

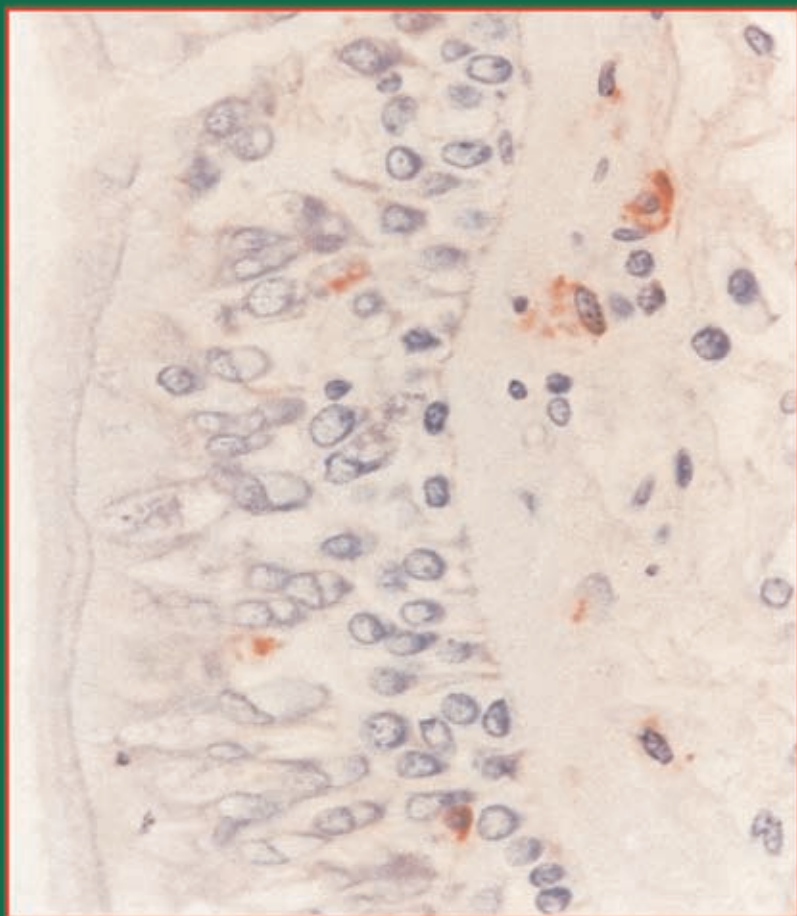
Therapeutic Strategies

# ASTHMA

Current Treatments

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R. Polosa • S. T. Holgate



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Therapeutic Strategies

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# ASTHMA: CURRENT TREATMENTS

Edited by

Riccardo Polosa  
Stephen T. Holgate

CLINICAL PUBLISHING

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## Preface

The discovery of adrenergic agonists and corticosteroids at the start of the 20th century has provided the basis for much of the treatment of asthma. The last 50 years has witnessed major advances in our understanding of asthma and significant improvement in these therapeutic agents with respect to safety, efficacy and duration of action. Inhaled corticosteroids (ICS), short and long acting  $\beta_2$ -agonists (SABAs and LABAs) are now the mainstay of asthma treatment as advocated by disease management guidelines. When used regularly, ICS reduce both morbidity and mortality and the addition of LABAs to the management plan appears to improve control of moderate-to-severe asthma. Yet, despite the undoubted efficacy of this combination for most patients, there remains ~10% of the asthmatic population in whom symptoms persist with considerable impact on quality of life and disproportionate use of healthcare resources.

While ICS are highly effective in suppressing airway inflammation in asthma, they do not influence the natural history of the disease even when started in early childhood and are largely ineffective in virus-induced exacerbations and in those asthmatics who smoke. There is also heterogeneous group of asthma patients who are genuinely refractory to corticosteroids. A few additional therapies are available and include methylxanthines, anticholinergics, cromones and leukotriene modifiers, but these are of variable efficacy. The introduction of a monoclonal antibody that is able to block IgE effects in severe allergic asthma is a breakthrough in asthma management but only for a limited number of patients. It should also be remembered that 'reagin', the biological activity of IgE was first discovered in 1922 by Prausnitz and Kustner and the biological activity of the leukotrienes, slow reacting substance (SRS), was recognised by Trethewie and Kellaway in 1938 and yet for both of these 'activities' a further 40–45 years elapsed before their molecular basis was discovered and another 15–40 years before the development of therapies that target these. One could legitimately ask why progress has been so slow in the development of new therapeutic agents in this field. Part of the difficulty may be in the high dependency that the pharmaceutical and biotechnology industries have placed on antigen challenge models both in animals and humans to screen for anti-asthma activity, whereas allergen/antigen driven responses represent only part of the asthmatic paradigm: diet, air pollutants, tobacco smoke, drugs and viruses are all known to impact on the origins and progression of asthma. Much of the testing of novel chemical activities has also been undertaken on 'acute' models, whereas asthma is often a chronic, albeit relapsing disease that often spreads across a lifetime. Some of the therapeutic targets identified in these models such as neuropeptide antagonists, PAF antagonists, bradykinin inhibitors, adhesion molecule antagonists, mast cell 'stabilising' agents and some cytokine blockers (e.g. anti-IL5) have all shown great promise in animal models but have failed when tested in humans with asthma. The time has therefore arrived to take a fresh look at asthma and at the novel strategies that are now appearing on the horizon.

*Asthma – Current Treatments* provides readers with an overview of possible novel approaches in a field in need of innovation. The book is divided into four sections, each of which covers a particular 'theme'. The book begins with a series of contributions on the modifications of current therapeutic approaches and a careful consideration of their wider

activities and possible side effects. The second section critically reviews possible alternative approaches targeted towards bronchodilation. The third section addresses some of the really innovative discoveries that have therapeutic implications in asthma to include novel immunomodulatory approaches targeting the innate immune response. The final section covers the specific aspects of asthma where there remains a major unmet clinical need, such as acute exacerbations and cigarette smoking in asthma that causes loss of response to corticosteroids.

The range of subjects covered and the level of imagination required to make each section a stimulating and educational read has called for remarkable commitment from a large number of leading experts from the pharmaceutical industry and academic world. We would like to acknowledge their considerable contributions to this book without whose help, this collection of informative and up-to-date reviews would not have been possible.

We hope that you will find this book interesting and helpful, and that it will give as much enjoyment to you, the reader, as we have had in its design and editing. Finally, and most importantly of all, our hope is that this new publication shows that the field of novel asthma therapies has a most promising future and that it may be of assistance in the process of finding better therapies for our patients with asthma both now and in the future.

*Riccardo Polosa  
Stephen T. Holgate*

# Section I

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**First line anti-inflammatory and  
bronchodilator drugs**

# 1

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## Novel mechanistic aspects of glucocorticosteroids in relation to asthma therapy

*I. M. Adcock*

### INTRODUCTION

The treatment of chronic inflammatory diseases was revolutionized by the discovery of the therapeutic utility of glucocorticosteroids in the 1950s. Since this time they have been the mainstay of treatment for chronic inflammatory diseases. Their utility has been tempered, however, by the increasing risk of debilitating side-effects with higher dose therapy. In the treatment of airway diseases side-effects can be limited by targeted delivery to the airway and lung and significant progress has been made through the use of increasingly selective molecules, and through a variety of lung targeting strategies. Moreover, the recent developments in our understanding of the molecular and structural mechanisms of action of glucocorticosteroid actions have suggested that it may be possible to develop new glucocorticosteroids with intrinsically different pharmacology which lacks the ability to induce many of the pathways involved in the manifestation of side-effects. A combination of these developments will enable the design of agents with an enhanced therapeutic index.

Many of the key processes underlying human physiology are regulated by glucocorticoids and their importance is demonstrated by the series effects of cortisol lack due to illness or of structural changes/mutations in the glucocorticosteroid receptor (GR) [1]. These include glucose homeostasis, after which they are named, and the regulation of metabolism, cell survival/death, development and response to stress. In the context of inflammation and the protective response to infection or noxious stimuli they also have important effects on the immune system [2].

All major chronic inflammatory diseases including inflammatory bowel disease, psoriasis, rheumatoid arthritis and asthma can be treated with glucocorticoids [3]. Indeed, these are the most effective anti-inflammatory agents currently available. However, since the elucidation of their clinical effectiveness in 1948 for the treatment of rheumatoid arthritis, by Kendall and Hench, who were awarded the Nobel Prize for Medicine for this work in 1950 [4], it has become clear that the beneficial effects of ever increasing doses of glucocorticosteroids is countered by the onset of severe debilitating side-effects [5]. It is this aspect of glucocorticosteroid actions that has limited their systematic use in a number of chronic diseases [5]. These side-effects (Table 1.1) include osteoporosis and suppression of the hypothalamic-pituitary-adrenocortical (HPA) axis, reduction of growth velocity in children, bone mineral loss, weight gain, ocular symptoms, and skin changes [5]. Although, over the years, newer glucocorticosteroids have been developed and improvements in topical delivery have reduced systemic exposure, steroid phobia in relation to potential adverse effects still

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**Table 1.1** Tissue/organ specific side-effects of topical and systemic corticosteroids

<i>Endocrine system, metabolism, electrolytes</i>
Cushing's syndrome
Diabetes mellitus
Adrenal atrophy
Growth retardation
Hypogonadism, delayed puberty
Increased sodium retention and potassium excretion
<i>Skeleton and muscle</i>
Muscle atrophy/myopathy
Osteoporosis
Bone necrosis
<i>Skin</i>
Atrophy, striae, distension
Delayed wound healing
Steroid acne, perioral dermatitis
Erythema, teleangiectasia, petechiae, hypertrichosis
<i>Eye</i>
Glaucoma
Cataract
<i>CNS</i>
Disturbances in mood, behaviour, memory and cognition
'Steroid psychosis', steroid dependence
Cerebral atrophy
<i>Immune system</i>
Increased risk of infection
Re-activation of latent viruses
<i>Gastrointestinal</i>
Peptic ulcer
Gastrointestinal bleeding
Pancreatitis
<i>Cardiovascular system</i>
Hypertension
Dyslipidaemia
Thrombosis
Vasculitis

remains. It is hoped that by understanding how glucocorticosteroids function at the cell and molecular level it will be possible to develop new, safer drugs in the future.

## AIRWAY INFLAMMATION IN ASTHMA

All patients with asthma have a specific pattern of inflammation in the airways that is characterized by degranulated mast cells, an infiltration of eosinophils and an increased number

of activated T helper 2 (Th2) cells [6]. It is believed that this specific pattern of inflammation underlies the clinical features of asthma, including intermittent wheezing, dyspnoea, cough and chest tightness. Suppression of this inflammation by corticosteroids controls and prevents these symptoms in the vast majority of patients. Multiple mediators are produced in asthma and approximately 100 known inflammatory mediators that are increased include: lipid mediators, inflammatory peptides, chemokines, cytokines and growth factors [7]. There is increasing evidence that structural cells of the airways, such as epithelial cells, airway smooth muscle cells, endothelial cells and fibroblasts are a major source of inflammatory mediators in asthma. Epithelial cells may play a particularly important role, as they may be activated by environmental signals and they may release multiple inflammatory proteins, including cytokines, chemokines, lipid mediators and growth factors [8].

Inflammation is mediated by the increased expression of multiple inflammatory proteins, including cytokines, chemokines, adhesion molecules, and inflammatory enzymes and receptors [9]. Most of these inflammatory proteins are regulated by increased gene transcription, which is controlled by proinflammatory transcription factors, such as nuclear factor- $\kappa$ B (NF- $\kappa$ B) and activator protein-1 (AP-1) that are activated in asthmatic airways [10]. For example, NF- $\kappa$ B is markedly activated in epithelial cells of asthmatic patients [11] and this transcription factor regulates many of the inflammatory genes that are abnormally expressed in asthma [12]. NF- $\kappa$ B may be activated by rhinovirus infection and allergen exposure, both of which exacerbate asthmatic inflammation [13, 14].

## NUCLEAR FACTOR- $\kappa$ B

NF- $\kappa$ B, consisting usually of the p65(RelA)/p50 heterodimer, normally resides in the cytoplasm held in an inactive state by its inhibitor chaperone, inhibitor  $\kappa$ B alpha ( $I\kappa$ B $\alpha$ ). Phosphorylation of  $I\kappa$ B $\alpha$  leads to ubiquitination and subsequent proteolysis of  $I\kappa$ B $\alpha$  thereby releasing active NF- $\kappa$ B enabling it to translocate into the nucleus. The  $I\kappa$ B kinase (IKK) complex responsible for  $I\kappa$ B $\alpha$  phosphorylation contains three catalytic subunits, termed IKK-1 (IKK $\alpha$ ), IKK2 (IKK $\beta$ ) and IKK $\gamma$  [15]. IKK2 is the important IKK for the control of these proinflammatory genes. In the nucleus, NF- $\kappa$ B binds to target DNA elements and upregulates the transcription of many genes encoding cytokines, chemokines, growth factors, enzymes, adhesion molecules, receptors/receptor antagonists involved in immune and inflammatory responses and potentially relevant to the pathogenesis of asthma. It is important to appreciate that the clusters of genes activated by NF- $\kappa$ B can be cell and stimulus dependent and therefore NF- $\kappa$ B activation is context dependent [16, 17].

In addition to NF- $\kappa$ B, other kinase pathways are probably essential for amplifying and perpetuating the inflammatory response in asthma, e.g. the mitogen-activated protein kinases (MAPKs), and more signal specific Janus kinases (JAKs)/signal transduction-activated transcription (STAT) factor pathways [18–20]. Although each pathway can activate specific downstream transcription factors, there is considerable cross-talk between kinase pathways both at the membrane proximal and the transcription factor proximal ends of each pathway which allows signal integration. The importance of the NF- $\kappa$ B pathways has been shown by the ability of inhibitors to modulate the expression of many inflammatory mediators and adhesion molecules, control granulocyte apoptosis and chemotaxis and T-cell, macrophage and epithelial cell function [18, 21–23]. Furthermore, NF- $\kappa$ B inhibitors have been reported to regulate airway smooth muscle (ASM) proliferation and various other factors involved in airway remodelling in an animal model of asthma [19].

## SEVERE TREATMENT OF INSENSITIVE ASTHMA

The combination of  $\beta_2$ -agonists and glucocorticosteroids is highly effective in treating about 95% of patients without problems in terms of adverse effects. However, 5–10% patients do

not respond well to these treatments and these patients account for ~50% of the healthcare costs of asthma [24, 25]. These subjects include severe asthmatics who are at increased risk of dying from asthma and who have continued morbidity from both their disease and the oral corticosteroids that are often used to treat it [24, 25]. Furthermore, despite the availability of effective and relatively cheap treatments, there is still a considerable degree of under-treatment of severe asthma. For example, a European survey showed that only ~25% of patients with severe asthma were receiving inhaled corticosteroids [26].

## **PATHOLOGY OF SEVERE ASTHMA**

In a cross-sectional study of 163 severe asthmatics (European Network for Understanding Mechanisms of Severe Asthma, ENFUMOSA) [27] it was found that these patients were predominantly female, were more aspirin sensitive and had lower levels of atopy than mild-to-moderate asthmatics. In addition, these subjects had greater airway obstruction, increased air-trapping and a slightly lower diffusing capacity. Sputum eosinophil numbers remained elevated in 30% of subjects despite high-dose inhaled and often oral steroids and, importantly, there was a marked increase in sputum neutrophilia. This study therefore suggested that severe asthma might be a separate disease from mild-to-moderate, therapy-responsive asthma [27].

Previous studies have shown that sputum and tissue eosinophilia vary in severe asthma with one subgroup showing 'normal' levels and the other 'elevated' levels. The subset of patients with high eosinophils had been shown previously to have a greatly thickened basement membrane suggesting a difference in airway remodelling [28] perhaps involving distinct types of collagen [29]. This difference may account for the profound differences seen in reversibility and other measures of lung function in some patients with severe asthma. However, there are no biological/disease markers that clearly differentiate one severe asthma group from the other and current 'markers' overlap.

Thus, a distinct pathophysiology present in the severe asthma population may account in part for these differences in responsiveness, i.e. neutrophilic inflammation, but other explanations may involve corticosteroid resistance that prevents corticosteroids from functioning effectively on the same pathological processes that occur in mild asthma [30] or that excessively remodelled airways are fixed and non-responsive to corticosteroids [31, 32]. An alternative explanation for the differences seen in inflammatory patterns seen in glucocorticosteroid-resistant (CR) asthma other than distinct diseases has been proposed by Hamid and co-workers [33]. Before oral steroid treatment, bronchial levels of T cells, eosinophils, mast cells, macrophages and neutrophils were similar in corticosteroid-sensitive (CS) and CR subjects. However, steroids decreased T cell and eosinophil counts in CS patients but not CR patients where mast cell numbers were decreased [33].

Further work is required to correlate clinical and inflammatory phenotypes of asthma with treatment response. The ENFUMOSA data suggest that, rather than severe asthma being a distinct disease, it may consist of several (at least two) different diseases. Ongoing studies such as the European 'Bio-Air' study, which will include biopsy data and the US 'The Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimens' (TENOR) study may provide an answer to this question.

## **MOLECULAR EVENTS IMPLICATED IN SEVERE ASTHMA**

Bronchoalveolar lavage of a group of CR subjects revealed an increased number of cells expressing interleukin (IL)-2, IL-4 and IL-13 mRNA compared to CS asthmatics and a lack of suppression of these cytokines by prednisolone therapy [33]. This suggested that the profile of cytokine expression may underlie the poor responsiveness to glucocorticosteroids in these patients. Biopsy studies are difficult to perform on these subjects and importantly for

understanding the molecular and biochemical mechanisms underlying insensitivity to corticosteroids, corticosteroids are also less effective in inhibiting the expression of inflammatory cytokines from circulating monocytes and T lymphocytes in CR compared with CS subjects [30, 34].

Leung and colleagues demonstrated that there was an altered affinity of dexamethasone for GR in subjects with CR asthma and that exposure of peripheral blood mononuclear cells (PBMC) to a combination of IL-2 and IL-4, or IL-13, reduces dexamethasone affinity for the GR and resistance to its anti-inflammatory action *in vitro* [30, 34]. However, it is unclear whether these changes in receptor affinity alter the function response to glucocorticosteroids at least with respect to changes in airway remodelling [35]. Initial results suggested that IL-2 and IL-4 were able to upregulate the expression of the dominant negative form of GR, GR $\beta$  [30, 34]. However, the role of GR $\beta$  in repressing GR function has been questioned in other groups of CR patients [36, 37] although more recent data suggest that tissue-selective expression of other GR isoforms may play a role in glucocorticosteroid insensitivity [38].

Previous data had also indicated that there was a reduction in GR binding to DNA in response to dexamethasone which was associated with excessive activation of AP-1, increased c-fos expression and JNK activity in response to inflammatory stimuli, such as tumour necrosis factor  $\alpha$  (TNF $\alpha$ ) [34, 35, 39, 40]. In another study, examining steroid responsiveness in asthma, expression of c-fos, but not c-jun or GR $\beta$ , inversely correlated with steroid sensitivity [39]. Finally, in Crohn's disease, steroid resistance is associated with increased epithelial activation of JNK, p38 MAPK, NF- $\kappa$ B and AP-1 [41] suggesting that drugs targeted towards these mediators may be useful in CR asthma.

Furthermore, other factors may also be important. For example, STAT5 phosphorylation under the control of JAK3 was shown to prevent GR nuclear import in murine HT-2 cells following IL-2 stimulation [42]. It is important to confirm these studies in human cells as there are distinct differences between human and rodent GR [43]. Th2 cytokines have also been proposed to play a role in severe CR asthma. A recent study has shown that CD4+ T cells from CR asthmatics are less able to produce the anti-inflammatory cytokine IL-10 in response to dexamethasone than cells from CS patients [44].

We have also demonstrated that IL-2 and IL-4 (but neither alone) activates p38 MAPK, phosphorylation of GR at Ser226, reduced GR affinity, reduced nuclear localization and reduced repression of stimulated granulocyte-macrophage colony-stimulating factor (GM-CSF) release in human PBMCs [45]. These effects were reversed by SB203580 an inhibitor of p38 MAPK. In addition, histone acetylation in response to high concentrations of corticosteroids is abnormally reduced in PBMC from CR and corticosteroid-dependent (CD) patients [46]. In approximately half of the patients this is a result of reduced nuclear localization of GR, whereas in the other half of patients there is *normal* nuclear localization but reduced activation of nuclear cofactors [46]. Therefore, drugs that enhance GR nuclear translocation are likely to be of benefit in 50% of these patients. One important enzyme that is rapidly induced by GR is MAPK phosphatase 1 (MKP-1) [47], which dephosphorylates and inactivates p38 MAPK. Thus changes in p38/MKP-1 homeostasis may be important in contributing to steroid insensitivity [48].

## EFFECT OF CIGARETTE SMOKING IN ASTHMA

Interestingly, patients with asthma who smoke cigarettes also show resistance to the anti-inflammatory actions of glucocorticosteroids and this persists to some extent even in ex-smokers [49, 50]. Cigarette smoking is an oxidative stress and may affect several aspects of glucocorticosteroid function including GR nuclear translocation and effects on nuclear cofactors. Intriguingly, there is a marked increase in oxidative stress in severe CR asthma [51, 52]. Increases in markers of oxidative stress such as 8-isoprostane also appear to be relatively resistant to treatment with steroids [53]. This suggests that anti-oxidants or nitric oxide

synthase (NOS) inhibitors, which would reduce the formation of peroxynitrite, may therefore be effective therapies in CR asthma.

## ENHANCING GLUCOCORTICOID RESPONSIVENESS IN ASTHMA

With airway diseases it has been possible to design and optimize glucocorticosteroids specifically for inhaled therapy [54]. Molecules such as fluticasone propionate (FP) are not only extremely potent but also have negligible oral bioavailability and undergo rapid hepatic inactivation to further reduce systemic exposure [54]. Furthermore, it is possible to target the activity of glucocorticosteroids preferentially to the lung [55, 56]. As such, both budesonide and ciclesonide can undergo fatty acid esterification within the lung resulting in a depot of highly lipophilic molecules that are retained in the lung and these depots are thought to slowly release active compound over time.

Other aspects of glucocorticosteroid pharmacology may be utilized to improve the treatment of airways disease in particular lung delivery and lung retention can be modified to enhance glucocorticosteroid efficacy. Currently used inhaled glucocorticosteroids have been developed with high potency for GR, thus FP and mometasone furoate (MF) are both considerably more potent than beclomethasone dipropionate (BDP), ciclesonide, and budesonide in *in vitro* assays of anti-inflammatory activity [57, 58]. This enables the use of lower doses to obtain equivalent clinical benefit. However, *in vitro* potency alone does not establish clinical dose and drug delivery devices and pharmacokinetics have a strong influence on therapeutic index [59]. The current drugs are probably as potent as necessary and the next stages in improving the therapeutic window for glucocorticosteroids will probably result from alterations in drug delivery, pharmacokinetics or from the development of selective agents based on the newer concepts of glucocorticosteroid mechanisms.

## REDUCED ORAL BIOAVAILABILITY AND METABOLIC INACTIVATION

The high systemic exposure seen with most current inhaler devices is due to the deposition of 60–90% of a given dose in the mouth and pharynx [60]. This has led to the search for modified glucocorticosteroids that have reduced oral uptake. The development of budesonide was a significant improvement in inhaled glucocorticosteroids by reducing oral bioavailability to 11% compared with that of BDP (46%) [60]. However, the most recent generation of compounds (FP, ciclesonide and MF) have an oral bioavailability of less than 1% [60]. Thus, essentially all the systemic exposure from these compounds is a result of uptake through the target organ, i.e. the lung.

Most glucocorticosteroids are cleared from the circulation by hepatic metabolism. This generally results in the formation of inactive metabolites such as the 17 $\beta$ -carboxylic acid metabolite of FP. However, in contrast, beclomethasone monopropionate (BMP), a potent glucocorticosteroid, is the metabolite of BDP and can be classified as the first successful glucocorticosteroid prodrug many years before the development of ciclesonide [61]. Further reductions in systemic bioavailability have been achieved by rapid inactivation in the plasma by serum paraoxonase [59]. For example, lactone (cyclic ester) glucocorticosteroids display the ideal combination of stability in lung tissue with extremely rapid ( $t_{1/2} \approx 24$  min) inactivation in plasma [62] due to the plasma and liver specific degradation by paraoxonase. The terms 'antedrug' [59] or 'soft' drug [59] have been applied to these compounds.

## LUNG RETENTION AND PRODRUGS

Modifications of the basic glucocorticosteroid backbone structure have resulted in improved lung retention and therefore reduced systemic bioavailability. Budesonide forms highly lipophilic fatty acid esters in the lung [55], which results in both prolonged tissue binding

and the slow release of active drug. This leads to an improved topical selectivity and increased duration of action. Ciclesonide and other glucocorticosteroids containing free hydroxyl groups at position 21 such as BMP will also undergo the same modification. An alternative strategy was used to enhance FP lung retention. Increasing the lipophilicity of FP resulted in a higher affinity for lung tissue due to exhibiting an 80-fold slower rate of dissolution within the lung compared with budesonide [54]. Inhaled glucocorticosteroids can induce irritation of the larynx and local immune suppression and as a result dyspnoea is a common side-effect [5]. To overcome this problem, prodrugs such as BDP and ciclesonide have been developed in order to reduce these local and systemic side-effects [59].

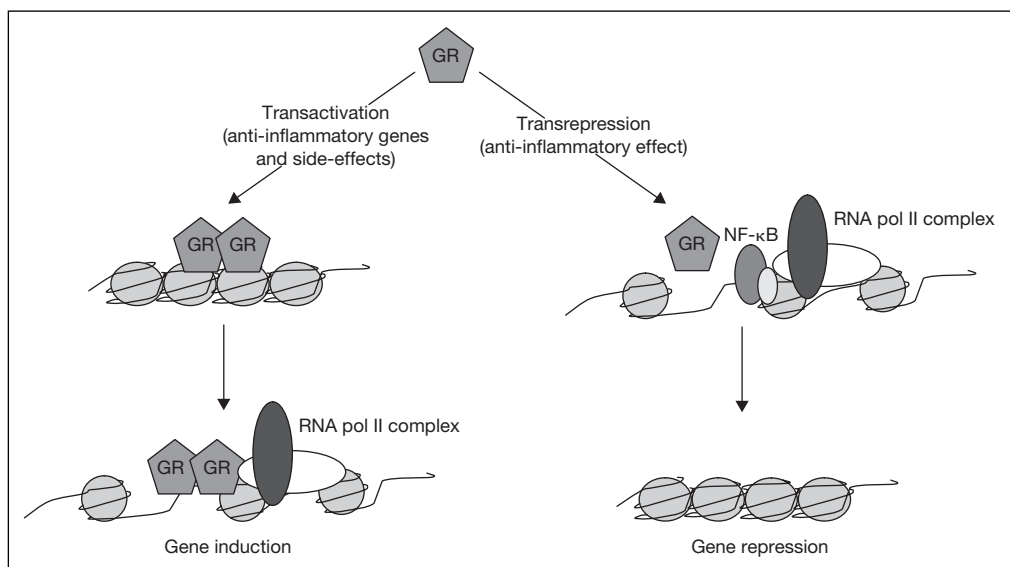
## DRUG DELIVERY

The change to CFC-free propellants in inhalers highlighted the effect of changes in the physicochemical properties of glucocorticosteroids on drug efficacy [63]. This became very evident with the demonstration that BDP delivered by QVAR® (beclomethasone dipropionate HFA) required half the daily dose of CFC-BDP [64]. Furthermore, liposomal formulations of budesonide and polylactic acid microspheres encapsulating BDP has been used to enhance lung retention and prolong duration [65]. Recent evidence has shown that changing the size of monodispersed particles of salbutamol can alter the site of deposition [66]. More importantly, 3 µm particles have a similar clinical efficacy as 20-fold greater doses of standard inhaler devices. If similar effects can be seen with inhaled glucocorticosteroids a 20-fold reduction in glucocorticosteroid dose will improve the therapeutic index [66]. Thus, further improvement in inhaler devices is likely to lead to better airway deposition of glucocorticosteroid at the site of disease and may allow clinically relevant improvements in lung function to be achieved with smaller doses of glucocorticosteroid, thereby reducing the incidence of lung disease.

## MECHANISMS OF GLUCOCORTICOSTEROID ACTION

GR is a ligand-activated transcription factor localized within the cytoplasm of virtually all cells [67]. Glucocorticosteroids freely diffuse from the circulation, bind to GR and induce a rapid translocation of the receptor into the nucleus. Many genes including liver-specific metabolic genes such as tyrosine aminotransferase (TAT) and the stress response genes such as metallothionein and MKP-1 have been shown to contain glucocorticosteroid response elements (GREs) in their promoter regions. Binding of the activated GR dimer to a GRE leads to recruitment of a number of transcriptional co-activators which possess intrinsic histone acetyltransferase (HAT) activity. This leads to acetylation of local histone residues, formation of bromodomains and further recruitment of chromatin remodelling complexes. Once these large transcriptional complexes have been co-ordinately activated, RNA polymerase II is stimulated and gene transcription is induced [67]. In addition, the expression of some genes such as prolactin and osteocalcin were found to be decreased upon GR-GRE binding due to steric hindrance since these GREs were located across the start site of transcription. However, generally the number of GREs and their position relative to the transcriptional start site are considered to be important determinants of the magnitude and direction of the transcriptional response to glucocorticosteroids [68].

Although the induction of anti-inflammatory genes was originally proposed to be the major mechanism of anti-inflammatory actions of GR in asthma, it became increasingly clear that other mechanisms of action must be important. Full expression of many pro-inflammatory genes including IL-6, IL-8, inducible nitric oxide synthase (NOS2) and intercellular adhesion molecule-1 (ICAM-1) requires the co-ordinated activation of a number of transcription factors including AP-1 and NF-κB acting together in a coordinated manner. It is now clear that GR, in this case acting as a monomer, was able to bind to, and suppress, NF-κB and AP-1 transcriptional



**Figure 1.1** Rationale for dissociated glucocorticosteroids. Most anti-inflammatory actions of glucocorticosteroids are mediated through the corticosteroid receptor (GR) monomer interacting with pro-inflammatory transcription factors such as AP-1 and NF- $\kappa$ B which activate gene expression by reversing the active state of chromatin. In contrast, gene induction events mediated by a GR homodimer are responsible for many of the detrimental side-effects of glucocorticosteroids as well as the induction of some anti-inflammatory genes.

activity [30]. The precise mechanism for this repression is still unclear and may include binding to, or recruiting, nuclear receptor co-repressors [69, 70], direct repression of co-activator complexes [70] or effects on RNA polymerase II phosphorylation [71]. For example, modification of tyrosine 735 selectively impairs transactivation without affecting transrepression via the differential recruitment of NCoR1 rather than SRC-1 allowing a molecular switch to occur [72]. These effects are context/gene dependent however as GR can combine with NF- $\kappa$ B to induce the expression of TLR2 and stem cell factor (SCF) [73, 74]. Glucocorticosteroids have also been reported to regulate the levels of cell ribonucleases and mRNA destabilizing proteins, thereby reducing the levels of mRNA [38, 75] although this generally occurs at high non-therapeutic concentrations.

### DISSOCIATED GLUCOCORTICOSTEROIDS (FIGURE 1.1)

Whilst the major anti-inflammatory effects of corticosteroids are almost certainly due to transrepression, the underlying molecular mechanisms for the side-effects of glucocorticosteroids are complex and not fully understood [5]. Certain side-effects such as diabetes, resulting from upregulation of hepatic phosphoenolpyruvate carboxykinase and glucose 6-phosphatase, and muscle wasting, a result of skeletal muscle glutamine synthetase induction, are due to transactivation events whilst others are due transrepression (HPA suppression) [5]. In addition, the precise molecular events underlying glucocorticosteroid induction of osteoporosis are unclear but probably require both gene induction and gene repression [5]. Support for this hypothesis comes from a series of elegant experiments in transgenic mice expressing mutated GRs unable to dimerize (GRdim). These mice are defective in their ability to induce pro-opiomelanocort (POMC) transactivation but maintain wild-type transrepression activity [76, 77]. Thus, a novel glucocorticosteroid that shows selectivity for



comparable *in vivo* anti-inflammatory activity to prednisolone, there was no improvement in side-effect parameters such as osteoporosis, weight reduction, or thymic involution [43]. Furthermore, more recent experiments were not able to confirm the dissociated properties of RU24858 *in vivo* [43, 78]. This raised questions either about the validity of the concept of 'dissociated' glucocorticosteroids or whether RU24858 was a good tool compound. It has been proposed that the results with RU24858 may either reflect differences in RU24858 metabolism or, more likely, problems inherent with differences between the different *in vitro* models used and between rodent and human GRs and subsequent lack of the correct complement of transcriptional co-modulator proteins [43, 79]. Evidence for the latter effect comes from analysis of 7- $\alpha$  esters of beclomethasone and betamethasone which show profound dissociation in rat systems, despite acting as classical corticosteroids in humans and mice [43]. These molecules have strong anti-inflammatory activity *in vivo* but fail to induce TAT and other liver enzymes.

Osteoprotegerin (OPG) and receptor activator of NF- $\kappa$ B ligand (RANKL) are osteoblast-derived proteins pivotal to the regulation of bone mass. RANKL stimulates bone resorption by increasing osteoclast differentiation, activation and survival, whereas OPG is the decoy receptor for RANKL and thus inhibits bone resorption. Humphrey *et al.* [80] showed that a series of 'dissociated' glucocorticosteroids suppress OPG production to a similar extent as dexamethasone and prednisolone but have a much reduced induction of RANKL in osteoblastic cells. This suggests that these agents, if their discriminative stimulus (DS) properties are continued through *in vivo*, should have reduced bone loss.

More recently, researchers have moved away from classical steroidal molecules in favour of non-steroidal GR ligands as these molecules appear to maintain the selective trans-repression profile *in vivo* as well as in *in vitro* assays. AL-438, for example, maintains its anti-inflammatory *in vivo* and displays a reduced side-effect profile compared with prednisolone [81]. Moreover, the non-terpenoid A276575 exhibited high affinity for GR and potently suppresses inflammatory gene expression in several cell types with a reduced induction of glucocorticosteroid-stimulated genes compared with dexamethasone [82]. However, the differential repression of RANTES and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) production in a cell by the two (-)-enantiomers of A276575 illustrates the complexity of repression by GR [82] and provides further evidence needed to examine global gene expression patterns in these types of studies.

Similar results with other compounds containing non-steroidal backbones have also been reported from several laboratories [83–86] and even from natural products [87]. Thus, a plant-derived phenyl aziridine precursor also shows clear dissociated properties at the GR in both *in vitro* and *in vivo* systems having good repression of NF- $\kappa$ B and lacking hyperglycaemic side-effects [87]. In addition, ZK216348 is equipotent to prednisolone but has a reduced side-effect profile with respect to blood glucose levels and spleen involution compared to that of prednisolone *in vivo* [88]. Interestingly, however, ZK216348 exhibited similar suppression of adrenocorticotrophic hormone (ACTH) *in vivo* as prednisolone with results similar to those seen with other steroid-based drugs. This suggests that dissociated glucocorticosteroids may not have an improved therapeutic index as far as HPA axis effects are concerned due to the requirement for a non-GRE-mediated effect controlling HPA function. Whether this will be a problem in man will be determined once Phase I studies are complete.

The recent resolution of the crystal structure of the GR [89] has also helped in the better design of dissociated glucocorticosteroids [90]. The overall structure is similar to that of other nuclear hormone receptor (NHR) ligand binding domains (LBDs), but contains a unique dimerization interface and a second charge clamp that might be important for co-factor selectivity. Unlike other NHR LBDs, the GR LBD also has a distinct binding pocket that might explain ligand selectivity and lead to rationale-based design of selective dissociated GR agonists. Overall, this suggests that the development of glucocorticosteroids with a

greater margin of safety is possible and may even lead to the development of oral glucocorticosteroids that do not have significant adverse effects.

## GR CROSS-TALK WITH OTHER NUCLEAR RECEPTORS AND CO-ACTIVATORS

GRs do not only affect gene expression through monomeric or homodimeric formation. Thus, GRs can associate with other transcription factors, such as members of the STAT family [91, 92] and the environmental tobacco smoke (ETS) transcription factors [93] on DNA as heterodimers, leading to the recruitment of distinct co-activator (e.g. GRIP-1) or co-repressor (e.g. RIP140 or HDAC) complexes [72, 94, 95]. In addition, several glucocorticosteroids currently used for asthma therapy also have an affinity for the progesterone receptor and the mineralocorticoid receptor with reduced affinity for androgen and oestrogen receptors [96] suggesting the possibility of cross-talk with other NHRs. Recent evidence from Chris Glass's laboratory indicates that dexamethasone-activated GR represses a large set of functionally related inflammatory genes stimulated by p65/IRF-3 complexes [97]. In contrast, peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) and liver X receptors (LXRs) repress overlapping transcriptional targets in a p65/IRF-3-independent manner and co-operate with GR to suppress distinct subsets of LPS-responsive genes [97]. In addition, vitamin B<sub>6</sub> can reduce dexamethasone-stimulated GRE activity without affecting transrepression [98]. Designing drugs with the capacity to activate GR and other NHRs may, therefore, enhance the anti-inflammatory profile of glucocorticosteroids. Moreover, as the expression of many cofactors and nuclear receptors are tissue specific, there is the attractive possibility of designing tissue specific ligands.

## OTHER APPROACHES TO ANTI-INFLAMMATORY THERAPY

The elucidation of the molecular mechanisms of glucocorticosteroids raises the possibility that novel non-steroidal anti-inflammatory treatments might be developed that mimic the actions of glucocorticosteroids on inflammatory gene regulation. Inhibition of specific co-activators activated by NF- $\kappa$ B may prove to be useful targets, especially if they also repress the action of other pro-inflammatory transcription factors [99]. Alternatively, activation of co-repressor molecules may have therapeutic potential [100]. Many of the anti-inflammatory effects of glucocorticosteroids appear to be mediated via inhibition of the transcriptional effects of NF- $\kappa$ B, and small-molecule inhibitors of IKK2, which activate NF- $\kappa$ B, are in development. However, glucocorticosteroids have additional effects, so it is uncertain whether IKK2 inhibitors will parallel the clinical effectiveness of glucocorticosteroids. They may have side-effects, such as increased susceptibility to infections; however, as a corollary to this, if glucocorticosteroids were discovered today, they would be unlikely to be used in humans because of the low therapeutic ratio and their side-effect profile.

## SUMMARY

Enormous progress has been made in improving glucocorticosteroid treatment since the introduction of hydrocortisone as the first clinically used corticosteroid. Extensive drug development has resulted in highly potent molecules, the pharmacokinetic profiles of which have been optimized to minimize systemic exposure and to target activity to the lung. Advances in delineating the fundamental mechanisms of glucocorticosteroid pharmacology, especially the concepts of transactivation and transrepression and cofactor recruitment, have resulted in better understanding of the molecular mechanisms whereby glucocorticosteroids suppress inflammation. This will undoubtedly lead to the rational design of drugs that target novel aspects of GR function in a cell-specific manner and potentially restore glucocorticosteroid sensitivity to diseases that are unresponsive to current therapeutic strategies.

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The literature in this area is extensive, and many important studies were omitted because of constraints on space, for which we apologize.

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