

Clinical and molecular aspects of glucocorticoid resistant asthma

Chris J Corrigan
Tuck-Kay Loke

Division of Asthma, Allergy and Lung Biology, King's College London, London, England, UK; MRC and Asthma UK Centre in Allergic Mechanisms of Asthma, King's College London, London, England, UK

Abstract: This paper is an overview of the diagnosis, differential diagnosis and cellular and molecular mechanisms of glucocorticoid resistant asthma. It addresses the clinical definition and rationale for the diagnosis of therapy resistant asthma. It purports that, since glucocorticoid resistant asthmatics are not globally physiologically glucocorticoid resistant, then the phenomenon is most likely acquired, probably in immune cells (and most probably in T cells and monocyte/macrophages), as a result of local inflammatory and environmental influences. The molecular mechanisms which have been uncovered to date which could account for glucocorticoid resistance are discussed, in particular the roles of AP-1 and p38 MAP kinase signaling, the role of the β -isoform of the glucocorticoid receptor and the role of histone proteins and DNA folding. Finally, there are suggestions for clinical management of these patients based on accumulated evidence.

Introduction

Glucocorticoids are very effective therapy for asthma and numerous studies have shown that they reduce asthma symptoms, exacerbations and bronchial hyperresponsiveness. The benefit/risk ratio of glucocorticoid therapy was enhanced further in the 1960's when topical delivery devices were introduced. Inhaled glucocorticoids are now the first line therapy in patients with all but the mildest disease.

The vast majority of asthmatics are controlled satisfactorily with regular inhaled glucocorticoids with or without the addition of short- or long-acting bronchodilators. In such patients, particularly those on low to moderate dosages of inhaled glucocorticoids, it is hard to conceive of a safer or more effective therapy.

Unfortunately, however, a proportion of patients develop severe disease which is relatively or totally refractory to glucocorticoid therapy. The possible etiology of this condition will be discussed in subsequent paragraphs, but the existence of the phenomenon implies that asthma is heterogeneous in terms of its susceptibility to inhibition by glucocorticoids, and possibly therefore also in terms of its etiology.

While the percentages of patients with glucocorticoid resistant asthma are small, these patients consume a significant proportion of medical resources in terms of both time and money (Buist 1995). Regardless of costs, there is an urgent need to provide alternative therapies for these patients, who often have severely impaired quality of life not only from the severity of their symptoms but from the effects of excessive glucocorticoid exposure.

Difficult asthma

Definition

Although any clinician managing asthmatic patients will have a clear picture of "difficult to treat" disease, it is in fact extraordinarily difficult to define this condition accurately, particularly for the purposes of research.

Correspondence: Chris Corrigan
5th Floor, Thomas Guy House, Guy's Hospital, London SE1 9RT, UK
Tel +44 207 188 0599
Fax +44 207 403 8640
Email chris.corrigan@kcl.ac.uk

Definition of “difficult asthma” could include the following:

- Confirmation of the diagnosis of asthma
- An unusually poor response to therapy
- Chronicity of the problem.

Problems arise with all of these concepts. Obviously it is essential to be as certain as possible of a primary diagnosis of asthma. This diagnosis of course rests on a clinical history of typical symptoms and physiological evidence of variable and reversible airways obstruction. Often, however, on presentation to a specialist, it may not be possible to obtain contemporary evidence of these features if the patient is already established on therapy. Clearly it is essential to search for and eliminate other possible alternative or associated diagnoses (Table 1). Allergic bronchopulmonary aspergillosis and pulmonary eosinophilic syndromes (for example, pulmonary eosinophilia or Churg-Strauss syndrome) may be considered unique diseases which encompass some of the clinical features of asthma and which are often difficult to treat: these are probably best considered outside the definition of “difficult” asthma. Vocal cord dysfunction characterized by paradoxical adduction of the vocal cords may masquerade as and co-exist with asthma (Newman et al 1995). Other respiratory conditions such as chronic bronchitis or bronchiectasis may also co-exist with asthma, but there are very few studies assessing the effect of these in the long term on asthma severity or control.

“Resistance to therapy” is usually identified as failure of asthma “control” despite therapy. This does, however, imply that all patients who are resistant to glucocorticoid therapy will necessarily have severe disease, which may not be the case. It also begs the question of what is used to define asthma “control”. This is usually assessed in terms of symptoms and requirement for short-acting β_2 -agonist medication. Patients vary in their perception of airflow limitation and poor perceivers may be particularly prone to severe attacks (Kikuchi et al 1994). Other techniques commonly used to define asthma “control” include objective monitoring of airways obstruction and the numbers of exacerbations experienced by patients defined according to various criteria (such as, for example, those requiring systemic glucocorticoid therapy). The international guidelines (Global Initiative for Asthma 1995) define asthma severity in terms of airways obstruction as measured by PEF or FEV₁. Nevertheless, the presence of persistent airways obstruction does not necessarily reflect loss of asthma “control”. The value of using asthma biomarkers to assess the amount of airways inflammation and the relationship of these measurements to asthma control is

Table 1 Diagnoses that may masquerade as difficult/therapy-resistant asthma

In children

Obliterative bronchiolitis
Vocal cord dysfunction
Bronchomalacia
Inhaled foreign bodies
Cystic fibrosis
Recent aspiration (particularly in handicapped children)
Developmental abnormalities of the upper airway
Immunoglobulin deficiencies
Primary ciliary dyskinesia

In adults

Cystic fibrosis
Bronchiectasis
Inhaled foreign body
Tracheobronchomalacia
Recurrent aspiration
Chronic obstructive pulmonary disease
Congestive cardiac failure
Tumours in or impinging on central airways
Obstructive bronchiolitis
Vocal cord dysfunction
Bronchial amyloidosis

As part of the asthmatic diathesis

Allergic bronchopulmonary aspergillosis
Pulmonary eosinophilic syndromes
(eg, Churg-Strauss, pulmonary eosinophilia)

also currently under close scrutiny. There is little evidence to date, however, that short-term measurements of biomarkers can predict the severity and chronicity of symptoms. Consequently, the concept of “difficult asthma” at present requires our third dimension of chronicity of observation. Increasingly, the amount of inhaled glucocorticoid and other therapy needed for the control of asthma, rather than quantification of symptoms or of lung function is being used to define asthma severity (Cockcroft and Swystun 1996).

A number of factors may contribute to poor asthma “control”, and must be taken into account before labeling the patient as glucocorticoid refractory or resistant (Table 2). Since response to therapy is part of the definition of “difficult” asthma, the question of patient adherence to therapy must be considered. Compliance with inhaled glucocorticoid therapy in asthma has been reported to be very poor (Kelloway et al 1994; Robinson et al 2003) and it might be expected, although there is actually little hard evidence (Cochrane 1992; Milgrom et al 1996) that this contributes to poor asthma control. Insufficient therapy is probably a much bigger contributor to poor asthma control worldwide (Rabe et al 2004). Psychosocial factors, which may be linked with or compounded by poor patient compliance and lack of appropriate medical care (Miller and Strunk 1989; Wareham

Table 2 Factors that may be responsible for poor asthma control Incorrect diagnosis

| |
|--------------------------------------|
| Poor compliance/adherence to therapy |
| Psychosocial and emotional factors |
| Inadequate medical facilities |
| Poor access to medical facilities |
| Inadequate treatment |
| Exposure to allergens |
| Viral respiratory tract infections |
| Indoor/outdoor pollution |
| Gastro-oesophageal reflux |
| Rhinosinusitis |
| Genetic factors |

et al 1993; Chung et al 1999; American Thoracic Society 2000) have also been implicated in poor asthma control and asthma deaths. Incorrect diagnosis of respiratory symptoms as asthma is a cause of apparent poor responsiveness to asthma therapy, and this is not as uncommon as might be expected, even in specialist asthma centres (Robinson et al 2000). Although a precise mechanistic link between gastro-oesophageal reflux and a decline in asthma control is not established, varying degrees of improvement in asthma have been observed when concomitant gastro-oesophageal reflux has been treated (Spaulding et al 1982). Targeted treatment of rhinosinusitis, often present concurrently with asthma, can lead to specific improvement in asthma control (Corren et al 1982). Although attention to these comorbid factors seems pertinent, there is little evidence that they exert a global impact on the response of asthmatics to therapy: indeed one study (Heaney et al 2003), although showing a prevalence of comorbid conditions in patients with poorly controlled asthma, failed to show that such conditions were more prevalent in asthmatics who responded poorly to glucocorticoids. Moreover, targeted treatment of identified comorbidities barely altered asthma-related quality of life in those patients with glucocorticoid-insensitive disease.

Clinical and pathophysiological phenotypes of “difficult” asthma

Studies attempting to delineate clinical phenotypes of “difficult” asthma are ongoing in the United States and Europe (ENFUMOSA 2003; Dolan et al 2004; Miller et al 2005). The ENFUMOSA study (ENFUMOSA 2003) showed that severe asthmatics were more likely to be female, more likely to be aspirin sensitive and less likely to be atopic. Although some clinical subgroups such as “brittle” asthmatics and glucocorticoid resistant asthmatics are recognized as being difficult to treat, these studies have not yet provided a viable

framework upon which to predict responsiveness to therapy on clinical grounds.

Attempts have also been made to delineate pathophysiological features of asthma which predict a poor response to therapy. The problem with such studies in severe asthmatics is that it is difficult to discriminate a priori abnormalities from the effects of the high dosages of glucocorticoid and other therapies that these patients are inevitably receiving. Much circumstantial evidence implicates eosinophils in asthma pathogenesis, although as with all inflammatory leukocytes the precise mechanisms by which these cells cause the clinical features of asthma, and why these mechanisms might be therapy resistant, remain unclear. One study described a group of apparently therapy resistant severe asthmatics with high numbers of airways eosinophils (Silkoff et al 2005), but there is evidence (ten Brinke et al 2004) that such patients may respond if treated with sufficiently high dosages of glucocorticoid. On the other hand, Wenzel and colleagues (Wenzel et al 1997, 1999) have shown that it is possible to delineate groups of severe, glucocorticoid dependent asthmatics with numbers of airways eosinophils within the range observed in normal controls. One of these studies suggested that these patients also had increased airways neutrophils, while the other did not. The airways changes associated with asthma which are collectively termed “remodeling” (lay down of new proteins, mucous hyperplasia, neovascularisation, smooth muscle hypertrophy/hyperplasia) could conceivably be resistant to therapy and cause irreversible airways blockage, although this has never been formally demonstrated. Clinically, patients with severe asthma and irreversible airways obstruction show tomographic abnormalities suggestive of remodeling (Bumbacea et al 2004). Whether remodeling is caused by inflammatory cells or cytokines, or both, is still not clear. In animals, over expression of the cytokine IL-13 alone in the airways reproduced all the features of airways remodeling (Kibe et al 2003); glucocorticoid administration abolished the associated cellular infiltrate but not the remodeling changes. In human studies, anti-IL-5 therapy was shown to reduce lay down of extracellular matrix proteins in the lungs while partly but not completely abolishing the infiltration of eosinophils (Flood-Page et al 2003), leaving the question open whether or not leukocytes such as eosinophils play an indispensable role in this process. It has been suggested that one etiological factor in intrinsic asthma is autoimmune attack of the airways, for example by autoantibodies (Lasalle et al 1993; Nahm et al 2002).

In summary, despite this wealth of possible aetiological factors which may contribute to loss of asthma control, it is

still not clear which of these can be clearly implicated in the genesis of glucocorticoid refractory or resistant asthma. It is furthermore not clear whether these aetiological factors lead to specific features of asthma pathology or contribute specifically to any feature of the natural history of the disease.

Environmental factors and “difficult” asthma

Oxidative stress and anti-oxidants

Markers of oxidative stress such as 8-isoprostane are elevated in severe asthma (Katsoulis et al 2003; Kharitonov and Barnes 2003). Cigarette smoking and poor dietary anti-oxidant intake are potential sources of oxidative stress, and it has been shown that asthmatics who smoke are clinically more resistant to glucocorticoid therapy (Chaudhuri et al 2003). Oxidative stress can affect several aspects of glucocorticoid activity, for example through activation of the transcriptional regulatory protein AP-1 or by reducing nuclear translocation of the glucocorticoid receptor (see below).

Viral and other infections

Recurrent respiratory tract infections, most of these viral, increase the risk of asthma exacerbation (ten Brinke et al 2005), although it is not clear whether they alter responsiveness to glucocorticoid therapy. Very little is known about the mechanisms by which viral infections exacerbate asthma, and this field deserves much more study. There is also some evidence for the involvement of latent chlamydial (Hahn et al 1991) and, at least in animal models, adenoviral infections (Yamada et al 2000).

Allergen exposure

There is abundant evidence (summarized by Leung and Bloom 2003; Busse et al 2005) that exposure of sensitized asthmatics to clinically relevant allergens requires increased glucocorticoid therapy for disease control. It has been shown (Nimmagadda et al 1997) that seasonal allergen exposure of atopic asthmatics increases refractoriness of their T cells to glucocorticoid in an allergen-specific and IL-2 and IL-4 dependent fashion (see also below).

Microbial superantigens

There is evidence in other atopic diseases, particularly atopic dermatitis that colonization of the target organ (which is common) or infection with *Staphylococcus aureus* confers resistance to glucocorticoid therapy. Preferential expansion of T cells expressing particular antigen receptor V β chains has been observed in poorly controlled asthmatics (Hauk et al 1999), suggesting activation by microbial superantigen. In addition to T cell activation, superantigen has been shown

to confer T cell resistance to glucocorticoid by induction of the β -isoform of the glucocorticoid receptor and by phosphorylation of the glucocorticoid receptor (Hauk et al 2000; Li et al 2004).

The concept of glucocorticoid resistant asthma

The diagnosis of glucocorticoid resistant asthma is essentially one of exclusion. Before it can be diagnosed, it must be ensured that the diagnosis of asthma is correct and that factors contributing to poor asthma control have been eliminated as far as is possible. These stages have been formalized (Woolcock 1993) as follows:

- Establish/confirm the diagnosis of asthma (consider differential diagnosis – see Table 1);
- Ensure that adequate dosages of glucocorticoids are reaching the airways (compliance, inhaler technique, metabolic factors which may increase glucocorticoid clearance);
- Exclude ongoing exposure to provoking agents (smoke, irritants, allergens, etc.);
- Rule out and eliminate as far as possible other potential aggravating conditions (chronic sinusitis, esophageal reflux, drugs which may exacerbate asthma, etc.);
- Rationalize inhaled β 2-agonist therapy;
- Introduce a strict management plan to assess response to therapy which will typically last for weeks or sometimes months.

Even when all factors which may abrogate the effects of glucocorticoid therapy are eliminated or minimized as far as possible, there remains a group of patients who show little or no response of airways obstruction to glucocorticoid therapy. This concept was first proposed over 30 years ago (Schwartz et al 1968). In this study, the authors described asthmatic patients in whom disease was poorly controlled, and the typical peripheral blood eosinopaenic response diminished, on large oral dosages of glucocorticoids. Later, it was recognised that there were some asthmatics in whom the diurnal pattern of airways obstruction is little altered by glucocorticoid therapy (Clark and Hetzel 1977).

The first attempt to define glucocorticoid resistant asthma in objective terms (Carmichael et al 1981) was based on changes in base line FEV₁ following a 14 day course of oral prednisolone (40 mg/day). Patients showing improvements of <15% of baseline were classified as resistant, whereas those showing improvements of 30% or more were considered glucocorticoid sensitive. All patients, in contrast, showed marked improvement in FEV₁ in response to inhaled

β_2 -agonists, indicating that, at least in this group of patients, the glucocorticoid response did not reflect “irreversible” airways obstruction (although, as discussed, this may be a factor in other patients). Clearly, these FEV₁ responses represent opposite ends of a continuum of clinical response. Most subsequent studies have employed definitions of glucocorticoid sensitive and resistant asthma similar or identical to the above, in both adults and children (Kamada et al 1992; Alvarez et al 1992). The possibility that glucocorticoid “resistant” subjects who show no clinical response following two weeks of oral glucocorticoid therapy might nevertheless respond following more protracted therapy has never been formally addressed, although it was shown (Kamada et al 1993) that 90% of severe asthmatic children showing an improvement in FEV₁ >15% of baseline on high-dosage oral glucocorticoid therapy did so within 10 days.

On the basis of these studies, a workshop of experts on glucocorticoid resistant asthma proposed that this should be defined by the failure to improve baseline morning pre-bronchodilator FEV₁ by more than 15% of the baseline value following at least 14 days of therapy with prednisolone 40 mg daily or its equivalent (Lee et al 1996). One weakness of this definition is that patients with refractory asthma are typically taking large dosages of inhaled glucocorticoid, the therapeutic effect of which may have reached a “ceiling” limiting further clinical response. Nevertheless, patients with complete glucocorticoid resistance show not only a failure of response in terms of FEV₁, but also, in general, an ability to tolerate reduction of glucocorticoid dosages without significant change in disease activity. In addition, they typically show little increase in FEV₁ even with more protracted, much higher dosages of systemic glucocorticoids. This is in distinction to glucocorticoid “dependent” asthmatics, who may not show a response in FEV₁ of 15% or more during a 14 day trial of systemic glucocorticoid therapy, but rapidly deteriorate when this therapy is withdrawn. Although it is assumed that these patients fall at the end and near end of a spectrum of glucocorticoid responsiveness in asthma, it is not certain whether these two groups of patients have fundamentally different abnormalities leading to glucocorticoid refractoriness. Although glucocorticoid resistant patients may show a degree of fixed airways obstruction, many show marked diurnal variability in PEF and a brisk bronchodilator response (Alvarez et al 1992; Kamada et al 1992).

It is inherent in the diagnosis of glucocorticoid resistant asthma that the diagnosis of asthma has been made correctly and that factors confounding therapy have been eliminated or reduced as far as possible. Most clinical descriptions of

glucocorticoid resistant asthma emphasize the following common clinical features (Spahn et al 1997):

- Persistent symptoms despite optimal therapy;
- Chronic airflow limitation with FEV₁ <60% predicted in adults and <80% predicted in children;
- Failure to achieve an increase in morning pre-bronchodilator FEV₁ of >60% predicted despite systemic glucocorticoid therapy (at least 40 mg/day of prednisolone or its equivalent given for at least 14 days);
- Frequent nocturnal symptoms with significant “morning dipping” of PEF;
- Poor clinical and spirometric response to oral glucocorticoid therapy, with <15% improvement in pre-bronchodilator FEV₁ following a trial of oral glucocorticoid therapy as specified.

It should be noted finally that, although glucocorticoid resistant patients are identified by definition on the basis of having severe disease which does not respond to therapy, it cannot be excluded that patients with milder disease may also nevertheless be glucocorticoid resistant. It would require a large and protracted study to investigate this hypothesis, but the point has a bearing on whether or not the severity of disease itself actually regulates, at least in part, glucocorticoid responsiveness (see below).

Mechanisms of glucocorticoid resistant asthma

Despite several decades of usage of glucocorticoids as anti-inflammatory agents, in general very little is known about the precise mechanisms by which they ameliorate inflammatory diseases. A good review is provided by Ito et al (2006). Glucocorticoids exert a number of generalized anti-inflammatory activities, such as capillary vasoconstriction and reduction of vascular permeability, which may be relevant to suppression of inflammation however caused. In the case of asthma, it is now generally accepted that bronchial mucosal inflammation is a fundamental feature of disease pathogenesis. Despite the doubts about the precise roles (if any) of effector inflammatory leukocytes discussed above, a working scenario is that eosinophils are regarded as pro-inflammatory effector cells the products of which damage the bronchial mucosa, causing variable airways obstruction and bronchial hyperresponsiveness. Selective eosinophil accumulation and activation in asthma is in turn brought about by the release of eosinophil-active cytokines, particularly IL-3, IL-5 and GM-CSF, principally from activated T cells but also partly from other inflammatory cells, including mast cells and eosinophils themselves (Corrigan and Kay 1992).

If a mechanistic role for leukocytes such as eosinophils in asthma has become less clear recently, there remains much better evidence that glucocorticoid therapy which results in amelioration of asthma is associated with reduced activation of, and synthesis of asthma-relevant cytokines by activated T cells. For example, elevated percentages of peripheral blood CD4, but not CD8 T cells from patients with exacerbation of asthma expressed mRNA encoding IL-3, IL-5 and GM-CSF but not IL-2 and IFN- γ as compared with controls (Corrigan et al 1995). Spontaneous secretion of the corresponding cytokines was also demonstrable in these patients using an eosinophil survival-prolonging assay. The percentages of CD4 T cells expressing mRNA encoding asthma-relevant cytokines, as well as spontaneous secretion of these cytokines was reduced in association with glucocorticoid therapy and clinical improvement. In a double-blind, parallel group study (Robinson et al 1993), therapy of mild atopic asthmatics with oral prednisolone, but not placebo, resulted in clinical improvement associated with a reduction in the percentages of BAL fluid cells expressing IL-5 and IL-4 and an increase in those expressing IFN- γ . These and other studies have provided overwhelming evidence in support of the general hypothesis that, in asthma, activated CD4 T cells secrete cytokines that are relevant to asthma pathogenesis, through direct actions on the airways mucosa or activation of inflammatory leukocytes such as eosinophils, and that glucocorticoids exert their anti-asthma effect at least partly by reducing the synthesis of cytokines by these cells.

Functional T cell abnormalities in glucocorticoid resistant asthma

A reasonable hypothesis is, then, that glucocorticoid resistant asthma may reflect refractoriness of T cells to glucocorticoid inhibition. A pioneering study in this field (Poznansky et al 1984) showed that when peripheral blood mononuclear cells from glucocorticoid sensitive and resistant asthmatics were cultured with the T cell mitogen phytohaemagglutinin (PHA) in soft agar in vitro, methylprednisolone (10^{-8} M) produced a lesser degree of inhibition of colony formation by the cells from the resistant asthmatics, suggesting impaired T cell inhibition by glucocorticoids in these patients. This observation was followed up with two reports from the author's laboratory (Corrigan et al 1991a, 1991b) characterizing peripheral blood T cells of glucocorticoid sensitive and resistant asthmatics. In summary, it was demonstrated in these reports that PHA-induced proliferation of peripheral blood T cells was inhibited by dexamethasone at therapeutic concentrations in glucocorticoid sensitive, but not resistant asthmatics. This

resistance was not absolute but relative, reflecting a shift in the concentration-response curve for inhibition. In other words, T cells from glucocorticoid resistant asthmatics can be inhibited by glucocorticoids, but only at concentrations requiring glucocorticoid dosages that most physicians would not contemplate using for protracted periods in clinical practice. Consistent with this, it was demonstrated (Corrigan et al 1991b) that elevated percentages of peripheral blood T cells expressed the activation markers in CD25 and HLA-DR in glucocorticoid resistant, as compared with sensitive asthmatics, with no differences in total cell numbers, suggesting persistent T cell activation in the resistant patients despite glucocorticoid therapy.

In glucocorticoid resistant asthmatics, clinical resistance to therapy could not be accounted for by differences in absorption and clearance of plasma prednisolone derived from orally administered prednisone. We have subsequently shown (Haczku et al 1994) that the inhibition of PHA-induced proliferation of peripheral blood T cells from asthmatics by glucocorticoids in vitro is reproducible both in the short term and when patients are re-tested after intervals of several months. This suggests that the degree of glucocorticoid sensitivity of peripheral blood T cells from asthmatics, and by inference their clinical sensitivity to glucocorticoid therapy, remains relatively constant, although there is evidence (see below) that sensitivity in individual patients may vary to some degree according to the ongoing severity of their disease.

Glucocorticoid resistant asthmatics are not Addisonian and do not have elevated plasma cortisol concentrations (Corrigan et al 1991a), suggesting that the impaired glucocorticoid responsiveness observed in their peripheral blood T cells is not a generalised, systemic phenomenon. One possibility is that impaired T cell glucocorticoid responsiveness in asthma may be induced by the action of pro-inflammatory cytokines within the local environment of the inflammatory process. In this regard, there exists evidence that the glucocorticoid receptor ligand binding affinity of peripheral blood T cells in asthmatics may be altered in the short term according to disease severity in vivo and by exposure to cytokines in vitro. The glucocorticoid receptor ligand binding affinities of peripheral blood T cells from a group of poorly controlled asthmatics were reduced as compared with normal controls (median K_d 29.0 vs 8.0 nM). Glucocorticoid therapy of these asthmatics, which was accompanied by clinical improvement, was associated with a significant increase in affinity of the T cell glucocorticoid receptors (Spahn et al 1995). In a detailed study of glucocorticoid receptor binding

in peripheral blood mononuclear cells from glucocorticoid sensitive and resistant asthmatics, two distinct abnormalities were observed (Sher et al 1994). The majority of the asthmatics (15 out of 17) demonstrated a significantly reduced receptor binding affinity (mean K_d 42.1 nM) as compared with sensitive patients (mean K_d 21.6 nM) and normal controls (mean K_d 7.9 nM). This abnormality was confined to T cells, reverted to normal after culture of the T cells *in vitro* for 48 hours, but could be sustained by culture in the simultaneous presence of high concentrations of IL-2 and IL-4. The remaining two glucocorticoid resistant asthmatics had abnormally low numbers of nuclear glucocorticoid receptors with normal binding affinity. This abnormality was not confined to T cells and was not influenced by the presence of exogenous cytokines. It was further shown (Kam et al 1993) that this abnormality could be induced by culture of peripheral blood T cells from normal donors with IL-2 and IL-4 *in vitro*, and that this induction was associated with a reduced inhibitory effect of methylprednisolone on the proliferation of the T cells induced by phorbol ester and ionomycin. A similar effect of exogenous IL-13 alone was subsequently demonstrated in monocytes (Spahn et al 1996). The intrinsic physiological significance of these relatively small changes in ligand binding affinity of the glucocorticoid receptor is difficult to assess, but clearly they may contribute to glucocorticoid refractoriness. More likely, they may represent an epiphenomenon reflecting more fundamental changes in the properties of the glucocorticoid receptor in T cells following exposure to cytokines (see below).

There exists similar evidence for a differential effect of glucocorticoids on T cells from sensitive and resistant asthmatics *in vivo* (Leung et al 1995). In this study, bronchoalveolar lavage was performed in glucocorticoid sensitive and resistant asthmatics before and after a course of oral prednisone, and expression by BAL cells of mRNA encoding cytokines was measured by *in situ* hybridization. Whereas prednisone therapy of the glucocorticoid sensitive asthmatics was associated with reductions in the percentages of BAL cells expressing mRNA encoding IL-4 and IL-5 and elevation of the percentages of cells expressing mRNA encoding IFN- γ , only a decrease in the percentages of cells expressing mRNA encoding IFN- γ was observed in association with prednisone therapy of the glucocorticoid resistant asthmatics. In addition, compared with the sensitive asthmatics, the resistant patients had elevated percentages of BAL cells expressing mRNA encoding both IL-2 and IL-4 at baseline. These data are compatible with the hypothesis that glucocorticoids exert differential effects on cytokine mRNA expression in

T cells from glucocorticoid sensitive and resistant asthmatics *in vivo*. The authors also speculated that the local elevated expression of IL-2 and IL-4, at least at the level of mRNA, in these patients might have been responsible for some of the glucocorticoid refractoriness of the cells described in the *in vitro* experiments above.

Although glucocorticoids generally reduce inflammation by inhibiting the production of pro-inflammatory cytokines, one interesting facet of glucocorticoid action, which is receiving increasing attention, is their ability to increase the production of the anti-inflammatory cytokine interleukin-10 (IL-10). Studies in patients, initially in transplantation (Wan et al 1996), and more recently in other conditions including asthma (John et al 1998), have demonstrated that administration of glucocorticoids, intravenously or by inhalation, results in a significant increase in systemic or local IL-10 synthesis respectively. Parallel studies from our own laboratories have shown a concentration-dependent induction by glucocorticoids of T cell IL-10 expression *in vitro* (Richards et al 2000). Interest in the possible therapeutic benefit of IL-10 in asthma already exists, based on its proposed role in regulating immune homeostasis in the lung (Akbari et al 2001). IL-10 inhibits pro-inflammatory cytokine production, antigen presentation, T cell activation and mast cell and eosinophil function (reviewed by Moore et al 2001). Furthermore, synthesis of IL-10 is deficient in the airways of asthmatics as compared with controls (Borish et al 1996), and polymorphisms of the IL-10 gene leading to impaired expression of IL-10 are associated with a more severe disease phenotype (Lim et al 1998). We have recently described a marked deficiency in the capacity of CD4⁺ T cells from glucocorticoid resistant asthmatics to synthesize IL-10 following *in vitro* stimulation in the presence of dexamethasone, as compared with sensitive patients of equivalent disease severity (Hawrylowicz et al 2002). We have furthermore shown that vitamin D3 in combination with glucocorticoids can restore the ability of blood CD4 T cells from patients with glucocorticoid resistant asthma to manufacture IL-10 in quantities comparable with those of sensitive patients, and that oral administration of vitamin D3 to glucocorticoid resistant asthmatics for seven days enhanced *ex vivo* responses of their T cells to glucocorticoid (Xystrakis et al 2006).

Molecular basis of glucocorticoid action and resistance

Observations made in the past few years have greatly increased our knowledge of how the GR regulates transcription, and how this process may be modified both

in vitro and in vivo. The GR comprises three domains, the N-terminal or immunogenicity domain, the central DNA-binding domain and the C-terminal ligand-binding domain (Kino and Chrousos 2001; Ito et al 2006). The GR in its ligand-unbound state is located primarily in the cytoplasm as part of hetero-oligomeric complexes containing the heat shock proteins 90, 70 and 50. Upon binding to ligand, the GR undergoes conformational changes, dissociates from the heat shock proteins, homodimerizes and translocates to the nucleus. There, the ligand-activated GR may interact with DNA sequences (glucocorticoid response elements) or with other transcriptional regulators through protein/protein interactions, directly influencing the activity of the latter on their target genes (Figure 1).

Proteins which may bind directly to the GR and modulate its activity in this way include AP-1, NF κ B, signal transduction and activators of transcription (STATs) and certain of

the CCAAT-enhancer binding proteins (C/EBP). These interactions appear to be particularly important in glucocorticoid-mediated suppression of inflammation, and may allow the activated GR to transrepress expression of cytokine genes without binding to DNA at all (Reichhardt et al 2001).

Interaction of GR with AP-1

The pro-inflammatory transcriptional element activator protein-1 (AP-1) is an important contributor to the expression of the asthma-relevant Th2 cytokines IL-4, IL-5 and IL-13. AP-1 comprises of variable heterodimers of Jun (c-Jun, JunB and JunD) and Fos (c-Fos, FosB, Fra1 and Fra2) family members. AP-1 is inducible by a variety of cytokines and growth factors (Lee et al 1987), and also by oxidative stress (see above). It is activated through the phosphorylation of c-Jun and the transcriptional regulation of c-Fos (Figure 2). Phosphorylation of c-Jun is the end result of the action of a

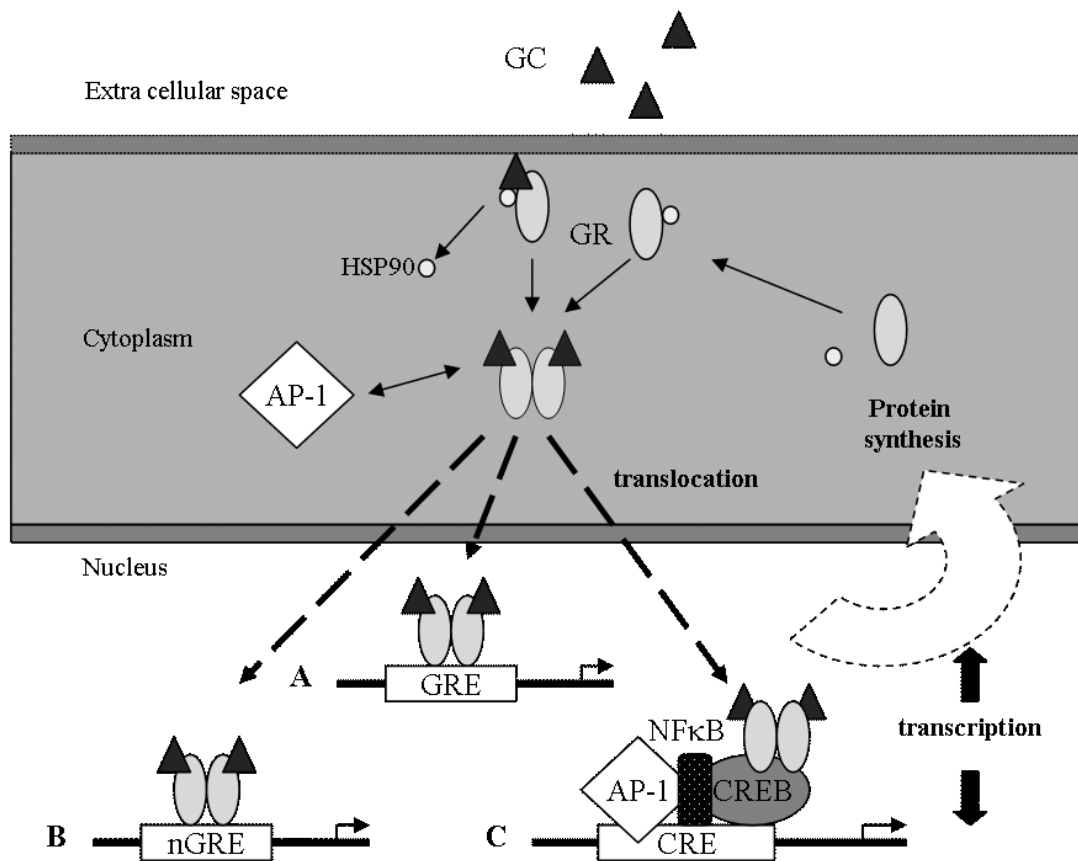


Figure 1 Mechanism of glucocorticoid action. After diffusion across the cytoplasmic membrane, glucocorticoid (GC) binds to the glucocorticoid receptor (GR) which dissociates from heat shock protein 90 (HSP90) then dimerises. In the cell cytoplasm it may interact with transcriptional regulatory proteins such as AP-1; such interactions are mutually inhibitory. Nuclear translocation of the ligand bound GR is regulated by chemical modifications, such as phosphorylation, and possibly other mechanisms. Once inside the nucleus, the ligand bound receptor may interact directly with binding regions (glucocorticoid regulatory elements, GRE) adjacent to genes, either increasing (A) or decreasing (B, negative GRE) transcription of these genes. More usually, however, the ligand-bound GR interacts with DNA as part of a highly dynamic complex of proteins including transcriptional regulatory proteins such as AP-1, NF κ B and cyclic AMP response element binding protein (CREB) (see text). The complexity of these interactions in individual cells accounts for the remarkable variability of glucocorticoid actions on cells according to their function and phenotype.

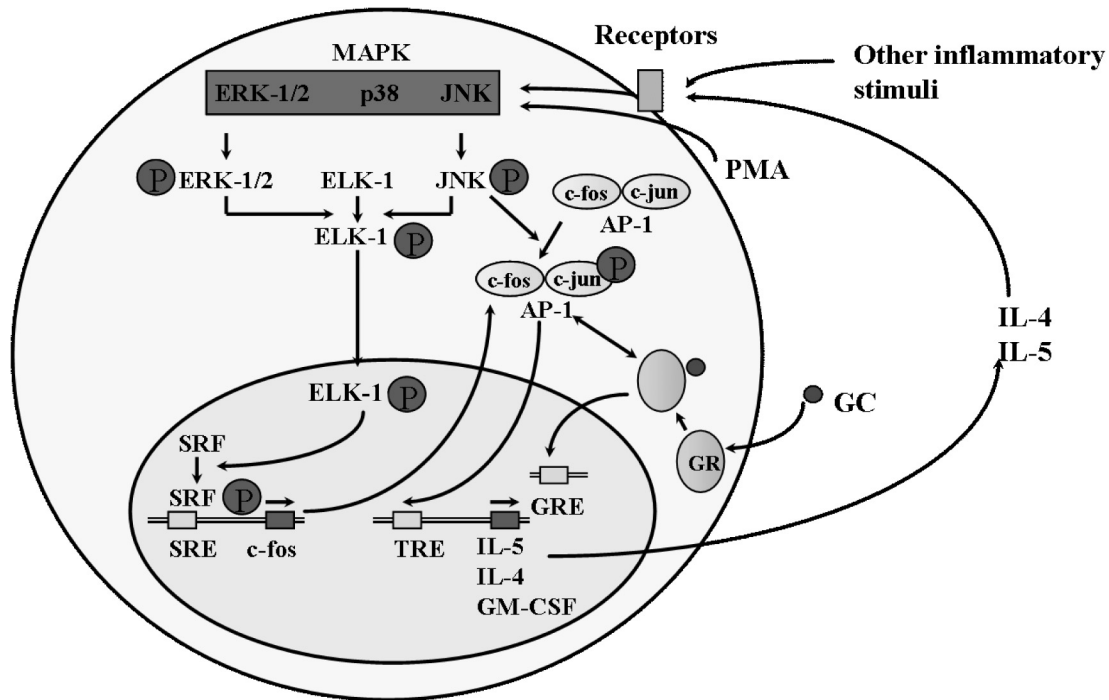


Figure 2 Regulation of activation of c-fos and c-jun, the components of AP-1. Transcription of c-fos is induced by serum response factor (SRF) which binds to the serum response element (SRE) adjacent to the c-fos gene. SRF is activated by phosphorylation by ELK-1, which is in turn activated by phosphorylation of a range of MAP kinases including ERK-1/2 and JNK (see text). Phosphorylated JNK also phosphorylates and activates c-jun, which together with c-fos forms the heterodimer complex AP-1. AP-1 increases the transcription of a number of asthma-relevant cytokine genes such as interleukin (IL)-4 and IL-5. This activity is inhibited by interaction with the ligand bound glucocorticoid receptor (GR) (see text and Figure 1 above). IL-4 and IL-5 may in turn further stimulate MAP kinase activity, forming a positive, feed-forward loop. Many other stimuli, including oxidative stress may also activate MAP kinases.

trilayer of kinases (English and Cobb 2002). c-Jun itself is phosphorylated by Jun-N-terminal kinase (JNK), a member of the extracellular signal-related kinases/mitogen-activated protein kinases (ERK/MAPK) family of serine/threonine kinases. JNK is in turn activated by phosphorylation by JNK kinase, a member of the MAPK/ERK kinase (MEK) family of kinases that phosphorylate on both a tyrosine and a threonine or serine residue. Of the seven members of the MEK family, MEK4 or Jun-N-terminal kinase kinase is principally responsible for the phosphorylation of JNK. At the top end of the trilayer, the most upstream kinases in the cascade, are the MEK kinases (MEKK), serine/threonine kinases that are diverse in sequence and structure (Figure 3).

In collaboration with Professor Ian Adcock at Imperial College, London, we have implicated abnormal regulation of AP-1 in the mechanism of glucocorticoid resistance. We showed that glucocorticoid-exposed peripheral blood mononuclear cells from glucocorticoid-resistant, as compared with glucocorticoid-sensitive asthmatics have fewer activated GR available for DNA binding (Adcock et al 1995a), but elevated DNA binding of AP-1 following phorbol ester stimulation (Adcock et al 1995b). These cells also demonstrated significantly elevated basal, as well as

phorbol ester-stimulated, transcription and translation of c-Fos. Furthermore, phorbol ester stimulation of cells from glucocorticoid-sensitive patients induced a glucocorticoid-resistant phenotype, which was associated with direct interaction between the activated GR and c-Fos, detected by co-immunoprecipitation (Lane et al 1998). Binding of GR to other pro-inflammatory transcriptional activators (such as CREB and NFκB) was unaffected. We interpret these data to suggest that mononuclear cells from glucocorticoid-resistant asthmatics inappropriately over-express AP-1, which sequesters and neutralises activated GR, thus causing refractoriness to glucocorticoid-induced inhibitory responses. More recently we have shown that (Loke et al 2006) that systemic glucocorticoid therapy of glucocorticoid sensitive, but not resistant, asthmatics is associated with down-regulation of phosphorylation of both c-Jun and JNK within inflammatory cells within the bronchial mucosa, further underlining a critical role for AP-1 components in regulating glucocorticoid response in asthma. The reason for this failure of glucocorticoid to down-regulate the c-Jun/JNK/MEK cascade in clinically resistant patients is presently unclear, but may reflect influences of the local cytokine milieu in these patients (such as elevated IL-2/IL-4 expression, as described above, which

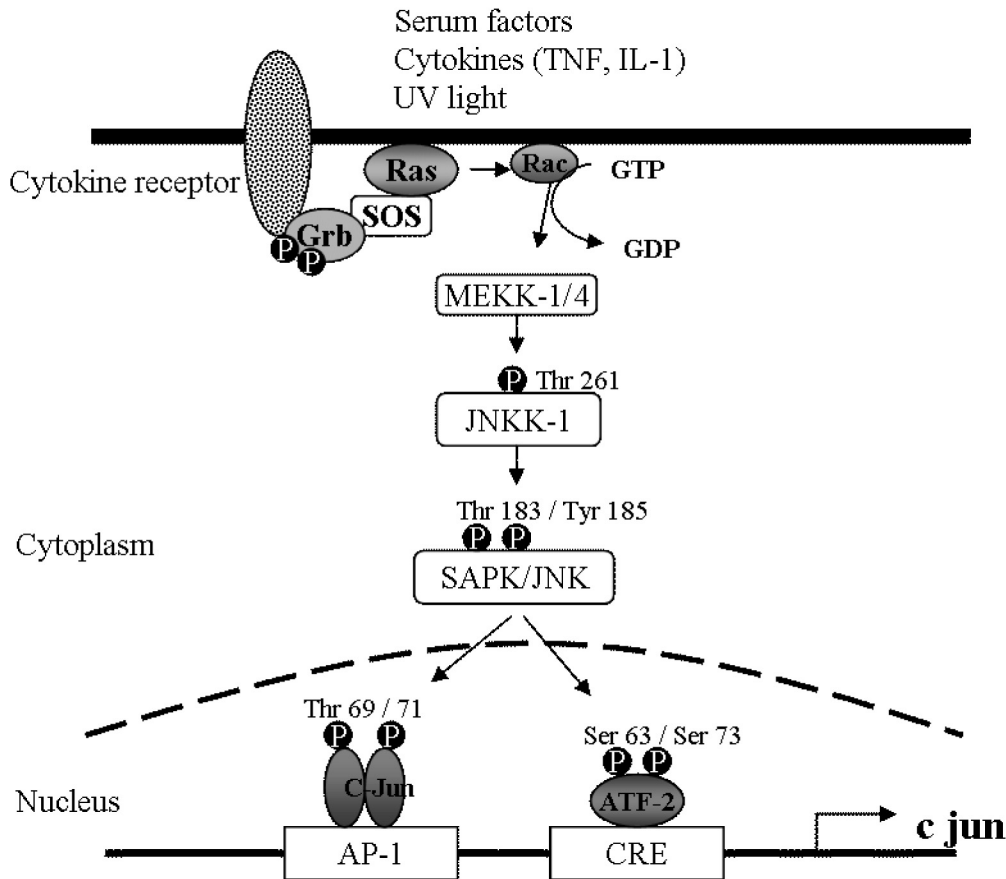


Figure 3 The tri-layer of kinases which activate c-jun. Environmental cytokines, ultraviolet light, serum factors, oxidative stress and many other stimuli phosphorylate and activate MEKK-1/4 through the Ras/Rho family of small GTP-binding proteins as shown. MEKK1 in turn phosphorylates JNKK1, which phosphorylates JNK, which binds to and phosphorylates c-jun, the active component of AP-1 (see text and Figure 2 above), and the regulatory protein activating transcription factor-2 (ATF-2). Phosphorylation may occur on threonine or tyrosine residues.

Abbreviations: GT(D)P, guanosine tri(di)phosphate; JNK, c-jun N-terminal kinase; JNKK1, c-jun N-terminal kinase kinase-1; MEKK-1/4, MAPK/ERK kinase kinase-1/4; SAPK, serum activated protein kinase; Ser, serine; SOS, son of sevenless; Thr, threonine; Tyr, tyrosine.

may enhance AP-1 production (Wang et al 1994)) and/or the influences of environmental stimuli such as oxidative stress or microbial superantigens as described above.

The role of p38 MAP kinase

p38 MAP kinase is another member of the family of MAPK/ERK molecules. Its activity is regulated by phosphorylation, principally by the p38 MAP kinases MEK3 and MEK6. A recent study (Irusen et al 2002) has raised the intriguing possibility that changes in the binding affinity of nuclear GR induced by exposure to IL-2/IL-4 may be caused by direct phosphorylation of the GR at serine 226 secondary to the resulting activation of p38 MAP kinase. Although this study did not directly demonstrate that p38 MAP kinase phosphorylation of the GR is responsible for its reduced binding affinity for glucocorticoids or its defective induction of an inhibitory signal, the hypothesis is certainly plausible. Similarly TNF- α has been shown to induce glu-

cocorticoid resistance in normal human monocytes, possibly through activation of p38 MAP kinase in addition to NF- κ B (Franchimont et al 1999). Several other protein kinases, such as JNK, may also modify activity of the GR in this way, either directly or through phosphorylation of co-factor molecules (Krstic et al 1997; Rogatsky et al 1998).

The findings with p38 MAP kinase raise the possibility that the small alteration in ligand binding affinity of the nuclear GR induced by IL-2/IL-4 exposure is an epiphenomenon reflecting more fundamental alterations in the function of the GR brought about by phosphorylation. This is in line with the fact that the observed changes in ligand-binding affinity of the GR are relatively small and of doubtful physiological significance per se. Such a fundamental alteration in GR function is also suggested by the fact that serine 226 is located in the N-terminal domain of the GR, remote from the ligand-binding pocket which resides in the C-terminal portion of the molecule. There is some precedent for the

possibility that remote regions of the GR may mutually interact. For example, there are two trans-activation domains, activation functions (AF) 1 and 2, situated respectively in the immunogenic and DNA-binding domains of the GR, which co-contribute to the full activity of the GR molecule on its responsive promoters. Conceivably, phosphorylation at a remote site might effect such interactions. Furthermore, both AF1 and AF2 interact with several other nuclear proteins and protein complexes, such as members of the p160 family and the co-activators p300/cyclic AMP-responsive element-binding protein (CREB)-binding protein (CRP) (Hittelman et al 1999) which can affect the glucocorticoid-titration response of GR transactivation of its responsive promoters (Szapary et al 1999).

The role of mitogen-activated extracellular signal-related kinases (MEK)

In one further recent study (Li et al 2004), stimulation of T cells with enterotoxin superantigen produced by *Staphylococcus aureus* rendered them resistant to GC inhibition in vitro, and this was accompanied by phosphorylation of both the mitogen-activated extracellular signal-related kinase (MEK/ERK) signaling protein and the GC receptor itself, as well as its impaired nuclear translocation. Treatment of cells with MEK/ERK inhibitors abrogated all of these effects. The authors inferred that activation of the MEK/ERK signaling pathway renders T cells resistant to GC inhibition by direct, ERK-mediated phosphorylation of the GC receptor which impairs its nuclear translocation. The effects of external environmental factors such as staphylococcal colonization on GC responsiveness emphasize the possibility that environmental factors may influence this responsiveness in individual patients.

The role of the GR β -isoform

The β -isoform of the GR is a splice variant of the "normal" GR, or GR α . The splicing varies in the choice of a splice acceptor site in exon 9. The cDNA sequence up until this point encodes a common region of 727 amino acids. Thereafter, the GR α splice adds 50 amino acids whereas the β isoform has only a further 15 residues. The consequence of this alternative splice was shown to be an inability of the β isoform to bind ligand (Oakley et al 1996). The reason for this has been clarified by the recent crystallization of the GR ligand-binding domain complexed with dexamethasone and a peptide that was homologous to the GR interaction domain of the co-activator TIF2 (Bledsoe et al 2002). This study demonstrated that the amino acids of the receptor bind

to ligand through either hydrogen bonds or hydrophobic interactions. One residue forming a hydrogen bond (T739) and seven residues forming hydrophobic interactions (L732, Y735, C736, T739, I747, F749 and L753) are present within the α , but are absent from the β isoform.

Artificial transfection of cells with GR β can inhibit GR α -mediated stimulation of gene expression, [Oakley et al 1996; Webster et al 2001]. The popular theory to explain this is that it acts as a dominant negative inhibitor of GR α activity (but see reservations below). Physiological expression of GR β in neutrophils has been suggested as a possible cause of their relative refractoriness to glucocorticoid inhibition (Strickland et al 2001). Several studies suggest that GR β may be induced in cell lines to an extent sufficient to induce glucocorticoid resistance by pro-inflammatory cytokines such as TNF- α (Webster et al 2001), possibly reflecting the location of a consensus NF- κ B binding sequence in the 5-flanking sequences of the GR gene. Furthermore, increased GR β immunoreactivity has been reported in peripheral blood mononuclear cells and bronchoalveolar lavage cells from patients with glucocorticoid-resistant asthma (Hamid et al 1999), although possible modulation of expression in association with glucocorticoid therapy was not explored. In studies from our own laboratory using a model of tuberculin-induced cutaneous inflammation, we reported elevated expression of GR β immunoreactivity in inflammatory cells infiltrating the skin lesions in glucocorticoid resistant, as compared with sensitive asthmatics (Sousa et al 2000). Treatment of the patients with systemic glucocorticoids was associated with down regulation of GR α expression in the glucocorticoid sensitive, but not the resistant patients.

Notwithstanding these observations, it seems likely that there is much yet to be learned about the possible functional role of GR β . In this context it is possible to speculate on how GR β may influence transcriptional activation mediated by GR α and its binding to the glucocorticoid response element (GRE), transcriptional repression mediated by GR α binding to negative GREs and inhibition of transcriptional activation mediated by sequestration of transcription factors:

Effects of GR β on transcriptional activation

In terms of transcriptional activation, the classical role of GR α is to bind ligand, to dimerize and then to bind to GRE. This binding permits the recruitment of co-activator complexes, because one of the consequences of ligand binding for nuclear hormone receptors is a reduction in their affinity for co-repressor complexes, and replacement of these complexes with co-activators. The crystallization data referred to above

(Bledsoe et al 2002) indicate that GR β lacks residues forming the charge clamp required for docking of the amino terminus TIF2 LXXLL motif, suggesting that GR β may be unable to recruit co-factors required for transcriptional activation. Thus GR β , by displacing GR α , could conceivably act as a dominant negative inhibitor of transcriptional activation. There are, however, uncertainties regarding this conclusion. In the first place, GR β seems to be expressed in much lower quantities (typically 10- to 100-fold less, at least at the level of mRNA expression) than GR α . In the second place, whereas in the case of GR α ligand binding appears to be a prerequisite for nuclear translocation, it is not known what governs the cytoplasmic/nuclear partitioning of GR β or if it is present in sufficient quantities in the cellular nucleus. Finally, it is unlikely that GR β could dimerise efficiently, so that its ability to compete for binding with high affinity to GREs is questionable.

Effects of GR β on transcriptional repression

Repression mediated by ligand-bound GR is thought to occur through binding to atypical sites on DNA, for example non-consensus NFAT/AP-1 sites, rather than binding to GREs. At these sites, it is thought that the conformation of the DNA binding site, and the influence of locally bound factors might cause the GR to adopt a structure that is not permissive for recruitment of co-activators, but rather permissive for recruitment of co-repressors. There is at present no information as to whether or not GR β can bind to such sites and, more importantly, recruit co-repressor complexes.

Inhibition of transcriptional activation mediated by sequestration of transcription factors

So far, there has been no clear demonstration for a role for the extreme carboxy-terminus of the GR in the sequestration (and inactivation) of transcriptional activating proteins such as AP-1. It is conceivable, therefore, that both GR α and GR β could exert repressive effects on gene expression by sequestration of such transcriptional activators.

The possible role of histone proteins

DNA is packaged into chromatin, a highly organized and dynamic protein-DNA complex. The N-terminal tails of the core histone proteins contain highly conserved lysine residues that are sites for post-translational acetylation. Acetylation of histone residues results in unwinding of the DNA coiled around the histone core. This process opens up the chromatin structure, allowing transcriptional factors and RNA polymerase II to bind more readily to DNA, thereby

increasing gene transcription (Imhof and Wolffe 1998). The large co-activator molecule CREB binding protein (CBP) that binds to the basal transcriptional apparatus has intrinsic histone acetyl transferase (HAT) activity. Additionally, associated co-activator proteins, including steroid receptor co-activator-1 (SRC-1), transcription factor intermediary factor-2 (TIF2), p300/CBP co-integrator-associated protein (p/CIP) and glucocorticosteroid receptor enhancing protein-1 (GRIP-1) may enhance local HAT activity. In genes which are induced by glucocorticoids, high concentrations of glucocorticoids cause binding of the activated GR to CBP and/or associated co-activators, resulting in histone acetylation on lysines 5 and 16 of histone H4 and increased gene transcription. HAT activity may be further enhanced by binding of transcriptional regulatory proteins such as AP-1 and NF- κ B (Janknecht and Hunter 1996; Imhof and Wolffe 1998). Repression of genes is conversely associated with a reversal of this process by histone deacetylation, mediated by histone deacetylases (HDACs) (Pazin and Kadonaga 1997). HDACs comprise of a growing family of enzymes of which at least 10 mammalian members have been described (Bertos et al 2001). Some, such as HDAC4 and HDAC8 are able to shuttle between the cellular nucleus and cytoplasm.

Histone acetylation is a dynamic process in which small changes in acetylase and deacetylase activity can considerably alter the net HAT activity at any particular gene promoter site. Suppression of HAT activity, as well as recruitment of HDAC activity to active transcriptional complexes may play a role in glucocorticoid regulation of gene transcription (Ito et al 2000). This may occur in a variety of ways. Glucocorticoid repression may reflect competition between the activated GR and the binding sites on CBP for other transcriptional activating proteins, such as AP-1 (Kamei et al 1996), NF- κ B, Sp1, Ets, NF-AT and STATs, which may alter local HAT activity. Alternatively, and not exclusively, the GR may bind to one of several co-repressor molecules such as RIP140, NCoR1 and GRIP1 which in turn associate with proteins having differing HAT activity (Ding et al 1998). These complex interactions probably play a major role in the genesis of the infinitely variable and subtle effects of glucocorticoids on individual target cells.

Clearly, then, intrinsic abnormalities of, or external influences on the regulation of HAT activity in individual cells may influence their glucocorticoid responsiveness. Already there are suggestions that external influences such as exposure to cigarette smoke, an oxidative stress, may inhibit the anti-inflammatory actions of glucocorticoids on cells in the lungs of smokers by reducing HDAC expression and

activity (Ito et al 2005). Similar phenomena may contribute to glucocorticoid resistance in asthma.

Genetic basis of glucocorticoid resistance

The glucocorticoid receptor gene itself is a plausible candidate for a genetic basis to glucocorticoid resistance. A missense mutation in this gene had previously been found to be responsible for the phenomenon of familial glucocorticoid resistance (Hurley et al 1991). These patients have a markedly reduced affinity of the glucocorticoid receptor for ligand and clinically have features of Addison's disease, which is not the case with glucocorticoid resistant asthmatics. Analysis of the sequence of the glucocorticoid receptor protein in six resistant and sensitive patients showed no mutations in any of the subjects (Lane et al 1994). Although this work is ongoing, there is no obvious mutation that might account for glucocorticoid resistance. Obviously, relevant mutations do not necessarily have to be within the coding region of the receptor gene itself, but could be in genes encoding products in downstream signaling pathways.

In addition to this, glucocorticoid responsiveness may be governed at least partly by genetic factors. For example, one study suggested that inherited anomalies might render approximately 7% of the normal population relatively hypersensitive to glucocorticoids (Lamberts et al 1996). Indeed, there is marked variability in the sensitivity of T cells even from normal individuals to glucocorticoid inhibition *in vitro*, suggesting that the phenomenon may not be induced entirely by inflammation but may be partly heritable.

The technique of positional cloning provides a way to identify genes or gene complexes which predispose to disease in an unbiased fashion. Using this technique, five genes or gene complexes (ADAM33, PHF11, DPP10, GPR4 and SPINK5) have been linked with predisposition to asthma (reviewed by Cookson and Moffatt 2004), and studies are ongoing to investigate the functions of these genes, some of which, along with others to be discovered, may play a role in therapy resistant asthma. These studies involve large numbers of patients, but larger studies still will be needed to discern the relative contribution of genetics and environment in regulating asthma severity and responsiveness to therapy in individual patients. A further complication is that different genes may contribute variably to asthma in different racial groups.

The alternative approach to genetic analysis is to look for variability in the expression of "candidate genes" (that is, those hypothesized to be an important part of the disease process or in governing responsiveness to therapy), for

example between asthmatics and controls or between glucocorticoid sensitive and resistant asthmatics. The list of such "candidate genes" is potentially very large, and the problem with this approach is that it is impossible to know the relative importance, and possible physiological significance of any differences uncovered. This process is being facilitated by microarray gene fingerprinting, which allows analysis of the differential expression of potentially many thousands of genes on a single chip. Using this technique in an Icelandic population it was possible to define a gene expression fingerprint which predicted glucocorticoid resistant, as compared with sensitive asthma not only in the population of origin but also in an independent cohort (Hakonarson et al 2005). Again such analysis might be confounded by ethnicity. Genes may also regulate drug metabolism variably in different individuals (Hall 1998).

Management of glucocorticoid resistant asthma

In view of the above, a proposed list of techniques for the investigation of "difficult" asthma, including glucocorticoid resistant asthma, is proposed in Table 3. Such investigation would also include a formal trial of glucocorticoid therapy to investigate possible glucocorticoid resistance defined by the criteria discussed above. Key features of the management of severe asthma have much in common with the management of any patient with asthma. It is critical to make an accurate diagnosis, minimize factors which may induce loss of asthma control and ensure good compliance with therapy.

By definition, the "difficult" asthmatic will be receiving high dosages of inhaled glucocorticoid therapy, often together with oral glucocorticoids. Generally all patients with severe asthma, particularly those admitted as an emergency will be treated with systemic glucocorticoids at high dosage for at least two weeks. It is only in the fullness of time that a pattern of glucocorticoid responsiveness is established. In patients thought to be truly glucocorticoid resistant (that is, patients showing the proscribed failure in FEV₁ response when the diagnosis is confirmed and aggravating conditions excluded), further long-term therapy with systemic glucocorticoids is probably inadvisable since there is little evidence that these drugs will influence asthmatic symptoms and disease activity, but may on the other hand cause considerable unwanted effects. In such cases it may be sensible to reduce or withdraw glucocorticoids and treat instead with adequate dosages of alternative anti-asthma drugs such as bronchodilators, leukotriene receptor antagonists and immunosuppressive drugs (see below). In clinical practice, however, because

Table 3 Investigation of difficult/therapy resistant asthma**Assessment of severity**

Symptom score chart and use of β_2 -agonist reliever therapy
 Spirometric measurement of lung volumes and gas transfer
 Bronchial responsiveness to methacholine/histamine
 Diurnal variation in peak expiratory flow
 Quality of life assessment

Determination of pharmacological responsiveness

Assessment of compliance to therapy and inhaler technique
 Bronchodilator response to β_2 -adrenergic agonists
 Response to prednisolone (corticosteroid-responsiveness)

Radiology

Chest radiograph
 Barium swallow
 Computed tomography of sinuses
 High-resolution computed tomography of sinuses

Blood tests

Full blood count, including eosinophil count
 Serum IgG, IgA, IgM and IgG subclasses*
 Serum total IgE
 Specific IgE to selected allergens
 Thyroid function tests

Other tests

Biomarkers of inflammation (exhaled nitric oxide, eosinophils in induced sputum)
 Sweat test and genetic assessment of CFTR** mutations (if indicated)
 24-h oesophageal pH monitoring
 Examination of nasopharyngeal airways
 Tests of ciliary function
 Skin prick test to common aeroallergens
 Fiberoptic bronchoscopy with bronchial biopsy and bronchoalveolar lavage
 Psychological assessment

Notes: *Ig – immunoglobulin;

**CFTR – cystic fibrosis transmembrane conductance regulator

there is little hard evidence to justify withdrawal of systemic glucocorticoids from any severe asthmatic patient, this process is often ignored or delayed. Furthermore, it is not clear on clinical grounds whether glucocorticoid responsiveness is induced or enhanced by the use of glucocorticoids themselves (although reduction of asthmatic inflammation would appear to be associated with an increased affinity of the glucocorticoid receptor for binding to its ligand, as discussed above). In view of the possibility that glucocorticoid sensitivity may change with time, it would seem prudent to re-assess glucocorticoid sensitivity periodically (Demoly et al 1998) although, as discussed above, within individuals T cell glucocorticoid sensitivity appears to be relatively stable at least over a period of months.

The observation that glucocorticoid responsiveness of asthmatics correlates with glucocorticoid inhibition of their T cells suggests that other drugs that inhibit T cells might be useful for asthma therapy, in particular drugs that inhibit

T cells by mechanisms distinct from those of glucocorticoids. We have shown (Corrigan et al 1991b; Haczku et al 1994) that the immunosuppressive drugs cyclosporin A, rapamycin and mycophenolate mofetil inhibit proliferation of T cells from glucocorticoid sensitive and resistant asthmatics to an equivalent extent. It has been shown (Alexander et al 1992; Lock et al 1996) that cyclosporin A, when administered to patients with poorly controlled asthma despite continuous systemic glucocorticoid therapy, improved lung function while allowing reduction of oral glucocorticoid dosages in a proportion of the patients. Similarly concomitant therapy of glucocorticoid dependent asthmatics with methotrexate (Shiner et al 1990) or gold salts (Klaustermeyer et al 1987) has been shown in some trials to spare glucocorticoid therapy, although no trials have suggested that these agents improve lung function. In general, none of these agents is particularly satisfactory in the sense that many patients fail to respond and it is impossible to predict responsiveness a priori. Furthermore, chronic immunosuppression raises the risk of development of serious infection or malignancy, and there is in addition a list of not insignificant unwanted effects associated, in some patients, with the use of each particular drug. An urgent appraisal of other immunosuppressive drugs or cytokine inhibitory strategies is needed in glucocorticoid dependent and glucocorticoid resistant asthma. It is a priority to produce a global definition of which patients are suitable for treatment, and what constitutes an adequate trial of therapy. It will also be essential to develop cheap and reliable biomarkers of airways inflammation and remodeling so that glucocorticoid responsiveness can be assessed accurately and objectively in the short and long term.

References

- Adcock IM, Lane SJ, Brown CR, et al. 1995. Defective binding of glucocorticoid receptor to DNA in steroid-resistant asthma. *J Immunol*, 154:3500–5.
- Adcock IM, Lane SJ, Brown CR, et al. 1995. Abnormal glucocorticoid receptor-activator protein 1 interaction in steroid-resistant asthma. *J Exp Med*, 182:1951–8.
- Akbari O, DeKruyff RH, Umetsu DT. 2001. Pulmonary dendritic cells producing IL-10 mediate tolerance induced by respiratory exposure to antigen. *Nature Immunol*, 2:725–31.
- Alexander AG, Barnes NC, Kay AB. 1992. Trial of cyclosporine in corticosteroid-dependent chronic severe asthma. *Lancet*, 339:324–8.
- Alvarez J, Surs W, Leung DYM et al. 1992. Steroid resistant asthma: immunologic and pharmacologic features. *J Allergy Clin Immunol*, 89:714–21.
- American Thoracic Society. 2000. Proceedings of the ATS workshop on refractory asthma: current understanding, recommendations and unanswered questions. *Am J Respir Crit Care Med*, 162:2341–51.
- Bertos NR, Wang AH, Yang XJ. 2001. Class II histone deacetylases: structure, function and regulation. *Biochem Cell Biol*, 79:243–52.
- Bledsoe RK, Montana VG, Stanley TB, et al. 2002. Crystal structure of the glucocorticoid receptor ligand binding domain reveals a novel mode of receptor dimerisation and coactivator recognition. *Cell*, 110:93–105.

- Borish L, Aarons A, Rumbly J, et al. 1996. Interleukin-10 regulation in normal subjects and patients with asthma. *J Allergy Clin Immunol*, 97:1288–96.
- Buist SA. 1995. Cost effectiveness of asthma management strategies. *Eur Respir Rev*, 5:292–4.
- Bumbacea D, Campbell D, Nguyen L, et al. 2004. Parameters associated with persistent airflow obstruction in chronic severe asthma. *Eur Respir J*, 24:122–8.
- Busse PJ, Wang JJ, Halm EA. 2005. Allergen sensitization evaluation and allergen avoidance education in inner-city adult cohort with persistent asthma. *J Allergy Clin Immunol*, 116:146–52.
- Carmichael J, Paterson IC, Diaz P, et al. 1981. Corticosteroid resistance in chronic asthma. *Br Med J*, 282:1419–22.
- Chaudhuri R, Livingston E, McMahon AD et al. 2003. Cigarette smoking impairs the therapeutic response to oral corticosteroids in chronic asthma. *Am J Respir Crit Care Med*, 168:1308–11.
- Clark TJH, Hetzel MR. 1977. Diurnal variation of asthma. *Br J Dis Chest*, 71:87–92.
- Cochrane GM. 1992. Therapeutic compliance in asthma: its magnitude and implications. *Eur Respir J*, 5:122–4.
- Cockcroft DW, Swystun VA. 1996. Asthma control versus asthma severity. *J Allergy Clin Immunol*, 98:1016–18.
- Cookson W, Moffatt M. 2004. Making sense of asthma genes. *N Engl J Med*, 351:1794–6.
- Corren J, Adinoff AD, Buchmeier AD, et al. 1992. Nasal beclomethasone prevents the seasonal increase in bronchial responsiveness in patients with allergic rhinitis and asthma. *J Allergy Clin Immunol*, 90:250–6.
- Corrigan CJ, Brown PH, Barnes NC, et al. 1991a. Glucocorticoid resistance in chronic asthma: glucocorticoid pharmacokinetics, glucocorticoid receptor characteristics and inhibition of peripheral blood T-cell proliferation by glucocorticoids *in vitro*. *A Rev Respir Dis*, 144:1016–25.
- Corrigan CJ, Brown PH, Barnes NC, et al. 1991b. Glucocorticoid resistance in chronic asthma: peripheral blood T-lymphocyte activation and a comparison of the T-lymphocyte inhibitory effects of glucocorticoids and cyclosporin A. *Am Rev Respir Dis*, 144:1026–32.
- Corrigan CJ, Kay AB. 1992. The role of T lymphocytes and eosinophils in the pathogenesis of asthma. *Immunol Today*, 13:501–7.
- Corrigan CJ, Hamid Q, North J, et al. 1995. Peripheral blood CD4 but not CD8 T-lymphocytes in patients with exacerbation of asthma transcribe and translate messenger RNA encoding cytokines which prolong eosinophil survival in the context of a TH2-type pattern: effect of glucocorticoid therapy. *Am J Respir Cell Mol Biol*, 12:567–78.
- English JM, Cobb MH. 2002. Pharmacological inhibitors of MAPK pathways. *Trends in Pharmacological Sciences*, 23:40–5.
- Demoly P, Jaffuel D, Mathieu M, et al. 1998. Glucocorticoid insensitive asthma: a one-year clinical follow up pilot study. *Thorax*, 53:1063.
- Ding XF, Anderson CM, Ma H, et al. 1998. Nuclear receptor-binding sites of coactivators glucocorticosteroid receptor interacting protein 1 (GRIP1) and steroid receptor coactivator 1 (SRC-1): multiple motifs with different binding specificities. *Mol Endocrinol*, 12:302–13.
- Dolan CM, Fraher KE, Bleecker ER et al. 2004. Design and baseline characteristics of the epidemiology and natural history of asthma: Outcomes and treatment regimens (TENOR) study: a large cohort of patients with severe or difficult-to-treat asthma. *Ann Allergy Asthma Immunol*, 92:32–9.
- ENFUMOSA. 2003. The ENFUMOSA cross-sectional European multi-centre study of the clinical phenotype of chronic severe asthma. *Eur Respir J*, 22:470–7.
- Flood-Page P, Menzies-Gow A, Phipps S, et al. 2003. Anti-IL-5 treatment reduces deposition of ECM proteins in the bronchial subepithelial basement membrane of mild atopic asthmatics. *J Clin Invest*, 112:1029–36.
- Franchimont D, Martens M, Hagelstein MT, et al. 1999. Tumour necrosis factor alpha decreases, and interleukin-10 increases, the sensitivity of human monocytes to dexamethasone: potential regulation of the glucocorticoid receptor. *J Clin Endocrinol Metab*, 84:2834–9.
- Haczku A, Alexander A, Brown P, et al. 1994. The effect of dexamethasone, cyclosporine and rapamycin on T-lymphocyte proliferation *in vitro*: comparison of cells from patients with glucocorticoid-sensitive and glucocorticoid-resistant chronic asthma. *J Allergy Clin Immunol*, 93:510–19.
- Hahn DL, Dodge RW, Golubjatnikov R. 1991. Association of *Chlamydia pneumoniae* (strain TWAR) infection with wheezing, asthmatic bronchitis, and adult-onset. *JAMA*, 266:225–30.
- Hakonarson H, Bjornsdottir US, Halapi E, et al. 2005. Profiling of genes expressed in peripheral blood mononuclear cells predicts glucocorticoid sensitivity in asthma patients. *Proc Natl Acad Sci USA*, 102:14789–94.
- Hall IP. 1998. Genetic factors in asthma severity. *Clin Exp Allergy*, 28(5 Suppl):16–20.
- Hamid QA, Wenzel SE, Hank PJ, et al. 1999. Increased glucocorticoid receptor β in airway cells of glucocorticoid-insensitive asthma. *Am J Respir Crit Care Med*, 59:1600–4.
- Hauk PJ, Wenzel SE, Trumble AE, et al. 1999. Increased T-cell receptor vbeta8+ T cells in bronchoalveolar lavage fluid of subjects with poorly controlled asthma: a potential role for microbial superantigens. *J Allergy Clin Immunol*, 104:37–45.
- Hauk PJ, Hamid QA, Chrousos GP, et al. 2000. Induction of corticosteroid insensitivity in human PBMCs by microbial superantigens. *J Allergy Clin Immunol*, 105:782–7.
- Hawrylowicz CM, Richards DF, Loke T-K, et al. 2002. A defect in corticosteroid-induced IL-10 production in T lymphocytes from corticosteroid resistant asthmatic patients. *J Allergy Clin Immunol*, 109:369–70.
- Heaney LG, Conway E, Kelly C, et al. 2003. Predictors of therapy resistant asthma: outcome of a systematic evaluation protocol. *Thorax*, 58:561–6.
- Hittelman AB, Burakov D, Iniguez-Lluki JA, et al. 1999. Differential regulation of glucocorticoid receptor transcriptional activation via AF-1-associated proteins. *EMBO J*, 18:5380–8.
- Hurley DM, Accili D, Stratakis CA, et al. 1991. Point mutation causing a single amino acid substitution in the hormone-binding domain of the glucocorticoid receptor in familial glucocorticoid resistance. *J Clin Invest*, 87:680–6.
- Imhof A, Wolffe AP. 1998. Transcription: gene control by targeted histone acetylation. *Curr Biol*, 8:R422–4.
- Irusen E, Matthews JG, Takaiashi A, et al. 2002. MAP kinase-induced glucocorticoid-receptor phosphorylation reduces its activity: role in steroid-insensitive asthma. *J Allergy Clin Immunol*, 109:649–57.
- Ito K, Barnes PJ, Adcock IM. 2000. Glucocorticoid receptor recruitment of histone deacetylase 2 inhibits interleukin-1 β -induced histone H4 acetylation on lysines 8 and 12. *Mol Cell Biol*, 20:6891–903.
- Ito K, Ito M, Elliott WM, et al. 2005. Decreased histone deacetylase activity in chronic obstructive pulmonary disease. *N Eng J Med*, 352:1967–76.
- Ito K, Chung KF, Adcock IM. 2006. Update on glucocorticoid action and resistance. *J Allergy Clin Immunol*, 117:522–43.
- Janknecht R, Hunter T. 1996. Versatile molecular glue: transcriptional control. *Curr Biol*, 6:951–4.
- John M, Lim S, Seybold J, et al. 1998. Inhaled corticosteroids increase interleukin-10 but reduce macrophage inflammatory protein-1 α , granulocyte-macrophage colony-stimulating factor and interferon- γ release from alveolar macrophages in asthma. *Am J Respir Crit Care Med*, 157:256–62.
- Kam JC, Szefer SJ, Surs W, et al. 1993. Combination IL-2 and IL-4 reduces glucocorticoid receptor-binding affinity and T cell response to glucocorticoids. *J Immunol*, 151:3460–6.
- Kamada AK, Leung DYM, Szefer SJ. 1992. Steroid resistance in asthma: our current understanding. *Pediatr Pulmonol*, 14:180–6.
- Kamada AK, Szefer SJ, Leung DYM. 1993. The growing problem of steroid resistant asthma. *Drug Ther*, 23:55–68.
- Kamei Y, Xu L, Heinzel T, et al. 1996. A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. *Cell*, 85:403–14.

- Katsoulis K, Kontakiotis T, Leonardopoulos I, et al. 2003. Serum total antioxidant status in severe exacerbation of asthma: correlations with the severity of the disease. *J Asthma*, 40:847–54.
- Kelloway JS, Wyatt RA, Adlis SA. 1994. Comparison of patients' compliance with prescribed oral and inhaled asthma medications. *Arch Intern Med*, 154:1349–52.
- Kharitonov SA, Barnes PJ. 2003. Nitric oxide, nitrotyrosine, and nitric oxide modulators in asthma and chronic obstructive pulmonary disease. *Curr Allergy Asthma Rep*, 3:121–9.
- Kibe A, Inoue H, Fukuyama S, et al. 2003. Differential regulation by glucocorticoid of interleukin-13-induced eosinophilia, hyperresponsiveness, and goblet cell hyperplasia in mouse airways. *Am J Respir Crit Care Med*, 167:50–6.
- Kikuchi Y, Okabe S, Tamura G, et al. 1994. Chemosensitivity and perception of dyspnea in patients with a history of near-fatal asthma. *N Engl J Med*, 330:1329–34.
- Kino T, Chrousos GP. 2001. Glucocorticoid and mineralocorticoid resistance/hypersensitivity syndromes. *J Endocrinol*, 169:437–45.
- Klaustermeyer WB, Noritake DT, Kwong FK. 1987. Chrysotherapy in the treatment of corticosteroid-dependent asthma. *J Allergy Clin Immunol*, 79:720–5.
- Krstic MD, Rogatsky I, Yamamoto KP, et al. 1997. Mitogen-activated and cyclin-dependent kinases selectively and differentially modulate transcriptional enhancement by the glucocorticoid receptor. *Mol Cell Biol*, 17:3947–54.
- Lamberts SWJ, Huizenga ATM, de Lande P, et al. 1996. Clinical aspects of glucocorticoid sensitivity. *Steroids*, 147:690–6.
- Lane SJ, Arm JP, Staynov DZ, et al. 1994. Chemical mutational analysis of the human glucocorticoid receptor cDNA in glucocorticoid-resistant bronchial asthma. *Am J Respir Cell Mol Biol*, 11:42–8.
- Lane SJ, Adcock IM, Richards D, et al. 1998. Corticosteroid-resistant bronchial asthma is associated with increased c-fos expression in monocytes and T lymphocytes. *J Clin Invest*, 102:2156–64.
- Lassalle P, Delneste Y, Gosset P, et al. 1993. T and B-cell immune response to a 55 kDa endothelial cell-derived antigen in severe asthma. *Eur J Immunol*, 23:796–803.
- Lee TH, Brattsand R, Leung D. 1996. Corticosteroid action and resistance in asthma. *Am J Respir Crit Care Med*, 154:S51.
- Lee W, Mitchell P, Tjian R. 1987. Purified transcription factor AP-1 interacts with TPA-inducible enhancer elements. *Cell*, 49:741–52.
- Leung DY, Martin RJ, Szefer SJ, et al. 1995. Dysregulation of interleukin 4, interleukin-5 and interferon gamma gene expression in steroid-resistant asthma. *J Exp Med*, 181:33–40.
- Leung DY, Bloom JW. 2003. Update on glucocorticoid action and resistance. *J Allergy Clin Immunol*, 111:3–22.
- Li LB, Goleva E, Hall CF, et al. 2004. Superantigen-induced corticosteroid resistance of human T cells occurs through activation of the mitogen-activated protein kinase kinase/extracellular signal-regulated kinase (MEK-ERK) pathway. *J Allergy Clin Immunol*, 114:1059–69.
- Lim S, Crawley E, Woo P, et al. 1998. Haplotype associated with low interleukin-10 production in patients with severe asthma. *Lancet*, 352:113.
- Lock SH, Kay AB, Barnes NC. 1996. Double-blind, placebo-controlled study of cyclosporine A as a corticosteroid-sparing agent in corticosteroid-dependent asthma. *J Allergy Clin Immunol*, 153:509.
- Loke TK, Mallett KH, Ratoff J, et al. 2006. Systemic glucocorticoid reduces bronchial mucosal activation of activator protein 1 components in glucocorticoid-sensitive but not glucocorticoid-resistant asthmatic patients. *J Allergy Clin Immunol*, 118:368–75.
- Milgrom H, Bender B, Ackerson L, et al. 1996. Noncompliance and treatment failure in children with asthma. *J Allergy Clin Immunol*, 98:1051–7.
- Miller BD, Strunk RC. 1989. Circumstances surrounding the deaths of children due to asthma. A case-control study. *Am J Dis Child*, 143:1294–9.
- Miller MK, Johnson C, Miller DP, et al. 2005. Severity assessment in asthma: an evolving concept. *J Allergy Clin Immunol*, 116:990–5.
- Moore KW, de Waal Malefyt R, Coffman RL, et al. 2001. IL-10 and the IL-10 receptor. *Annu Rev Immunol*, 19:683–765.
- Nahm DH, Lee YE, Yim EJ, et al. 2002. Identification of cytokeratin 18 as a bronchial epithelial autoantigen associated with nonallergic asthma. *Am J Respir Crit Care Med*, 165:1536–9.
- National Institutes of Health. 1995. NHLB/WHO Workshop Report. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention: No. 95–3659. p. 1–176.
- Newman KB, Mason UG, Schmaling KB. 1995. Clinical features of vocal cord dysfunction. *Am J Respir Crit Care Med*, 152:1382–6.
- Nimmagadda SR, Szefer SJ, Spahn JD, et al. 1997. Allergen exposure decreases glucocorticoid receptor binding affinity and steroid responsiveness in atopic asthmatics. *Am J Respir Crit Care Med*, 155:87–93.
- Oakley RH, Sar M, Cidlowski JA. 1996. The human glucocorticoid receptor β isoform: expression, biochemical properties, and putative function. *J Biol Chem*, 271:9550–9.
- Oakley RH, Jewell CM, Yudit MR, et al. 1999. The dominant negative activity of the human glucocorticoid receptor β isoform. *J Biol Chem*, 274:27857–66.
- Pazin MJ, Kadonaga JT. 1997. What's up and down with histone deacetylation and transcription? *Cell*, 89:325–8.
- Poznansky MC, Gordon ACH, Douglas JG, et al. 1984. Resistance to methylprednisolone in cultures of blood mononuclear cells from glucocorticoid-resistant asthmatic patients. *Clin Sci*, 67:639–45.
- Rabe KF, Adachi M, Lai CK, et al. 2004. Worldwide severity and control of asthma in children and adults: the global asthma insights and reality surveys. *J Allergy Clin Immunol*, 114:40–7.
- Reichhardt HM, Tuckermann JP, Gottlicher M, et al. 2001. Repression of inflammatory responses in the absence of DNA binding by the glucocorticoid receptor. *EMBO J*, 20:7168–73.
- Richards DF, Fernandez M, Caulfield J, et al. 2000. Glucocorticoids drive human CD8+ T cell differentiation towards a phenotype with high IL-10 and reduced IL-4, IL-5 and IL-13 production. *Eur J Immunol*, 30:2344–54.
- Robinson DS, Hamid Q, Ying S, et al. 1993. Prednisolone treatment in asthma is associated with modulation of bronchoalveolar lavage cell interleukin-4, interleukin-5 and interferon-gamma cytokine gene expression. *Am Rev Respir Dis*, 148:402–6.
- Robinson DS, Campbell DA, Durham SR, et al. 2003. Systematic assessment of difficult-to-treat asthma. *Eur Respir J*, 22:478–83.
- Rogatsky I, Logan SK, Garabedian MJ. 1998. Antagonism of glucocorticoid receptor transcriptional activation by the c-Jun N-terminal kinase. *Proc Natl Acad Sci USA*, 95:2050–5.
- Schwartz HL, Lowell FC, Melby JC. 1968. Steroid resistance in bronchial asthma. *Ann Intern Med*, 69:493–9.
- Sher ER, Leung DM, Surs W, et al. 1994. Steroid-resistant asthma cellular mechanisms contributing to inadequate response to glucocorticoid therapy. *J Clin Invest*, 93:33–9.
- Shiner RJ, Nunn AJ, Chung KF, et al. 1990. Randomised, double-blind, placebo-controlled trial of methotrexate in steroid-dependent asthma. *Lancet*, 336:137–40.
- Silkoff PE, Lent AM, Busacker AA, et al. 2005. Exhaled nitric oxide identifies the persistent eosinophilic phenotype in severe refractory asthma. *J Allergy Clin Immunol*, 116:1249–55.
- Sousa AR, Lane SJ, Cidlowski JA, et al. 2000. Glucocorticoid resistance in asthma is associated with elevated *in vivo* expression of the glucocorticoid receptor beta-isoform. *J Allergy Clin Immunol*, 105:943–50.
- Spahn JD, Leung DY, Surs W, et al. 1995. Reduced glucocorticoid binding affinity in asthma is related to ongoing allergic inflammation. *Am J Respir Crit Care Med*, 151:1709–14.
- Spahn JD, Szefer SJ, Surs W, et al. 1996. A novel action of IL-13: induction of reduced monocyte glucocorticoid receptor-binding affinity. *J Immunol*, 157:2654–9.
- Spahn JD, Leung DY, Szefer SJ. 1997. New insights into the pathogenesis and management of steroid-resistant asthma. *J Asthma*, 34:177–94.
- Spaulding HS Jr, Mansfield LE, Stein MR, et al. 1982. Further investigation of the association between gastro-oesophageal reflux and bronchoconstriction. *J Allergy Clin Immunol*, 69:516–21.

- Strickland I, Kisich K, Hauk PJ, et al. 2001. High constitutive glucocorticoid receptor beta in human neutrophils enables them to reduce their spontaneous rate of cell death in response to corticosteroids. *J Exp Med*, 193:585–94.
- Szapary D, Huang Y, Simons SS. 1999. Opposing effects of corepressors and coactivators in determining the dose-response curve of agonists, and residual agonist-activity of antagonists, for glucocorticoid receptor-regulated gene expression. *Mol Endocrinol*, 13:2108–21.
- ten Brinke A, Zwinderman AH, Sterk PJ, et al. 2004. “Refractory” eosinophilic airway inflammation in severe asthma: effect of parenteral corticosteroids. *Am J Respir Crit Care Med*, 170:601–5.
- ten Brinke A, Sterk PJ, Masclee AA, et al. 2005. Risk factors of frequent exacerbations in difficult-to-treat asthma. *Eur Respir J*, 26:812–18.
- Wan S, DeSmet JM, Antoine M, et al. 1996. Steroid administration in heart and heart-lung transplantation: is the timing adequate? *Ann Thoracic Surg*, 61:674–8.
- Wang CY, Bassuk AG, Boise LH, et al. 1994. Activation of the granulocyte-macrophage colony-stimulating factor promoter in T cells requires cooperative binding of Elf-1 and AP-1 transcription factors. *Mol Cell Biol*, 14:1153–9.
- Wareham NJ, Harrison BD, Jenkins PF, et al. 1993. A district confidential enquiry into deaths due to asthma. *Thorax*, 48:1117–20.
- Webster JC, Oakley RH, Jewell CM, et al. 2001. Proinflammatory cytokines regulate human glucocorticoid receptor gene expression and lead to the accumulation of the dominant negative β -isoform: a mechanism for the generation of glucocorticoid resistance. *Proc Natl Acad Sci USA*, 98:6865–70.
- Wenzel SE, Szeffler SJ, Leung DY, et al. 1997. Bronchoscopic evaluation of severe asthma. Persistent inflammation associated with high dose glucocorticoids. *Am J Respir Crit Care Med*, 156:737–43.
- Wenzel SE, Schwartz LB, Langmack EL, et al. 1999. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. *Am J Respir Crit Care Med*, 160:1001–8.
- Woolcock AJ. 1993. Steroid-resistant asthma: what is the clinical definition? *Eur Respir J*, 6:743–7.
- Xystrakis E, Kusumakar S, Boswell S, et al. 2006. Reversing the defective induction of IL-10-secreting regulatory T cells in glucocorticoid-resistant asthma patients. *J Clin Invest*, 116:146–55.
- Yamada K, Elliott WM, Hayashi S, et al. 2000. Latent adenoviral infection modifies the steroid response in allergic lung inflammation. *J Allergy Clin Immunol*, 106:844–51.

