

Review Article

New insights into steroid resistant asthma

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The term “steroid resistant (SR) asthma” refers to a group of asthmatics who have persistent airway obstruction and immune activation despite treatment with high doses of systemic glucocorticoids. There are at least two forms of SR asthma, i.e. primary and acquired types. Type I SR asthma is acquired and is associated with abnormally reduced glucocorticoid receptor (GCR) ligand and DNA binding affinity. Type II SR asthma appears to be due to a constitutive defect and is associated with low numbers of GCRs. An important distinction between these two types of SR asthma is that the GCR defect in Type I, but not Type II, SR asthma is reversible in culture and is sustained by incubation with combination IL-2 and IL-4. Recent studies suggest that the abnormal GCR binding in Type I SR asthma may be due to cytokine-driven alternative splicing of the GCR pre-mRNA to a novel isoform called GCR β which does not bind glucocorticoids but antagonizes the transactivating activity of the classic GCR. These GCR changes along with recent evidence for increased transcription factor activation in SR asthma which may inhibit GCR/DNA interactions as well as the selective recruitment of neutrophils into the airways of certain patients with severe asthma contribute to the heterogeneity of mechanisms underlying steroid resistance.

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Numerous studies have established that inflammation and immune activation play a key role in the pathogenesis of airway obstruction, bronchial hyperreactivity and airway remodeling in chronic asthma (1, 2). Recent guidelines on the treatment of asthma have therefore recommended early intervention with the use of anti-inflammatory therapy, particularly inhaled glucocorticoid(GC)s (3, 4). Although the majority of patients respond to regular inhaled GC therapy, a subset of asthmatics have poorly controlled asthma even when treated with high doses of oral prednisolone or its equivalent. These patients are frequently on complex medication regimens and account for a large proportion of the hospitalizations and emergency room visits due to asthma.

With the increasing focus on reduction of health care costs, patients with a history of so-called “ster-

oid resistant” (SR) asthma provide a significant challenge to the clinician managing patients with severe asthma. The present review will examine the clinical definition of SR asthma, as well as the role of immune mechanisms and altered glucocorticoid receptors (GCR) in the pathophysiology of patients who appear to be resistant or insensitive to the immunosuppressive effects of GCs. Since these patients are likely to evolve over time, understanding mechanisms involved in steroid resistance or GC insensitivity is important to identify them early and develop new approaches for the treatment of their illness.

Defining steroid resistant asthma

The term “steroid resistant” asthma was first coined in 1968 by Schwartz and his colleagues (6) reporting a group of asthmatics who failed to respond to high dose systemic GC therapy. Interestingly, following the intravenous infusion of 40 mg hydrocortisone into these asthmatics, a blunted peripheral blood eosinopenic response was observed. Although these investigators initially suggested that accelerated plasma cortisol clearance could account for the apparent steroid resistance, recent studies have dem-

Abbreviations: BAL: bronchoalveolar lavage fluid; GC: glucocorticoid; GCR: glucocorticoid receptor; FEV₁: forced expiratory volume in 1 second; IL: interleukin; LPS: lipopolysaccharide; mRNA: messenger RNA; NF- κ B: nuclear factor kappa B; PBMC: peripheral blood mononuclear cells; PHA: phytohemagglutinin; SR: steroid resistant; SS: steroid sensitive; TNF: tumor necrosis factor.

onstrated that most SR asthmatics have normal GC pharmacokinetics (7, 8).

In 1981, Carmichael and colleagues described a group of 58 patients with baseline FEV₁'s of less than 60% predicted, and a 30% or greater increase in FEV₁ after bronchodilator therapy (9). However, their FEV₁ failed to increase by greater than 15% of predicted values after a course of prednisolone 20 mg daily for 7 days. They were therefore considered "steroid resistant". In contrast, steroid sensitive (SS) asthmatics had an increase in FEV₁ by >30% with this treatment. Other factors which also distinguished SR patients from SS asthmatics included a longer duration of symptoms, relatively lower morning peak expiratory flow rates, and increased bronchial hyperresponsiveness.

There is currently no universally accepted definition of GC resistance in asthma. For example, in a report by Corrigan et al. (8), SR asthma was defined as the failure to demonstrate an increase in baseline FEV₁ by greater than 15% after a course of 20 mg daily oral prednisolone for 1 week followed by 40 mg daily for a second week. To better define what constituted an adequate trial of systemic GC therapy, Kamada et al. (10) studied the response of asthmatic children whose baseline morning pre-bronchodilator FEV₁ was <70% predicted following a course of prednisone therapy. Over 60% of patients demonstrated a >15% improvement of their morning pre-bronchodilator FEV₁ within 3 days, and 93% showed a significant improvement within 10 days of starting GC therapy. In this study, extending the course of therapy over 10 days did not lead to a significantly greater improvement in FEV₁. From this it was concluded that 7–14 days of high dose systemic GC therapy (≥20 mg twice daily) was an adequate trial to identify patients with poor steroid response.

In a recent workshop on GC action and resistance in asthma, it was proposed that SR asthma could be defined by the failure to improve baseline morning pre-bronchodilator FEV₁ by greater than 15% predicted following at least 7–14 days of 20 mg twice daily oral prednisone or its equivalent (11). Although some patients might respond to higher doses of prednisone given for longer periods of time, such doses would be undesirable because they would be associated with marked adverse effects. Nevertheless, it should also be pointed out that in most patients, steroid resistance is not absolute but a shift in the dose-response curve such that they require a much higher quantity of GCs for optimal response to therapy as compared to patients with SS asthma (12). It has therefore been proposed that it would be more accurate to refer to this group of severe asthmatics as "GC insensitive asthmatics" (11). This distinction is important as optimizing response to

Table 1. Considerations in the evaluation of steroid resistant asthma

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- Another respiratory disorder presenting as recurrent wheezing/dyspnea
 - Concomitant medical problems affecting asthma care such as:
 - gastroesophageal reflux
 - chronic sinusitis or other respiratory infections
 - allergic bronchopulmonary aspergillosis
 - chronic airway infection
 - Psychosocial factors affecting self-care:
 - poor adherence with medications
 - emotional factors
 - Persistent inflammation due to:
 - allergen exposure
 - drug interactions
 - abnormal glucocorticoid pharmacokinetics or receptor defects
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GCs is one important strategy in the management of this severe group of asthmatics.

Importantly, patients with SR asthma fulfill the American Thoracic Society criteria for diagnosis of asthma and have a bronchodilator response of greater than 15% improvement in FEV₁. In the evaluation of asthmatics who present with poor response to steroids, it is important to rule out other respiratory conditions which may mimic the symptoms of asthma or confounding factors which contribute to difficulty in their management. Their potential role must be thoroughly studied and appropriate therapy instituted prior to a diagnosis of SR asthma. Gastroesophageal reflux and/or aspiration, sinusitis, ongoing exposure to environmental allergens or food allergy are some of the concomitant factors that must be ruled out (Table 1). In addition, psychological factors may interfere with optimal asthma management and should be appropriately addressed. Inadequate medication technique, e.g. with metered dose inhalers, or poor patient compliance with use of prescribed medications, particularly inhaled or systemic steroids, are also common reasons for treatment failure.

When a patient fails to respond or is unable to tolerate steroid doses lower than 20 mg every other day with either prednisone or its equivalent, we also evaluate their GC pharmacokinetics. The purpose of these studies is to determine whether there is incomplete GC absorption, failure to convert an inactive form (prednisone) to an active form (prednisolone), or the presence of rapid GC elimination. This evaluation is particularly important in a patient who fails to demonstrate the anticipated adverse effects because measurements of plasma GC concentrations can also be used to assess patient compliance with steroid therapy. Although the majority of patients have normal GC absorption, conversion to the active form and elimination, in our experience nearly 25% of patients who present with poorly controlled asthma will have pharmacokinetic abnormalities.

The most frequent finding is rapid elimination. This usually occurs as the result of a corticosteroid-drug interaction, particularly due to the use of anticonvulsants, e.g. phenytoin, carbamazepine, or phenobarbital. Occasional patients may also demonstrate poor absorption. Interestingly, substitution with an alternative GC such as methylprednisolone, can result in improved response in some of these patients.

Actions of glucocorticoids in asthma

The effectiveness of GCs in asthma is thought to result from a combination of inhibitory or down-regulatory effects on the airway inflammatory process. Early studies of GC action focused on their ability to increase the synthesis of lipocortin, an inhibitor of phospholipase A2, which controls the production of prostaglandins, leukotrienes, and platelet activating factor (13). The importance of this proposed mechanism, however, has been questioned. Although it has been shown that GCs induce lipocortin-1 in alveolar epithelial cells and inhibit eicosanoid release in the lung, studies in other cell types show no induction of lipocortin-1 by GCs (14). GCs may also act by increasing transcription of other genes with anti-inflammatory effects. For example, expression of neutral endopeptidase is increased in bronchial epithelial cells by GCs. This could lead to enhanced degradation of tachykinins, which play a role in neurogenic inflammation (15). GCs may also act by increasing expression of beta-adrenergic receptors in the airways (16).

More recently, studies on the anti-inflammatory action of GCs have focused on their inhibitory effects on cytokine gene transcription. A variety of proinflammatory cytokines and chemokines, including TNF- α , GM-CSF, IL-1 through IL-6, IL-8, RANTES and eotaxin, are involved in the inflammatory response of the lung (17, 18). GCs are likely to act in part by inhibiting transcription of these cytokines and their receptors in a variety of cell types thought to be involved in airway inflammation of asthma. Inhibition of cytokine production results in reduced expression of leukocyte-endothelial adhesion molecules, such as E-selectin and ICAM-1, as well as decreased cell survival of inflammatory effector cells. These studies predict that a major action of GCs is to down-regulate the infiltration and activation of inflammatory cells such as eosinophils and T lymphocytes which have a pivotal role in the pathogenesis of chronic asthma (19).

A number of controlled clinical studies have demonstrated that treatment of most asthmatics with inhaled and/or systemic GCs is accompanied by a decrease in BAL concentrations of proinflammatory cytokines and eosinophil cationic proteins accompanied by a reduction in the numbers of BAL eosi-

nophils and activated T lymphocytes in asthmatic airways (20, 21). In atopic patients, GC treatment blocks the appearance of the late phase asthmatic response following experimental airway allergen challenge (22). An additional aspect of GC action may occur from modulating the pattern of cytokine secretion by T lymphocytes. In this regard, studies on SS asthmatics indicate that response to oral prednisolone therapy results in a decreased number of activated T lymphocytes expressing IL-4 and IL-5 mRNA transcripts but a concomitant increase in interferon-gamma (IFN- γ) mRNA expression (23). Thus, there is considerable evidence that the primary beneficial effect of GCs lies in their potent anti-inflammatory and immunomodulatory properties.

At a cellular level, GCs exert their biological effects by freely penetrating the plasma membrane and binding to a specific intracellular receptor, i.e. the GCR. The unbound GCR is thought to be a heterohexamer containing the receptor with a single GC binding and DNA binding subunit, two molecules of heat shock protein (hsp) 90, and one molecule each of hsp 70, hsp 56, and hsp 26 (24). The binding of GC to its receptor results in the dissociation of the hsp subunits and exposure of the DNA binding site on the GCR. The activated GCR nuclear complex regulates transcription by binding to specific DNA sequences, called GC-responsive elements (GRE), of palindromic structures. Usually, two GRE half sites are arranged as inversed palindromes allowing the receptor to bind the DNA as a homodimer.

To date, GC-inducible genes have been characterized by a cluster of multiple GREs upstream of their promoter regions. The induction or repression of GCR target genes ultimately results in the altered expression of GC regulated proteins (25). This latter action is mediated at least in part by direct binding of the GCR with transcription factors, such as AP-1 or nuclear factor kappa B (NF- κ B). An additional mechanism is based on the established role of transcription factors, AP-1 and NF- κ B, in the activation of many cytokine genes and cell adhesion genes. The activation of NF- κ B requires the targeted degradation of its cytoplasmic inhibitor, I κ B α , followed by the translocation of NF- κ B to the nucleus. Two recent studies demonstrated that dexamethasone induces transcription of the I κ B α gene resulting in increased synthesis of I κ B α protein (26, 27). Stimulation by TNF- α , a proinflammatory cytokine, causes the release of NF- κ B from I κ B α . However, in the presence of dexamethasone this newly released NF- κ B rapidly reassociates with newly synthesized I κ B α , thus markedly reducing the amount of NF- κ B that translocates to the nucleus. This decrease in NF- κ B decreases cytokine secretion and thereby blocks activation of the immune response.

Mechanisms of glucocorticoid resistance

Cellular responses to glucocorticoids in SR asthma

Patients with SR asthma have a number of abnormalities in cellular immune responses (see Table 2). In 1981, Kay et al. (28) reported that peripheral blood monocytes from SR, as compared to SS, asthmatics failed to demonstrate a reduction in complement receptor expression following prednisolone therapy. In 1984, Poznansky and colleagues reported that freshly isolated PBMC from SS and SR asthmatics had no significant differences in their proportions of T cell subsets, monocytes, and B lymphocytes (29). However, when mononuclear cells were co-incubated with methylprednisolone, at doses as low as 10^{-8} M, phytohemagglutinin (PHA)-induced T lymphocyte proliferation in the SS asthma group was inhibited by greater than 60%. In contrast, T lymphocytes from SR asthmatics did not demonstrate a significant decrease in proliferation at the same concentration of methylprednisolone. This study suggested that there was a functional abnormality in PBMC responsiveness to GCs, but no difference in the surface phenotype of cells from SR asthmatics.

In 1989, Wilkinson and colleagues examined the possibility that GCs did not inhibit proinflammatory cytokines or mediator production by cells from SR asthmatics (30). In this study, PBMC from SS asthmatics were cultured in the absence and presence of hydrocortisone. When the supernatants of these cultures were added to calcium-ionophore-activated neutrophils, leukotriene B_4 production was suppressed by supernatants from the cells previously incubated with hydrocortisone. In contrast, supernatants from hydrocortisone-treated cells derived from SR asthmatics did not suppress leukotriene B_4 pro-

duction. The authors suggested that, based on these data, a monocyte-derived factor from SR asthmatics enhanced the proinflammatory activity of neutrophils and its production was not inhibited by GCs.

Further evidence that monocytes from SR asthmatics have altered responses to GCs was provided by Vecchiarelli et al. (31), who studied lipopolysaccharide (LPS)-induced production of $TNF-\alpha$ by PBMC from 14 acute asthmatics before and after a 1-week course of prednisolone. At baseline, levels of LPS-induced $TNF-\alpha$ production were similar in both groups of asthmatics. Following GC treatment, $TNF-\alpha$ production by PBMC from SS, but not SR, patients was significantly reduced. Furthermore, in SS patients the degree of $TNF-\alpha$ inhibition correlated with clinical response to steroids.

Since T lymphocytes play a critical role in the pathogenesis of asthma, there have been an increasing number of studies examining their role in SR asthma. In 1991, Corrigan and colleagues reported that PHA-induced proliferation of peripheral blood T lymphocytes in SS, but not in SR, asthmatics was inhibited by dexamethasone (8). Subsequently, these investigators also demonstrated an increased expression of CD25 (IL-2 receptor) and HLA-DR activation antigens on peripheral blood T cells of SR asthmatics (32). They also found that dexamethasone inhibited PHA-induced IL-2 and IFN- γ production in cells from SS, but not SR, asthmatics. In contrast, cyclosporin A was able to inhibit proliferation and cytokine production by T cells from both SS and SR asthmatics.

Alvarez et al. (7) also reported that PBMC from SR, as compared to SS, asthmatics were significantly less responsive to the inhibitory effects of methylprednisolone on PHA-induced lymphocyte proliferation. Of note, when co-incubated with troleandomycin (TAO), a macrolide antibiotic that has anti-inflammatory actions, proliferation was reduced in a dose-dependent fashion in T cells from both SS and SR asthmatics. This is of clinical importance because, while SR asthma is associated with poor responsiveness to GCs, these patients can be sensitive to other drugs used in the treatment of asthma, including β -agonists, cyclosporin and TAO.

Recently, Leung and colleagues (33) also examined bronchoalveolar lavage (BAL) airway cells from SS and SR asthmatics, prior to and after a 1-week course of daily high dose (40 mg daily) prednisone. At baseline, there were no significant differences between BAL total eosinophil counts or numbers of activated T cells in SR vs SS asthmatics. However, after prednisone therapy, there was a significant decrease in numbers of BAL eosinophils as well as BAL activated T cells in SS, but not SR, asthmatics.

Table 2. Immunologic features of steroid resistant asthma

1. T cells:

- Increased levels of T cell activation unresponsive to glucocorticoids (GC)
- Failure of glucocorticoids to inhibit PHA-induced T cell proliferation *in vitro*
- Increased IL-2 and IL-4 gene expression in the airways
- Failure to decrease production of airway IL-2, IL-4, IL-5, & IL-13 after GCs
- Increased expression of GCR β

2. Eosinophils

- Blunted eosinopenic response following treatment with GC

3. Monocytes

- Failure of GCs to suppress production of a monocyte derived factor which primes neutrophils for enhanced LTB_4 production
- Persistent IL-8 production despite GC treatment
- Increased expression of AP-1

4. Neutrophils

- Increased airway neutrophilia
 - Increased LTB_4 production
-

Since cytokines play a critical role in airway inflammation, studies were carried out to determine the expression of cytokine mRNA in BAL cells. At baseline, BAL cells from SR, as compared to SS, asthmatics had a significantly higher number of cells expressing mRNA for IL-2 and IL-4. However, no significant differences between these two patient populations were observed in the expression of IL-5 mRNA at baseline. After prednisone therapy, BAL cells from SS, but not SR, asthmatics demonstrated a significant decrease in cells expressing IL-4 mRNA and IL-5 mRNA. Although small reductions in the number of BAL cells expressing IL-2 mRNA accompanied prednisone therapy in each patient group, these differences were not significant. These data demonstrate that the airway cells from patients with SR asthma have much higher levels of IL-2 and IL-4 gene expression than in SS asthma. In addition, prednisone inhibits cytokine gene expression in SS, but not SR, asthma.

GC resistance in non-asthmatic conditions

Steroid resistance has been described in various inflammatory diseases including chronic sinusitis, atopic dermatitis, chronic urticaria, inflammatory bowel disease, systemic lupus erythematosus, rheumatoid arthritis and lymphomas (34–42). A syndrome of familial end organ GC resistance is particularly well described (35, 41). These patients usually have elevated total plasma cortisol concentrations, but do not develop cushingoid features. The molecular basis for end organ GC resistance in the various reported families includes either reduced GCR numbers, decreased binding affinity for GCs or poor DNA binding of the GCR to GC-responsive elements (GRE) (41).

Bloomfield et al. (34) found that malignant lymphoma cells from patients who respond to dexamethasone treatment had more GCR sites per cell and greater *in vitro* sensitivity to GC-induced inhibition of proliferation than did GC-resistant tumor cells. Yamamoto studied the isolation of four classes of SR S49 lymphoma cell lines after selection for growth in 10^{-6} M dexamethasone (43). SR cell lines expressed the following GCR abnormalities: a) GCR defective in dexamethasone binding, b) GCR defective in DNA binding and nuclear localization, c) a 40-kd GCR that bound steroid and DNA but did not contain the domain that modulates transcription, and d) *deathless* mutants that apparently expressed a normal GCR, but were defective in some other step of the lysis pathway. These studies suggested the molecular basis for GC resistance can be quite heterogeneous and involves multiple processes in the interaction of GCs with its receptor and GRE DNA binding sites.

GCR abnormalities in SR asthma

GCs bind to a specific intracellular receptor to inhibit cellular activation (44). Since patients with familial GC resistance and steroid resistant malignancies have alterations in their GCR, it was of interest to determine whether poor GC responses in SR asthma were due to an alteration in GCR number or binding affinity. In this regard, we have identified two forms of SR asthma (Table 3): an acquired form (Type I SR asthma) and a primary form (Type II SR asthma) (45). The majority of patients with SR asthma have the Type I or acquired defect. These patients develop significant side effects, including adrenal suppression, cushingoid changes and osteoporosis, when treated chronically with high dose systemic steroids. They have a decreased ligand and DNA GCR binding defect affecting their mononuclear cells, particularly T cells (45–47).

To date, there has only been one human GCR gene described (48). The clinical observation that patients with Type I SR asthma can develop severe side effects from chronic treatment with systemic steroids suggests that GCRs in non-immune cells have normal sensitivity to steroids. These clinical observations suggest that this form of SR asthma is acquired, possibly as the result of a particular pathway of immune activation. To test this possibility, Sher et al. (45) measured GCR binding in PBMC prior to and following incubation for 48 hours in culture medium. When PBMC from patients with Type I SR asthma were incubated in medium alone they showed a significant improvement in GCR binding with normalization of GCR K_d after 48 hours of incubation. Since airway cells from patients with SR, as compared to SS, asthma express increased levels of IL-2 and IL-4 mRNA (33), it was of interest to determine whether the combination of IL-2 and IL-4 could alter GCR binding affinity in normal PBMC. In this regard, Kam et al. (49) demonstrated that GCR abnormalities similar to those observed in cells from SR asthmatics can be induced in PBMC from normal donors by incubation of their cells with the combination of IL-2 and IL-4.

Table 3. Features of steroid resistant asthma

Features	Type I (acquired)	Type II (primary)
Chronic airway obstruction	Yes	Yes
Cortisol*	Suppressed	Normal
Cushingoid side effects*	Yes	No
GCR ligand and DNA binding affinity	Reduced	Normal
GCR number	Normal or elevated	Low
Reversibility of GCR defect	Yes	No

* Characteristic on high dose systemic steroids.

These data suggest that the GCR binding alterations in Type I SR asthma are acquired as a response to increased secretion of certain cytokines. Consistent with this concept, Lane et al. found no evidence for a structural abnormality in the GCR of SR asthmatics analyzed using a technique of PCR amplification and chemical mutational analysis (50). We have also examined the effects of IL-2 and IL-4 on GCR binding parameters in patients with Type I SR asthma (45). In these studies, the reduced GCR binding affinity in PBMC from Type I SR asthmatics was sustained in culture over the 48-hour period when their cells were incubated in the presence of combination IL-2 + IL-4.

In contrast to the Type I SR asthma patients, PBMC from patients with Type II SR asthma have normal GCR binding affinity but extremely low numbers of GCRs (45). Furthermore, they did not demonstrate any changes in GCR binding when incubated in culture medium alone. Of note, although the combination of IL-2 and IL-4 increased the number of GCRs in normal PBMC, these cytokines had no effect on GCR binding parameters from patients with Type II SR asthma. Thus, the low GCR number in Type II SR asthma patients is an irreversible defect likely with a genetic basis whereas the reduced GCR binding affinity in Type I SR asthma patients is an acquired reversible defect.

Since Type I SR asthma has been found to be associated with persistent T cell activation despite treatment with high doses of GCs, it was of interest to determine whether the altered GCR binding parameters were localized to specific subsets of PBMC (45). A four-fold increase in T cell, as compared to non-T cell, GCR Kd was observed in the PBMC from Type I SR asthma patients. The T cells from Type I SR asthma patients also had a significantly higher GCR Kd than T cells from normal controls. In contrast, the abnormally low number of GRs observed in Type II SR asthmatics was not limited to T cells but was also present in non-T cells as well (45).

Since patients with SR asthma are frequently treated with high doses of systemic GCs, concern has been raised that the abnormalities in GCR binding parameters observed in SR asthma might be induced by previous exposure to high dose systemic GC therapy. Several observations suggest that this is unlikely: First, in a group of stable patients with SR asthma who were not receiving maintenance oral prednisone therapy, there was no significant difference in GCR binding observed prior to and following treatment with a 1-week course of high dose prednisone (45). Second, patients with interstitial lung disease on chronic high dose prednisone therapy do not have the same degree of abnormal GCR binding as found in SR asthma. Third, we have

found a number of patients presenting with SR asthma and abnormal GCR binding who have not previously received systemic GC therapy. Fourth, previous studies have reported that GC treatment induces a modest down-regulation of the number of GCR which differs from the alterations seen in Type I SR asthma (51). Finally, Spahn et al. (52) examined GCR binding affinity in ten patients with symptomatic asthma prior to and following high dose prednisone therapy. These patients demonstrated significantly decreased GCR binding affinity at baseline. However, after prednisone treatment, there was an associated reduction in airway inflammation and a significant improvement in GCR binding affinity.

Molecular basis of SR asthma

The precise mechanisms by which cytokines such as the combination of IL-2 and IL-4 may decrease steroid responsiveness in patients with Type I SR asthma are unknown. There have been two postulated mechanisms neither of which is mutually exclusive. The first is based on *in vitro* observations that cytokines induce the activation of transcription factors and that overexpression of AP-1 or other transcription factors interferes with GCR binding and function (53). Indeed, Adcock et al. (54) have reported increased AP-1 expression in the PBMC of patients with SR asthma, and have suggested that abnormal GCR/AP-1 interactions leading to reduced GCR DNA binding might contribute to steroid resistance in asthma. However, further studies are needed to demonstrate direct interactions between AP-1 and the GCR in the cells of SR asthmatics.

A second potential mechanism is based on studies demonstrating that alternative splicing of the GCR pre-mRNA generates a second GCR, termed GCR β , which does not bind to GCs and antagonizes the transactivating activity of the classic GCR (47). The increased expression of GCR β could therefore account for SR asthma. Indeed, we have found that SR asthma is associated with a significant increase in the number of peripheral blood and BAL cells expressing GCR β (Fig. 1 and refs 47, 56). The expression of GCR β is inducible by the combination of IL-2 and IL-4, and markedly increased in BAL cells as compared to PBMC of SR asthmatics (Fig. 2). Interestingly, GCR β expression is markedly increased in the T cell subset as compared to other cell types found in BAL or PBMC of patients with SR asthma (Hamid and Leung, unpublished observations).

Factors which contribute to GC resistance

Since the majority of patients with SR asthma have an acquired form of GC resistance induced by immune activation, it is of interest to ascertain whether

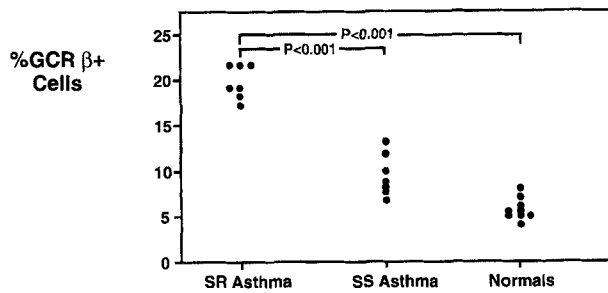


Fig. 1. Percent GCRβ⁺ cells in freshly isolated PBMC from steroid resistant (SR) asthmatics vs steroid sensitive (SS) asthmatics and control subjects. PBMC were processed and stained for GCRβ-immunoreactivity using an immunocytochemistry technique with a specific antibody to GCRβ. Reproduced with permission from reference 47.

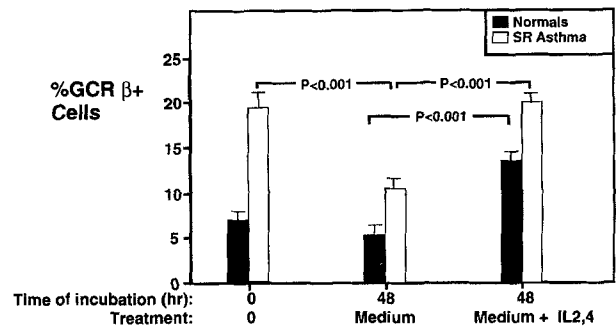


Fig. 2. Effect of combination IL-2 and IL-4 on percent GCRβ-immunoreactive cells in PBMC from normal subjects, steroid resistant (SR) asthmatics. PBMC from four normal donors and three SR asthma patients were analyzed for GCRβ immunoreactivity, freshly isolated (Time 0) and after incubation in culture medium or the combination of IL-2 and IL-4 for 48 hours. Reproduced with permission from reference 47.

factors, e.g. allergen exposure or microbial products, known to contribute to poorly controlled asthma and increased GC requirements, have an effect on GCR binding affinity or response to GCs. Indeed, Nimmagadda et al. (57) have recently examined whether exposure to allergens can alter GCR binding affinity in PBMC from atopic asthmatics. PBMC GCR binding affinity from 12 ragweed sensitive asthmatics were measured prior to, during the peak, and after ragweed season. A significant reduction in PBMC GCR binding affinity was seen during ragweed pollen season as compared to before and after ragweed season.

In vitro effects of allergen treatment on GCR binding affinity were also examined on PBMC from ragweed or cat allergic asthmatics by incubating their cells with ragweed or cat allergen. GCR binding affinity was significantly reduced after a 48-hour incubation with ragweed or cat allergen as compared to baseline. These effects were allergen-specific because *C. albicans* had no such effect on GCR binding affinity. Furthermore, allergen-induced reductions in GCR binding affinity were associated with significant decreases in the inhibitory effects of dexamethasone and hydrocortisone on T cells from these atopic asthmatics. The induction of GCR binding abnormalities was also found to be IL-2 and IL-4 dependent.

To further analyze the nature of the T cell activation in patients with poorly controlled asthma, we recently analyzed the T cell receptor repertoire in poorly controlled, as compared to well-controlled asthmatics, and normal controls (58). PBMC and BAL cells from 12 poorly controlled asthmatics (FEV_1 <75% predicted despite use of inhaled GCs), four well-controlled asthmatics (FEV_1 >85% predicted) and ten normal controls were stained for the

expression of different T cell receptor-BV regions on T cells using immunofluorescence and flow cytometry. In poorly controlled asthmatics, TCR-BV8+ (but not other TCR-BV+) T cells were significantly increased. BV8+ BAL T cells were abnormally increased in both the CD4+ and the CD8+ T cell subsets of poorly controlled asthmatics suggesting activation by a microbial superantigen (59).

To determine whether microbial superantigens alter steroid sensitivity, we assessed the capacity of 10^{-6} M dexamethasone to inhibit the stimulation of normal PBMC with a prototypic superantigen, staphylococcal enterotoxin E (SEE), vs phytohemagglutinin (PHA) (58). Dexamethasone caused a 99% inhibition of PHA-induced PBMC proliferation, but only a 29% inhibition of SEE-induced PBMC proliferation (PHA vs SEE, $p < 0.01$). Taken together, these observations suggest that bacterial or viral agents secreting superantigens may contribute to poorly controlled asthma and reduced GC sensitivity.

Persistent inflammation associated with high dose GCs

The above studies on SR asthma have generally involved patients with ongoing airway obstruction, i.e. FEV_1 <70% predicted, who were not on chronic oral GCs but demonstrated a lack of response to a 7–14-day course of oral GCs. However, many patients with severe asthma and chronic airway obstruction are treated with prolonged courses of high dose oral GCs. It is important to determine the nature of inflammation in such individuals because, if inflammation were absent, aggressive anti-inflammatory therapy would place patients at risk of side effects without providing any therapeutic benefit. Recently, Wenzel et al. (60) analyzed the inflamma-

tory cell infiltrate of BAL and bronchial biopsies in patients with severe asthma who were dependent on high dose oral GCs (mean $\pm$ SEM FEV₁ = 58 $\pm$ 6% predicted despite daily prednisone treatment of >20 mg/d for more than 1 year), and compared them to patients with moderate asthma (mean $\pm$ SEM FEV₁ = 65 $\pm$ 3% predicted but on no prednisone) and normal controls.

The concentration of eosinophils in BAL fluid was highest in the moderate asthmatics not on GCs, with little difference between normal controls and severe asthmatics. In contrast, the severe asthmatics demonstrated a significantly higher concentration of neutrophils in BAL than either the moderate asthmatics or the normal controls. Similar results were observed using bronchial biopsies from these three study populations. Patients with severe asthma were also found to have significantly higher levels of leukotriene B₄ and Thromboxane, which respectively can induce neutrophil chemotaxis and airway hyper-reactivity (61). These studies indicate a novel form of inflammation in severe symptomatic asthmatics despite treatment with high dose oral GCs. It remains to be determined whether these findings are due to a different pathologic form of asthma, a direct response to high dose GCs or an indirect response to a novel trigger, e.g. an infectious process in the airways. As noted above, so-called SR asthma is associated with a relative GC-insensitivity of T lymphocytes. Thus, it might be expected that treatment of such patients with a prolonged course of high dose systemic GCs could reduce the eosinophil/T lymphocyte driven process but leave behind, or even augment, a neutrophil-mediated process. Indeed, it is well established that neutrophils are steroid resistant and may even activate neutrophils (62, 63). Therefore treatment of these severe patients must take this into consideration.

Conclusions

In summary, SR asthma is associated with persistent immune activation and airway inflammation which is refractory to the immunosuppressive and anti-inflammatory effects of inhaled and/or systemic GCs. The large majority of patients with SR asthma appear to have an acquired GC ligand and DNA binding defect occurring as the result of cytokine stimulation. The molecular basis of these GC binding defects appear to be related to cytokine-induced expression of GCR β , a molecule which interferes with the actions of the classic GCR, and potentially the activation of transcription factors such as AP-1 which interfere with GCR DNA binding. The second group of SR asthmatics appear to have a primary GCR defect which is irreversible and not affected by cytokines. In severe asthmatics treated for pro-

longed periods with high dose oral GCs, neutrophils are the dominant cell in the inflammatory cell infiltrate of the airway. These data indicate that the basis for lack of response to GCs in patients with chronic asthma is quite complex.

An understanding of the mechanisms by which GCs fail to resolve inflammation in SR asthma are likely to provide important new insights into the cellular and molecular mechanisms which perpetuate chronic airway inflammation. Although this review has focused on patients with SR asthma it is likely that such patients are simply at the extreme end of a spectrum of disease. New information on mechanisms for GC resistance may therefore provide new directions for future strategies in management of the full spectrum of chronic asthma. These possibilities could include the use of GCs with higher binding affinity or resorting to alternative anti-inflammatory or immunomodulator therapy.

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