Overcoming steroid unresponsiveness in airways disease

Ian M. Adcock*,†, Pai-Chien Chou*,†, Andrew Durham*† and Paul Ford*†

*Airways Disease Section, National Heart and Lung Institute, Imperial College London, Dovehouse Street, London SW3 6LY, U.K., and †MRC-Asthma UK Centre for Allergic Mechanisms in Asthma, 5th Floor, Tower Wing, Guy’s Hospital, London SE1 9RT, U.K.

Abstract

Most of the patients with asthma are found to be successfully treated with conventional therapy. However, there are a small proportion of asthmatic patients who fail to respond to corticosteroids even at high doses or with supplementary therapy. In addition, even high doses of corticosteroids have a minimal effect on the inexorable decline in lung function in COPD (chronic obstructive pulmonary disease) and only a small effect in reducing exacerbations. Corticosteroid-insensitivity therefore presents a profound management problem. Corticosteroids act through a cytosolic receptor [GR (glucocorticoid receptor)], which is activated and translocates to the nucleus. Once in the nucleus, it either binds to DNA and switches on the expression of anti-inflammatory genes or represses the activity of distinct signalling pathways such as NF-κB (nuclear factor κB), AP-1 (activator protein-1) or MAPKs (mitogen-activated protein kinases). This latter step requires the recruitment of co-repressor molecules. A failure to respond to corticosteroids may therefore result from lack of binding to GR, reduced GR expression, lack of co-repressor activity or enhanced activation of inflammatory pathways. These events can be modulated by oxidative stress or high levels of inflammatory cytokines, which may lead to a reduced clinical outcome. Understanding the molecular mechanisms of GR action, and inaction, may lead to the development of new anti-inflammatory drugs or reverse the relative corticosteroid-insensitivity that is characteristic of these diseases.

Introduction

Asthma is a common chronic inflammatory disease and affects approx. 300 million people worldwide [1]. Asthma accounts for ~1 out of every 250 deaths worldwide and also has profound health care and societal costs in terms of emergency room visits, hospitalizations and work or school absenteeism. Most patients with asthma respond well to current therapies; however, a small percentage (5–10%) have severe disease that often fails to respond but these patients account for >50% of the total asthma health care costs [2]. Improved knowledge of asthma pathophysiological mechanisms and in particular the differences seen in groups of patients with severe disease is urgently needed. It is now recognized that there may be distinct asthma phenotypes [2] and that distinct therapeutic approaches may only impinge upon some aspects of the disease process, or at least outcome measures, within each subgroup. Thus, although a potential new drug may work in some patients, this is not necessarily the case when studied in larger cohorts as reported recently.

Key words: asthma, chronic obstructive pulmonary disease (COPD), corticosteroid, mitogen-activated protein kinase (MAPK), steroid, theophylline.

Abbreviations used: AP-1, activator protein-1; BAI, bronchoalveolar lavage; Bq/g, Blood-related gene; C/EBPα, CCAAT/enhancer-binding protein α; COPD, chronic obstructive pulmonary disease; CSD, corticosteroid-dependent; CSR, corticosteroid-resistant; FEV1, forced expiratory volume in 1 s; GR, glucocorticoid receptor; GRE, glucocorticoid response element; HDAC, histone deacetylase; IL, interleukin; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MPAK, MAPK phosphatase; NF-κB, nuclear factor κB; NOS2, nitric oxide synthase 2; PI3K, phosphoinositide 3-kinase; PPAR, peroxisome proliferator-activated receptor; TNFα, tumour necrosis factor α.

1 To whom correspondence should be addressed (email ian.adcock@imperial.ac.uk).

Asthma pathophysiology

Even before asthma becomes symptomatic, exposure to an allergen produces structural changes to the airways, including subepithelial fibrosis, airway smooth-muscle hypertrophy and hyperplasia, goblet cell hyperplasia and epithelial denudation. Inhaled allergens encounter antigen-presenting cells (dendritic cells, macrophages) that line the airways and mast cells that release mediators, such as histamine. The antigen-presenting cells migrate to the lymph nodes, where they induce the proliferation and activation of CD4+ T-lymphocytes (Th2 cells). These structural and infiltrating cells express a variety of mediators that lead to pathological effects, such as airway hyperresponsiveness, vasodilatation and angiogenesis [6].

Current asthma therapies

The mainstay of asthma therapy is bronchodilators, particularly β2-adrenergic agonists and anti-inflammatory drugs such as ICS (inhaled corticosteroids) [6]. Asthmatic inflammation is generally exquisitely sensitive to corticosteroids.
Biochemical Basis of Respiratory Disease 825

**Figure 1 | Mechanisms of corticosteroid function**

Corticosteroids act through a cytosolic receptor (GR) that is activated by ligand binding and translocates to the nucleus. Once in the nucleus it can bind to DNA as a dimer and, in combination with co-activator proteins, switch on the expression of anti-inflammatory genes such as MKP-1. Alternatively, activated GR can recruit co-repressor proteins and repress the activity of pro-inflammatory transcription factors such as NF-κB and AP-1. These activation steps may be attenuated in severe asthma and COPD in response to oxidative stress or cigarette smoking, viral infection, allergen challenge or chronic inflammation. Thus high levels of IL-2 and IL-4 can repress GR ligand binding, MAPK activation is able to reduce GR nuclear translocation and PI3K activation can reduce GR co-repressor activity.

Furthermore, long-term maintenance treatment with ICS reverses airflow obstruction, reduces exacerbations of asthma and the need for hospitalization and improves the quality of life [6]. The combination of LABAs (long-acting β<sub>2</sub> agonists) with low to moderate doses of ICS enhances the effectiveness of ICS, particularly with respect to asthma control and reducing exacerbations, and is better than that shown by higher doses of ICS taken alone [7]. Combination therapy has been established as the most efficacious treatment for all types of asthma [1].

**The need for new therapies**

However, not all asthmatics respond well to combination therapy and it is important to understand how and why these patients respond differently from other asthmatic patients [8]. One characteristic is the relatively corticosteroid-insensitive asthmatic patient, also labelled the CSD (corticosteroid-dependent) patient, which is part of a spectrum of patients that ends with the extreme CSR (corticosteroid-resistant) asthmatics [2]. Recent data from the SARP (Severe Asthma Research Program) [9] have begun to delineate some of the aberrant molecular and cellular mechanisms present in severe asthma that may lead to the identification of novel targets [10].

Patients with severe asthma generally have a thickened airway epithelium, basement membrane and more airway smooth muscle, which is associated with increased expression of Ki67 and reduced RB (retinoblastoma) and Bcl-2 expression [11]. In addition, hierarchical clustering of BAL (bronchoalveolar lavage) and sputum cytokine expression, the analysis of volatile organic components using an electronic nose, have supported the possibility of several distinct phenotypes of patients with severe asthma [2,10]. Such differences indicate that severe asthma-specific drug targets may exist for subgroups of these patients.

**Molecular actions of glucocorticoids**

Glucocorticoids act by binding to and activating specific cytosolic receptors [GR (glucocorticoid receptor)] (Figure 1). These receptors then translocate to the nucleus where they regulate gene expression [7]. GR is able to either induce a number of key anti-inflammatory genes after direct association with GREs (glucocorticoid response elements) in the promoter regions of these genes or selectively repress specific
inflammatory genes by a number of pleiotropic actions on promoter-specific components of transcription factor activation complexes including, but not limited to, NF-κB (nuclear factor κB) and AP-1 (activator protein-1) [7]. Importantly, these actions are mutually inhibitory. Thus the pleiotropic effects of GR may underlie their effectiveness but suggest that abnormal activation of other signalling pathways that impinge upon GR action may result in glucocorticoid refractoriness.

**Molecular mechanisms of corticosteroid resistance in asthma**

Understanding why corticosteroids are ineffective in CSD/CSR asthma at a molecular level has been aided by the fact that corticosteroid responsiveness of peripheral blood and sputum/lavage cells mimics that of the patient. Thus, in both peripheral blood monocytes and BAL macrophages from CSD/CSR patients, the ability of dexamethasone to suppress the induction of some, but not all, inflammatory mediators stimulated by TNFα or LPS (lipopolysaccharide) is attenuated [12–14]. This reduction in corticosteroid responsiveness has been ascribed, by us and by other groups, to a number of steps in the GR activation pathway including reduced GR expression, reduced GR-co-repressor expression, altered affinity of the ligand for GR, reduced ability of the GR to bind to DNA or increased expression of inflammatory transcription factors, such as NF-κB, AP-1 and C/EBPα (CCAAT/enhancer-binding protein α) [15,16] (Figure 1). The loss of C/EBPα in airway smooth-muscle cells of asthmatics may be critical for the loss of GR function since, in these cells, GR forms a critical complex with C/EBPα that enables the induction of key anti-inflammatory mediators [16].

Furthermore, unlike familial corticosteroid resistance where there are mutations in GR and a subsequent resetting of the basal cortisol level, CSR patients have normal cortisol levels and are not Addisonian [15]. There is some evidence, however, for a genetic link with the p50 subunit of NF-κB in CSR asthma [17]. A limiting factor in most of these mechanistic studies on CSD/CSR asthma is that they are conducted on limited numbers of subjects, generally six to twelve in each. Changes in ligand binding are restored when cells are incubated in serum from normal subjects, and they may reflect a spill-over effect of CSR inflammation [15].

**Defects in ligand binding, nuclear translocation and cofactor association**

Exposure to allergens and to important asthma cytokines such as IL-2, IL-4 and IL-13 reduces nuclear ligand binding affinity in T-lymphocytes, nuclear translocation and GR-GRE binding. This may account for the local resistance to the anti-inflammatory actions of corticosteroids [12,15,18]. Changes in ligand binding are restored when cells are incubated in serum from normal subjects, and they may reflect a spill-over effect of CSR inflammation [15]. Increased expression of the dominant-negative isoform of GR, GRβ, may be important in some CSD/CSR patients. Knockdown of GRβ using siRNA (small interfering RNA) can up-regulate GRα function in BAL cells from CSR asthmatics [19]. The ligand for GRβ has recently been determined and this may lead to new therapeutic strategies [20].

p38 MAPK (mitogen-activated protein kinase) activity is increased in severe asthma [14,21] and it has been proposed that GR phosphorylation induced by p38 MAPK activation may induce a loss of GR nuclear translocation and function [14,22]. This may be an indirect effect as mutation of potential p38 MAPK sites in the C-terminus of GR did not affect the ability of p38 MAPK to modulate GR function [22]. The important role of dual MKP-1 (MAPK phosphatase-1) expression in regulating p38 MAPK activity and the fact that it is induced by corticosteroids has raised the possibility that changes in p38/MKP-1 homoeostasis may be important in contributing to corticosteroid-insensitivity in CSD/CSR asthma [23].

Depending on the stimulus used, other MAPKs or kinase pathways may also regulate GR function, e.g. ERK (extracellular-signal-regulated kinase) MAPK, when T-cells are stimulated by co-receptor activation or superantigen [15]. In addition, excessive JNK (c-Jun N-terminal kinase) and AP-1 activity may affect GR function in severe asthma in response to inflammatory stimuli, such as TNFα [15]. A 2 week treatment with prednisolone (40 mg/day) did not affect the numbers of phospho-c-Jun-, activated JNK- or c-Fos-positive cells in CSR asthma biopsies [24].

It is unclear whether increased p38 MAPK and JNK activation is a primary or secondary defect caused by excessive production of a unique pattern of cytokines in asthmatic airways or whether it too can affect GR phosphorylation [15]. Furthermore, whether these kinases directly phosphorylate GR or act via phosphorylation of GR cofactors has not been systematically studied in severe asthma [15].

Other groups of CSR asthmatic subjects show no loss of nuclear translocation and no defect in side-effect profile but a loss in anti-inflammatory properties [15]. This may reflect a reduced ability of GR to associate with key transcriptional repressors such as HDAC2 (histone deacetylase 2) or Brg1 (Brahma-related gene 1) [15]. HDAC2 expression is reduced in PBMCs (peripheral blood mononuclear cells) from patients with CSR asthma [25], whereas Brg1 is absent in many cases of glucocorticoid-insensitive corticocorticoid adenocarcinomas found in subjects with Cushing’s disease [26].

**Viral infection**

Recurrent exacerbations are a major cause of morbidity and have a high health care cost in patients with asthma. Recent clinical evidence indicates that viral-induced wheezing is not responsive to oral or inhaled corticosteroids [27,28], perhaps due to the fact that rhinoviral infection can reduce GR nuclear translocation suppression of cytokine release through an NF-κB-dependent process [29]. Therapeutic interventions aimed at treating viral or bacterial infections may therefore restore steroid responsiveness in these patients.
Cigarette smoking
Patients with asthma who smoke have a local and systemic resistance to the anti-inflammatory actions of corticosteroids [15]. This persists to some extent even in ex-smokers although the effects may be lost after smoking cessation [30]. Smoking cessation improves basal lung function but requires at least a year to demonstrate any improvement in corticosteroid responsiveness with respect to morning PEF (peak expiratory flow), but not FEV1, after a high dose of prednisolone [30]. PPARs (peroxisome-proliferator-activated receptors) are nuclear receptors activated by polyunsaturated fatty acid derivatives, oxidized fatty acids and phospholipids. PPARγ agonists produce a distinct anti-inflammatory profile in macrophages compared with that seen with dexamethasone, and the combination of both a PPARγ agonist and a corticosteroid may have a greater anti-inflammatory effect [31,32]. This becomes more interesting as corticosteroids can induce PPARγ expression [31]. Indeed, Rosiglitazone has been reported to increase lung function in smoking asthmatics who do not respond well to either inhaled or oral corticosteroids [33]. The combination of a PPARγ agonist and a corticosteroid may provide additional and more complete suppression of inflammation in severe asthma compared with monotherapy with either drug, and single molecules that can activate both receptors are in preclinical development [32].

The effects of cigarette smoking on corticosteroid function are due predominantly to oxidative stress and the molecular mechanisms underlying this are discussed in the next section on COPD (chronic obstructive pulmonary disease).

COPD
COPD, unlike asthma, is related mainly to cigarette smoking [34]. The cigarette smoke activates macrophages and airway epithelial cells to release mediators that recruit and activate CD8+ T-lymphocytes (CD8+ Tc cells) and neutrophils. Macrophages and neutrophils release proteases that break down connective tissue, producing emphysema and stimulating mucus hypersecretion [34]. Although COPD is a chronic inflammatory disease, the type of inflammation and its location in the lung are distinct from that of asthma. In contrast with asthma, no currently available treatments slow the progression of COPD and corticosteroids fail to reduce the inflammatory process in COPD and have a minimal effect on the inexorable decline in lung function and have only a small effect in reducing COPD exacerbations [34].

Furthermore, neither inhaled nor oral steroids are able to significantly attenuate inflammatory cell numbers or the expression of most key cytokines, chemokines or proteases in induced sputum or airway biopsies of patients with COPD [34]. Corticosteroids are also less effective at suppressing the release of many of these inflammatory mediators from COPD alveolar macrophages in vitro compared with cells from normal smokers and non-smokers [34]. This may reflect an intrinsic defect in corticosteroid function since other anti-inflammatory therapies, such as theophylline and resveratrol, are able to suppress the release of inflammatory mediators from these cells [34]. Glucocorticoid-insensitivity therefore presents a profound management problem in COPD and these patients account for a disproportionate amount of health care costs due to frequent hospital admissions [34].

Oxidative stress
Oxidative stress has been implicated as a driving force behind the inflammatory response and lack of corticosteroid-sensitivity evident in patients with COPD and severe asthma [34]. Moreover, antioxidants are able to restore corticosteroid functions that were reduced in response to cigarette smoke or other oxidative stresses [35]. These antioxidants have included NAC (N-acetylcysteine), NAL (nacystelyn) and the SOD (superoxide dismutase) mimetic AEOL 10150 studied in both primary human cells and in animal models [35]. High levels of oxidative stress in combination with high nitric oxide levels are seen in patients with asthma and will result in the formation of peroxynitrite, tyrosine nitrination and lipid peroxidation products [35] and this has been linked to corticosteroid-insensitivity. NOS2 (nitric oxide synthase 2) inhibitors have recently been shown to be safe, but ineffective, in mild asthma [36], but they may play an important role in the treatment of patients with COPD, severe asthma and in smoking asthmatics who have higher levels of oxidative stress [35]. The activity of p38 MAPK is also enhanced in COPD and whether this contributes towards corticosteroid-insensitivity as described for asthma is under investigation [37].

Resveratrol (3,5,4′-trihydroxystilbene), a component of red wine, has anti-inflammatory and antioxidant properties [38]. Although resveratrol inhibits cytokine release in vitro by alveolar macrophages from patients with steroid-resistant COPD, it is not clear whether this is due to its antioxidant properties. Nevertheless, this compound may also be beneficial for patients with severe asthma [38]. Curcumin is a compound that has many therapeutic properties due to its antioxidative, anti-inflammatory and anticancerous effects and can restore corticosteroid responsiveness in cells exposed to H2O2 or cigarette smoke by preventing HDAC2 degradation [39].

HDAC2 expression in COPD
HDAC2 activity is reduced in BAL macrophages of smokers and inversely correlates with glucocorticoid-sensitivity [40]. HDAC2 expression and activity is further reduced in BAL macrophages, bronchial biopsies and peripheral lung tissue of patients with COPD [41] and in peripheral blood cells of asthmatics that smoke compared with non-smokers [42]. Importantly, overexpression of HDAC2 in corticosteroid-insensitive BAL macrophages from COPD subjects restored corticosteroid responsiveness, and conversely, suppression of HDAC2 expression using RNA interference in BAL macrophages from normal subjects attenuated corticosteroid-sensitivity [40]. The suppression of HDAC2 activity may be due to tyrosine nitration, implicating a potential therapeutic
role for antioxidants or NOS2 inhibitors in restoring corticosteroid responsiveness.

**HDAC2 activation by theophylline**

Theophylline has been used as a bronchodilator in COPD asthma for many years due to its weak actions compared with β2-agonists and its side-effect profile. However, it is becoming clear that theophylline has significant anti-inflammatory effects on COPD at lower plasma concentrations that are distinct from its effects on airway calibre (phosphodiesterase 4 inhibition) or from its classical side-effect mechanisms (adenosine receptor antagonism) [43], which are seen at higher plasma concentrations. Furthermore, adding a low dose of theophylline is more effective than increasing the dose of inhaled corticosteroids in patients who are not adequately controlled and is why theophylline withdrawal worsens the control of patients with severe asthma [43].

Theophylline activates HDAC2 preferentially under conditions of oxidative stress and potentiates the anti-inflammatory effects of corticosteroids in vitro [44]. Using iTRAQ (isobaric tag for relative and absolute quantification) technology we demonstrated that this was due to marked changes in the pattern of proteins associated with theophylline under conditions of oxidative stress [44]. On the basis of this knowledge, studies are now being conducted examining the efficacy of theophylline as a corticosteroid add-on therapy in COPD. In a smoking mouse model of COPD, theophylline and dexamethasone were able enhance HDAC2 activity in the lung and attenuate BAL macrophage and neutrophil recruitment in contrast with the lack of effect seen with either drug alone [45]. More recently, the combination of low-dose theophylline and standard therapy in 39 COPD patients improved the exacerbation rate and reduced sputum IL-8 and TNFα [46]. Furthermore, we have demonstrated that lung function, sputum cell counts and cytokine levels were significantly improved in COPD patients taking a combination of ICS and slow-release theophylline subsequent to an increase in HDAC activity [47]. A similar study in smoking asthmatics has also shown a significant increase in lung function with the combination of drugs only [48].

The exact mechanism whereby theophylline and other agents activate HDAC2 is not yet certain, but it may be through an effect on PI3K (phosphoinositide 3-kinase) [49]. Mice that express a PI3Kδ (phosphoinositide 3-kinase) [49], which are seen at higher plasma concentrations. Furthermore, adding a low dose of theophylline is more effective than increasing the dose of inhaled corticosteroids in patients who are not adequately controlled and is why theophylline withdrawal worsens the control of patients with severe asthma [43].

Theophylline activates HDAC2 preferentially under conditions of oxidative stress and potentiates the anti-inflammatory effects of corticosteroids in vitro [44]. Using iTRAQ (isobaric tag for relative and absolute quantification) technology we demonstrated that this was due to marked changes in the pattern of proteins associated with theophylline under conditions of oxidative stress [44]. On the basis of this knowledge, studies are now being conducted examining the efficacy of theophylline as a corticosteroid add-on therapy in COPD. In a smoking mouse model of COPD, theophylline and dexamethasone were able enhance HDAC2 activity in the lung and attenuate BAL macrophage and neutrophil recruitment in contrast with the lack of effect seen with either drug alone [45]. More recently, the combination of low-dose theophylline and standard therapy in 39 COPD patients improved the exacerbation rate and reduced sputum IL-8 and TNFα [46]. Furthermore, we have demonstrated that lung function, sputum cell counts and cytokine levels were significantly improved in COPD patients taking a combination of ICS and slow-release theophylline subsequent to an increase in HDAC activity [47]. A similar study in smoking asthmatics has also shown a significant increase in lung function with the combination of drugs only [48].

The exact mechanism whereby theophylline and other agents activate HDAC2 is not yet certain, but it may be through an effect on PI3K (phosphoinositide 3-kinase) [49]. Mice that express a PI3Kδ (phosphoinositide 3-kinase) [49], which are seen at higher plasma concentrations. Furthermore, adding a low dose of theophylline is more effective than increasing the dose of inhaled corticosteroids in patients who are not adequately controlled and is why theophylline withdrawal worsens the control of patients with severe asthma [43].

**Conclusion**

More evidence is required to elucidate the pathophysiological changes in the airway that define CSD/CSR asthma. Increasing knowledge of the molecular mechanisms by which CSD/CSR asthmatics lose responsiveness to glucocorticoids opens up the possibility of defined, patient-specific therapy. Not all CSD/CSR patients have glucocorticoid-insensitive for the same reason and some drugs will therefore be more effective than others in each group of CSR patients. Similarly, understanding the molecular mechanisms underlying relative corticosteroid-insensitivity in COPD will allow the development of novel anti-inflammatory agents or better corticosteroid-sparing drugs. Rapid tests that distinguish some of these molecular defects in cells from these patients may make the advent of selective therapy closer and more effective.

**Acknowledgment**

We apologize to those authors whose work was not cited due to limitations on the number of references.

**Funding**

Work in our laboratories is funded by the European Union, Medical Research Council, The Wellcome Trust, AstraZeneca, Covance, GlaxoSmithKline and Pfizer.

**References**


