Journal of Steroid Biochemistry & Molecular Biology 120 (2010) 76–85

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Mechanisms and resistance in glucocorticoid control of inflammation☆

Peter J. Barnes*

National Heart & Lung Institute, Imperial College, London, UK

ARTICLE INFO

Article history:
Received 18 November 2009
Received in revised form 15 February 2010
Accepted 16 February 2010

Keywords:
COPD
Asthma
p38 MAP kinase
Histone deacetylase
Steroid resistance

ABSTRACT

Glucocorticoids are the most effective anti-inflammatory therapy for many chronic inflammatory and immune diseases, such as asthma, but are relatively ineffective in other diseases such as chronic obstructive pulmonary disease (COPD). Glucocorticoids suppress inflammation by several mechanisms. Glucocorticoids suppress the multiple inflammatory genes that are activated in chronic inflammatory diseases, such as asthma, by reversing histone acetylation of activated inflammatory genes through binding of liganded glucocorticoid receptors (GR) to coactivator molecules and recruitment of histone deacetylase-2 (HDAC2) to the activated transcription complex. At higher concentrations of glucocorticoids GR homodimers interact with DNA recognition sites to activate transcription through increased histone acetylation of anti-inflammatory genes and transcription of several genes linked to glucocorticoid side effects. Decreased glucocorticoid responsiveness is found in patients with severe asthma and asthmatics who smoke, as well as in all patients with COPD and cystic fibrosis. Several molecular mechanisms of glucocorticoid resistance have now been identified. HDAC2 is markedly reduced in activity and expression as a result of oxidative/nitrative stress so that inflammation becomes resistant to the anti-inflammatory actions of glucocorticoids. Dissociated glucocorticoids have been developed to reduce side effects but so far it has been difficult to dissociate anti-inflammatory effects from adverse effects. In patients with glucocorticoid resistance alternative anti-inflammatory treatments are being investigated as well as drugs that may reverse the molecular mechanism of glucocorticoid resistance.

1. Introduction

Glucocorticoids are the most effective anti-inflammatory drugs available for the treatment many chronic inflammatory and immune diseases, including asthma, rheumatoid arthritis, inflammatory bowel disease and autoimmune diseases. However, a minority of patients with these diseases show little or no response even to high doses of glucocorticoids. Several other inflammatory diseases, including chronic obstructive pulmonary disease (COPD), interstitial pulmonary fibrosis and cystic fibrosis, appear to be largely steroid-resistant. There is now a much better understanding of how glucocorticoids suppress chronic inflammation and this has given insights into the mechanisms and potential therapy of glucocorticoid resistance [1]. Glucocorticoid-resistance or insensitivity is an important barrier to effective therapy and accounts for considerable and increasing health care spending. This review describes the molecular mechanisms whereby corticosteroids so effectively suppress inflammation and then discusses the molecular basis for glucocorticoid resistance and the implications for therapy.

2. How glucocorticoids suppress inflammation

There have been major advances in understanding the molecular mechanisms whereby glucocorticoids suppress inflammation [2,3]. Glucocorticoids activate and suppress many pro- and anti-inflammatory genes, as well as having post-transcriptional effects. Understanding the molecular mechanism of glucocorticoid action also provides new insights into molecular mechanisms of glucocorticoid resistance [1].

2.1. Gene activation

Glucocorticoids diffuse across the cell membrane and bind to glucocorticoid receptors (GR) in the cytoplasm [3]. Upon ligand binding, GR are activated and released from chaperone proteins (heat shock protein 90 and others) and rapidly translocate to the nucleus where they exert their molecular effects. The mechanism of nuclear translocation involves the nuclear import protein importin-α (karyopherin-β) and importin-13 [4,5]. There is only one form of GR that binds glucocorticoids termed GRα. GRβ is an alternatively spliced form of GR that interacts with DNA but not with glucocorticoids, so may theoretically act as a dominant-negative inhibitor of

☆ Article from the special issue on Steroids: modulators of inflammation and immunity.

* Correspondence address: Airway Disease Section, National Heart & Lung Institute, Dovehouse Street, London SW3 6LY, UK.
Tel.: +44 207 351 8174; fax: +44 207 351 5675.
E-mail address: p.j.barnes@imperial.ac.uk.

0960-0760/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jsbmb.2010.02.018

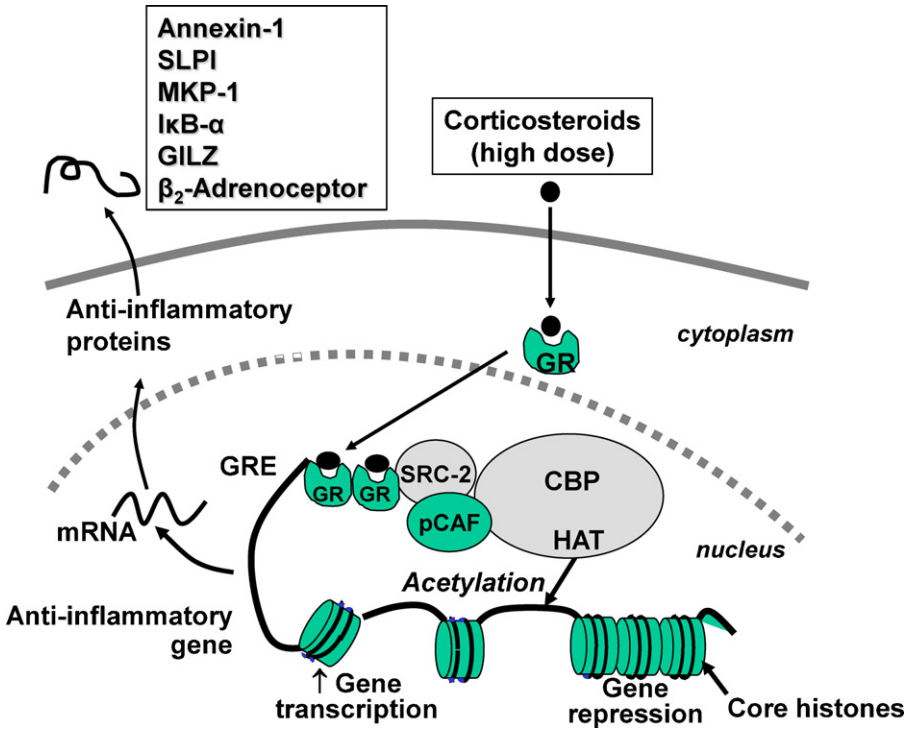

Fig. 1. Glucocorticoid activation of anti-inflammatory gene expression. Glucocorticoids bind to cytoplasmic glucocorticoid receptors (GR) which translocate to the nucleus where they bind to glucocorticoid response elements (GRE) in the promoter region of steroid-sensitive genes and also directly or indirectly to coactivator molecules such as CREB-binding protein (CBP), p300/CBP activating factor (pCAF) or steroid receptor coactivator-2 (SRC-2), which have intrinsic histone acetyltransferase (HAT) activity, causing acetylation of lysines on histone H4, which leads to activation of genes encoding anti-inflammatory proteins, such as secretory leukoprotease inhibitor (SLPI), mitogen-activated kinase phosphatase-1 (MKP-1), inhibitor of NF-κB (IκB-α) and glucocorticoid-induced leucine zipper protein (GILZ).

glucocorticoid action by interfering with the binding of GR to DNA [6]. GR homodimerises and binds to glucocorticoid response elements (GRE) in the promoter region of glucocorticoid-responsive genes and this interaction switches on (or occasionally switches off) gene transcription. Activation of glucocorticoid-responsive genes occurs via an interaction between the DNA-bound GR and transcriptional coactivator molecules such as CREB-binding protein (CBP), which have intrinsic histone acetyltransferase activity and cause acetylation of core histones (particularly histone-4). This tags histones to recruit chromatin remodelling engines such as SWI/SNF and subsequent association of RNA polymerase II resulting in gene activation (Fig. 1) [7]. Genes that are switched on by glucocorticoids include genes encoding β_2-adrenergic receptors and the anti-inflammatory proteins secretory leukoprotease inhibitor and mitogen-activated protein kinase phosphatase-1 (MKP-1), which inhibits MAP kinase pathways. These effects may contribute to the anti-inflammatory actions of glucocorticoids [8,9]. GR interaction with negative GREs, or to GREs that cross the transcriptional start site, may suppress gene transcription and this may be important in mediating many of the side effects of glucocorticoids, such as inhibition of osteocalcin that is involved in bone synthesis (Fig. 2) [10].

2.2. Switching off inflammatory genes

The major action of glucocorticoids is to switch off multiple activated inflammatory genes that encode for cytokines, chemokines, adhesion molecules inflammatory enzymes and receptors [11]. These genes are switched on in the airways by proinflammatory transcription factors, such as nuclear factor-κB (NF-κB) and activator protein-1 (AP-1), both of which are usually activated at sites of inflammation, resulting in the switching on of multiple inflammatory genes. These genes are activated through interactions with transcriptional coactivator molecules in a similar manner to that described above for GR-mediated gene transcription [12].

Activated GR interact with corepressor molecules to attenuate NF-κB-associated coactivator activity, thus reducing histone acetylation, chromatin remodelling and RNA polymerase 2 actions [2,7]. Reduction of histone acetylation more importantly occurs through the specific recruitment of histone deacetylase-2 (HDAC2) to the activated inflammatory gene complex by activated GR, thereby resulting in effective suppression of activated inflammatory genes within the nucleus (Fig. 3). This may account for why glucocorticoids are so effective in the control of inflammation, but also why they are relatively safe, since other genes are not affected. GR becomes acetylated upon ligand binding allowing it to bind to GREs and HDAC2 can target acetylated GR thereby allowing it to associate with the NF-κB complex [13] (Fig. 4). The site of acetylation of GR is the lysine rich region −492–495 with the sequence KKTK, which is analogous to the acetylation sites identified on other nuclear hormone receptors. Site-directed mutagenesis of the lysine residues K494 and K495 prevents GR acetylation and reduces the activation of the SLPI gene by corticosteroids, whereas repression of NF-κB is unaffected. HDAC6 has also been implicated in GR function by modulating hsp90 acetylation status and thereby GR nuclear translocation [14].

Additional mechanisms are also important in the anti-inflammatory actions of glucocorticoids. Glucocorticoids have potent inhibitory effects on mitogen-activated protein kinase (MAPK) signalling pathways through the induction of MKP-1 and this may inhibit the expression of multiple inflammatory genes [8,9] (Fig. 5).

2.3. Post-transcriptional effects

Some proinflammatory genes, such as TNF-α, have unstable messenger RNA that is rapidly degraded by certain RNAses but stabilised when cells are stimulated by inflammatory mediators. Glucocorticoids reverse this effect, resulting in rapid degrada-

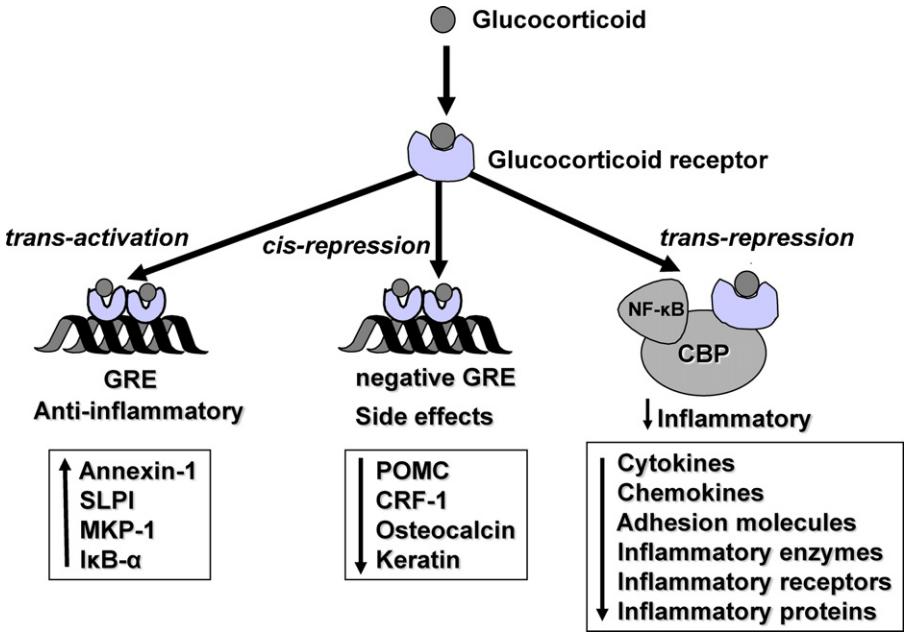

Fig. 2. Glucocorticoids regulate gene expression in several ways. Glucocorticoids enter the cell to bind to glucocorticoid receptors (GR) in the cytoplasm that translocate to the nucleus. GR homodimers bind to glucocorticoid-response elements (GRE) in the promoter region of steroid-sensitive genes, which may encode anti-inflammatory proteins. Less commonly, GR homodimers interact with negative GREs to suppress genes, particularly those linked t. Nuclear GR also interact with coactivator molecules, such as CREB-binding protein (CBP), which is activated by proinflammatory transcription factors, such as nuclear factor-κB (NF-κB), thus switching off the inflammatory genes that are activated by these transcription factors. *Other abbreviations:* SLPI: secretory leukoprotease inhibitor; MKP-1: mitogen-activated kinase phosphatase-1; IκB-α: inhibitor of NF-κB; GILZ: glucocorticoid-induced leucine zipper protein; POMC: proopiomelanocortin; CRH: corticotrophin releasing factor.

tion of mRNA and reduced inflammatory protein secretion [15] (Fig. 5). This may be mediated through the increased gene expression of proteins that destabilize mRNAs of inflammatory proteins, such as the zinc finger protein tristetraprolin, which binds to the 3′ AU-rich untranslated region of mRNAs [16].

3. Molecular mechanisms of glucocorticoid resistance

Several distinct molecular mechanisms contributing to decreased anti-inflammatory effects of glucocorticoids have now been identified, so that there is heterogeneity of mechanisms even within a single disease (Table 1 and Fig. 6) [1]. However,

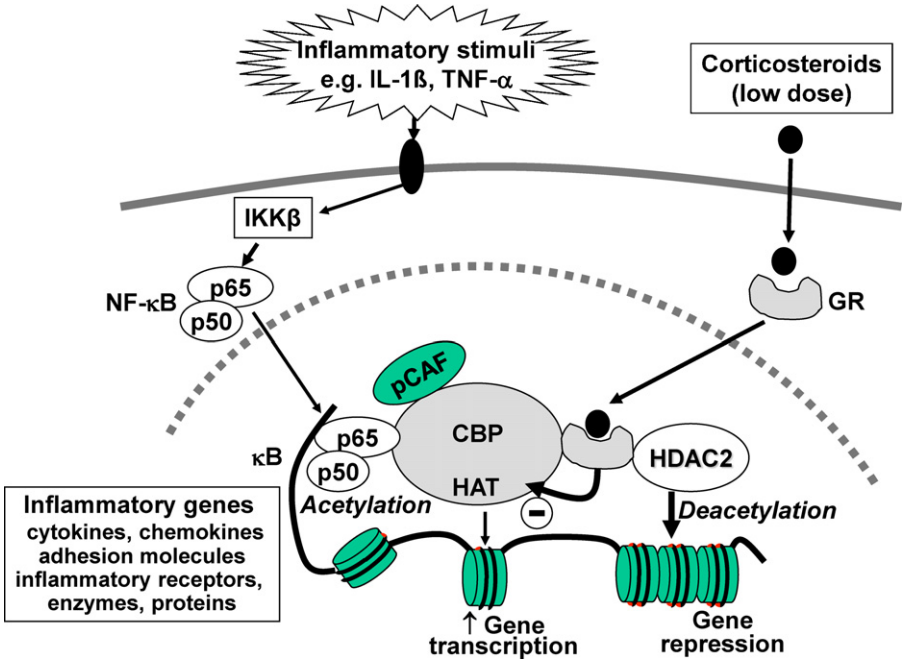

Fig. 3. Glucocorticoid suppression of activated inflammatory genes. Inflammatory genes are activated by inflammatory stimuli, such as interleukin-1β (IL-1β) or tumour necrosis factor-α (TNF-α), resulting in activation of IKKβ (inhibitor of I-κB kinase-β), which activates the transcription factor nuclear factor κB (NF-κB). A dimer of p50 and p65 NF-κB proteins translocates to the nucleus and binds to specific κB recognition sites and also to coactivators, such as CREB-binding protein (CBP) or p300/CBP-activating factor (pCAF), which have intrinsic histone acetyltransferase (HAT) activity. This results in acetylation of core histone H4, resulting in increased expression of genes encoding multiple inflammatory proteins. Glucocorticoid receptors (GR) after activation by corticosteroids translocate to the nucleus and bind to coactivators to inhibit HAT activity directly and recruiting histone deacetylase-2 (HDAC2), which reverses histone acetylation leading in suppression of these activated inflammatory genes.

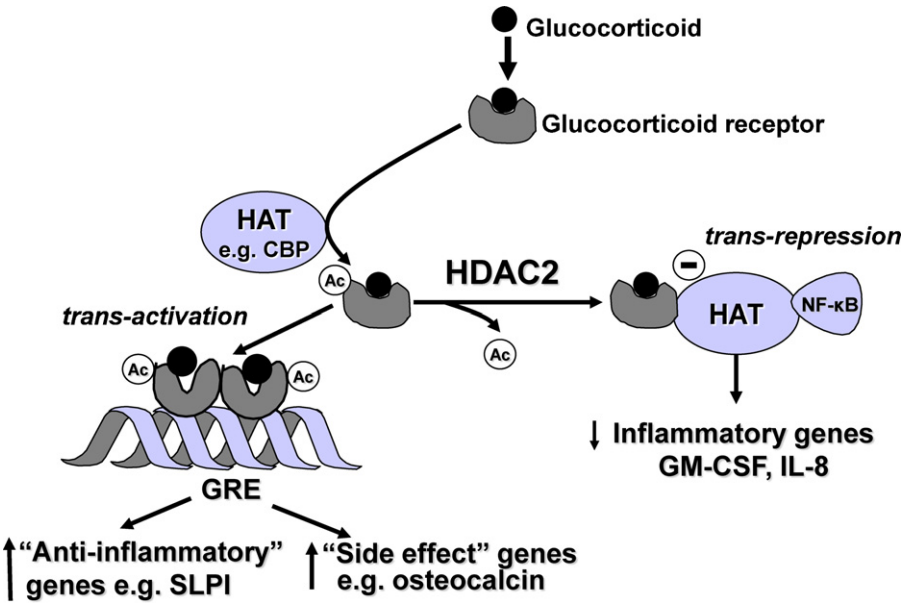

Fig. 4. Acetylation of glucocorticoid receptors (GR). Binding of a corticosteroid to GR results in its acetylation by histone acetyltransferases (HAT), such as CREB-binding protein (CBP), and a dimmer of acetylated GR then binds to glucocorticoid response elements (GRE) to activate or suppress genes (such as side effect genes). Deacetylation of GR by histone deacetylase-2 (HDAC2) is necessary for GR to interact with CBP and inhibit nuclear factor-κB (NF-κB) to switch off inflammatory genes.

similar molecular mechanisms have also been identified in different inflammatory diseases indicating that there may be common therapeutic approaches to glucocorticoid-resistant diseases in the future.

3.1. Genetic susceptibility

The early descriptions of glucocorticoid-resistant asthma suggested that it was more commonly found within families [17], indicating that there may genetic factors may determine glucocorticoid responsiveness. Microarray studies of peripheral blood mononuclear cells (PBMC) from glucocorticoid-sensitive and glucocorticoid-resistant asthma patients has identified 11 genes that discriminate between these patients [18], suggesting that it might be possible to develop a genomic test for glucocorticoid resistance. However, in normal subjects differential gene expression between the 10% with the greatest and least glucocorticoid responsiveness of circulating genes has identified 24 genes of which the

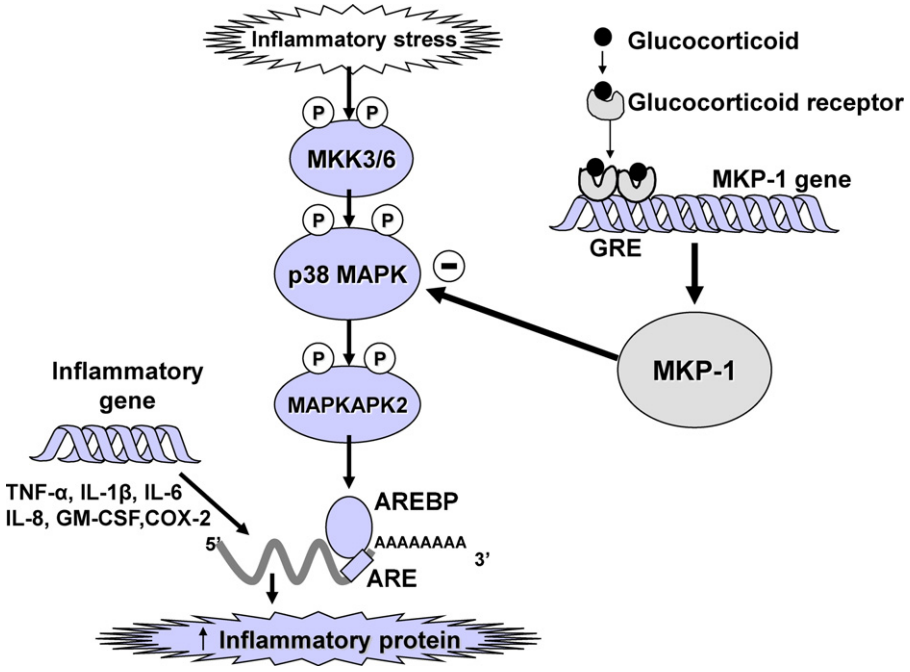

Fig. 5. Inhibition of p38 mitogen-activated protein (MAP) kinase by glucocorticoids. p38 MAP kinase is activated by inflammatory stresses though activation of MAP kinase kinase(MKK)-3 and -6. p38 phosphorylates (P) MAP kinase-activated protein kinase(MAPKAPK)-2, which plays a role in stabilising messenger RNA (mRNA) encoding several inflammatory proteins, such as tumour necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, IL-8, granulocyte-macrophage colony stimulating factor (GM-CSF) and cyclo-oxygenase(COX)-2. This mRNA is characterised by AU-rich elements (ARE) in the 3′-untranslated region, which make the mRNA unstable and rapidly degraded. ARE-binding proteins (AREBP) stabilise these proteins and may be activated (probably indirectly) by MAPKAPK-2. Corticosteroids induce the expression of MAP kinase phosphatase (MKP)-1, which inhibits p38 and thus prevents the stabilisation of multiple inflammatory proteins.

Table 1
Molecular mechanisms of glucocorticoid resistance.

- Familial glucocorticoid resistance
- Glucocorticoid receptor modification
  Phosphorylation: decreased nuclear translocation
    p38 MAP kinase due to IL-2 + IL-4 or IL-13 in severe asthma
    p38 MAP kinase due to MIF in several inflammatory diseases
    JNK due to proinflammatory cytokines
    ERK due to microbial superantigens
  Nitrosylation: ↑ NO from inducible NO synthase
  Ubiquitination: ↑ degradation by proteasome
- Increased GRβ expression
- Increased proinflammatory transcription factors
  Activator protein-1, JNK
  STAT5, JAK3
- Defective histone acetylation
  Decreased acetylation of lysine-5 on histone 4
  Decreased histone deacetylase-2
    ↑ Oxidative stress
    ↑ Phosphoinositide-3-kinase-δ activation
- Increased P-glycoprotein
  Increased efflux of steroids

Abbreviations: IL = interleukin, MAP = mitogen-activated protein, MIF = macrophage migration inhibitory factor, JNK = c-Jun *N*-terminal kinase, ERK = extracellular signal-regulated kinase, NO = nitric oxide, STAT = signal transduction activated transcription factor.

most discriminant is bone morphogenetic protein receptor type II, which enhances glucocorticoid responsiveness when transfected into cells [19].

The very rare inherited syndrome familial glucocorticoid resistance (FGR) is characterised by high circulating levels of cortisol without signs or symptoms of Cushing's syndrome [20]. Clinical manifestations, which may be absent, are due to an excess of non-corticosteroid adrenal steroids, stimulated by high adrenocorticotrophin levels, resulting in hypertension with hypokalaemia and/or signs of androgen excess (usually hirsutism and menstrual abnormalities in females). Inheritance appears to be dominant with variable expression, but only about a dozen cases have so far been reported. Sporadic cases have also been described. Several abnormalities in GR function have been described in peripheral blood leukocytes or fibroblasts from patients with FGR, including decreased binding for cortisol, reduced numbers, thermolability and an abnormality binding to DNA, all of which are due to mutations of GR. These patients are clearly different from patients with glucocorticoid-resistant inflammatory diseases and in patients with glucocorticoid-resistant asthma mutational analysis demonstrated no obvious abnormality in GR structure [21]. Various single nuclear polymorphisms of GR have been linked to altered cellular responses to glucocorticoids and a polymorphism of GRβ (GR-9β) is associated with a reduced trans-repressional response to glucocorticoids [22]. These polymorphisms have yet to be associated with glucocorticoid resistance in inflammatory diseases, however.

3.2. Defective GR binding and translocation

There is increased expression of IL-2 and IL-4 in the airways of patients with glucocorticoid-resistant asthma [23] and *in vitro* these cytokines in combination reduce GR nuclear translocation and binding affinity within the nucleus of T-cells [24–26]. IL-13 alone mimics this effect in monocytes [25,27]. The mechanism whereby these cytokines reduce GR function may be mediated via phosphorylation of GR by p38MAPK and their effect is blocked by a p38MAPK-α inhibitor [25]. In support of this p38MAPK shows a greater degree of activation in alveolar macrophages from asthmatics with a poor response to glucocorticoids than patients who show a normal response [28]. GR may be phosphorylated by several kinases that may alter its binding, stability, translocation to the nucleus, binding to DNA and interaction with other proteins, such as transcription factors and molecular chaperones [29]. The serine residue phosphorylated by p38MAPK is not yet certain and may be Ser226 or Ser 211, or this may be an indirect effect [25,30,31]. IL-2 may also cause reduced nuclear translocation in murine T-cells through a mechanism involving interaction to the transcription factor STAT5 under the control of JAK3 [32]. In patients with

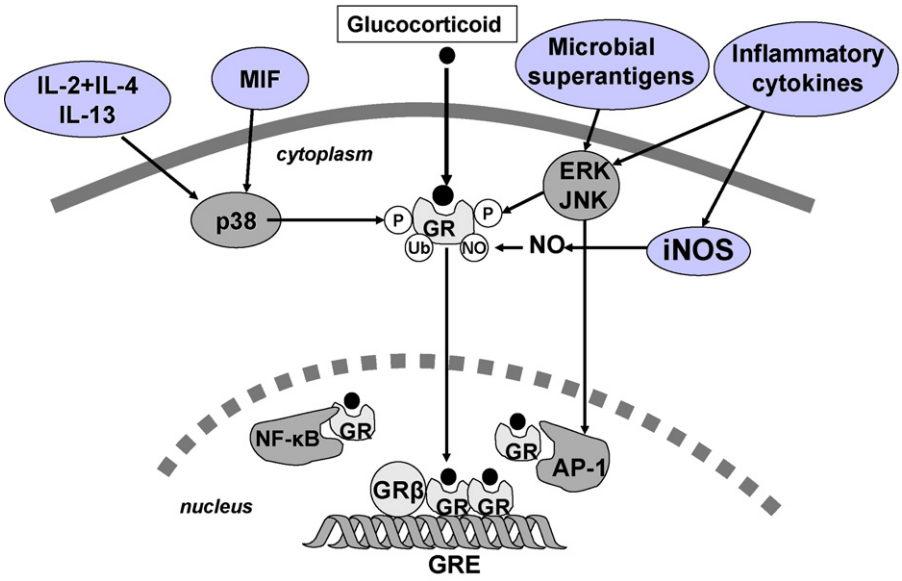

Fig. 6. Possible molecular mechanisms of glucocorticoid resistance. Glucocorticoid receptors (GR) may be modified in several ways to reduce their nuclear translocation and transactivational efficacy. Phosphorylation may occur as a result of activation of p38 mitogen-activated protein kinase (MAPK), which may be activated by the cytokines interleukin (IL)-2 plus IL-4 or IL-13, or by macrophage migration inhibitory factor (MIF), of c-Jun *N*-terminal kinase (JNK) activated by proinflammatory cytokines or of extracellular signal-regulated kinase (ERK) activated by microbial superantigens. GR may also be nitrosylated by nitration of tyrosine residues as a result of increased nitric oxide (NO) from inducible NO synthase (iNOS) or ubiquitinated (Ub), resulting in degradation of GR by the proteasome. GR may prevented from binding to glucocorticoid response elements (GRE) or inhibiting nuclear factor-κB (NF-κB) by sequestration by the transcription factor activator protein-1 (AP-1), which is activated by JNK, or by the binding of increased GRβ to GRE binding sites.

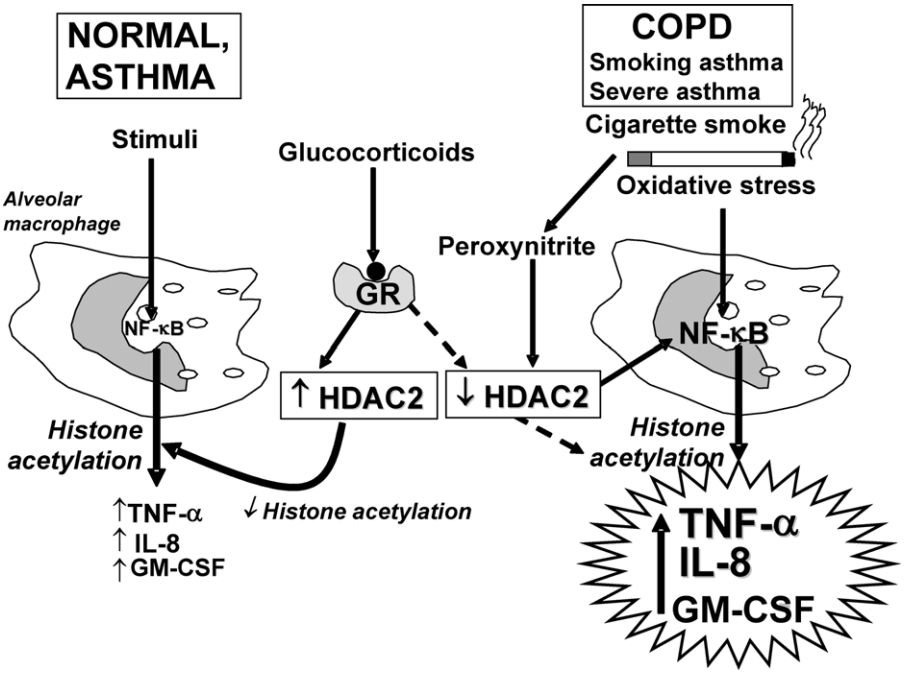

Fig. 7. Mechanism of glucocorticoid resistance in COPD, severe asthma and smoking asthma. Stimulation of normal and asthmatic alveolar macrophages activates nuclear factor-κB (NF-κB) and other transcription factors to switch on histone acetyltransferase leading to histone acetylation and subsequently to transcription of genes encoding inflammatory proteins, such as tumour necrosis factor-α (TNF-α), CXCL8 (IL-8) and granulocyte-macrophage colony stimulating factor (GM-CSF). Glucocorticoids reverse this by binding to glucocorticoid receptors (GR) and recruiting histone deacetylase-2 (HDAC2). This reverses the histone acetylation induced by NF-κB and switches off the activated inflammatory genes. In COPD and smoking asthmatic patients cigarette smoke and activated neutrophils generate oxidative stress which impairs the activity of HDAC2. This amplifies the inflammatory response to NF-κB activation, but also reduces the anti-inflammatory effect of glucocorticoids, as HDAC2 is now unable to reverse histone acetylation. A similar mechanism may operate in severe asthma where increased oxidative stress is generated by airway inflammation.

glucocorticoid-resistant asthma a large proportion show reduced nuclear translocation of GR and reduced GRE binding in PBMC following glucocorticoid exposure and this may be explained by GR phosphorylation [26,31]. Another MAPK c-Jun N-terminal kinase (JNK), which is activated by TNF-α and other proinflammatory cytokines, also directly phosphorylates GR at Ser-226 and inhibits GRE binding [33]. Microbial superantigens induce glucocorticoid resistance in T cells *in vitro* via activation of extracellular receptor kinase (ERK) pathways, resulting in GR phosphorylation [34]. MKP-1 is an endogenous inhibitor of MAPK which is activated by glucocorticoids, as discussed above. Macrophages from MKP-1 gene knock-down mice show reduced anti-inflammatory responses to glucocorticoids *in vitro* [35]. In asthmatic patients with poor glucocorticoid responses there is a significant reduction in MKP-1 expression in alveolar macrophages after glucocorticoid exposure and this is correlated with increased p38 MAPK activity [28].

In vitro GR may be nitrosylated by nitric oxide (NO) donors resulting in reduced binding affinity for glucocorticoids [36]. In inflammatory diseases there is often increased expression of inducible NO synthase (iNOS) which produces large amounts of NO that could reduce glucocorticoid responsiveness. Whether this mechanism is relevant in glucocorticoid-resistant patients has not yet been evaluated by the use if iNOS inhibitors, for example. GR may also be ubiquitinated and tagged for proteasomal degradation implying that proteasome inhibitors may increase glucocorticoid responsiveness, although this has not yet been demonstrated in glucocorticoid-resistant disease [37].

3.3. Increased GRβ

Increased expression of GRβ has been reported in glucocorticoid-resistant patients of several diseases, including asthma, rheumatoid arthritis and inflammatory bowel disease [38–41], but this has not been confirmed in several other studies [42,43]. GRβ is induced by proinflammatory cytokines and has the capacity to compete for the binding of GRα to GRE, thus acting as a dominant-negative inhibitor [44]. GRβ expression is also increased by microbial superantigens, such as staphylococcal enterotoxins, which may account for glucocorticoid resistance in atopic dermatitis [45]. However, in most cell types, apart from neutrophils, the expression of GRβ is much lower than GRα, making this mechanism unlikely [42]. Another mechanism may be through interference with GRα nuclear translocation, since knockdown of GRβ in alveolar macrophages from glucocorticoid-resistant asthma patients results in increased GRα nuclear localisation and increased glucocorticoid responsiveness [46]. While glucocorticoids do not bind to GRβ it is transcriptionally active and the GR antagonist mifepristone (RU-486) binds to GRβ, making it translocate to the nucleus, but the endogenous ligand of GRβ is currently unidentified [47].

3.4. Transcription factor activation

Excessive activation of AP-1 has been identified as a mechanism of glucocorticoid resistance in asthma as AP-1 binds GR and thus prevents its interaction with GRE and other transcription factors [48,49]. AP-1 is a heterodimer of Fos and Jun proteins and may be activated by proinflammatory cytokines such as TNF-α, acting through the JNK pathway. JNK is activated to a greater extent and there is increased expression of c-Fos in PBMC and bronchial biopsies of glucocorticoid-resistant compared to sensitive asthma, with no reduction of JNK activity or c-Jun after high doses of oral glucocorticoids [50]. This may explain why the increased inflammation found in severe inflammatory disease results in secondary glucocorticoid-resistance and is a mechanism for perpetuating resistance whatever the initial mechanism. Increased c-Jun results in depolymerisation of the cytoskeleton, which may also reduce GR trans-activating activity [51]. Cofilin-1 is an actin-binding pro-

tein that depolymerases the cytoskeleton and in gene array studies has been identified as showing increased expression in T-cells from glucocorticoid-resistant compared to sensitive asthma [52]. Overexpression of cofilin-1 results in glucocorticoid resistance in T-cells.

3.5. Abnormal histone acetylation

Histone acetylation plays a critical role in the regulation of inflammatory genes and the mechanism of action of glucocorticoids. Glucocorticoids switch on glucocorticoid-responsive genes, such as MKP-1, via acetylation of specific lysine residues (K5 and K16) on histone-4 [7]. In a small proportion of patients with glucocorticoid-resistant asthma, GR translocates normally to the nucleus after glucocorticoid exposure but fails to acetylate K5 so that transactivation of genes does not occur [26]. These patients show a poor response to high dose inhaled glucocorticoids, but unlike most patients with glucocorticoid resistance seem to have fewer side effects as many of these are mediated via GREs [10].

Recruitment of HDAC2 to activated inflammatory genes is a major mechanism of inflammatory gene repression by glucocorticoids [53] and reduced HDAC2 activity and expression is reduced in some diseases where patients respond poorly (Fig. 7). For example, HDAC2 is markedly reduced in alveolar macrophages, airways and peripheral lung in patients with COPD [54], and similar changes are found in PBMC and alveolar macrophages of patients with refractory asthma [55] and in the airways of smoking asthmatics [56]. The glucocorticoid resistance of COPD bronchoalveolar macrophages is reversed by overexpressing HDAC2 (using a plasmid vector) to the level seen in control subjects [13]. Whether HDAC2 is also reduced in glucocorticoid-resistant patients with other inflammatory diseases, such as rheumatoid arthritis has not yet been determined. The mechanisms for HDAC2 reduction in COPD are now being elucidated [57]. Oxidative and nitrative stress result in the formation of peroxynitrite, which nitrates tyrosine residues on HDAC2 resulting in its inactivation, ubiquitination and degradation [58]. Oxidative stress also activates phosphoinositide-3-kinase (PI3K)-δ, which leads to phosphorylation and inactivation of HDAC2 [59]. This suggests that oxidative stress may be an important mechanism of glucocorticoid resistance and is increased in most severe and glucocorticoid-resistant inflammatory diseases.

3.6. Decreased regulatory T cells

IL-10 is an important anti-inflammatory and immunoregulatory cytokine and secreted by regulatory T cells (Treg) in response to glucocorticoids [60]. In patients with glucocorticoid-resistant asthma there is a failure of T-helper cells to secrete IL-10 but this is restored to normal by vitamin D3 (calcitriol) *in vitro* [61]. Furthermore, administration of vitamin D3 to three glucocorticoid-resistant asthmatics also restored the T-cell IL-10 response to glucocorticoids, suggesting that this might be a useful therapeutic approach in the future.

3.7. Increased P-glycoprotein

The multidrug resistance gene MDR1 (ABCB1) encodes the drug efflux pump P-glycoprotein 170, a member of the ATP-binding cassette (ABC) transporters, which transports drugs, including glucocorticoids, out of cells. It has therefore been implicated as a mechanism for glucocorticoid resistance in inflammatory diseases. High levels of expression of MDR1 have been reported in circulating lymphocytes from patients with glucocorticoid-resistant inflammatory bowel disease [62,63] and rheumatoid arthritis [64]. Furthermore, certain single nucleotide polymorphisms of MDR1 have been linked to glucocorticoid resistance in these diseases [65,66]. However, these observations have not been confirmed in other studies and this mechanism has not been explored in glucocorticoid-resistant pulmonary disease.

3.8. Macrophage migration inhibitory factor

MIF is a proinflammatory cytokine that has potent anti-glucocorticoid effects and has been associated with several inflammatory diseases [67]. MIF is induced by glucocorticoids and inhibits their anti-inflammatory effects mainly through inhibiting the induction of MKP-1 [68]. Increased MIF expression has been reported in colonic mononuclear cells from patients with glucocorticoid-resistant ulcerative colitis and a MIF antibody restores the anti-inflammatory response to glucocorticoids in these cells [69]. Similar findings are reported in glucocorticoid-resistant rheumatoid arthritis and systemic lupus erythematosis. Polymorphisms of the MIF gene have also been reported in association with glucocorticoid resistance [70,71], although this is disputed [72]. MIF has also been implicated in the glucocorticoid resistance in asthma [73], suggesting the potential for anti-MIF therapies in glucocorticoid-resistant diseases.

4. Therapeutic implications

Although glucocorticoids are highly effective in treating many inflammatory and immune diseases the major problem is side effects. Although this may be overcome by topical application, such as inhaled or dermal administration this is not suitable for all diseases. There has been a concerted effort to develop glucocorticoids that have reduced side effects, while retaining anti-inflammatory efficacy.

4.1. Dissociated steroids

Selective glucocorticoid receptor agonists (SEGRAs or dissociated steroids) are more effective in trans-repression than trans-activation so have less side effects [74]. Several dissociated steroids have now been developed, including non-glucocorticoid agonists, but there is uncertainly about the efficacy of these drugs as anti-inflammatory therapies. In a mouse knock-in strain with dimerization-deficient GR some inflammatory processes can be suppressed by glucocorticoids, whereas others cannot [75]. This may reflect the anti-inflammatory effects of glucocorticoid mediated through transactivation of genes such as MKP-1. Furthermore, side effects of glucocorticoids may also occur in these mice.

Resistance to the anti-inflammatory effects of glucocorticoids is a major barrier to effective control of many common diseases and enormously increases their morbidity and medical costs. There are several therapeutic strategies to manage glucocorticoid-resistant diseases, but the most important general approaches are to use alternative anti-inflammatory ("steroid-sparing") treatments or to reverse the molecular mechanisms of glucocorticoid resistance if these are identified.

4.2. Alternative anti-inflammatory treatments

There are several alternative anti-inflammatory drugs currently available to treat certain glucocorticoid-resistant diseases, but these may have a toxicity of their own. Calcineurin inhibitors, such as cyclosporin A and tacrolimus, may be effective in some patients with glucocorticoid-resistant rheumatoid arthritis, but have not been found to be very effective in glucocorticoid-resistant asthma [76,77]. This has led to a search for novel anti-inflammatory treatments, particularly for diseases with marked glucocorticoid resistance, such as COPD, where no effective anti-inflammatory treatments are currently available.

Phosphodiesterase-4 inhibitors are broad spectrum anti-inflammatory treatments that are now in clinical development for several inflammatory diseases, such as COPD and inflammatory bowel disease [78]. However, systemic doses have been limited by side effects as side effects, such as nausea, diarrhoea and headaches.

Several p38 MAPK inhibitors have been in clinical development and theoretically could be particularly effective in asthma with glucocorticoid resistance due to IL-2 and IL-4, as this is reversed *in vitro* by selective p38 MAPK inhibitors [25]. These drugs may also be useful in other glucocorticoid-insensitive inflammatory diseases such as COPD where p38 MAPK is activated and they have been shown to have efficacy in glucocorticoid-resistant animal models of these diseases [79]. However these drugs have had problems with toxicity and side effects.

Blocking NF-κB by selective inhibitors of inhibitor of NF-κB kinase (IKKβ, IKK2) is another way of treating glucocorticoid-resistant inflammation, but it is likely that these drugs will also have toxicity and side effects so may only be suitable for topical application.

4.3. Reversing glucocorticoid resistance

Another therapeutic option for treating glucocorticoid resistance is to reverse the cause of resistance if it can be identified. This is possible with smoking cessation in smoking asthmatics [80] and might be possible for some patients with glucocorticoid-resistant asthma with p38 MAPK, JNK inhibitors and vitamin D3 in the future [25,49,61]. In patients with glucocorticoid-resistant ulcerative colitis a monoclonal antibody to IL-2 receptors (anti-CD25, basiliximab) was clinically effective in an uncontrolled study [81]. There are several therapeutic strategies for inhibiting P-glycoprotein to prevent the efflux of glucocorticoids, some of which are based on the observations that verapamil and quinidine are efflux blockers; several novel drugs are now in development [82]. Increased MIF has been implicated in glucocorticoid-resistance in several diseases, so strategies to inhibit MIF, including small molecule inhibitors and monoclonal antibodies, are currently being explored [83].

Selective activation of HDAC2 can be achieved with theophylline, which restores HDAC2 activity in COPD macrophages back to normal and reverses glucocorticoid resistance [84]. In mice exposed to cigarette smoke which develop glucocorticoid-resistant inflammation oral theophylline is also effective in reversing glucocorticoid resistance [59,85]. The molecular mechanism of action of theophylline in restoring HDAC2 appears to be via selective inhibition of PI3Kδ, which is activated by oxidative stress in COPD patients [59,86]. This suggests that selective PI3Kδ inhibitors may also be effective and these drugs are currently in clinical development for other diseases. Since oxidative stress appears to be an important mechanism in reducing HDAC2 and leads to glucocorticoid resistance, antioxidants should also be effective. Unfortunately currently available antioxidants are not very effective and several more potent antioxidants are in clinical development. In the future novel drugs which increase HDAC2 may be developed when the molecular signalling pathways that regulate HDAC2 are better understood [87].

References

[1] P.J. Barnes, I.M. Adcock, Glucocorticoid resistance in inflammatory diseases, Lancet 342 (2009) 1905–1917.
[2] P.J. Barnes, How corticosteroids control inflammation, Br. J. Pharmacol. 148 (2006) 245–254.
[3] T. Rhen, J.A. Cidlowski, Antiinflammatory action of glucocorticoids—new mechanisms for old drugs, N. Engl. J. Med. 353 (2005) 1711–1723.
[4] D.S. Goldfarb, A.H. Corbett, D.A. Mason, M.T. Harreman, S.A. Adam, Importin alpha: a multipurpose nuclear-transport receptor, Trends Cell Biol. 14 (2004) 505–514.
[5] T. Tao, J. Lan, G.L. Lukacs, R.J. Hache, F. Kaplan, Importin 13 regulates nuclear import of the glucocorticoid receptor in airway epithelial cells, Am. J. Respir. Cell. Mol. Biol. 35 (2006) 668–680.
[6] L.J. Lewis-Tuffin, J.A. Cidlowski, The physiology of human glucocorticoid receptor beta (hGRbeta) and glucocorticoid resistance, Ann. N. Y. Acad. Sci. 1069 (1–9) (2006) 1–9.
[7] K. Ito, P.J. Barnes, I.M. Adcock, Glucocorticoid receptor recruitment of histone deacetylase 2 inhibits IL-1β-induced histone H4 acetylation on lysines 8 and 12, Mol. Cell. Biol. 20 (2000) 6891–6903.
[8] P.J. Barnes, Corticosteroid effects on cell signalling, Eur. Respir. J. 27 (2006) 413–426.
[9] A.R. Clark, MAP kinase phosphatase 1: a novel mediator of biological effects of glucocorticoids? J. Endocrinol. 178 (2003) 5–12.
[10] A. Dostert, T. Heinzel, Negative glucocorticoid receptor response elements and their role in glucocorticoid action, Curr. Pharm. Des. 10 (2004) 2807–2816.
[11] P.J. Barnes, I.M. Adcock, How do corticosteroids work in asthma? Ann. Intern. Med. 139 (2003) 359–370.
[12] P.J. Barnes, I.M. Adcock, K. Ito, Histone acetylation and deacetylation: importance in inflammatory lung diseases, Eur. Respir. J. 25 (2005) 552–563.
[13] K. Ito, S. Yamamura, S. Essilfie-Quaye, B. Cosio, M. Ito, P.J. Barnes, I.M. Adcock, Histone deacetylase 2-mediated deacetylation of the glucocorticoid receptor enables NF-κB suppression, J. Exp. Med. 203 (2006) 7–13.
[14] J.J. Kovacs, P.J. Murphy, S. Gaillard, X. Zhao, J.T. Wu, C.V. Nicchitta, M. Yoshida, D.O. Toft, W.B. Pratt, T.P. Yao, HDAC6 regulates Hsp90 acetylation and chaperone-dependent activation of glucocorticoid receptor, Mol. Cell. 18 (2005) 601–607.
[15] M.W. Bergmann, K.J. Staples, S.J. Smith, P.J. Barnes, R. Newton, Glucocorticoid inhibition of GM-CSF from T cells is independent of control by NF-κB and CLE0, Am. J. Respir. Cell. Mol. Biol. 30. (2004) 555–563.
[16] K. Smoak, J.A. Cidlowski, Glucocorticoids regulate tristetraprolin synthesis and posttranscriptionally regulate tumor necrosis factor alpha inflammatory signaling, Mol. Cell. Biol. 26 (2006) 9126–9135.
[17] J. Carmichael, I.C. Paterson, P. Diaz, G.K. Crompton, A.B. Kay, I.W.B. Grant, Corticosteroid resistance in asthma, Br. Med. J. 282 (1981) 1419–1422.
[18] H. Hakonarson, U.S. Bjornsdottir, E. Halapi, J. Bradfield, F. Zink, M. Mouy, H. Helgadottir, A.S. Gudmundsdottir, H. Andrason, A.E. Adalsteinsdottir, K. Kristjansson, I. Birkisson, T. Arnason, M. Andresdottir, D. Gislason, T. Gislason, J.R. Gulcher, K. Stefansson, Profiling of genes expressed in peripheral blood mononuclear cells predicts glucocorticoid sensitivity in asthma patients, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 14789–14794.
[19] R. Donn, A. Berry, A. Stevens, S. Farrow, J. Betts, R. Stevens, C. Clayton, J. Wang, L. Warnock, J. Worthington, L. Scott, S. Graham, D. Ray, Use of gene expression profiling to identify a novel glucocorticoid sensitivity determining gene, BMPRII, FASEB J. 21 (2007) 402–414.
[20] S.W. Lamberts, Hereditary glucocorticoid resistance, Ann. Endocrinol. (Paris) 62 (2001) 164–167.
[21] S.J. Lane, J.P. Arm, D.Z. Staynov, T.H. Lee, Chemical mutational analysis of the human glucocortiocoid receptor cDNA in glucocorticoid-resistant bronchial asthma, Am. Am. J. Respir. Cell Mol. Biol. 11 (1994) 42–48.
[22] E.L. van den Akker, H. Russcher, E.F. van Rossum, A.O. Brinkmann, F.H. de Jong, A. Hokken, H.A. Pols, J.W. Koper, S.W. Lamberts, Glucocorticoid receptor polymorphism affects transrepression but not transactivation, J. Clin. Endocrinol. Metab. 91 (2006) 2800–2803.
[23] D.Y.M. Leung, R.J. Martin, S.J. Szefler, E.R. Sher, S. Ying, A.B. Kay, Q. Hamid, Dysregulation of interleukin 4, interleukin 5, and interferon y gene expression in steroid-resistant asthma, J. Exp. Med. 181 (1995) 33–40.
[24] E.R Sher, Y.M. Leung, W. Surs, J.C. Kam, G. Zieg, A.K. Kamada, S.J. Szefler, Steroid-resistant asthma. Cellular mechanisms contributing to inadequate response to glucocorticoid therapy, J. Clin. Invest. 93 (1994) 33–39.
[25] E. Irusen, J.G. Matthews, A. Takahashi, P.J. Barnes, K.F. Chung, I.M. Adcock, p38 Mitogen-activated protein kinase-induced glucocorticoid receptor phosphorylation reduces its activity: role in steroid-insensitive asthma, J. Allergy Clin. Immunol. 109 (2002) 649–657.
[26] J.G. Matthews, K. Ito, P.J. Barnes, I.M. Adcock, Defective glucocorticoid receptor nuclear translocation and altered histone acetylation patterns in glucocorticoid-resistant patients, J. Allergy Clin. Immunol. 113 (2004) 1100–1108.
[27] J.D. Spahn, S.J. Szefler, W. Surs, D.E. Doherty, S.R. Nimmagadda, D.Y. Leung, A novel action of IL-13: induction of diminished monocyte glucocorticoid receptor-binding affinity, J. Immunol. 157 (1996) 2654–2659.
[28] P. Bhavsar, M. Hew, N. Khorasani, T. Alfonso, P.J. Barnes, I. Adcock, K.F. Chung, Relative corticosteroid insensitivity of alveolar macrophages in severe asthma compared to non-severe asthma, Thorax 63 (2008) 784–790.
[29] N.L. Weigel, N.L. Moore, Steroid receptor phosphorylation: a key modulator of multiple receptor functions, Mol. Endocrinol. 21 (2007) 2311–2319.
[30] A.L. Miller, M.S. Webb, A.J. Copik, Y. Wang, B.H. Johnson, R. Kumar, E.B. Thompson, p38 Mitogen-activated protein kinase (MAPK) is a key mediator in glucocorticoid-induced apoptosis of lymphoid cells: correlation between p38 MAPK activation and site-specific phosphorylation of the human glucocorticoid receptor at serine 211, Mol. Endocrinol. 19 (2005) 1569–1583.
[31] Z. Szatmary, M.J. Garabedian, J. Vilcek, Inhibition of glucocorticoid receptor-mediated transcriptional activation by p38 mitogen-activated protein (MAP) kinase, J. Biol. Chem. 279 (2004) 43708–43715.
[32] E. Goleva, K.O. Kisich, D.Y. Leung, A role for STAT5 in the pathogenesis of IL-2-induced glucocorticoid resistance, J. Immunol. 169 (2002) 5934–5940.

[33] N. Ismaili, M.J. Garabedian, Modulation of glucocorticoid receptor function via phosphorylation, Ann. N. Y. Acad. Sci. 1024 (2004) 86–101.
[34] L.B. Li, E. Goleva, C.F. Hall, L.S. Ou, D.Y. Leung, 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 (2004) 1059–1069.
[35] S.M. Abraham, T. Lawrence, A. Kleiman, P. Warden, M. Medghalchi, J. Tuckermann, J. Saklatvala, A.R. Clark, Antiinflammatory effects of dexamethasone are partly dependent on induction of dual specificity phosphatase 1, J. Exp. Med. 203 (2006) 1883–1889.
[36] M.D. Galigniana, G. Piwien-Pilipuk, J. Assreuy, Inhibition of glucocorticoid receptor binding by nitric oxide, Mol. Pharmacol. 55 (1999) 317–323.
[37] A.D. Wallace, J.A. Cidlowski, Proteasome-mediated glucocorticoid receptor degradation restricts transcriptional signaling by glucocorticoids, J. Biol. Chem. 276 (2001) 42714–42721.
[38] Q.A. Hamid, S.E. Wenzel, P.J. Hauk, A. Tsicopoulos, B. Wallaert, J.J. Lafitte, G.P. Chrousos, S.J. Szefler, D.Y. Leung, Increased glucocorticoid receptor beta in airway cells of glucocorticoid-insensitive asthma, Am. J. Respir. Crit. Care Med. 159 (1999) 1600–1604.
[39] A.R. Sousa, S.J. Lane, J.A. Cidlowski, D.Z. Staynov, T.H. Lee, Glucocorticoid resistance in asthma is associated with elevated in vivo expression of the glucocorticoid receptor beta-isoform, J. Allergy Clin. Immunol. 105 (2000) 943–950.
[40] D.L. Kozaci, Y. Chernajovsky, I.C. Chikanza, The differential expression of corticosteroid receptor isoforms in corticosteroid-resistant and -sensitive patients with rheumatoid arthritis, Rheumatology (Oxford) 46 (2007) 579–585.
[41] F. Orii, T. Ashida, M. Nomura, A. Maemoto, T. Fujiki, T. Ayabe, S. Imai, Y. Saitoh, Y. Kohgo, Quantitative analysis for human glucocorticoid receptor alpha/beta mRNA in IBD, Biochem. Biophys. Res. Commun. 296 (2002) 1286–1294.
[42] L. Pujols, J. Mullol, C. Picado, Alpha and beta glucocorticoid receptors: relevance in airway diseases, Curr. Allergy Asthma Rep. 7 (2007) 93–99.
[43] R. Gagliardo, P. Chanez, A.M. Vignola, J. Bousquet, I. Vachier, P. Godard, G. Bonsignore, P. Demoly, M. Mathieu, Glucocorticoid receptor α and β in glucocorticoid dependent asthma, Am. J. Respir. Crit. Care Med. 162 (2000) 7–13.
[44] J.C. Webster, R.H. Oakley, C.M. Jewell, J.A. Cidlowski, Proinflammatory cytokines regulate human glucocorticoid receptor gene expression and lead to the accumulation of the dominant negative beta isoform: a mechanism for the generation of glucocorticoid resistance, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 6865–6870.
[45] S. Fakhri, M. Tulic, P. Christodoulopoulos, M. Fukakusa, S. Frenkiel, D.Y. Leung, Q.A. Hamid, Microbial superantigens induce glucocorticoid receptor beta and steroid resistance in a nasal explant model, Laryngoscope 114 (2004) 887–892.
[46] E. Goleva, L.B. Li, P.T. Eves, M.J. Strand, R.J. Martin, D.Y. Leung, Increased glucocorticoid receptor beta alters steroid response in glucocorticoid-insensitive asthma, Am. J. Respir. Crit. Care Med. 173 (2006) 607–616.
[47] L.J. Lewis-Tuffin, C.M. Jewell, R.J. Bienstock, J.B. Collins, J.A. Cidlowski, Human glucocorticoid receptor beta binds RU-486 and is transcriptionally active, Mol. Cell Biol. 27 (2007) 2266–2282.
[48] I.M. Adcock, S.J. Lane, C.A. Brown, T.H. Lee, P.J. Barnes, Abnormal glucocorticoid receptor/AP-1 interaction in steroid resistant asthma, J. Exp. Med. 182 (1995) 1951–1958.
[49] T.K. Loke, K.H. Mallett, J. Ratoff, B.J. O'Connor, S. Ying, Q. Meng, C. Soh, T.H. Lee, C.J. Corrigan, 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 (2006) 368–375.
[50] S.J. Lane, I.M. Adcock, D. Richards, C. Hawrylowicz, P.J. Barnes, T.H. Lee, Corticosteroid-resistant bronchial asthma is associated with increased *c-Fos* expression in monocytes and T-lymphocytes, J. Clin. Invest. 102 (1998) 2156–2164.
[51] L. Vardimon, I. Ben Dror, A. Oren, P. Polak, Cytoskeletal and cell contact control of the glucocorticoid pathway, Mol. Cell Endocrinol. 252 (2006) 142–147.
[52] N. Vasavda, T. Eichholtz, A. Takahashi, K. Affleck, J.G. Matthews, P.J. Barnes, I.M. Adcock, Expression of nonmuscle cofilin-1 and steroid responsiveness in severe asthma, J. Allergy Clin. Immunol. 118 (2006) 1090–1096.
[53] P.J. Barnes, K. Ito, I.M. Adcock, A mechanism of corticosteroid resistance in COPD: inactivation of histone deacetylase, Lancet 363 (2004) 731–733.
[54] K. Ito, M. Ito, W.M. Elliott, B. Cosio, G. Caramori, O.M. Kon, A. Barczyk, M. Hayashi, I.M. Adcock, J.C. Hogg, P.J. Barnes, Decreased histone deacetylase activity in chronic obstructive pulmonary disease, N. Engl. J. Med. 352 (2005) 1967–1976.
[55] M. Hew, P. Bhavsar, A. Torrego, S. Meah, N. Khorasani, P.J. Barnes, I. Adcock, K.F. Chung, Relative corticosteroid insensitivity of peripheral blood mononuclear cells in severe asthma, Am. J. Respir. Crit. Care Med. 174 (2006) 134–141.
[56] A. Murahidy, M. Ito, I.M. Adcock, P.J. Barnes, K. Ito, Reduction is histone deacetylase expression and activity in smoking asthmatics: a mechanism of steroid resistance, Proc. Am. Thorac. Soc. 2 (2005) A889.
[57] P.J. Barnes, Role of HDAC2 in the pathophysiology of COPD, Ann. Rev. Physiol. 71 (2009) 451–464.
[58] K. Ito, T. Tomita, P.J. Barnes, I.M. Adcock, Oxidative stress reduces histone deacetylase (HDAC)2 activity and enhances IL-8 gene expression: role of tyrosine nitration, Biochem. Biophys. Res. Commun. 315 (2004) 240–245.
[59] M. To, K. Ito, Y. Kizawa, M. Failla, M. Ito, T. Kusama, M. Elliot, J.C. Hogg, I.M. Adcock, P.J. Barnes, Targeting phosphoinositide-3-kinase-δ with theophylline reverses corticosteroid insensitivity in COPD, Am. J. Respir. Crit. Care Med. (2010).
[60] C.M. Hawrylowicz, Regulatory T cells and IL-10 in allergic inflammation, J. Exp. Med. 202 (2005) 1459–1463.
[61] E. Xystrakis, S. Kusumakar, S. Boswell, E. Peek, Z. Urry, D.F. Richards, T. Adikibi, C. Pridgeon, M. Dallman, T.K. Loke, D.S. Robinson, F.J. Barrat, A. O'Garra, P. Lavender, T.H. Lee, C. Corrigan, C.M. Hawrylowicz, Reversing the defective induction of IL-10-secreting regulatory T cells in glucocorticoid-resistant asthma patients, J. Clin. Invest. 116 (2006) 146–155.
[62] R.J. Farrell, A. Murphy, A. Long, S. Donnelly, A. Cherikuri, D. O'Toole, N. Mahmud, P.W. Keeling, D.G. Weir, D. Kelleher, High multidrug resistance (P-glycoprotein 170) expression in inflammatory bowel disease patients who fail medical therapy, Gastroenterology 118 (2000) 279–288.
[63] R.J. Farrell, D. Kelleher, Glucocorticoid resistance in inflammatory bowel disease, J. Endocrinol. 178 (2003) 339–346.
[64] S. Tsujimura, K. Saito, M. Nawata, S. Nakayamada, Y. Tanaka, Overcoming drug resistance induced by P-glycoprotein on lymphocytes in patients with refractory rheumatoid arthritis, Ann. Rheum. Dis. 67 (2008) 380–388.
[65] U. Potocnik, I. Ferkolj, D. Glavac, M. Dean, Polymorphisms in multidrug resistance 1 (MDR1) gene are associated with refractory Crohn disease and ulcerative colitis, Genes Immun. 5 (2004) 530–539.
[66] M. Drozdzik, T. Rudas, A. Pawlik, M. Kurzawski, B. Czerny, W. Gornik, M. Herczynska, The effect of 3435C>T MDR1 gene polymorphism on rheumatoid arthritis treatment with disease-modifying antirheumatic drugs, Eur. J. Clin. Pharmacol. 62 (2006) 933–937.
[67] H. Flaster, J. Bernhagen, T. Calandra, R. Bucala, The macrophage migration inhibitory factor-glucocorticoid dyad: regulation of inflammation and immunity, Mol. Endocrinol. 21 (2007) 1267–1280.
[68] T. Roger, A.L. Chanson, M. Knaup-Reymond, T. Calandra, Macrophage migration inhibitory factor promotes innate immune responses by suppressing glucocorticoid-induced expression of mitogen-activated protein kinase phosphatase-1, Eur. J. Immunol. 35 (2005) 3405–3413.
[69] Y. Ishiguro, T. Ohkawara, H. Sakuraba, K. Yamagata, H. Hiraga, S. Yamaguchi, S. Fukuda, A. Munakata, A. Nakane, J. Nishihira, Macrophage migration inhibitory factor has a proinflammatory activity via the p38 pathway in glucocorticoid-resistant ulcerative colitis, Clin. Immunol. 120 (2006) 335–341.
[70] J.A. Baugh, S. Chitnis, S.C. Donnelly, J. Monteiro, X. Lin, B.J. Plant, F. Wolfe, P.K. Gregersen, R. Bucala, A functional promoter polymorphism in the macrophage migration inhibitory factor (MIF) gene associated with disease severity in rheumatoid arthritis, Genes Immun. 3 (2002) 170–176.
[71] T. Griga, C. Wilkens, N. Wirkus, J. Epplen, W. Schmiegel, W. Klein, A polymorphism in the macrophage migration inhibitory factor gene is involved in the genetic predisposition of Crohn's disease and associated with cumulative steroid doses, Hepatogastroenterology 54 (2007) 784–786.
[72] S. Ayoub, M.J. Hickey, E.F. Morand, Mechanisms of disease: macrophage migration inhibitory factor in SLE, RA and atherosclerosis, Nat. Clin. Pract. Rheumatol. 4 (2008) 98–105.
[73] A.G. Rossi, C. Haslett, N. Hirani, A.P. Greening, I. Rahman, C.N. Metz, R. Bucala, S.C. Donnelly, Human circulating eosinophils secrete macrophage migration inhibitory factor (MIF). Potential role in asthma, J. Clin. Invest. 101 (1998) 2869–2874.
[74] H. Schacke, M. Berger, H. Rehwinkel, K. Asadullah, Selective glucocorticoid receptor agonists (SEGRAs): novel ligands with an improved therapeutic index, Mol. Cell Endocrinol. 275 (2007) 109–117.
[75] A. Kleiman, J.P. Tuckermann, Glucocorticoid receptor action in beneficial and side effects of steroid therapy: lessons from conditional knockout mice, Mol. Cell Endocrinol. 275 (2007) 98–108.
[76] K. Kitahara, S. Kawai, Cyclosporine and tacrolimus for the treatment of rheumatoid arthritis, Curr. Opin. Rheumatol. 19 (2007) 238–245.
[77] D.J. Evans, P. Cullinan, D.M. Geddes, Cyclosporin as an oral corticosteroid sparing agent in stable asthma (Cochrane Review), Cochrane Database Syst. Rev. 2 (2001), CD002993.
[78] S.G. Dastidar, D. Rajagopal, A. Ray, Therapeutic benefit of PDE4 inhibitors in inflammatory diseases, Curr. Opin. Investig. Drugs 8 (2007) 364–372.
[79] S. Medicherla, M. Fitzgerald, D. Spicer, P. Woodman, J.Y. Ma, A.M. Kapoun, S. Chakravarty, S. Dugar, A.A. Protter, L.S. Higgins, p38α selective MAP kinase inhibitor, SD-282, reduces inflammation in a sub-chronic model of tobacco smoke-induced airway inflammation, J. Pharmacol. Exp. Ther. 324 (2007) 921–929.
[80] R Chaudhuri, E. Livingston, A.D. McMahon, J. Lafferty, I. Fraser, M. Spears, C.P. McSharry, N.C. Thomson, Effects of smoking cessation on lung function and airway inflammation in smokers with asthma, Am. J. Respir. Crit. Care Med. 174 (2006) 127–133.
[81] T.J. Creed, C.S. Probert, M.N. Norman, M. Moorghen, N.A. Shepherd, S.D. Hearing, C.M. Dayan, Basiliximab for the treatment of steroid-resistant ulcerative colitis: further experience in moderate and severe disease, Aliment. Pharmacol. Ther. 23 (2006) 1435–1442.
[82] S. Nobili, I. Landini, B. Giglioni, E. Mini, Pharmacological strategies for overcoming multidrug resistance, Curr. Drug Targets 7 (2006) 861–879.
[83] A.Y. Hoi, M.N. Iskander, E.F. Morand, Macrophage migration inhibitory factor: a therapeutic target across inflammatory diseases, Inflamm. Allergy Drug Targets 6 (2007) 183–190.
[84] B.G. Cosio, L. Tsaprouni, K. Ito, E. Jazrawi, I.M. Adcock, P.J. Barnes, Theophylline restores histone deacetylase activity and steroid responses in COPD macrophages, J. Exp. Med. 200 (2004) 689–695.

[85] J.C. Fox, D. Spicer, K. Ito, P.J. Barnes, M.F. Fitzgerald, Oral or inhaled corticosteroid combination therapy with low dose theophylline reverses corticosteroid insensitivity in a smoking mouse model, Proc. Am. Thorac. Soc. 2 (2007) A637.
[86] J.A. Marwick, G. Caramori, C.C. Stevenson, P. Casolari, E. Jazrawi, P.J. Barnes, K. Ito, I.M. Adcock, P.A. Kirkham, A. Papi, Inhibition of PI3Kδ restores glucocorticoid function in smoking-induced airway inflammation in mice, Am. J. Respir. Crit. Care Med. 179 (2009) 542–548.
[87] P.J. Barnes, Targeting histone deacetylase 2 in chronic obstructive pulmonary disease treatment, Expert. Opin. Ther. Targets 9 (2005) 1111–1121.