

Endogenous glucocorticoids and antigen-induced acute and late phase pulmonary responses

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Summary

Background Several studies suggest that endogenous glucocorticoids can dampen the severity of experimental allergic reactions in animals.

Objective To investigate the influence that endogenous glucocorticoids have on the course of IgE-mediated pulmonary early and late phase reactions.

Methods Twenty-one allergic asthmatic and six healthy control subjects underwent inhaled antigen challenge with measurements of plasma cortisol and cortisone by gas chromatography-mass spectrometry.

Results There were no differences between the asthmatic and control groups in the baseline levels of cortisol or cortisone. However, the asthmatic subjects had significantly higher cortisol levels (67.2 ± 8.6 vs 35.1 ± 4.5 ng/mL; $P = 0.04$) and had higher cortisol/cortisone ratios (4.8 ± 0.6 vs 3.0 ± 0.2 ; $P = 0.01$) 8 h after challenge compared to the control subjects. Among the asthmatic subjects, those whose FEV₁ recovered rapidly had higher baseline levels of cortisol and those who displayed a late phase reaction had lower levels of cortisol during the late phase period.

Conclusion The results suggest that endogenous glucocorticoids may play a significant role in the modulation of airway responses to antigen challenge, and that antigen challenge may induce cortisol production in allergic subjects.

Keywords: asthma, glucocorticoids, allergen challenge, late phase response, bronchospasm

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Introduction

Glucocorticoids are potent anti-inflammatory and immunosuppressive hormones. Inhaled and oral glucocorticoids are among the most effective medications for the treatment of allergic diseases of the airways. Glucocorticoid treatment exerts both physiological and immunological effects in asthma therapy, improving lung function, enhancing the bronchodilating activity of β -agonists, blocking mucus secretion, and inhibiting macromolecular leak into the airways. At

the cellular level, glucocorticoids significantly decrease the accumulation and activity of eosinophils, lymphocytes, macrophages, and other cell types [1]. Several studies suggest that endogenous glucocorticoids can dampen the severity of experimental allergic reactions. For example, treatment of dogs with metyrapone, a drug that blocks the adrenal synthesis of active glucocorticoids, increases the incidence and severity of late phase reactions (LPR) after antigen challenge in the lung and skin [2]. Restoration of the endogenous glucocorticoid cortisol after metyrapone administration reverses the increase in late phase responses, suggesting that the endogenous cortisol maintains a suppressive effect on the late phase response [2]. Cutaneous antigen challenge studies in humans have shown that late phase reactions are more pronounced when the challenge is performed late in the day, when endogenous glucocorticoid levels are low [3].

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Numerous antigen challenge studies have definitively established that administration of oral or inhaled glucocorticoids profoundly suppresses the late phase response [4–7].

The role of endogenous glucocorticoids in mitigating antigen-induced lung function changes in humans has not been established. Endogenous glucocorticoids are increased (perhaps as a protective response) after physiological stress associated with septic shock, surgery, thermal injury, and strenuous exertion [8–11]. Whether antigen challenge of the airways increases endogenous glucocorticoids in humans is unknown. Increases may be anticipated based on studies *in vitro* and in animals demonstrating that glucocorticoid secretion is increased by inflammatory cytokines [12–16], histamine [17,18] and prostaglandins [19,20] that are known to be generated after IgE-mediated challenge.

We hypothesized that (a) whole lung antigen challenge induces increases in endogenous glucocorticoid levels which in turn protect against the development of late phase reactions, and that (b) baseline endogenous glucocorticoid levels influence the bronchoconstriction that results from whole lung antigen challenge. In order to begin to determine the potential influence of endogenous glucocorticoids on the course of an experimental antigen challenge, we measured cortisol, cortisone, and the ratio of cortisol/cortisone in the plasma of both allergic asthmatics and control subjects during the course of an antigen challenge response. Cortisol (i.e. hydrocortisone) is an active glucocorticoid, whereas cortisone is an inactive metabolite of cortisol which is formed in the liver and elsewhere [1]. The relative quantities of these two steroids give indirect information regarding the status of new synthesis and metabolism of cortisol [1]. We found that asthmatic subjects challenged with antigen had a significant elevation of plasma cortisol levels compared to antigen-challenged nonallergic controls. Among asthmatics, cortisol levels were considerably higher in those who recovered from the acute response rapidly and in those who failed

to show a late phase response. These results suggest that the absolute levels of endogenous glucocorticoids, or increases in glucocorticoid levels induced by antigen challenge, can influence the severity of the physiological response of the airways to antigen challenge.

Methods

Subjects

Subjects were men and women between the ages of 19 and 47 years with no smoking history (Table 1). Asthmatic subjects reported episodic wheezing and shortness of breath in response to seasonal or intermittent antigen exposure, exercise, and/or cold air exposure and had a positive methacholine challenge to a dose less than or equal to 25 mg/mL [21,22]. No patients had used either oral or inhaled steroids for 2 weeks prior to entry, and inhaled β -agonist usage was discontinued 1 day before the studies. Control subjects had no rhinitic or asthmatic symptoms and had a negative methacholine challenge ($FEV_1 \geq 80\%$ of baseline following inhalation of 75 mg/mL of methacholine). All subjects were asymptomatic at the time of the study. All atopic status was confirmed by prick skin testing with a battery of nine common inhalant antigens. All skin tests in this study were read 15 min after the skin test was placed. Atopic subjects had at least one positive skin test and no control subject had a positive skin test.

Study protocol

The study protocol was approved by the Institutional Review Board of the Johns Hopkins Bayview Medical Center, and all subjects gave written informed consent. For the main study (Study 1, Table 1), on day 1, the subjects came to the laboratory at 8 a.m. and blood was drawn by venipuncture for determination of glucocorticoid levels

Table 1. Subject characteristics

Study	<i>n</i>	(M/F)	Age*	FEV1*		PC ₂₀ *
				Baseline	Immediate reaction	(mg/mL methacholine)
1						
Controls	6	3/3	28.5 ± 1.9			> 75
Allergic Asthmatics	21	9/12	28.5 ± 1.7	79.9 ± 10.1	55.7 ± 9.5	2.84 ± 2.24
2						
Controls	15	4/11	36.2 ± 11.0			
Allergic Asthmatics	15	6/9	32.5 ± 6.9			

* Values represent mean ± SD.

(baseline). Pulmonary function testing was performed to ensure that FEV₁ was greater than 60% of predicted. The subjects then underwent antigen challenge which involved inhalation of five breaths of phosphate buffered saline (diluent challenge) followed by half-log₁₀ (equating to 3.16-fold dose) increasing concentrations of ragweed ($n = 17$) (Greer Laboratories, Inc., Lenoir, NC, USA), timothy grass ($n = 1$) (Greer Laboratories, Inc., Lenoir, NC, USA), or cat ($n = 3$) (ALK, Wallingford, CT, USA) extract. The initial inhaled dose was determined from skin test titrations performed on each patient at an earlier occasion. Serial dilutions of concentrated antigen extract were made to a final concentration of 10⁻³ PNU/mL for ragweed and timothy grass and 1 AU/mL for cat. Skin test titrations were performed with a relevant antigen determined during the initial screening process as described above. Serial skin tests were performed with antigen extract concentrations that were increased by half log dilutions until a weal of 10 mm was obtained. The dilution that caused a 10-mm weal was the initial concentration used for the antigen inhalational procedure. The inhaled antigen provocation was terminated when at least a 20% reduction in FEV₁ occurred from the values obtained after the diluent challenge. The control subjects inhaled a single dose of ragweed antigen at least 10 times greater than the largest dose of ragweed required to cause a 20% reduction in FEV₁ in any of the ragweed allergic asthmatic subjects. All inhaled antigen provocation studies were completed by 9 a.m. and this was defined as the 0 h timepoint. Starting at 10 a.m., pulmonary function testing was performed each hour for 8 h. Blood was drawn at 10 a.m. (1 h timepoint) and at 5 p.m. (8 h timepoint) for determination of cortisol and cortisone levels. All blood was drawn by separate venipunctures, and blood was drawn only at one point during the late phase to avoid the potentially confounding variable of stress induced by repeated phlebotomies. On day 2 at 8 a.m., 24 h after the initiation of the whole lung antigen challenge, pulmonary function tests were performed and blood was again drawn for determination of glucocorticoid levels (23 h timepoint).

To eliminate the possibility that the differences in plasma cortisol observed in our study at 8 h between asthmatics and normals resulted from an intrinsic difference between these two groups rather than from antigen-induced changes in plasma cortisol, we performed a second study in which we measured plasma cortisol levels at 5 p.m. (i.e. the same time as the 8 h timepoint) in 15 normal subjects and 14 asthmatics.

Measurement of endogenous glucocorticoid levels

Endogenous plasma cortisol and cortisone levels were determined via combined gas chromatography-mass spectrometry as described [23]. In study 1, glucocorticoid levels were assessed in plasma samples taken at the baseline, 1, and 8 h

time points from all 21 allergic subjects. The blood from two of the asthmatic subjects was not analysable at the 23 h timepoint because of excessive lipemia which reduces the recovery of the stable isotope analogues of cortisol and cortisone to unacceptable levels. Glucocorticoid levels assessed at all four time points were available for all of the control subjects. All samples from study 2 were assayed for cortisol and cortisone.

Statistical analyses

The Mann-Whitney *U*-test was used for unpaired between-group comparisons; within-group analysis employed Friedman ANOVA followed by the Wilcoxon-signed rank statistic as a *post-hoc*. The Spearman rank correlation coefficient analysis was utilized to assess the correlations between glucocorticoid levels at 8 a.m. on days 1 and 2. For all tests, *P*-values ≥ 0.05 were considered significant. Values presented are mean $\pm$ SEM. All computations were performed using Statview II Software (Abacus Concepts, Inc., Berkeley, CA, USA).

Results

The influence of antigen challenge on pulmonary function in asthmatics is shown in Fig. 1. All subjects had acute declines in FEV₁; late phases ($\geq 15\%$ decline in FEV₁ from baseline, 4–8 h after the end of the challenge) were observed in nine asthmatic subjects. The challenge had no influence on pulmonary function in control subjects at any timepoint (not shown). Plasma cortisol and cortisone levels are shown numerically in Table 2 and graphically in Fig. 2.

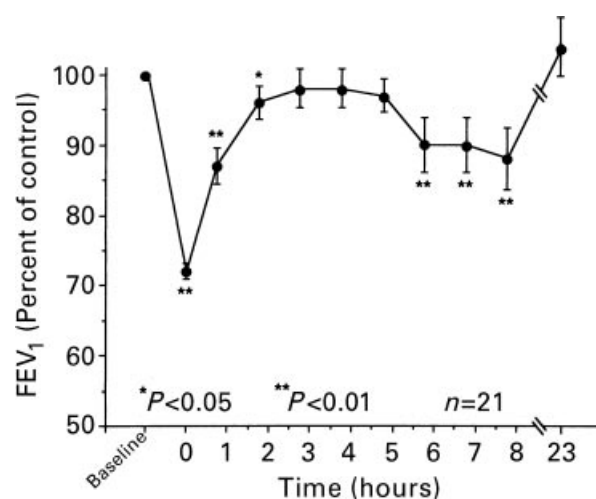


Fig. 1. Airway response to whole lung challenge with antigen. Antigen was administered by inhalation to 21 asthmatics as described in Methods section. FEV₁ was monitored hourly for 23 h. **P* < 0.05 and ***P* < 0.01 vs baseline.

Table 2. Plasma levels of cortisone and cortisol, and the ratio of cortisol/cortisone immediately before (baseline) and 1, 8, and 24 h after whole lung antigen challenge in both allergic asthmatics and in nonallergic nonasthmatic controls. The values expressed are mean ng/mL $\pm$ SEM. The *P*-values were calculated by using the Mann–Whitney *U*-test.

	Asthmatics	Controls	<i>P</i> -values
Baseline cortisone	21.9 $\pm$ 1.4	23.5 $\pm$ 3.3	0.64
Baseline cortisol	125.0 $\pm$ 12.3	112.6 $\pm$ 14.2	0.82
Cortisol/cortisone	5.9 $\pm$ 0.6	4.9 $\pm$ 0.5	0.72
1 h Cortisone	16.9 $\pm$ 1.2	22.9 $\pm$ 5.9	0.50
1 h Cortisol	77.6 $\pm$ 9.7	73.4 $\pm$ 10.1	0.91
Cortisol/cortisone	4.5 $\pm$ 0.4	3.6 $\pm$ 0.4	0.22
8 h Cortisone	14.3 $\pm$ 1.3	12.6 $\pm$ 2.5	0.58
8 h Cortisol	67.2 $\pm$ 8.6	35.1 $\pm$ 4.5	0.04
Cortisol/cortisone	4.8 $\pm$ 0.6	3.0 $\pm$ 0.2	0.01
23 h Cortisone	24.1 $\pm$ 1.2	23.0 $\pm$ 2.8	0.77
23 h Cortisol	124.9 $\pm$ 15.5	100.1 $\pm$ 7.8	0.54
Cortisol/cortisone	5.1 $\pm$ 0.6	4.6 $\pm$ 0.4	0.87

No differences between the allergic asthmatics and the control subjects in the levels of cortisol, cortisone, and total glucocorticoids, or the ratio of cortisol/cortisone were found at baseline or 1 h after inhaled antigen challenge (Table 2, Fig. 2). However, at 8 h after challenge, significantly higher levels of cortisol and higher ratios of cortisol/cortisone were found in the allergic asthmatics compared to

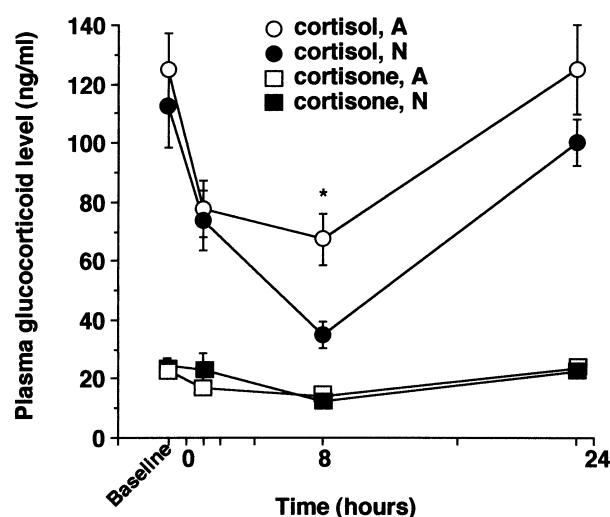


Fig. 2. Assessment of plasma cortisol and cortisone in asthmatic and control subjects following challenge with antigen. Plasma was drawn either immediately before (time = 0 h) or 1 h, 8 h or 23 h after inhalation challenge with antigen. Values shown are mean $\pm$ SEM of *n* = 6 for controls, and *n* = 19–21 for asthmatics as described in the Methods section. **P* < 0.05 asthmatics vs normals.

the control group. This difference in cortisol and cortisol/cortisone ratio in the allergic asthmatic subjects compared to controls was concurrent with a significant late reduction in FEV₁ compared to the diluent challenge values (Fig. 1). Twenty-three hours after challenge, the cortisol levels and the cortisol/cortisone ratio were not significantly different between the allergic asthmatic subjects and the normal control subjects. In the asthmatic group at 23 h, FEV₁ had returned to 3.23 ± 0.15 (mean $\pm$ SEM, range 1.84 ± 4.58); a value not significantly different from baseline. A tight correlation between the cortisol ($r_s = 0.87$; *P* = 0.0002) and cortisone ($r_s = 0.72$; *P* = 0.002) levels at 8 a.m. on day 1

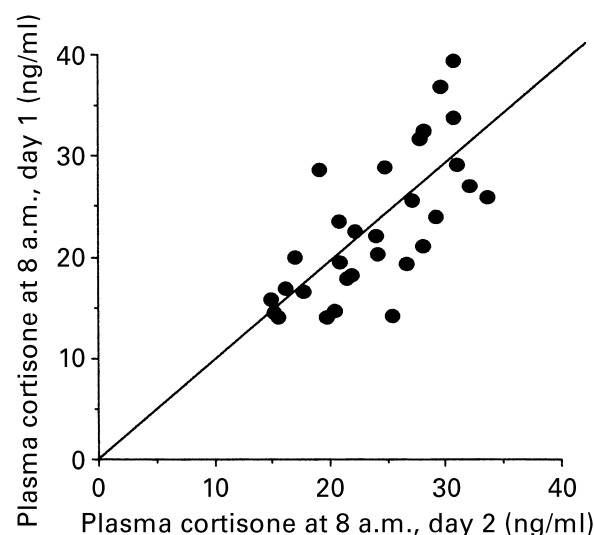
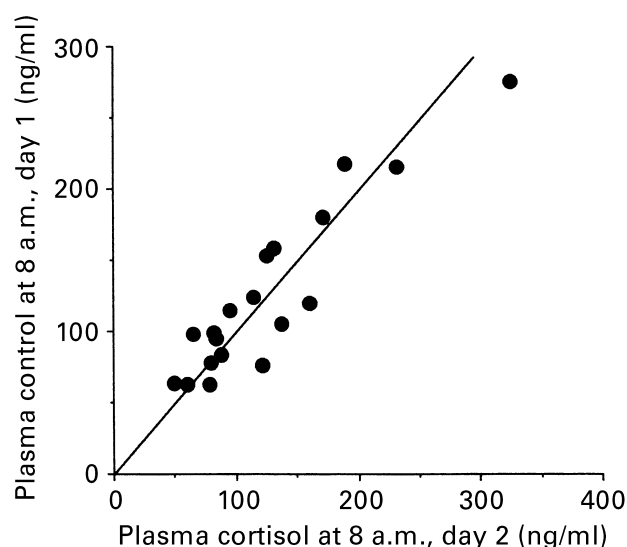


Fig. 3. Relationship between plasma cortisol and cortisone levels at time = 0 and time = 23 h after antigen challenge. For both glucocorticoids a strong correlation was observed. The line of identity is depicted.

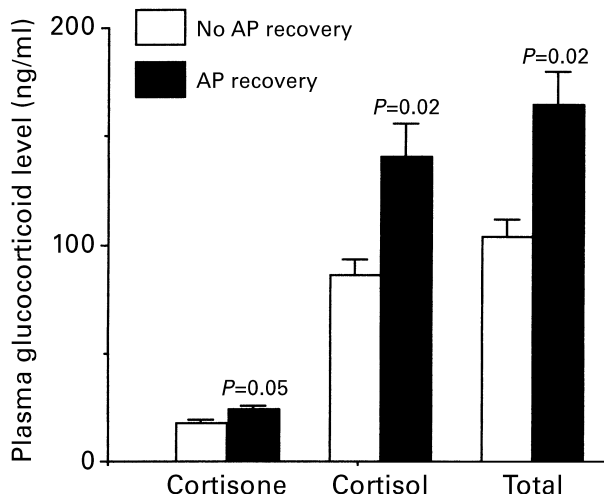


Fig. 4. Relationship between recovery from acute phase response and endogenous glucocorticoid levels. Asthmatic subjects were divided into those who recovered from the acute phase bronchoconstriction by 50% or greater in the first hour ($n = 15$) and those who did not recover by 50% ($n = 6$). Data shown are mean $\pm$ SEM levels for cortisone, cortisol, and total glucocorticoid from subjects as described in Methods. P -values refer to the comparison between the two groups of asthmatics.

and those 24 h later was observed, suggesting that any alterations of glucocorticoid levels were shortlived (Fig. 3).

To determine if the circulating endogenous glucocorticoid levels might relate to the recovery of pulmonary function after inhaled antigen challenge, asthmatics were separated into two groups by a *post-hoc* analysis; those subjects whose FEV₁ recovered spontaneously by greater than 50% from the antigen-induced fall in the first hour after inhaled antigen challenge ($n = 15$), and those whose FEV₁ did not recover by 50% ($n = 6$) (Fig. 4). Comparison between these two groups shows that the subjects with the greatest recovery of pulmonary function after inhaled antigen challenge had higher baseline levels of endogenous cortisol and total glucocorticoids. This result suggests that the level of endogenous circulating glucocorticoids might influence the recovery from the physiological response to antigen. No relationships between baseline levels of glucocorticoids and antigen PD20 was found (data not shown).

In order to analyse the possible influence that endogenous glucocorticoid levels have on the late phase response (or vice versa), the allergic asthmatic subjects were again divided into two groups; those subjects who had late phase pulmonary reactions, defined as an FEV₁ that was at least 15% lower than the baseline measurement at one or more time points in the 4–8 h after the inhaled antigen challenge (late phase positive, $n = 12$), and those whose FEV₁ never reached a 15% reduction over baseline in the same period (late phase negative, $n = 9$). A comparison was made of the

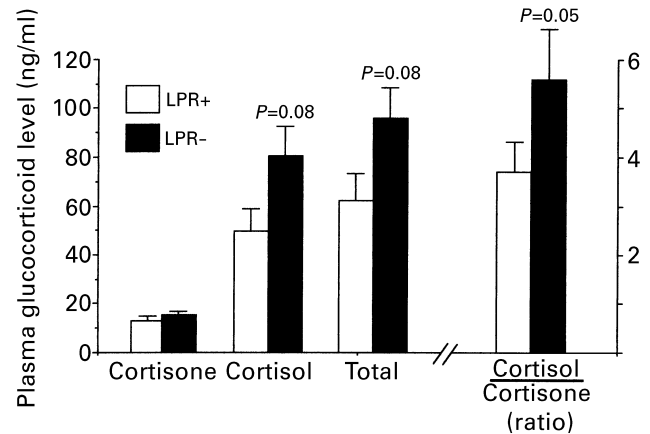


Fig. 5. The relationship between plasma glucocorticoid levels and the late phase response. Values shown are mean $\pm$ SEM levels for cortisone, cortisol, total glucocorticoids and the ratio of cortisol to cortisone from asthmatic subjects who were late phase responders (open bars, $n = 12$) and those who did not have late phase (solid bars, $n = 9$) as described in Materials and Methods.

glucocorticoid levels between these two groups at the 8 h timepoint after inhaled antigen challenge (Fig. 5). There was no difference in the plasma cortisone levels between those with late phase reactions and those without. However, there was a trend for the subjects without late phase responses to have both higher cortisol and higher total glucocorticoid levels at 8 h compared to those subjects who had late phase reactions. There was also a significant increase in the cortisol/cortisone ratio in the subjects without late phase reactions compared to late phase responders. Comparison of these two groups at other time points did not reveal any significant differences.

We also compared the late phase responders with the late phase nonresponders with regard to the fall in plasma cortisol during the period of the challenge. Since values vary among individuals (e.g. see Fig. 3), we calculated a ratio between the cortisol levels at 8 h and the cortisol levels at 0 h. Late phase responders had a significantly lower ratio than nonresponders (LPR+ 0.39 ± 0.06 vs LPR- 0.76 ± 0.15 , $P < 0.03$ and $P < 0.06$ for one-tailed and two-tailed t -tests, respectively) indicating that the daily (normalized) fall in plasma cortisol was greater in the late phase responders.

Of the 15 subjects whose FEV₁ recovered spontaneously in the first hour by 50% from the antigen-induced fall in FEV₁, 10 subjects (67%) did not have late phase reactions while five subjects (33%) had late phase reactions. In contrast, in those six subjects whose FEV₁ did not spontaneously recover after the first hour, four subjects experienced late phase reactions while two did not. Of the four subjects whose FEV₁ did not spontaneously recover after the first hour and who experienced late phase reactions, two never had a recovery of FEV₁ to greater than 85% of

baseline during the 8 h of FEV₁ measurement, while the other two had dual phase reactions (i.e. immediate phase and recovery, followed by a late phase reaction).

A second study was performed to eliminate the possibility that the differences in plasma cortisol observed in our study at 8 h between asthmatics and normals (see Fig. 2) resulted from an intrinsic difference between these two groups. We measured plasma cortisol levels at 5 p.m. (i.e. the same time as the 8 h timepoint) in 15 normal subjects and 14 asthmatics. We observed slightly lower levels of cortisol in asthmatics which was not statistically significant compared to normals (26.7 ± 3.1 ng/mL vs 21.2 ± 4.0 in normals and asthmatics, respectively, $P = 0.06$).

Discussion

Studies in animals and humans indicate that endogenous glucocorticoids suppress inflammatory responses, including those elicited via an IgE-dependent mechanism [1]. It remains unknown, however, to what extent endogenous glucocorticoids regulate inflammation or airways function in asthma. The present study was designed with two questions in mind. First, to determine whether challenge of asthmatic subjects by inhalation with antigen can lead to an increase in circulating glucocorticoid levels, i.e. a potential feedback response. The second question is whether endogenous glucocorticoids (either pre-existing or induced by antigen challenge) can influence the physiological outcomes of antigen challenge — the so-called acute and late phase responses. The results show that asthmatics challenged with antigen have elevated levels of cortisol 8 h following challenge compared to challenged control subjects. Among asthmatics, those whose lung function recovered quickly after the acute phase had higher baseline levels of cortisol and those who did not develop a late phase reaction had higher levels of glucocorticoids at the 8 h timepoint. In aggregate, these results suggest that antigen challenge results in elevated cortisol levels and that elevated endogenous cortisol can suppress the physiological responses to antigen challenge.

Plasma glucocorticoids are produced by the adrenal gland in response to adrenocorticotrophic hormone (ACTH) produced by the pituitary gland. ACTH production is stimulated by corticotropin releasing hormone (CRH) produced primarily in the hypothalamus [24]. The hypothalamic production of CRH in response to stress such as hypoglycaemia can occur within 15 min [24]. Since studies in animals have shown that histamine, prostanoids and cytokines can stimulate adrenal glucocorticoid release both indirectly and directly, and since bronchospasm may fall under the general category of 'stress', we hypothesized that the reaction to inhalation challenge with antigen would lead to an elevation of glucocorticoids. As data in Fig. 2 show, no

differences between asthmatics and healthy controls were observed 1 h after antigen challenge. However, measurements made 8 h after antigen challenge showed a clear elevation of circulating cortisol, but not cortisone, in asthmatics compared to normal subjects. These higher levels of cortisol are unlikely to be due to an intrinsic difference in cortisol levels between asthmatics and normals since they had similar resting values at 8 a.m. (Fig. 2). The same was true at 5 p.m. in a second study in which we compared cortisol levels in 14 asthmatics and 15 normals. One explanation for this finding is that either the changes in pulmonary physiology induced by antigen challenge or the mediators released as a result of antigen challenge increase plasma cortisol. While this could occur either via stimulated production or decreased metabolism of cortisol, we believe that increased synthesis is the more likely explanation since the ratio of cortisol/cortisone, an indirect indicator of production of cortisol, was higher at 8 h in the asthmatics compared to the controls (4.8 ± 0.6 vs 3.0 ± 0.2 , respectively, Table 2). This ratio was higher yet in asthmatics who failed to have a LPR vs those who had a LPR (5.6 ± 1 vs 3.7 ± 0.3 , respectively, $P < 0.05$, Fig. 5).

Normal glucocorticoid levels vary in a circadian pattern, with peak levels occurring just before awakening each morning in persons with standard night time sleep cycles. Cortisol levels then decline through the day to their lowest levels in the evening [24]. It is therefore difficult to compare glucocorticoid levels in a given subject at different times in the day. The nonallergic subjects in the present study served two purposes. First, as a control for the normal circadian variation in glucocorticoids and second, as a control for nonspecific effects of antigen challenge. It is possible however, that asthmatic subjects and normal individuals differ in their circadian pattern or glucocorticoid level independent of the influence of antigen. Durham *et al.* found no differences between asthmatics challenged with antigen and asthmatics challenged with saline in circulating cortisol levels [25]. The explanation for the differences between our results and those of Durham *et al.* are unclear. They found no relationship between changes in cortisol or absolute levels of cortisol and either blood eosinophil counts or changes in FEV₁ induced by allergen challenge. One half (7/14) of their subjects were receiving treatment with inhaled glucocorticoids while none of our subjects were using inhaled steroids. Although Durham *et al.* used RIA to detect cortisol and we used combined gas chromatography mass spectrometry, it is difficult to explain how the divergent results may have arisen purely from the use of different assay techniques. We have great confidence in the use of GC-MS, which is a highly reproducible and sensitive assay.

There was no difference between the glucocorticoid levels in the asthmatic and control subjects 24 h after the challenge. This fact, and the high degree of correlation between 8 a.m.

measurements on two successive days suggest that the stimulus to increase glucocorticoid production had disappeared by the day after inhaled antigen challenge. We were impressed that endogenous cortisol levels varied over a sixfold range among subjects (Fig. 3). It would not be surprising if this variation produces variation in the influence of endogenous glucocorticoids on glucocorticoid-sensitive responses.

The influence of endogenous glucocorticoids on the physiological response to inhaled antigen challenge within the allergic asthmatic group was studied. Those subjects in whom FEV₁ recovered by greater than 50% by 1 h after the immediate reaction had had significantly higher levels of cortisol, and total glucocorticoid levels, at the 0 h timepoint than those whose FEV₁ remained low. Since glucocorticoid levels at the 0 h time period were obtained prior to antigen challenge, these results suggest that higher circulating glucocorticoid levels facilitate recovery from the pulmonary response to the allergic stimulus. The mechanism of this effect on lung function recovery is not clear. Clearly the level of endogenous glucocorticoids is not the sole determinant of lung function recovery from acute bronchoconstriction. Not surprisingly, there was a significant correlation between the magnitude of the acute fall in FEV₁ and the recovery of lung function 1 hour following the end of challenge ($r_s = 0.46$; $P = 0.04$). However, there was no correlation between the acute fall of FEV₁ from baseline and either baseline plasma cortisol ($r_s = 0.07$; $P = 0.76$) or cortisone ($r_s = 0.152$; $P = 0.49$) levels prior to antigen challenge at baseline. This indicates that the relationship between baseline glucocorticoid levels and lung function recovery that we have observed is not the result of a reduced fall in FEV₁ acutely in individuals with high endogenous glucocorticoids.

Another interesting finding of this study was the higher cortisol/cortisone ratio and the trend toward an increase in cortisol and total glucocorticoid levels 8 h after challenge in the asthmatic subjects who did not have late phase reactions compared to those who did. If the higher levels of cortisol in the asthmatic individuals were due to increased synthesis, one would expect an increase in the ratio of cortisol/cortisone. As one can see from Table 2, the ratio of cortisol/cortisone declines throughout the day in normals (4.9 at 8 a.m., 3.6 at 10 a.m., and 3.0 at 5 p.m.), presumably reflecting the decrease in production of cortisol during the day. The increase in the ratio of cortisol/cortisone in asthmatics (4.8 ± 0.6 at 5 p.m. vs 3.0 ± 0.2 in controls) and especially in asthmatics with no LPR (5.6 ± 1 vs 3.7 ± 0.3 in LPR responders, Fig. 5) supports the hypothesis that greater cortisol production explains the higher cortisol levels in the asthmatics (especially the LPR nonresponders at 5 p.m.). Alternatively, the increase in glucocorticoid may only be a marker for another unidentified physiological event which suppresses the LPR. The speculation that

increased endogenous glucocorticoids suppress late phase responses is supported by the many reports that exogenous glucocorticoids have a protective effect on these reactions (reviewed in [1]).

There are several mechanisms by which antigen challenge could stimulate adrenal glucocorticoid production. As mentioned above, histamine is known to directly and rapidly activate adrenal glucocorticoid release [17]. Although histamine may be responsible for the observed increase of cortisol in the present study, the lack of difference in cortisol in our asthmatic subjects at 1 h after challenge, a time at which peak plasma histamine levels may already have passed, makes this possibility less likely. Other amines, including 5-hydroxytryptamine as well as prostanoids have also been shown to stimulate cortisol release [18–20]. Inflammatory cytokines, including IL-1, IL-6 and TNF have been shown to activate the HPA axis in animals [13–16]. Since previous studies from our laboratory and others have shown these cytokines to be expressed during allergic challenge responses, their role must be considered [26]. Alternatively, other soluble mediators, or neuronal mechanisms triggered by the stress of compromised lung function or its perception, could mediate the apparent adrenal activation. Recently, Landstra *et al.* published an abstract demonstrating lower serum cortisol levels in asthmatic children compared with controls [27]. We found that cortisol levels at 4 p.m. in asthmatic adults were lower than those of controls (21.2 ± 4 ng/mL vs 26.7 ng/mL) with a P -value of 0.06. This observation adds modest support to the conclusion of Landstra *et al.* and also supports the interpretation of our results that higher cortisol levels eight hours after antigen challenge of asthmatics compared to challenged controls are the result of increased synthesis or decreased metabolism of cortisol in the asthmatics.

The exact mechanism of bronchospasm in the late phase reaction is unknown; however, one characteristic of the late phase response in the lung is the migration and degranulation of eosinophils in the lung airways and parenchyma [28]. Eosinophils produce leukotriene C₄, a potent bronchoconstrictor, and eosinophil major basic protein, which can produce epithelial cell desquamation and increases in the reactivity of respiratory smooth muscle to cholinergic agonists [29]. Previous studies have shown that oral steroids are effective late phase inhibitors but do not influence the immediate phase reaction in pulmonary challenge models [30]. Inhaled glucocorticoids have been reported to inhibit both the acute phase and late phase reactions [26,31]. Glucocorticoids are believed to interfere with late phase reactions in part through their actions on eosinophils, which include decreased eosinophil numbers in the circulation, decreased eosinophil survival, and decreased eosinophil recruitment to the airways [32]. The recognized circadian variation in eosinophil numbers is likely to result in part

from the influence of endogenous glucocorticoids, adding support to the suggestion that endogenous glucocorticoids regulate allergic responses in an ongoing manner and that eosinophils may be an important target of endogenous glucocorticoids. Like eosinophils, basophils are suppressed by glucocorticoids, display a circadian variation, and have been proposed to be important in antigen-induced late phase reactions [33–35].

The present finding may assist in explaining the findings of Mohiuddin *et al.* who noted a temporal variation in the prevalence of late phase reactions [36]. When they performed inhaled antigen challenge at 8 a.m., four of 10 subjects experienced late phase reactions. However, when the challenges were performed in the evening, a time when endogenous glucocorticoid levels are at their lowest, nine of 10 of the same subjects had late phase reactions. In conclusion, our data indicate that antigen challenge can cause levels of glucocorticoids to be increased in allergic asthmatic subjects and that endogenous glucocorticoids may influence the outcome of allergic reactions in the lower airways.

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