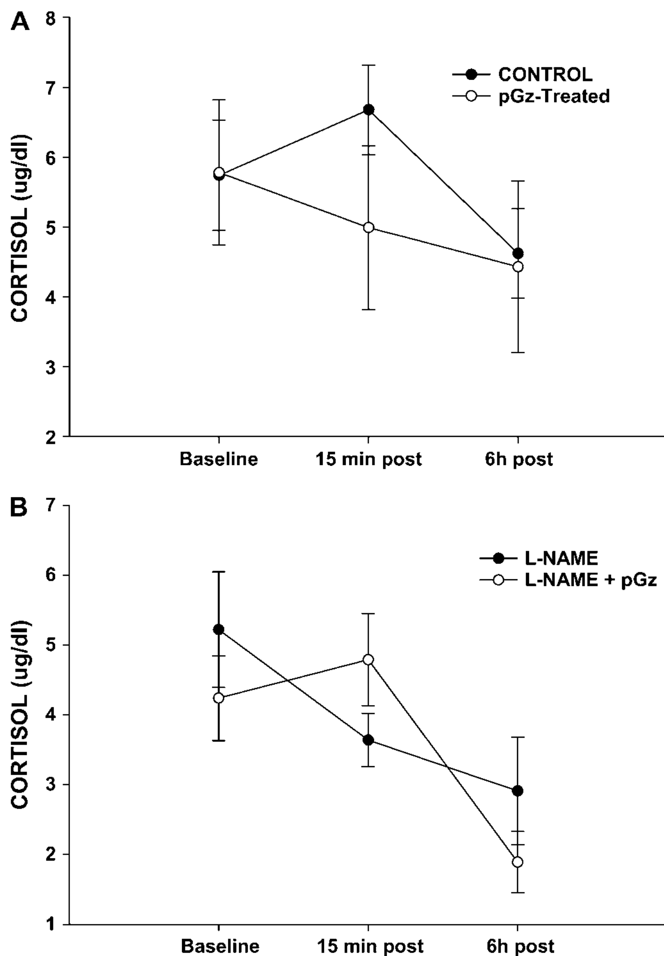

Figure 2. pGz does not increase serum cortisol. Values are mean ± SEM for six sheep without and with pretreatment with L-NAME.

The Effect of pGz on LAR Is Not Dependent on Blocking EAR (Protocol 3)

To determine if the effects on the LAR seen in Figure 1A were dependent on the reduction in the EAR, animals were treated with pGz between 2 and 4 h after antigen challenge, and the effects on the LAR and AHR were determined. The antigen-induced peak EAR in the control and treatment trials were not different, but pGz treatment provided 81% protection against the LAR ($p < 0.05$) (Figure 4, Table 1). Nevertheless, as was seen in the pretreatment study, the posttreatment effect of pGz did not affect the antigen-induced AHR (Figure 4, Table 1).

Multiple Treatments with pGz Block Antigen-induced AHR (Protocol 4)

To determine if the effects of pGz could be extended so that treatment would also block the antigen-induced AHR, we used a 4-d treatment paradigm, which has been used in previous pharmacologic studies to provide more prolonged and improved efficacy against these antigen-induced airway responses (30). When sheep were treated with pGz twice a day for 3 d and then 1 h before antigen challenge on Day 4, there was protection not only against the antigen-induced EAR and LAR but also against AHR (Figure 5, Table 1). In the control trial, the EAR AUC and the LAR AUC were 872 ± 36 and 374 ± 33, respectively. When these animals were treated with pGz, the EAR AUC and the LAR AUC were significantly reduced ($p < 0.05$) to 283 ± 8 and 52 ± 11, respectively. The degree of inhibition of the EAR (68 ± 1%) and LAR (87 ± 2%) seen with the extended pretreatment time was not different from that seen with the 1-h pretreatment. Nevertheless, the effect on AHR was evident because the PC400 after challenge (23.3 ± 2.3 BU) was not different from the prechallenge value of 24.0 ± 2.8 BU. This contrasts with the control response where PC400 after challenge fell to 12.0 ± 0.6 BU from a prechallenge value of 24.5 ± 2.1 BU ($p < 0.05$).

To determine if the multiple treatments with pGz affected the antigen-induced leukocyte infiltration into the airways, we compared the changes in the BAL cell response after antigen challenge in multiple-pGz–treated and control animals. Animals that received multiple pGz treatments had a significantly lower BAL neutrophilia (5.7-fold) compared with untreated animals (Figure 6). Total cells (2.7-fold) and lymphocytes (2.4-fold) were

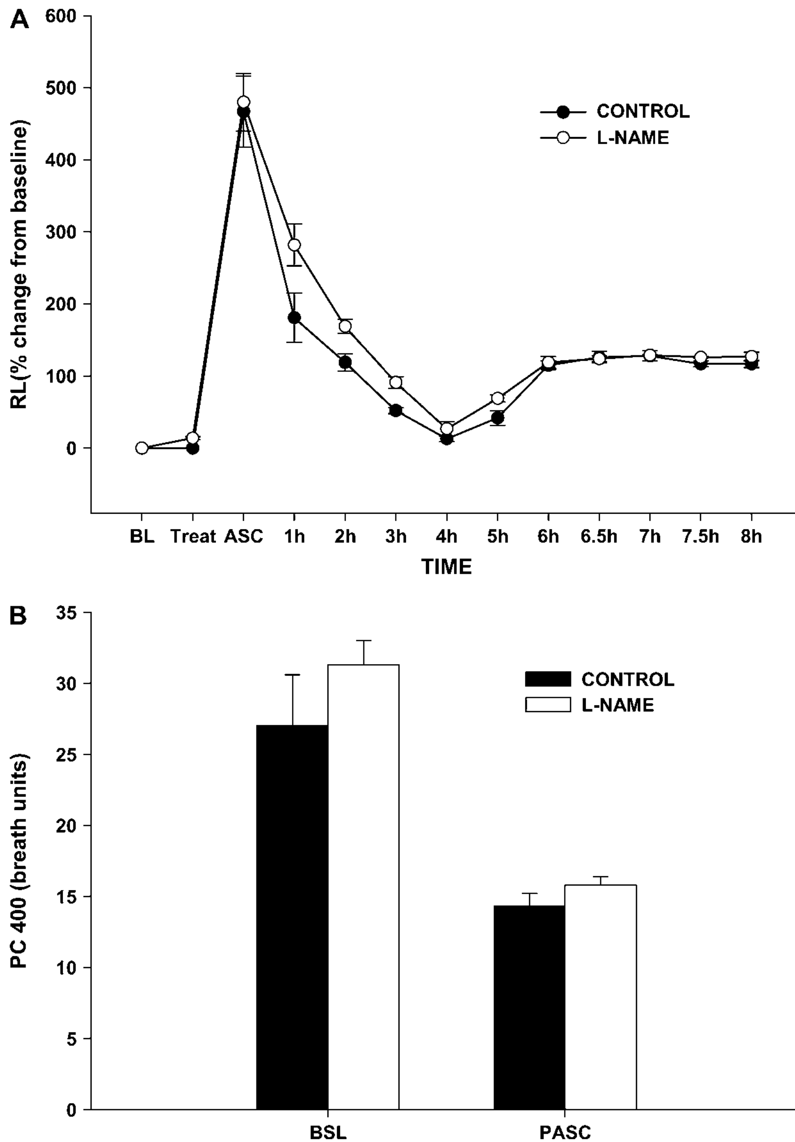

Figure 3. L-NAME does not affect antigen-induced early airway response or late airway response in allergic sheep (*A*), nor does it affect AHR (*B*). Values are mean ± SEM for four sheep. BL, BSL = baseline; PASC = 24 h after antigen challenge; RL = lung resistance.

also reduced in the treated animals, but the relative changes were not significantly different between the groups.

pGz Reduces Antigen-induced Increases in NF-κB Activity in BAL Cells (Protocol 5)

The ability of acute pGz treatments to block the LAR and multiple treatments to block the LAR and the AHR and to reduce the inflammatory cell influx after antigen challenge suggests that pGz modulates inflammatory pathways. NF-κB is a key transcription factor regulating inflammatory gene expression of proinflammatory cytokines and adhesion molecules. Because eNO is reported to inhibit NF-κB (8) and because pGz stimulates eNOS, we reasoned that NF-κB activity in animals treated with pGz should be lower than in untreated animals after antigen challenge. Furthermore, if pGz reduced NF-κB activity through an NO-dependent pathway, this effect should be abolished by L-NAME. To test this hypothesis, we repeated the antigen challenge studies with and without a 1-h pGz treatment in the presence or absence of L-NAME, except that the sheep were lavaged immediately after the 6-h measurement of RL. The recovered cell pellet was assayed for NF-κB activity. For this study, the respective peak EAR and LAR (at 6 h) for the control group was 481 ± 38% and 118 ± 3%; as shown in the previous study, the EAR (222 ± 27%) and LAR (21 ± 4%) were significantly reduced in the pGz-treated animals, and this protection was lost in the L-NAME + pGz–treated animals (EAR 598 ± 64% and LAR 130 ± 4%). Figure 7 shows that the cells recovered from the control group and the pGz + L-NAME group had 1.9- and 1.8-fold increases ($p < 0.05$), respectively, in NF-κB activity over the pGz-treated sheep. Thus, pGz not only blocked the functional airway effects and the inflammatory cell response to antigen challenge but also was a key inflammatory signaling factor. This protection was lost in the presence of L-NAME.

DISCUSSION

This study provides the first evidence that whole-body pGz can modulate antigen-induced functional airway responses and airway inflammatory cell recruitment in an animal model of asthma. This protection is blocked by L-NAME, suggesting that the effect of pGz is NO dependent. Because previous studies in animals and humans demonstrate that pGz releases eNO (15, 20, 22) and that, at least in animal studies, this eNO can reduce pathophysiologic responses to inflammatory stimuli (17, 24), one can

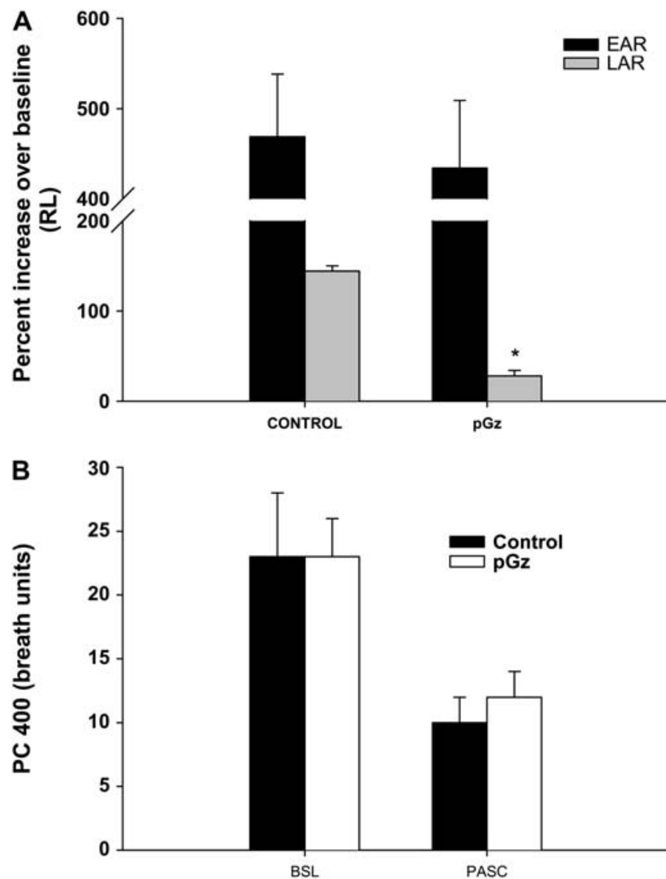

Figure 4. pGz treatment 2–4 h after antigen challenge blocks late airway response (*A*) but not AHR (*B*). Values are mean ± SEM for five sheep. *$p < 0.05$ versus control (*see* Table 1 for statistical analysis of peak responses). EAR = early airway response; LAR = late airway response; PASC = 24 h after antigen challenge; RL = lung resistance; BSL = baseline.

speculate that the pGz-induced protection observed in the present study occurs through a similar mechanism. Although there is evidence from previous animal and human studies (15, 20) that pGz stimulates eNOS resulting in NO release, our conclusions must remain speculative because direct measurements of eNO were not made in the present study. What is not speculative, however, are the novel findings that pGz inhibits antigen-induced functional and inflammatory responses in the sheep model of asthma and that the extent of the protection is dependent on the number of treatments.

The evidence that pGz can stimulate eNOS resulting in NO release is based on multiple sources. It is well known that in response to blood flow–induced changes in laminar and pulsatile shear stress the vascular endothelium releases NO through the action of eNOS (14–16). pGz has been shown to increase vascular shear stress, resulting in decreased pulmonary and systemic vascular resistances, which is consistent with the release of NO from endothelial sources. Direct evidence that pGz can stimulate the release of eNO comes from studies in anesthetized, supine piglets, where the pulsatile shear stress produced by 60 min pGz significantly increased serum nitrite, a marker of eNOS activity (35), and attenuated the vascular constrictor responses caused by L-NAME infusion (15). Adams and colleagues (15) confirmed these *in vivo* observations by applying pGz to an isolated vessel preparation *in vitro*. Effluents from porcine aortas exposed to pulsatile flow and pulsatile flow plus pGz showed 300 and 1,000% greater increases in nitrite levels, respectively, when compared with nitrite levels in effluents collected from vessels exposed to nonpulsatile flow (15). In human subjects, Sackner and colleagues provided indirect evidence that pGz released NO by measuring the extent of the descent of the dicrotic notch down the diastolic limb of the finger pulse (19, 20), a measure that reflects the vasodilator action of NO on the resistance vessels owing to the delay of pulse wave reflection (36, 37). This study further showed that the eNOS-stimulating effects of pGz were maintained throughout the 45 min of treatment (20), which, if extended to the current study, would translate to pGz stimulating eNOS activity throughout the 1-h treatment time.

The speculation in the present study that pGz stimulates release of NO from eNOS is also based on the pharmacologic abolition of the protective effects of pGz with L-NAME. This, in conjunction with the finding that L-NAME does not affect antigen-induced responses in non-pGz–treated animals, a result consistent with previous findings in patients with asthma (12), suggests that the NO generated by pGz is critical to the protective effects seen in our study. It is unlikely that iNOS was involved in the pGz protective effect because iNOS is thought to contribute to, but not block, antigen-induced inflammatory processes (1, 38). Furthermore, in patients with asthma, L-NAME significantly reduced exhaled NO, which is thought to reflect iNOS activity, but not the EAR or LAR. These observations led to the conclusion that iNOS expression and elevated iNO are not causally related to antigen-induced bronchoconstriction and that increased iNOS activity and iNO levels are a consequence of, rather than the cause of, the LAR and subsequent inflammatory response. In the present study, we extend the observations made in subjects with asthma and non-pGz–treated allergic sheep that L-NAME has no effect on antigen-induced responses by showing that, in pGz-treated animals, L-NAME reverses the protective effect of pGz. These findings indicate that the pGz-stimulated NO, which we suggest is due to increased eNOS activity, is responsible for the observed beneficial effects and that this NO is different from that in untreated animals. In the presence of L-NAME, this pGz-dependent NO is blocked, resulting in antigen-induced airway responses that are superimposed with the control (untreated) responses. L-NAME is widely used to block NO synthesis *in vivo* and *in vitro* (39), and although it is not a specific inhibitor of eNOS, L-NAME has a preference for eNOS over iNOS (40).

Our findings also make it unlikely that nNOS was stimulated by pGz. Neuronal NOS is thought to be a bronchodilator and to reduce airway responsiveness (1). As seen in our study, pGz had no effect on baseline tone or baseline bronchial responsiveness, which suggests that nNOS was not affected by pGz. Collectively, this evidence supports our contention that the pGz-induced effects are mediated through eNOS, a conclusion that is consistent with the reported beneficial antiinflammatory actions of eNO.

The primary aim of this study was to determine if pGz could modify allergen-induced airway responses in allergic sheep. This model is well suited for these studies because one is able to repeatedly and accurately assess changes in measures of pulmonary function after provocation with antigen (26). The functional changes (i.e., the EAR, LAR, and AHR) are similar to the respective pathophysiologic changes seen in human subjects in clinical antigen challenge studies, and the mechanisms responsible for the antigen-induced EAR, LAR, and AHR are well described (26). Within this context, two factors are critical for interpreting the present results. First, the mediators recovered from sheep airways in association with the antigen-induced changes in airway function are consistent with those recovered in patients with asthma after allergen challenge (41, 42). Second,

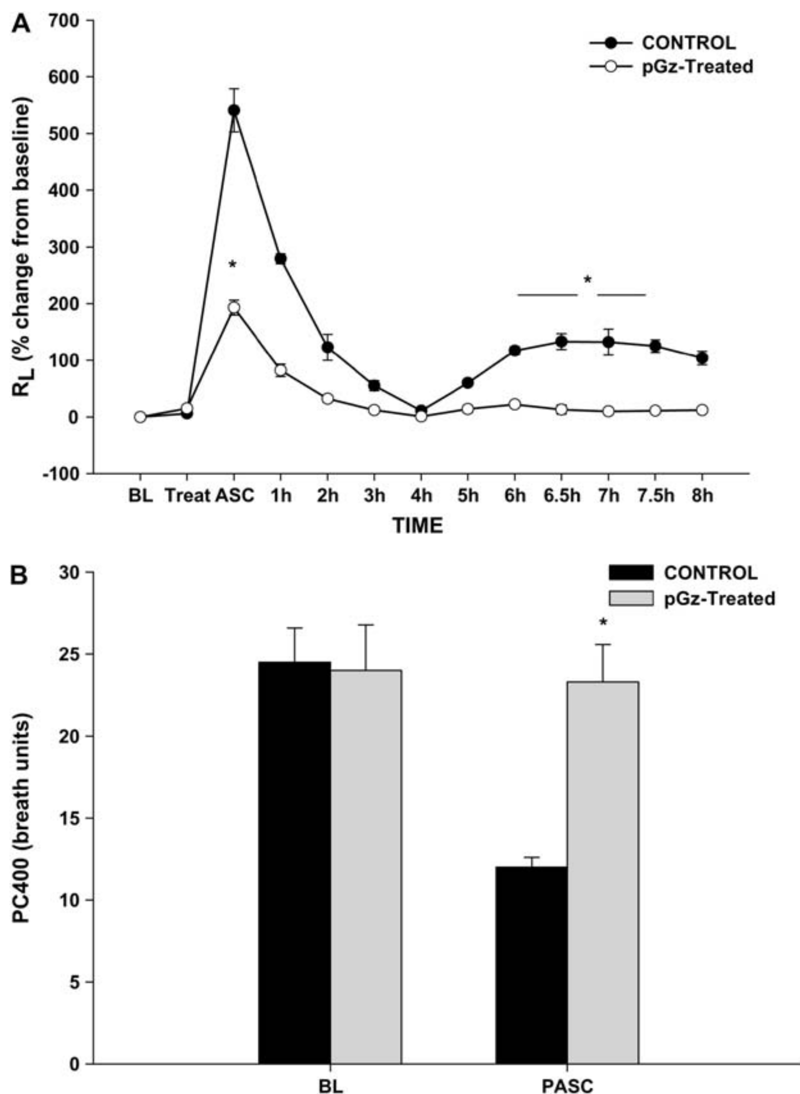

Figure 5. Multiple treatments with pGz block EAR, LAR, and AHR. pGz treatment was given for 1 h twice a day for 3 d and then 1 h before antigen challenge on Day 4. pGz reduced EAR and blocked LAR (*A*) and AHR (*B*). Values are mean ± SE for four sheep. *p < 0.05 versus control (*see* Table 1 for statistical analysis of peak responses and text for analysis of area under the curve).

when these responses (EAR, LAR, and/or AHR) are blocked pharmacologically, the abrogation of the antigen-induced airway response is accurately reflected by the reduction in relevant mediator levels (41–43).

NO can inhibit mast cell secretion *in vitro* and *in vivo* (3). The NO donor sodium nitroprusside inhibits anti-IgE–induced histamine and tryptase release from human skin mast cells (2) and histamine release from human basophils *in vitro* (44). In rats, an NO donor inhibited mast cell degranulation, mast cell–dependent granulocyte adhesion, and microvascular leakage *in vivo* (5). These latter findings are consistent with studies in pigs in which inhibition of NOS enhanced allergen-induced histamine release (45). Because the EAR in sheep, as in humans, is mast-cell dependent (42, 46, 47) and because pGz has no effect on airway tone and/or airway responsiveness, our findings support the concept that the inhibitory action of pGz on the EAR is through modulation of mast cell activation rather than bronchodilation. The protective effect of pGz was lost if the animals were treated with L-NAME before administering pGz. If we accept the argument that pGz can stimulate eNOS, the protective effects of pGz are consistent with the reported ability of NO to inhibit mast cell/basophil histamine and tryptase release (2), protective actions that would be lost in the presence of L-NAME.

The development of antigen-induced LAR is the initial sign of a heightened inflammatory process that leads to a prolonged increase in AHR (48). Acute pretreatment with pGz blocked the antigen-induced LAR, an effect that could be a linked to the initial reduction in the EAR. However, the finding that pGz affects the LAR independent of the EAR (Figure 4) suggests that pGz has antiinflammatory effects as well. Our previous work demonstrates that the LAR can be blocked by adhesion molecule inhibitors and/or specific mediator antagonists when given after the EAR, indicating that interfering with the recruitment of activated inflammatory cells to the airways is important for the development of the LAR (29, 30, 32, 49). That pGz can block the LAR when given after the EAR is consistent with reports that NO can block mast cell–dependent inflammatory processes (3), IgE-dependent cytokine production (4), and inflammatory cell recruitment and adhesion (5).

Although acute pretreatment and/or acute post-treatment with pGz protected against the antigen-induced EAR and/or LAR, both acute treatments were ineffective in preventing the post–antigen-induced AHR. The lack of protection for the latter can be attributed to the fact that a single pGz treatment is unlikely to sustain a sufficient signal to counteract the AHR that occurs 24 h after challenge. Therefore, in an attempt to extend the protective effects of pGz, we used a treatment paradigm that

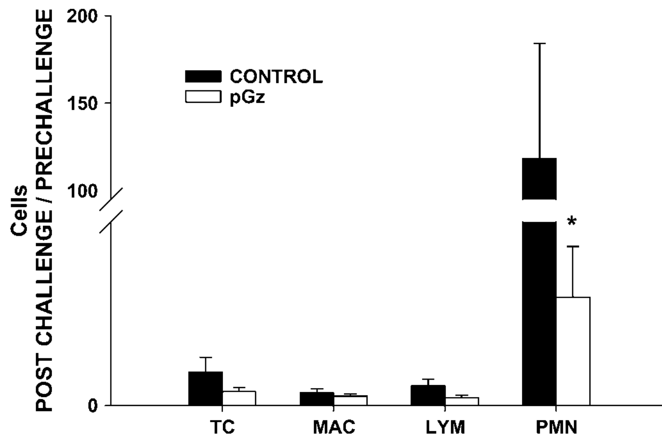

Figure 6. Multiple treatments with pGz block allergen-induced inflammation. pGz treatment was given for 1 h twice a day for 3 d and then 1 h before antigen challenge on Day 4. pGz significantly reduced the number of neutrophils recovered in the bronchoalveolar lavage fluid. Data are expressed as mean ± SEM ratio of the post-challenge value/prechallenge cell number for nine pGz-treated and nine control sheep. *$p < 0.05$ versus control. LYM = lymphocytes; MAC = macrophages; PMN = neutrophils; TC = total cells.

we demonstrated to be successful in blocking antigen-induced airway responses with other pharmacologic agents (30, 50). By using multiple pretreatments on the days before antigen challenge, we uncovered increased efficacy of compounds that were marginally effective and/or ineffective with acute dosing (30, 50). This treatment paradigm was successful in the present study because multiple twice-daily treatments with pGz successfully blocked the AHR that occurred 24 h after antigen challenge.

In the *Ascaris*-induced sheep model of asthma, the neutrophil is the most prominent leukocyte found in BAL fluid after antigen challenge, which signals an active inflammatory process (30–32, 51, 52). In the present study, multiple pGz treatments reduced

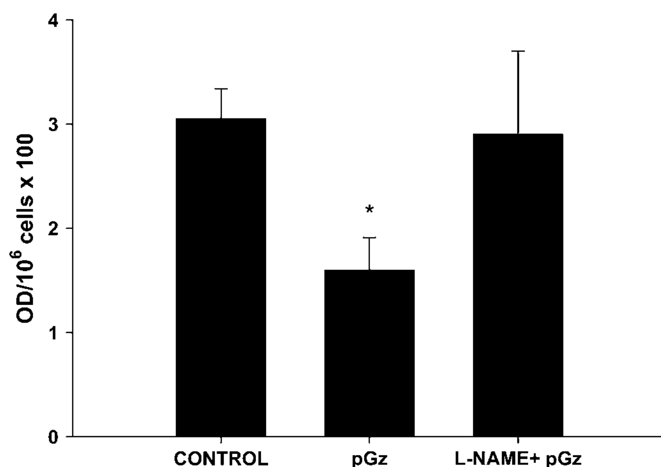

Figure 7. pGz treatment blocks the antigen-induced increase in nuclear factor-κB (NF-κB). Blockade of the late airway response (LAR) by pGz is associated with inhibition of the antigen-induced increase in NF-κB activity in bronchoalveolar lavage cells. The protective effect on the LAR and the NF-κB activity is reversed in the presence of L-NAME. Values are mean ± SEM for six sheep. *$p < 0.05$ versus control and L-NAME + pGz treatment groups.

this neutrophil influx into the airways; this finding is consistent with adhesion molecule inhibitors that have blocked the inflammatory cell influx and antigen-induced AHR (30–32, 51, 52).

In spite of the extended protective effect seen with multiple pGz treatments over 4 d, there was no statistically significant improvement in the degree of inhibition of the EAR or LAR. The reasons for this are not clear, although one could speculate that because the level of pGz was the same, the resultant stimulation of eNOS was similar in the two treatment regimens, but with the chronicity of multiple treatments, different molecular events, including the up-regulation of eNOS protein levels (14) and/or the increase the cellular S-nitrosylation, could occur (53). Both of these mechanisms could theoretically contribute to a prolonged release of NO (14).

Increased NF-κB activity results in the up-regulation of genes encoding inflammatory cytokines that are critical to inflammatory cell recruitment (6, 7). This transcription factor is activated by antigen-induced release of inflammatory cytokines and/or the generation of reactive oxygen species (54–57). Studies designed to elucidate the role of NF-κB using knockout mice (58, 59) indicate that disruption of NF-κB activity inhibits inflammation. Other reports detailing the time course of the activation show that this can occur as soon as 6 h after challenge (56). These findings are consistent with the timing of the increased NF-κB activity found in the present study. In horses affected with heaves, an active pulmonary inflammatory condition, epithelial cells (60) and cells recovered from BAL (61) showed increases in NF-κB activity, and these increases were correlated with the level of bronchial obstruction. The NF-κB in these heaves-affected horses was predominantly composed of p65 homodimers, rather than the classic p65-p50 heterodimer (61). This is significant because the detection system used in the present investigation measures p65. Our findings show that NF-κB activity in BAL cells recovered at the start of the LAR (i.e., 6 h after antigen challenge) in untreated animals was 1.9-fold higher than cells recovered from the pGz-treated group when the LAR was blocked. That NO was responsible for the suppression of NF-κB activity is supported by the restoration of NF-κB activity (and the LAR) in the presence of L-NAME. These results are in agreement with previous studies examining the role of NF-κB in asthmatic airways; in addition, these results extend those findings by showing that pGz can inhibit NF-κB activity *in vivo*. It has been reported that NO inhibition of NF-κB down-regulates vascular cell adhesion molecule-1, the ligand of the integrin $\alpha_4 \beta_1$ (14). Such a mechanism is consistent with our previous work, which demonstrated that blockade of this adhesion pathway with antibodies or small molecule inhibitors of $\alpha_4 \beta_1$ or antibodies to vascular cell adhesion molecule-1 blocked the LAR (30, 31, 62). The observed inhibition of NF-κB could also contribute to the reduced inflammatory response and the resultant abrogation of antigen-induced AHR seen in the multiple-pGz–treated animals. Our results are consistent with the reported inhibition of antigen-induced AHR in an ovalbumin-sensitized mouse model in which NF-κB decoys were used to block the effects of the natural transcription factor (58, 59) and in transgenic mice that overexpress eNOS (13), where antigen-induced AHR and lung inflammation were blocked.

Dogs treated with the cortisol synthesis inhibitor metyrapone were found to develop LAR after airway challenge with *A. suum*, suggesting that elevations in systemic cortisol levels could modulate the LAR response (63). In sheep, Apple and colleagues (34) found an immediate and sustained rise (up to 6 h) in plasma cortisol after the initiation of stress (leg restraint). Thus, if pGz were initiating a generalized stress response, it should be apparent immediately after treatment and should be sustained over the time course of our studies. Based on the dog

study (63), such a rise in systemic cortisol could explain the protective effects of pGz on the LAR. No such stress response was evident in the pGz-treated or nontreated sheep in the presence or absence of L-NAME (*see* Figure 2). Therefore, it is unlikely that the protective effects of pGz can be attributed to a generalized stress response in these animals.

In conclusion, our findings indicate that pGz has beneficial effects on the pathophysiologic airway responses and inflammatory cascades associated with allergen challenge in an experimental model of asthma. These observations, if confirmed in patients with asthma, suggest that chronic pGz treatment, a noninvasive modality, could provide an alternative medicine–adjunctive therapy approach that could be beneficial as a means of asthma control.

***Conflict of Interest Statement*:** W.M.A. does not have a financial relationship with a commercial entity that has an interest in the subject of the manuscript. A.A. does not have a financial relationship with a commercial entity that has an interest in the subject of the manuscript. I.S. does not have a financial relationship with a commercial entity that has an interest in the subject of the manuscript. I.T.L. does not have a financial relationship with a commercial entity that has an interest in the subject of the manuscript. J.B. owns shares of Non-Invasive Monitoring Systems, Inc. (NIM) stock. J.A.A. owns shares of NIM stock, which manufactures AT-101. M.A.S. is the chairman of the board of directors of NIM. He received no salary in 2004 and $26,000 in 2005. He holds approximately 22% shares of the company.

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References

1. Ricciardolo FL, Sterk PJ, Gaston B, Folkerts G. Nitric oxide in health and disease of the respiratory system. *Physiol Rev* 2004;84:731–765.
2. Van Overveld FJ, Bult H, Vermeire PA, Herman AG. Nitroprusside, a nitrogen oxide generating drug, inhibits release of histamine and tryptase from human skin mast cells. *Agents Actions* 1993;38:C237–C238.
3. Coleman JW. Nitric oxide: a regulator of mast cell activation and mast cell-mediated inflammation. *Clin Exp Immunol* 2002;129:4–10.
4. Davis BJ, Flanagan BF, Gilfillan AM, Metcalfe DD, Coleman JW. Nitric oxide inhibits IgE-dependent cytokine production and Fos and Jun activation in mast cells. *J Immunol* 2004;173:6914–6920.
5. Gaboury JP, Niu XF, Kubes P. Nitric oxide inhibits numerous features of mast cell-induced inflammation. *Circulation* 1996;93:318–326.
6. D'Acquisto F, May MJ, Ghosh S. Inhibition of nuclear factor Kappa B (NF-B): an emerging theme in anti-inflammatory therapies. *Mol Interv* 2002;2:22–35.
7. Ghosh S, May MJ, Kopp EB. NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses. *Annu Rev Immunol* 1998;16:225–260.
8. Peng HB, Libby P, Liao JK. Induction and stabilization of I kappa B alpha by nitric oxide mediates inhibition of NF-kappa B. *J Biol Chem* 1995;270:14214–14219.
9. Colasanti M, Persichini T, Menegazzi M, Mariotto S, Giordano E, Caldarera CM, Sogos V, Lauro GM, Suzuki H. Induction of nitric oxide synthase mRNA expression: suppression by exogenous nitric oxide. *J Biol Chem* 1995;270:26731–26733.
10. Park SK, Lin HL, Murphy S. Nitric oxide regulates nitric oxide synthase-2 gene expression by inhibiting NF-kappaB binding to DNA. *Biochem J* 1997;322:609–613.
11. Chung KF, Barnes PJ. Cytokines in asthma. *Thorax* 1999;54:825–857.
12. Taylor DA, McGrath JL, O'Connor BJ, Barnes PJ. Allergen-induced early and late asthmatic responses are not affected by inhibition of endogenous nitric oxide. *Am J Respir Crit Care Med* 1998;158:99–106.
13. Ten Broeke R, De CR, Van HR, Verweij V, Leusink-Muis T, Van Ark I, De Clerck F, Nijkamp FP, Folkerts G. Overexpression of endothelial nitric oxide synthase suppresses features of allergic asthma in mice. *Respir Res* 2006;7:58.
14. Boo YC, Jo H. Flow-dependent regulation of endothelial nitric oxide synthase: role of protein kinases. *Am J Physiol Cell Physiol* 2003;285: C499–C508.
15. Adams JA, Moore JE Jr, Moreno MR, Coelho J, Bassuk J, Wu D. Effects of periodic body acceleration on the in vivo vasoactive response to N-omega-nitro-L-arginine and the in vitro nitric oxide production. *Ann Biomed Eng* 2003;31:1337–1346.
16. Hutcheson IR, Griffith TM. Release of endothelium-derived relaxing factor is modulated both by frequency and amplitude of pulsatile flow. *Am J Physiol* 1991;261:H257–H262.
17. Adams JA, Mangino MJ, Bassuk J, Sackner MA. Hemodynamic effects of periodic G(z) acceleration in meconium aspiration in pigs. *J Appl Physiol* 2000;89:2447–2452.
18. Adams JA, Mangino MJ, Bassuk J, Inman DM, Sackner MA. Noninvasive motion ventilation (NIMV): a novel approach to ventilatory support. *J Appl Physiol* 2000;89:2438–2446.
19. Sackner MA, Gummels E, Adams JA. Effect of moderate-intensity exercise, whole-body periodic acceleration, and passive cycling on nitric oxide release into circulation. *Chest* 2005;128:2794–2803.
20. Sackner MA, Gummels E, Adams JA. Nitric oxide is released into circulation with whole-body, periodic acceleration. *Chest* 2005;127:30–39.
21. Sackner MA, Gummels EM, Adams JA. Say NO to fibromyalgia and chronic fatigue syndrome: an alternative and complementary therapy to aerobic exercise. *Med Hypotheses* 2004;63:118–123.
22. Adams JA, Bassuk J, Wu D, Grana M, Kurlansky P, Sackner MA. Periodic acceleration: effects on vasoactive, fibrinolytic, and coagulation factors. *J Appl Physiol* 2005;98:1083–1090.
23. Adams JA, Mangino MJ, Bassuk J, Kurlansky P, Sackner MA. Novel CPR with periodic Gz acceleration. *Resuscitation* 2001;51:55–62.
24. Adams JA, Bassuk J, Wu D, Kurlansky P. Survival and normal neurological outcome after CPR with periodic Gz acceleration and vasopressin. *Resuscitation* 2003;56:215–221.
25. Trochu JN, Bouhour JB, Kaley G, Hintze TH. Role of endothelium-derived nitric oxide in the regulation of cardiac oxygen metabolism: implications in health and disease. *Circ Res* 2000;87:1108–1117.
26. Abraham WM. Animal models of asthma. In: Busse WW, Holgate ST, editors. Asthma & rhinitis. Oxford: Blackwell Science; 2000. pp. 1205–1227.
27. Abraham WM, Ahmed A, Serebriakov I, Bassuk J, Adams JA, Sackner MA. Periodic acceleration via nitric oxide release modifies antigen-induced airway responses in sheep [abstract]. *Am J Respir Crit Care Med* 2004;169:A321.
28. Sackner MA, Lauredo IT, Serebriakov I, Ahmed A, Adams JA, Bassuk J, Abraham WM. Periodic acceleration modifies antigen-induced airway responses in sheep by nitric oxide (NO)-mediated down regulation of nuclear factor kappa beta (NF-kB) [abstract]. *Eur Respir J* 2004; 24:128s.
29. Abraham WM, Delehunt JC, Yerger L, Marchette B. Characterization of a late phase pulmonary response following antigen challenge in allergic sheep. *Am Rev Respir Dis* 1983;128:839–844.
30. Abraham WM, Gill A, Ahmed A, Sielczak MW, Lauredo IT, Botvinnikova Y, Lin KC, Pepinsky B, Leone DR, Lobb RR, *et al.* A small-molecule, tight binding inhibitor of the integrin $\alpha(4)\beta(1)$ blocks antigen-induced airway responses and inflammation in experimental asthma in sheep. *Am J Respir Crit Care Med* 2000;162:603–611.
31. Abraham WM, Sielczak MW, Ahmed A, Cortes A, Lauredo IT, Kim J, Pepinsky B, Benjamin CD, Leone DR, Lobb RR, *et al.* Alpha$_4$-integrins mediate antigen-induced late bronchial responses and prolonged airway hyperresponsiveness in sheep. *J Clin Invest* 1994;93:776–787.
32. Abraham WM, Ahmed A, Sabater JR, Lauredo IT, Botvinnikova Y, Bjercke RJ, Hu X, Revelle BM, Kogan TP, Scott IL, *et al.* Selectin blockade prevents antigen-induced late bronchial responses and airway hyperresponsiveness in allergic sheep. *Am J Respir Crit Care Med* 1999;159:1205–1214.
33. Hinder F, Meyer J, Booke M, Ehardt JS, Salsbury JR, Traber LD, Traber DL. Endogenous nitric oxide and the pulmonary microvasculature in healthy sheep and during systemic inflammation. *Am J Respir Crit Care Med* 1998;157:1542–1549.
34. Apple JK, Minton JE, Parsons KM, Unruh JA. Influence of repeated restraint and isolation stress and electrolyte administration on pituitary-adrenal secretions, electrolytes, and other blood constituents of sheep. *J Anim Sci* 1993;71:71–77.
35. Lauer T, Preik M, Rassaf T, Strauer BE, Deussen A, Feelisch M, Kelm M. Plasma nitrite rather than nitrate reflects regional endothelial nitric oxide synthase activity but lacks intrinsic vasodilator action. *Proc Natl Acad Sci USA* 2001;98:12814–12819.
36. Chowienczyk PJ, Kelly RP, MacCallum H, Millasseau SC, Andersson TL, Gosling RG, Ritter JM, Anggard EE. Photoplethysmographic assessment of pulse wave reflection: blunted response to endothelium-dependent beta2-adrenergic vasodilation in type II diabetes mellitus. *J Am Coll Cardiol* 1999;34:2007–2014.
37. Lund F. Digital pulse plethysmography (DPG) in studies of the hemodynamic response to nitrates: a survey of recording methods and principles of analysis. *Acta Pharmacol Toxicol (Copenh)* 1986;59:79–96.

38. Trifilieff A, Fujitani Y, Mentz F, Dugas B, Fuentes M, Bertrand C. Inducible nitric oxide synthase inhibitors suppress airway inflammation in mice through down-regulation of chemokine expression. *J Immunol* 2000;165:1526–1533.
39. Brown RH, Mitzner W. Airway response to deep inspiration: role of nitric oxide. *Eur Respir J* 2003;22:57–61.
40. Southan GJ, Szabo C. Selective pharmacological inhibition of distinct nitric oxide synthase isoforms. *Biochem Pharmacol* 1996;51:383–394.
41. Abraham WM, Burch RM, Farmer SG, Sielczak MW, Ahmed A, Cortes A. A bradykinin antagonist modifies allergen-induced mediator release and late bronchial responses in sheep. *Am Rev Respir Dis* 1991; 143:787–796.
42. Clark JM, Abraham WM, Fishman CE, Forteza R, Ahmed A, Cortes A, Warne RL, Moore WR, Tanaka RD. Tryptase inhibitors block allergen-induced airway and inflammatory responses in allergic sheep. *Am J Respir Crit Care Med* 1995;152:2076–2083.
43. Ahmed A, D'Brot J, Abraham WM, Lucio J, Mendelssohn R, Robinson MJ, Shakir S, SanPedro B. Heterogeneity of allergic airway responses in sheep: differences in signal transduction? *Am J Respir Crit Care Med* 1996;154:843–849.
44. Iikura M, Takaishi T, Hirai K, Yamada H, Iida M, Koshino T, Morita Y. Exogenous nitric oxide regulates the degranulation of human basophils and rat peritoneal mast cells. *Int Arch Allergy Immunol* 1998;115:129–136.
45. Middelveld RJ, Zetterquist WC, Bergman D, Alving K. Nitric oxide synthase inhibition augments acute allergic reactions in the pig airways in vivo. *Eur Respir J* 2000;16:836–844.
46. Russi EW, Perruchoud AP, Yerger LD, Stevenson JS, Tabak J, Marchette B, Abraham WM. Late phase bronchial obstruction following nonimmunologic mast cell degranulation. *J Appl Physiol* 1984;57:1182–1188.
47. Ahmed T, Syriste T, Mendelssohn R, Sorace D, Mansour E, Lansing M, Abraham WM, Robinson MJ. Heparin prevents antigen-induced airway hyperresponsiveness: interference with IP_3-mediated mast cell degranulation. *J Appl Physiol* 1994;76:893–901.
48. O'Byrne PM, Dolovich J, Hargreave FE. Late asthmatic responses. *Am Rev Respir Dis* 1987;136:740–751.
49. Delehunt JC, Perruchoud AP, Yerger L, Marchette B, Stevenson JS, Abraham WM. The role of SRS-A in the late bronchial response following antigen challenge in allergic sheep. *Am Rev Respir Dis* 1984;130:748–754.
50. Wright CD, Havill AM, Middleton SC, Kashem MA, Lee PA, Dripps DJ, O'Riordan TG, Bevilacqua MP, Abraham WM. Secretory leukocyte protease inhibitor prevents allergen-induced pulmonary responses in animal models of asthma. *J Pharmacol Exp Ther* 1999;289:1007–1014.
51. Abraham WM, Ahmed A, Serebriakov I, Carmillo AN, Ferrant J, de Fougerolles AR, Garber EA, Gotwals PJ, Koteliansky VE, Taylor F, *et al.* A monoclonal antibody to $\alpha 1\beta 1$ blocks antigen-induced airway responses in sheep. *Am J Respir Crit Care Med* 2004;169:97–104.
52. Rosen SD, Tsay D, Singer MS, Hemmerich S, Abraham WM. Therapeutic targeting of endothelial ligands for L-selectin (PNAd) in a sheep model of asthma. *Am J Pathol* 2005;166:935–944.
53. Marshall HE, Stamler JS. Inhibition of NF-kappa B by S-nitrosylation. *Biochemistry* 2001;40:1688–1693.
54. Bantel H, Schmitz ML, Raible A, Gregor M, Schulze-Osthoff K. Critical role of NF-kappaB and stress-activated protein kinases in steroid unresponsiveness. *FASEB J* 2002;16:1832–1834.
55. Gagliardo R, Chanez P, Mathieu M, Bruno A, Costanzo G, Gougat C, Vachier I, Bousquet J, Bonsignore G, Vignola AM. Persistent activation of nuclear factor-kappaB signaling pathway in severe uncontrolled asthma. *Am J Respir Crit Care Med* 2003;168:1190–1198.
56. Poynter ME, Irvin CG, Janssen-Heininger YMW. Rapid activation of nuclear factor-κB in airway epithelium in a murine model of allergic airway inflammation. *Am J Pathol* 2002;160:1325–1334.
57. Barnes PJ, Adcock IM. Transcription factors and asthma. *Eur Respir J* 1998;12:221–234.
58. Das J, Chen CH, Yang L, Cohn L, Ray P, Ray A. A critical role for NF-kappa B in GATA3 expression and TH2 differentiation in allergic airway inflammation. *Nat Immunol* 2001;2:45–50.
59. Donovan CE, Mark DA, He HZ, Liou HC, Kobzik L, Wang Y, De Sanctis GT, Perkins DL, Finn PW. NF-kappa B/Rel transcription factors: c-Rel promotes airway hyperresponsiveness and allergic pulmonary inflammation. *J Immunol* 1999;163:6827–6833.
60. Bureau F, Bonizzi G, Kirschvink N, Delhalle S, Desmecht D, Merville MP, Bours V, Lekeux P. Correlation between nuclear factor-kappaB activity in bronchial brushing samples and lung dysfunction in an animal model of asthma. *Am J Respir Crit Care Med* 2000;161:1314–1321.
61. Sandersen C, Bureau F, Turlej R, Fiévez L, Dogné N. p65 homodimer activity in distal airway cells determines lung dysfunction in equine heaves. *Vet Immunol Immunopathol* 2001;80:315–326.
62. Lobb RR, Abraham WM, Burkly LC, Gill A, Ma W, Knight JA, Leone DR, Antognetti G, Pepinsky RB. Pathophysiologic role of $\alpha 4$ integrins in the lung. *Ann N Y Acad Sci* 1996;796:113–123.
63. Sasaki H, Yanai M, Shimura S, Okayama H, Aikawa T, Sasaki T, Takishima T. Late asthmatic response to Ascaris antigen challenge in dogs treated with metyrapone. *Am Rev Respir Dis* 1987;136:1459–1465.