Steroid-resistant Asthma

Cellular Mechanisms Contributing to Inadequate Response to Glucocorticoid Therapy

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Abstract

The current study examined whether alterations in glucocorticoid receptor (GR) binding contribute to poor response to glucocorticoid therapy in asthma. 29 asthma patients with forced expiratory volume in 1 s (FEV_1) < 70% predicted were studied. Patients were classified as steroid sensitive (SS) if their morning FEV_1 increased > 30% after a 1-wk course of oral prednisone 20 mg twice daily and steroid resistant (SR) if they failed to increase > 15%. PBMC obtained from these two groups, 17 SR and 12 SS, as well as 12 normal controls were analyzed.

SR patients had two distinguishable GR binding abnormalities: 15 of the 17 SR patients demonstrated a significantly reduced GR binding affinity, as compared with SS patients (P = 0.0001) and normal controls (P = 0.0001). This defect was localized to T cells and reverted to normal after 48 h in culture media. However, incubation with a combination of IL-2 and IL-4 sustained this abnormality. The other two SR patients had an abnormally low GR number with normal binding affinity that was not limited to T cells. Furthermore, GR number failed to normalize after incubation in media alone or IL-2 and IL-4. Therefore, SR asthma may be due to more than one abnormality, the majority related to a reversible cytokine-induced reduction in GR binding affinity and the second related to an irreversible reduction in GR number. These findings may have important implications for the design of alternative treatment approaches for recalcitrant asthma. (*J. Clin. Invest.* 1994. 93:33–39.) Key words: steroid-resistant asthma • glucocorticoid receptors • glucocorticoids • cytokines • immune activation

Introduction

Recent studies have demonstrated the importance of airway inflammation and immune activation in the pathogenesis of asthma (1, 2). Glucocorticoids are the most potent antiinflammatory therapy commonly used in this disease (3, 4). Certain asthma patients, however, fail to respond to combined systemic and inhaled glucocorticoid treatment despite very high doses over extended treatment periods (5, 6). Many of these patients continue treatment with glucocorticoids despite the onset of serious adverse effects and poor clinical response. These patients require alternative approaches to treatment. It is therefore important to understand the mechanisms underlying this apparent steroid resistance.

Recent studies indicate that steroid-resistant (SR)[1] asthma is associated with a failure of glucocorticoids to inhibit their in vitro T cell proliferation and cytokine secretion (7, 8). More importantly, T cells from peripheral blood of SR asthmatics, but not steroid-sensitive (SS) asthmatics, are persistently activated despite continued treatment with aggressive steroid therapy (9). Glucocorticoids bind to a specific intracellular receptor to inhibit activation of T cells by various stimuli (10, 11). It is therefore possible that the poor glucocorticoid response in SR asthma is due to an alteration in glucocorticoid receptor (GR) number or binding affinity. In this study, we examined GR binding parameters in PBMC from SR asthmatics to determine whether abnormalities in GR binding may contribute to their inadequate response to glucocorticoid therapy.

Methods

Patient selection. Patients with a diagnosis of asthma, based on American Thoracic Society criteria (12), were selected for evaluation. They were included if they had a morning prebronchodilator forced expiratory volume in 1 s (FEV_1) < 70% of predicted values and a ≥ 15% increase in FEV_1 after two inhalations of albuterol (90 μg per actuation). Patients were excluded if they had evidence for other types of lung disease, pregnancy, suspected noncompliance with medical care, or concurrent therapy with medications that alter glucocorticoid metabolism, such as anticonvulsants or erythromycin. A complete set of pulmonary function tests with lung volume, methacholine bronchial challenge, and diffusion capacity/total lung capacity ratio were obtained if the diagnosis of asthma required confirmation. Informed consent, approved by the National Jewish Institutional Review Board, was obtained from all patients before their entry into this study.

Patients were classified as SS or SR based on their prebronchodilator morning FEV_1 and their response to a course of oral prednisone. Asthmatic patients were defined as SR if they failed to improve their morning prebronchodilator FEV_1 by ≥ 15% after a 1-wk course of prednisone at a minimum oral dose of 40 mg/d (13). Patients were classified as SS if they had an increase in baseline FEV_1 of 30% or greater. All SR asthma patients had glucocorticoid pharmacokinetic studies to exclude those patients with an abnormality in prednisone absorption or metabolism (14).

Cell isolation. Peripheral blood was collected in heparinized syringes and PBMC were isolated by gradient centrifugation (Ficoll-Paque®; Pharmacia LKB Biotechnology, Inc., Piscataway, NJ). Further isolation for the T cell component was performed by sheep red cell (E)

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Received for publication 10 May 1993 and in revised form 13 August 1993.

J. Clin. Invest.

0021-9738/94/01/0033/07 $2.00
Volume 93, January 1994, 33–39

1. *Abbreviations used in this paper:* AP-1, activation protein-1; FEV_1, forced expiratory volume in 1 s; GR, glucocorticoid receptor; GRE, glucocorticoid-responsive elements; SR, steroid resistant; SS, steroid sensitive.

rosetting (Colorado Serum Co., Denver, CO), and the (E−) non–T cell population was purified further by lysis with anti-CD3 (Ortho Diagnostic Systems, Inc., Raritan, NJ) and rabbit complement (Gibco Labs., Grand Island, NY). This procedure yielded an E (+) fraction > 97% T cell purity and < 1% B cells. The E (−) fraction contained < 5% T cells.

All blood samples were collected between 7 and 8 a.m., before medications and at least 24 h after any oral glucocorticoid therapy. A total blood sample of 80 ml was required for a standard binding assay and 400 ml for assay that required isolation of purified T cells.

Glucocorticoid receptor binding analysis. [^{3}H]dexamethasone (Amersham Corp., Arlington Heights, IL) radioligand binding assay and Scatchard analysis, based on the method of Crabtree et al. (15), were used to measure nuclear and cytosolic GR binding parameters in PBMC from normal donors and asthma patients. Cells (2×10^6) were incubated in RPMI 1640 (Gibco Labs.) at 37°C for 1 h with 10 different concentrations of [^{3}H]dexamethasone in duplicate ranging from 0.8 to 400 nM in the presence and absence of 1,000-fold excess of unlabeled dexamethasone. Measurements of glucocorticoid bound to nuclear receptors were obtained by hypotonic lysis of one set of PBMC with 1.5 mM $MgCl_2$ at 3°C for 30 min followed by centrifugation at 12,000 *g* for 4 min. The supernatant was removed to isolate the nuclear fraction for radioligand binding analysis. Cytosolic receptors were obtained after hypotonic lysis of the other set of PBMC with 1.5 mM $MgCl_2$ containing dextran-coated charcoal at 3°C for 30 min. The cells were then centrifuged at 12,000 *g* for 4 min and 100 μl of supernatant was removed for cytosolic receptor binding parameters. Analyzing the two fractions, we assumed that GR binding is saturable while nonspecific binding is nonsaturable. For measurement of nonspecific binding, a single measurement with a solution of 20 nM [^{3}H]dexamethasone and 2 μM of unlabeled dexamethasone (Sigma Chemical Co., St. Louis, MO) was used.

All values obtained for both cytoplasmic and nuclear-bound glucocorticoid were corrected for nonsaturable binding for each respective concentration. Saturation binding analysis was performed assuming a linear binding plot of the bound divided by the free [^{3}H]-dexamethasone concentration versus the amount bound and extrapolating to the amount bound at an infinite free hormone concentration. A least-squares linear regression fit was used to define the binding parameters, specifically, receptor sites per cell and binding affinity.

Reversibility and cytokine incubation protocols. PBMC from normal donors and SR asthma patients were isolated and resuspended at a concentration of 1×10^6 cells/ml in RPMI 1640 medium (Gibco Labs.) containing 10% heat-inactivated fetal calf serum (Hyclone Labs., Logan, UT). Cells were incubated in the absence and presence of IL-2 (50 U/ml; Cetus Corp., Emeryville, CA) and/or IL-4 (50 U/ml; gift of Dr. Paul Trotta, Schering-Plough Research Institute, Bloomfield, NJ) for 48 h at 37°C in 5% CO_2. PBMC were then analyzed for GR binding parameters.

Results

Patient characteristics. Patient characteristics are summarized in Table I. SR and SS asthmatics were similar for all parameters with two exceptions. First, although the SR asthmatics had a higher baseline FEV_1 ($P = 0.025$) before the prednisone course as compared with the SS asthma patients, their FEV_1 after treatment with prednisone was significantly lower than SS asthmatics ($P = 0.0001$). Second, 12 of the 17 SR asthma patients received maintenance oral prednisone (mean daily dose = 24 mg) at the time of their GR assay as compared with none of the SS asthma patients. Of note, the majority of SR patients (type I SR asthma, see below) developed cushingoid features during prednisone therapy. In contrast, one of the patients subsequently labeled type II SR asthma (see below) maintained a normal plasma cortisol concentration (12 μg/dl) and did not develop cushingoid features despite continuous treatment with oral prednisone (20 mg daily). The other type II SR asthma patient did not receive maintenance prednisone therapy because of poor clinical response.

Table I. Patient Characteristics

Parameter	SS Asthma Patients	SR Asthma Patients
Number of subjects	12	17
Age (*yr*)*	24	24
Sex (*m/f*)	8/4	12/5
FEV_1 before BD (*percent predicted*)*	47	58
FEV_1 after BD (*percent predicted*)*	69	74
FEV_1 after steroid burst	82	57
Duration of asthma (*yr*)*	16	19
Inhaled steroids (*yes/no*)	6/6	14/3
Systemic steroids (*yes/no*)	0/12	12/5
Systemic steroid dose‡	N/A	24
Atopy (*positive/negative*)	11/1	16/1

* Mean value of patients before burst.
‡ Mean daily prednisone dose in milligrams. *BD*, bronchodilator dose.

PBMC glucocorticoid receptor binding parameters in steroid-resistant asthma patients. Representative Scatchard plots are presented in Fig. 1 from PBMC [^{3}H]dexamethasone radioligand binding data from a normal donor, a patient with SR asthma, and a patient with SS asthma. PBMC GR binding parameters were examined in three study groups: 17 SR asthma patients, 12 SS asthma patients, and 12 normal nonasthmatic controls. Each study group showed distinctive binding patterns for their respective nuclear GR. Nonspecific [^{3}H]-dexamethasone binding was low in all three study groups (2.6±0.5 vs 5.2±1.6 vs 3.5±1.0, respectively, for normals, SS asthmatics, and SR asthmatics) and no significant difference ($P = 0.2$, ANOVA) was observed in nonspecific binding among the three groups.

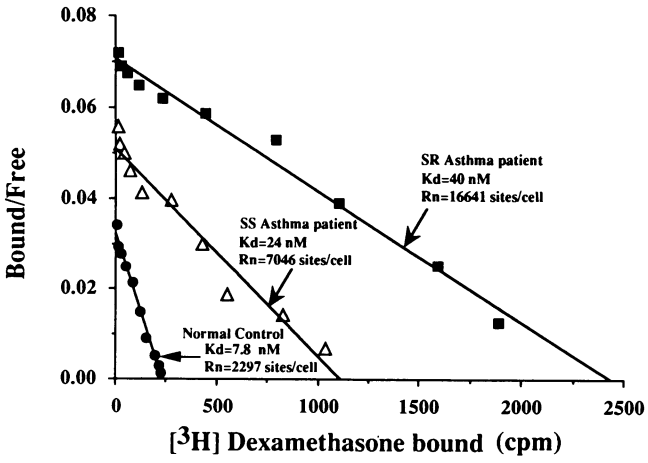

Figure 1. Scatchard analysis of [^{3}H]dexamethasone radioligand binding data of PBMC from a normal donor, an SS asthmatic, and a type I SR asthmatic. Glucocorticoid receptor number is determined by the intercept of the *x* axis and is normalized for the number of cells and the dissociation constant is determined by the reciprocal of the linear slope.

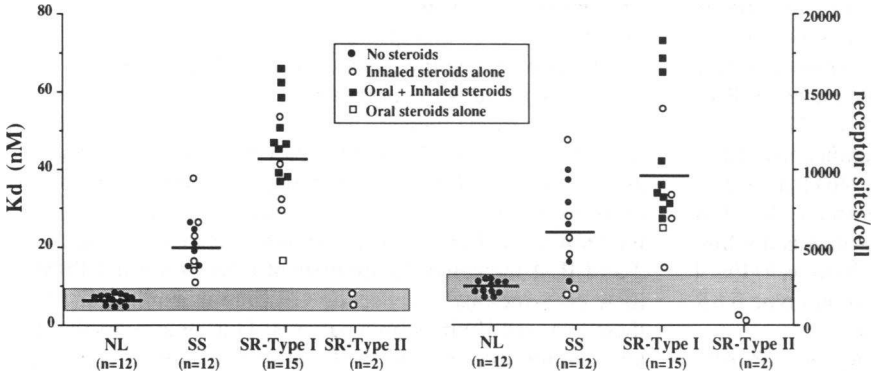

Figure 2. The GR binding parameters of 12 normal (*NL*) controls, 17 SR asthma patients, and 12 SS asthma patients. Normal ranges for both GR parameters are indicated by the shaded bar. The solid bar indicates the mean for each group. All groups were significantly different ($P = 0.0001$, ANOVA) from each other.

As described in Fig. 2, 15 of the 17 patients with SR asthma had a significant increase in their GR K_d i.e., a decrease in binding affinity for glucocorticoids, and an increased GR number in the nuclear fraction of their PBMC when compared with normal subjects or SS asthma patients ($P = 0.0001$, ANOVA). This defect was designated as type I SR asthma. Two other patients with SR asthma had normal GR K_d values but a markedly decreased number of GR binding sites per cell, i.e., < 3 SD below the normal range, compared with all other groups. Both patients were examined in the absence of maintenance prednisone therapy. This defect was designated as type II SR asthma. Of note, SS asthma patients also had a decreased GR binding affinity as compared with normals. Their increased GR K_d and GR number were significantly less than those observed in the SR asthma group but significantly more than the normal control group ($P = 0.0001$, ANOVA). Patients with SR asthma or SS asthma did not have any significant abnormality in cytosolic GR K_d or GR number (Table II).

Since 12 of the 17 patients with SR asthma received oral prednisone (mean daily dose = 24 mg) at the time of their GR assay, the effect of systemic steroids on GR binding parameters was examined. Three approaches were used. First, we measured GR binding parameters of PBMC from five SS asthma patients before and immediately after a 1-wk course of 20 mg twice daily prednisone. No significant changes occurred in either GR number ($P = 0.26$) or GR K_d ($P = 0.31$) after this 1-wk course of high dose oral prednisone (Fig. 3). Second, we assayed PBMC from seven patients who were on 3–12 mo of 20–100 mg prednisone daily for treatment of other diseases associated with tissue inflammation (multiple sclerosis [$n = 1$], pulmonary berylliosis [$n = 3$], nummular eczema [$n = 1$], chronic obstructive pulmonary disease [$n = 1$], and interstitial lung disease [$n = 1$]). None of these patients showed the degree of GR abnormality observed in the SR asthma patients. The GR number (5,591±361) and GR K_d (22.9±1.40) of these seven patients were comparable with those of SS asthma patients and were significantly less than the type I SR asthma patients ($P = 0.0001$). Finally, we examined whether there was any difference in GR binding parameters in type I SR asthma patients who did or did not receive maintenance oral prednisone therapy. Four of the type I SR asthmatics were not taking oral steroids (Fig. 2). Their PBMC still exhibited the same GR K_d (40.8 nM) as the other 11 type I SR asthmatics (GR K_d = 42.6 nM) who received maintenance oral prednisone therapy. All of the above suggest that the significantly reduced GR binding affinity in type I SR asthma is not induced by glucocorticoid therapy.

Localization of GR binding affinity defect to T cells in type I SR asthma patients. To determine whether the altered GR binding parameters were restricted to specific subsets of mononuclear cells in the two subtypes of SR asthma patients, we analyzed GR binding parameters in their T cell and non-T cell

Table II. Glucocorticoid Receptor Binding Parameters

Study group	Nuclear binding sites		Cytosolic binding sites	
	K_d	GR	K_d	GR
	nM	*sites/cell*	*nM*	*sites/cell*
Normal controls (n = 12)	7.94±0.37*	2,514±173*	4.34±0.50	290±39
Asthma patients (n = 12) steroid sensitive	21.6±2.10*	6,130±891*	8.53±2.13	460±124
Asthma patients (n = 15) steroid resistant-type I	42.1±3.07*	9,807±1146*	6.81±1.50	383±87
Asthma patients (n = 2) steroid resistant-type II	7.35±0.78	572±64	5.35±0.40	253±14

Values are ± SEM.
* Significantly different from other two groups ($P = 0.0001$, ANOVA) type II SR asthma patients were not included in statistical analysis.

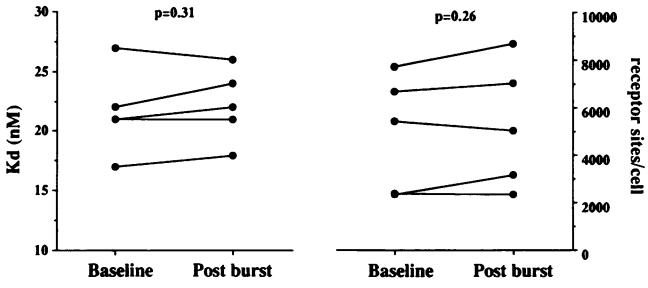

Figure 3. Effect of a 1-wk course of oral prednisone, 40 mg/day, on GR binding parameters in five steroid-sensitive asthma patients. None of the steroid-sensitive asthma patients were receiving maintenance oral prednisone therapy before evaluation.

subpopulations. A fourfold significant increase ($P = 0.0009$) in T cell nuclear GR K_d over non-T cells was observed in the population of type I SR asthma patients as compared with a small but significantly ($P = 0.02$) greater T cell nuclear GR K_d over non-T cells in normal controls (Fig. 4 *A*). The T cells from type I SR asthma patients also had a significantly higher GR K_d ($P = 0.0001$) and GR number ($P = 0.046$) than T cells from normal controls. In contrast, the abnormally low GR number observed in a type II SR asthma patient was not limited to T cells but was also present in non-T cells as well (Fig. 4 *B*). Insufficient cells were available from the second type II SR asthmatic for mononuclear cell subset analysis.

Effect of in vitro incubation on PBMC GR from SR asthmatics. To determine whether the alterations in PBMC GR binding parameters from patients with SR asthma were reversible, GR binding was measured before and after incubation of PBMC in culture medium (Fig. 5). When PBMC from five patients with type I SR asthma were incubated in medium alone they showed a significant ($P = 0.0001$) change in GR binding with normalization of both GR K_d and GR number after 48 h of incubation as compared with values after immediate analysis. Incubation of PBMC from seven normal donors (Fig. 5) under similar culture conditions did not result in any significant change in either GR K_d or GR number.

These data support the concept that the GR binding alterations in type I SR asthma patients were acquired. Since asthma is associated with increased immune activation and cytokine secretion, we examined the effects of IL-2 and IL-4, two cytokines reported to be increased in asthma (16), on GR binding parameters in patients with type I SR asthma and normal donors. When normal PBMC were incubated with the combination of IL-2 and IL-4, a significant increase was observed both in GR K_d ($P = 0.0001$) and in the number of GR sites per cell ($P = 0.001$) at 48 h as compared with values after immediate analysis. In contrast, the reduced GR binding affinity in PBMC from type I SR asthmatics was sustained in culture over the 48-h period when their cells were incubated in the presence of combination IL-2 and IL-4 (Fig. 5). Of interest, incubation of normal PBMC with IL-1, IL-5, and interferon-γ for 48 h, as compared with culture medium alone, did not induce a significant elevation in GR K_d. Based on a minimum of three experiments, mean±SEM, GR K_d after incubation with medium alone was 7.6±0.4, after IL-1 was 3.6±1.2, after IL-5 was 9.1±0.7, and after interferon γ was 6.2±0.4.

We also studied the effect of in vitro 48-h incubation with media alone and with combination IL-2 and IL-4 on the two type II SR asthma patients with low GR number (Fig. 5). In contrast to the type I SR asthma patients with elevated GR K_d and GR number, PBMC from these two patients did not demonstrate any changes in GR binding when incubated in culture medium alone. Furthermore, although the combination of IL-2 and IL-4 increased the number of GRs in normal PBMC, these cytokines had no effect on GR binding parameters from these two type II SR asthma patients. Thus, it appears that the low GR number in type II SR asthma patients is a primary irreversible defect, whereas the reduced GR binding affinity in type I SR asthma patients is an acquired reversible defect.

Discussion

Recent proposed guidelines for the treatment of asthma focus on early intervention with antiinflammatory therapy, especially inhaled glucocorticoids (3). However, a subset of asthma patients fails to demonstrate a satisfactory response to glucocorticoid therapy (5, 6). It is important to recognize these patients early because failure to respond often leads to courses of very high dose glucocorticoid therapy and consequent adverse effects despite persistent airway compromise. Indeed, the documented rise in asthma mortality during the past decade despite the use of glucocorticoids for asthma therapy in many countries raises the question of whether a proportion of these patients was indeed steroid resistant. Since these patients likely evolve over time, understanding mechanisms involved in glucocorticoid resistance could lead to earlier identification and could provide insight for new directions in management.

After careful clinical assessment for poor response, such as other pulmonary abnormalities, poor technique or adherence to medication schedules, pharmacokinetic abnormalities in medication absorption, distribution, or elimination, and concomitant medical or psychological disorders, questions may be raised whether persistent airway inflammation itself or genetic abnormalities in GR binding contributes to poor glucocorticoid response. Earlier studies have investigated potential mech-

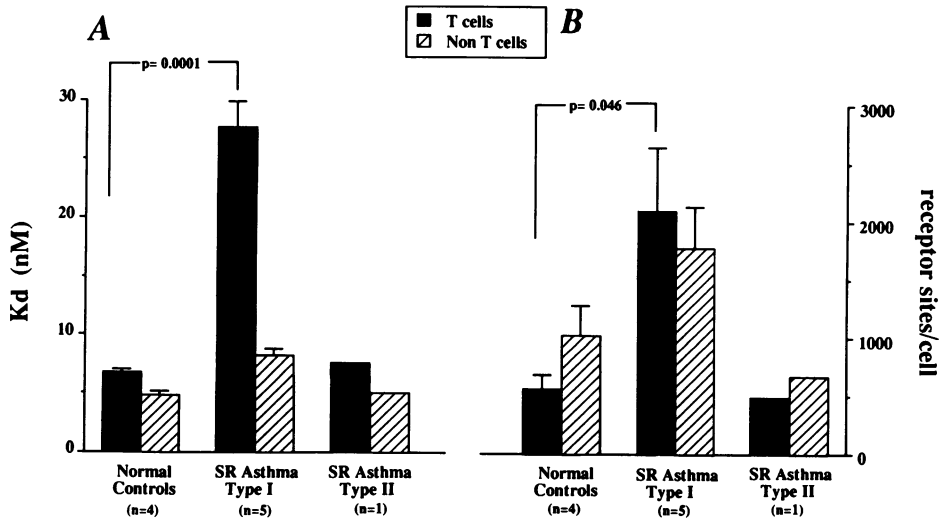

Figure 4. The GR number and binding affinity were measured in fractionated T and non-T cell populations. The increased K_d and receptor sites per cell of nuclear GR are limited to the T cell population of SR asthma patients and are significantly different from normal controls ($P = 0.0001$ K_d; $P = 0.046$ GR sites/cell). No difference in nuclear GR binding parameters in the non-T cell fraction was observed. Each bar represents the mean±SEM.

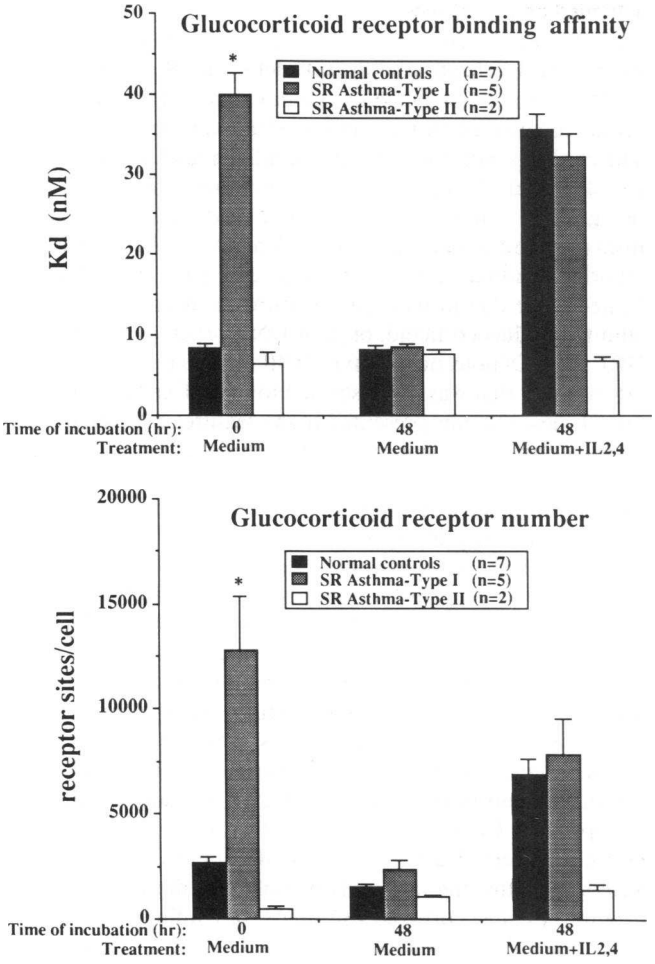

Figure 5. Time course of nuclear GR binding parameters in PBMC from type I and type II SR asthma patients and normal donors. Freshly isolated PBMC from five type I SR asthmatics (time 0 hours) have significantly elevated GR K_d (*$P = 0.0001$) and GR numbers (*$P = 0.002$) as compared with normals and revert to normal GR binding parameters when cultured in medium alone for 48 h. Incubation of PBMC from seven normal subjects and two type II SR asthma patients shows no change in GR binding parameters when cultured in medium alone for 48 h. Combination IL-2 and IL-4 significantly increases GR K_d and GR number ($P = 0.0001$) of normal donors and alters GR binding parameters in PBMC from type I SR asthmatics but not from type II SR asthmatic patients. Each bar represents the mean±SEM.

anisms for glucocorticoid resistance (17). Corrigan et al. (7) demonstrated that PHA-induced proliferation of peripheral blood T cells was inhibited by dexamethasone in SS asthma patients but not SR asthma patients. They also demonstrated that dexamethasone inhibits IL-2 and interferon-gamma production in cells from SS asthma patients but not in cells from SR asthma patients. In this study, we examined the possibility that alterations in GR binding may contribute to the apparent refractoriness to glucocorticoid therapy. Our operational definition of SR asthma was the failure to improve morning prebronchodilator FEV_1 by ≥ 15% after a 1-wk course of prednisone at a minimum oral dose of 40 mg/d. This definition was based on our previous observation that over 90% of asthmatics show a ≥ 15% improvement in their morning prebronchodilator FEV_1 within 1 wk of oral prednisone (40 mg daily) (13). We limited the course of prednisone to 1 wk so we could simultaneously examine a group of SS asthma patients on a similar time course of prednisone therapy.

Our observations indicate that there are at least two mechanisms present in this population of SR asthma patients. Type I SR asthma patients have an abnormally reduced GR binding affinity and an increased number of receptor sites per cell compared with our normal population and our SS asthma patients. It is interesting that the defect in type I SR asthma PBMC GR binding is limited to the T cell population. Conversely, SR asthma patients with the type II binding abnormality have normal binding affinity with a markedly reduced number of glucocorticoid receptors per cell. An important distinction between these two types of SR asthma was that the GR defect in type I SR asthma was reversible in culture but was sustained with the coincubation of combination IL-2 and IL-4. In contrast, the binding defect in type II SR asthma was irreversible and did not respond to coincubation with combination IL-2 and IL-4. Furthermore, the PBMC GR defect in this latter form of SR asthma was not restricted to T cells.

An obvious question is whether the abnormalities in GR binding parameters observed in our patients could be induced by systemic glucocorticoid therapy. Several observations suggest that this is not a relevant mechanism. Specifically, we observed: (*a*) no significant change in GR binding parameters after 1 wk of high dose "burst" prednisone in five SS asthma patients who were not receiving oral prednisone maintenance therapy; (*b*) that a disease control group receiving chronic high dose prednisone therapy did not show the same degree of abnormality in GR K_d as the type I or GR number as the type II SR asthmatics; and (*c*) type I SR asthma patients who were not receiving oral prednisone had abnormal GR binding affinity in the same range as type I SR asthma patients receiving oral prednisone maintenance therapy. Of note, both the type II SR asthmatics were studied while not taking systemic glucocorticoids. Finally, previous studies have reported that glucocorticoid treatment induces a modest downregulation of the number of GR, which differs from the alterations seen in type I SR asthma (11). Although these observations suggest that the phenomenon is not medication induced, we cannot completely exclude the possibility that previous in vivo treatment may affect the GR binding in these patients.

The SS patients also have reduced GR binding affinity as compared with normal donors but less than that observed in type I SR asthma patients. These data are of particular interest because they suggest that chronic asthma may be associated with a spectrum of GR binding defects with SR asthma at the extreme end of this range. The degree of change in GR binding affinity may be related to the magnitude of airway inflammation. This phenomenon along with the possibility of irreversible changes in the airway structure could explain refractoriness to conventional therapy. Although not specifically addressed, it is possible that the significantly reduced GR binding affinity in SS asthma patients may have clinical relevance since recent studies indicate that endogenous cortisol levels regulate IgE-dependent late phase allergic reactions (18). Since endogenous cortisol levels are significantly lower than levels achieved after the administration of therapeutic doses of steroids, even the moderate abnormalities in GR found in SS asthmatics may have an influence on the ability of endogenous glucocorticoids to suppress airway inflammation. Furthermore, even modest doses of inhaled glucocorticoids have the potential to reduce

nocturnal plasma cortisol concentrations, further compromising the availability of endogenous cortisol during the critical nighttime period when pulmonary function is lowest in patients with severe asthma (19, 20). Consequently, inhaled glucocorticoids may provide symptomatic relief while incompletely resolving the inflammatory process. Thus, an understanding of the mechanisms which underlie SR asthma may have important implications for controlling inflammation in milder forms of asthma.

Since there is only one GR gene, our observations suggest that type I SR asthma, which accounts for the large majority of patients with SR asthma, is acquired and restricted to T cells, whereas type II SR asthma is a form of primary cortisol resistance and is not limited to T cells. We demonstrated that the reduced GR binding affinity can be induced in normal PBMC with cytokines, i.e., combination IL-2 and IL-4. This raises the intriguing hypothesis that SR asthma may be the end result of poorly controlled asthma and ongoing immune activation. It has been previously demonstrated that PHA-induced T cell proliferation and cytokine production by PBMC from patients with SR asthma are poorly inhibited by the addition of dexamethasone or methylprednisolone in vitro (8, 9). Recently, however, we reported on several SR patients whose asthma came under control with the combination of troleandomycin and methylprednisolone therapy, resulting in normalization of T cell sensitivity in vitro to the inhibitory effects of methylprednisolone on T lymphocyte proliferation (8). Furthermore, cyclosporin A, a drug whose major action is inhibition of T cell proliferation and cytokine secretion, was reported to improve the clinical symptoms of several patients with SR asthma (9). These observations support the hypothesis that ongoing asthma inflammation and cytokine secretion may contribute to the acquired GR defect found in type I SR asthma.

The actual mechanisms by which cytokines or immune activation might induce a decrease in GR binding affinity are unknown. One possible insight may relate to the marked difference between the nuclear and cytosolic GR binding parameters of PBMC from SR asthma or normal PBMC treated with combination IL-2 and IL-4. In this regard, it is well known that the GR changes its structure and/or conformation when translocated between the nucleus and cytosol.

At a cellular level, glucocorticoids exert their biological effects by freely penetrating the plasma membrane and binding to a specific intracellular receptor, i.e., the GR (11). The unliganded receptor is thought to be a heteromer composed of a single steroid- and DNA-binding subunit and two 90-kD heat-shock proteins. The binding of glucocorticoid to its receptor results in the dissociation of the 90-kD heat-shock protein subunits and exposure of the DNA-binding site on the receptor. This activated GR complex then translocates into the nucleus and regulates transcription by binding to specific DNA sequences called glucocorticoid-responsive elements (GRE). Many of the glucocorticoid-inducible genes which have been identified are characterized by a cluster of multiple GREs upstream of their promoter and enhancer regions. The induction or repression of GR target genes ultimately results in the altered expression of glucocorticoid-regulated proteins (21). This latter action appears to be mediated via interaction of the modulatory domain of the GR with transcriptional factors, such as activation protein-1 (AP-1). Overexpression of AP-1 interferes with the function of the modulatory domain of the GR (22). Since T cells from SR asthmatics are chronically activated and cytokines can induce elevated AP-1 levels (23), the latter observation may provide a plausible explanation for the nuclear localization of the GR defect in SR asthma.

Primary cortisol resistance is a rare, but well described syndrome reported in humans and nonhuman primates (24, 25). The clinical syndrome is usually familial and characterized by elevated total plasma cortisol concentrations and the absence of signs and symptoms of Cushing's syndrome. The mechanisms for end organ glucocorticoid resistance in the various reported kindreds are heterogenous and have been demonstrated to be due to reduced GR number, decreased binding affinity for glucocorticoid, or poor DNA binding of the GR to GRE (26). Of note, our two type II SR asthma patients had low GR number that was not restricted to their T cells. Clinically, one of these patients presented many features consistent with primary cortisol resistance including the abilities to maintain normal plasma cortisol concentrations and to remain free of glucocorticoid adverse effects despite daily prednisone therapy in doses exceeding 20 mg/day.

In summary, we found that patients with SR asthma have alterations in PBMC GR number or binding affinity. With the increasing use of systemic steroids to treat airway inflammation in asthma, it is likely that more of these patients will be identified. While these patients may respond to extremely high dose glucocorticoid therapy, recent studies have identified several promising treatment regimens as alternatives to systemic glucocorticoid therapy. These include cyclosporin, troleandomycin, methotrexate, gold, and intravenous gamma globulin therapy (27). Characteristic of these treatments is the variable response observed among individual patients. Our observations suggest that the variability in molecular mechanisms contributing to glucocorticoid resistance may influence response to therapeutic intervention. Continued research is needed to define the mechanisms of action for ongoing immune activation and the correlation to response to individual treatment strategies. An understanding of the mechanisms by which glucocorticoids fail to resolve inflammation in asthma may provide important insight into the pathogenesis of asthma, especially as related to progressive deterioration, and may result in the rational design of innovative treatment approaches.

Acknowledgments

The authors thank Drs. Allen Adinoff, Lee Newman, and Talmadge King for referring patients to our study. We also thank Maureen Plourd-Sandoval for assistance in preparing this manuscript.

This work was supported in part by U. S. Public Health Service grants HL-36577 and AR-41256.

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