Allergology International (2000) **49**: 225–230

Review Article

Current understanding and problems of steroid-resistant asthma

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Abstract

Topical and systemic glucocorticoids have long been considered the most effective therapy for chronic asthma. Asthma patients who do not respond to systemic administration of high-dose glucocorticoids are termed 'steroid-resistant asthmatics'. Recent progress in the mechanisms of glucocorticoid action goes a long way to the understanding of steroid resistance. Several cellular responses to glucocorticoids have been characterized in steroid-resistant asthma. Based on molecular biological studies, excessive expression of glucocorticoid receptor β and *c-fos*, an inducible transcription factor, was suggested to result in steroid resistance. A thorough understanding of the pathophysiology of steroid-resistant asthma would facilitate the development of more powerful agents for the treatment of severe asthma.

Key words: bronchial asthma, cytokine, glucocorticoid, T cell.

Introduction

The role of persistent eosinophilic inflammation in the pathogenesis of bronchial asthma has been well documented.[1] For the management of chronic asthma, the use of anti-inflammatory agents, including inhaled glucocorticoids (GC), is advocated as the first-line treatment in various asthma guidelines.[2] However, it is well-known that asthmatic patients vary in their responsiveness to GC therapy.[3] 'Steroid-resistant' (SR) asthma represents a group of patients who respond to GC less effectively in comparison with others. Recent investigations have been directed towards the cellular and molecular alterations associated with SR asthma.[4–6] Elucidation of the precise mechanisms involved in steroid resistance may clarify what mode of GC action(s) is really relevant in the control of asthma.

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Received 5 June 2000.

Steroid-sensitive vs -resistant asthma

A group of asthmatic patients who had a poor clinical response to high-dose systemic GC therapy was first reported by Schwartz *et al*.[7] Later, Corrigan *et al*. and Alvarez *et al*. reported that the difference in patient responsiveness to GC could not be explained by pharmacokinetic characteristics, including oral absorption or clearance, of the agents.[8,9] To date, there is no established definition of steroid resistance, although most recent investigations have defined SR asthma as asthma in those patients who demonstrated a less than 15% improvement in forced expiratory volume in 1 s ($FEV_{1.0}$) after a course of oral prednisolone of 20–40 mg daily or equivalent doses for 10–14 days. Those patients who demonstrated more than 30% improvement tended to be classified as steroid-sensitive (SS) asthma patients. Kamada *et al*. studied the effect of oral GC on asthmatic children whose morning prebronchodilator $FEV_{1.0}$ was less than 70% predicted. They reported that more than 60% of patients who eventually responded to therapy demonstrated a more than 15% increase in their $FEV_{1.0}$ within 2–3 days and 93% showed a significant improvement within 10 days.[10] Prolongation of the course of therapy beyond 10 days did not bring about a significantly greater

improvement. Based on this finding, 2 weeks of high-dose prednisolone therapy has been considered appropriate to diagnose SR asthma.

Is steroid resistance a systemic or local phenomenon?

Familial GC resistance has been described.[11–13] Genomic mutation in the glucocorticoid receptor (GR) gene has been reported in several cases. These patients are characterized by extremely high serum cortisol concentrations and the absence of excessive GC action. They exhibited either reduced GR numbers, decreased binding affinity for GC or poor DNA binding of their GR to the glucocorticoid-responsive elements (GRE). However, in SR asthma, no such genomic alterations have been identified.

It is assumed that the effects of GC on the airway, but not its systemic effects, are impaired in SR asthma patients, thereby resulting in equivalent side effects compared with SS asthma patients.[14] It has been suggested that steroid resistance stems from a functional alteration induced locally by ongoing inflammation via yet unknown mechanisms. In contrast, SR asthma patients exhibited significantly less vasoconstrictor response to GC compared with SS asthma patients, suggesting that steroid resistance of SR asthmatics reflects a systemic phenotype.[15] So, steroid resistance cannot be explained by a single theory at present. Multiple pathogeneses may be involved.

Is SR asthma more severe than SS asthma?

One could imagine that SR asthma may represent patients suffering from severe asthma. Based on the severity classification of current asthma guidelines, steroid-dependent asthmatics requiring continuous systemic steroid therapy represent the most severe patients (step IV). This raises the question of whether or not SR asthma defined by the aforementioned criteria is identical to steroid-dependent asthma. The severity of the patients and the proportion of those on systemic (oral) steroids used in recent publications on SR asthma is shown in Table 1. Many studies on SR asthma apparently recruited patients with mild asthma. Based on this finding, it is assumed that steroid resistance *per se* does not mean that the patient has severe asthma. However, in several studies, the SR asthma group did contain more severe and steroid-dependent patients, suggesting that steroid resistance may be related to steroid dependence to some extent.

The clinical and pathophysiologic relationship between steroid-dependent (severe) asthma and SR asthma must be clarified to a greater extent. The criteria used for defining steroid resistance seem unequivocal, whereas the diagnosis for so-called steroid-dependent asthma has been hampered by many factors, including several differential diagnoses, confounding pulmonary and non-pulmonary disorders, patient compliance etc.[16]

Table 1 Is steroid-resistant asthma more severe than steroid-sensitive asthma?

Reference	Severity or patient characteristics	SR more severe than SS?	No. patients*	
			SR	SS
8	Moderate to severe %$FEV_{1.0}$: 49 vs 47	No	13 (3)	24 (4)
37	Mild %$FEV_{1.0}$: 71 vs 76	No	7 (0)	6 (0)
38	Mild (No BDI) %$FEV_{1.0}$: 61 vs 64	No	12 (0)	12 (0)
29	No oral steroids %$FEV_{1.0}$: 56 vs 47	No	6 (0)	6 (0)
30	Young atopics %$FEV_{1.0}$: 57 vs 47	Yes	17 (12)	12 (0)
43	Severe (high-dose BDI)	No	11 (2)	8 (2)
15	Severe and mild %$FEV_{1.0}$: 54 vs 82	Yes	15 (8)	31 (4)

*The number shown in the parenthesis is the number of patients who were taking oral steroids.
%$FEV_{1.0}$, forced expiratory volume in 1 s; BDI, beclomethasone dipropionate.

Mechanism of GC action

The understanding of GC action seems mandatory to clarify the pathogenesis of steroid resistance (Fig. 1). Because GC are highly lipid soluble, they can easily diffuse across the plasma membrane and enter the cytoplasm.[17] The existence of specific receptors on the cell surface is still controversial.[18] Two splice variants, GR-α and GR-β, have been identified in the cytoplasm, although only GR-α has the potential of ligand binding and transcriptional activation.[19] Without its ligands, GR-α forms an inactive complex with two molecules of heat shock protein (hsp) 90 and other associated molecules. When GR-α encounters GC, hsp are dissociated from GR and then the GC–GR complex is formed. The GC–GR complex translocates into the nucleus and binds as a homodimer to GRE located in the regulatory regions of the target genes to cause up- or downregulation of transcription. It has been reported that the GC–GR complex also interacts with other transcription factors, such as activator protein (AP)-1 and nuclear factor (NF)-κB,[20–23] thereby interfering with the transcriptional activation of several genes through these elements. So, multiple processes may become the candidates responsible for GC resistance.

Cellular and molecular investigations of SR asthma

Current diagnosis of SR asthma is based solely on the therapeutic effects of *in vivo* administration of GC. Great efforts have been made to characterize SR asthma by biological responses. One of the earliest studies reported a defect in the suppression of complement receptor expression on monocytes following GC therapy.[24] The proliferation of peripheral blood T cells in response to *Phaseolus vulgaris* agglutinin (PHA) and the production of monocyte-derived neutrophil chemotactic factors by peripheral blood mononuclar cells (PBMC) of SR asthmatics have also been reported to be less sensitive to GC.[25–27] Tumor necrosis factor (TNF) production by lipopolysaccharide-stimulated PBMC was also less sensitive to steroid action.[28]

Based on the fact that T lymphocytes are essentially involved in asthma, recent investigations have focused on possible T cell dysfunction in SR asthma. Corrigan *et al*. have reported that peripheral blood T cells of SR asthma expressed increased amounts of activation markers, such as CD25 and human leukocyte antigen (HLA)-DR, and not only PHA-induced proliferation but also interleukin (IL)-2 and interferon (IFN)-γ production was less sensitive to GC action.[8] These findings were confirmed by an *in vivo* study.[29] After a 1 week course of 40 mg daily prednisone therapy, the number of IL-4 and IL-5 mRNA-positive cells recovered in the bronchoalveolar lavage fluid was decreased in SS asthma but not in SR asthma. The number of IFN-γ mRNA-positive cells was increased in SS asthma but decreased in SR asthma. Taken together, these findings strongly suggest

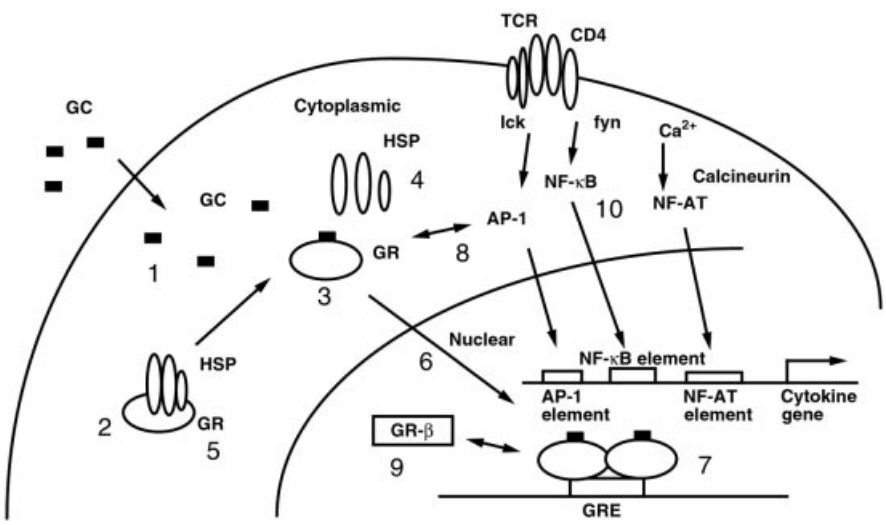

Fig. 1 Molecular understandings of glucocorticoid (GC) action. Glucocorticoid resistance may result from multiple steps. 1. Decreased cytoplasmic concentration of GC due to the accelerated clearance etc. 2. A lower number of glucocorticoid receptors (GR). 3. A lower affinity between the GC and GR. 4. Impaired dissociation of heat shock protein (hsp) 90. 5. Destabilization of GR molecules by dephosphorylation. 6. Impaired translocation of the GC–GR complex. 7. A lower affinity between GC–GR and glucocorticoid-responsive elements (GRE). 8. An increase in the molecular interaction between GR and activator protein (AP)-1 or GR and nuclear factor (NF)-κB etc. 9. An increased expression of GR-β. 10. An increased expression of activating transcription factors. TCR, T cell receptor; NF-AT, nuclear factor for activated T cells.

that T cells of SR asthma have a different responsiveness to GC action both *in vitro* and *in vivo*.

Alteration in GC–GR affinity, its reversibility and GR-β

Sher *et al*. performed a [^{3}H]-dexamethasone radioligand binding assay using PBMC obtained from 17 SR asthma patients.[30] They described type I SR asthma (15/17), in which GC–GR binding affinity was decreased three- to four-fold compared with SS asthma, whereas the total GC binding sites were increased three- to four-fold. Type II SR asthma (2/17) was characterized by the reduced number of total GC binding sites with apparently normal GC–GR binding affinity. The type I SR abnormality was reversed when cells were cultured *in vitro* for 48 h. Later, Kam *et al*. reported that the *in vitro* incubation of normal (SS) PBMC with IL-2 and IL-4 for 48 h reduced the GC–GR binding affinity in the T cell population (e.g. induction of type II SR abnormality).[31] In contrast, Spahn *et al*. demonstrated that IL-13 reduced GC–GR binding affinity in monocytes but not in T cells.[32] These findings suggest that the alteration in the type I SR asthma was a reversible phenomenon, possibly induced by the exposure of T cells and/or monocytes to inflammatory cytokines *in vivo*. Leung *et al*. have reported that the number of GR-β-positive cells was increased in PBMC of SR asthma patients.[33] Culturing cells in the presence of both IL-2 and IL-4 increased the number of GR-β-positive cells, consistent with the report of Kam *et al*.[31] The GR-α and GR-β are splice variants of a single GR gene and have N-terminal amino acids in common. The GR-β is only different from GR-α in its C-terminal 15 amino acids instead of the 50 amino acids present in GR-α. Because GR-β acts as a dominant negative form of GR-α,[19,34,35] the increase in GR-β content may explain the apparent reduction in GC–GR binding affinity.

Alteration in GR–GRE affinity and AP-1 induction

Adcock *et al*. reported that the amounts of GC–GR complexes that bind GRE were significantly less in the PBMC of SR asthmatics compared with those of SS asthmatics, whereas the affinity between the GC–GR complex and GRE was equivalent.[36] They also reported that the binding activity to the AP-1 element in the PBMC of SR asthmatics was twice as high as that of SS asthmatics, which could not be suppressed by dexamethasone treatment.[37] More recently, they found that *c-fos* expression of phorbol myristate acetate (PMA)-induced PBMC of SR asthmatics was four-fold higher than that of SS asthmatics.[38] These results collectively suggest that upregulation of *c-fos*, one member of the AP-1 family, may result in the lower responsiveness to GC in SR asthma. The functional relationship between the level of *c-fos* and the GC responsiveness warrants further investigation.

Other mechanisms potentially involved in GC resistance

Cyclic AMP-responsive element binding protein (CREB)-binding protein (CBP) and steroid receptor coactivator-1 (SRC-1) are the transcriptional coactivators that associate with GR.[39,40] They also associate with the transcription factor NF-κB. The competition for the transcriptional coactivators between the GR and NF-κB may be another mechanism for steroid resistance, because NF-κB is induced by inflammatory cytokines such as IL-1 and TNF-α.

Future directions

At this moment, immunosuppressants, including cyclosporine and FK506, seem promising for the treatment of SR asthma.[25,41,42] Increasing the dose of steroids by applying agents with fewer side effects (soft steroids) may be another practical approach. Upregulation of *c-fos* and GR-β seem good candidates responsible for the development of steroid resistance, but do not exclude other mechanisms. The number of molecules involved in the signal transduction, gene transcription and proliferation of inflammatory cells is growing rapidly. Elucidation of such molecules that lead to steroid-dependent asthma may help greatly the development of novel therapeutic agents in the near future.

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