# Glucocorticosteroids

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**Abstract**

Glucocorticosteroids are the most effective anti-inflammatory therapy for asthma but are relatively ineffective in COPD. Glucocorticoids are broad-spectrum anti-inflammatory drugs that suppress inflammation via several molecular mechanisms. Glucocorticoids suppress the multiple inflammatory genes that are activated in asthma by reversing histone acetylation of activated inflammatory genes through binding of ligand-bound glucocorticoid receptors (GR) to coactivator molecules and recruitment of histone deacetylase-2 (HDAC2) to the activated inflammatory gene transcription complex (trans-repression). 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 (trans-activation). Glucocorticoids also have post-transcriptional effects and decrease stability of some proinflammatory mRNAs. Decreased glucocorticoid responsiveness is found in patients with severe asthma and asthmatics who smoke, as well as in all patients with COPD. Several molecular mechanisms of glucocorticoid resistance have now been identified which involve phosphorylation and other post-translational modifications of GR. HDAC2 is markedly reduced in activity and expression as a result of oxidative/nitrative stress and pi3 kinase-δ inhibition, so that inflammation is resistant to the anti-inflammatory actions of glucocorticoids. Dissociated glucocorticoids and selective GR modulators which show improved trans-repression over trans-activation effects 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 mechanisms of glucocorticoid resistance.

**keywords**

Anti-inflammatory • Corticosteroid resistance • Glucocorticoid receptor • Glucocorticoid receptor-beta • Histone deacetylase-2 • p38 MAP kinase

## 1 Introduction

Glucocorticosteroids (also called glucocorticoids, corticosteroids or steroids) are the most effective anti-inflammatory drugs available for the treatment of many chronic inflammatory and immune diseases, including asthma (Barnes 2011). 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 glucocorticoid-resistant (Barnes and Adcock 2009). Both asthma and COPD involve chronic inflammation of the respiratory tract, with the activation and recruitment of many inflammatory cells and orchestrated by a complex network of inflammatory mediators (Barnes 2008).

However, there are differences in the nature of this inflammation and its inflammatory consequences between these diseases and this is demonstrated best by the differing response to glucocorticoids, which is excellent in most patients with asthma but very poor in most patients with COPD. There is now a much better understanding of how glucocorticoids suppress chronic inflammation in asthma and also why they fail to work in some patients with asthma and most patients with COPD, despite the fact that inflammatory genes are activated in these two diseases by similar molecular mechanisms. This has given insights into how glucocorticoids might be improved in the future and how glucocorticoid resistance may be overcome with new classes of therapy.

## 2 Clinical Use in Asthma

The widespread use of inhaled corticosteroids (ICS) has revolutionised the management of asthma, with marked reductions in asthma morbidity and mortality in patients of all severity. ICS are now recommended as first-line therapy for all patients with persistent asthma, including children (Reddel et al. 2015). Several topically acting glucocorticoids are now available for inhalation and all have similar efficacy, but there are pharmacokinetic differences that account for differences in therapeutic ratio between these drugs. ICS are very effective in controlling asthma symptoms in asthmatic patients of all ages and severity. ICS improve the quality of life of patients with asthma and allow many patients to lead normal lives, improve lung function, reduce the frequency of exacerbations and may prevent irreversible airway changes with long-term use (Barnes et al. 1998; O'Byrne et al. 2006). ICS were initially introduced to reduce the dose of oral glucocorticoids in patients with severe asthma and many studies have confirmed that the great majority of patients can be weaned off oral glucocorticoids. Only about 1% of asthmatic patients now require maintenance treatment with oral glucocorticoids for control of asthma ("steroid-dependent" asthmatics), but short courses of oral glucocorticoids are still needed to treat exacerbations of asthma. There are local side effects of ICS, including increased oral candidiasis and dysphonia, but these are rarely a major problem. Systemic side effects, largely arising from absorption of ICS from the lung, are not a problem in patients treated with the usually required doses, but may become a problem in patients with severe asthma who require larger doses for asthma control.

## 3 Clinical Use in COPD

Most patients with COPD have a poor response to glucocorticoids in comparison to asthma with little improvement in lung function or symptoms (Barnes 2010a). High doses of ICS have shown a reduction (20–25%) in exacerbations in patients with severe disease and this is the main clinical indication for their use (Vestbo et al. 2013). However, even the effect on exacerbations has been questioned as it may be explained by an artefact in trial design (Suissa and Barnes 2009). Several

large studies have shown that glucocorticoids fail to reduce the progression in COPD (measured by annual fall in $FEV_1$) or its mortality (Yang et al. 2012). This is likely to reflect the resistance of pulmonary inflammation to glucocorticoids in COPD patients (as discussed below). Current guidelines suggest that high doses of ICS should be used only in patients with severe disease ($FEV_1 < 50\%$ predicted) who have frequent exacerbations ($\leq$2/year) which would comprise about 10% of patients, whereas currently high dose ICS are used in approximately 80% of patients with a clinical diagnosis of COPD. This overuse of glucocorticoids is likely to produce several long-term side effects, such as osteoporosis, diabetes, cataracts and hypertension. In addition there is now considerable evidence that high doses of ICS in COPD increase the risk of pneumonia (Finney et al. 2014). Oral glucocorticoids are used to treat acute exacerbations, although they are poorly effective. Some patients with COPD, who also have concomitant asthma (termed "overlap syndrome"), benefit from ICS and these patients may be recognised by increased sputum and blood eosinophils and exhaled nitric oxide and by a greater bronchodilator reversibility (Postma and Rabe 2015).

## 4 Anti-Inflammatory Mechanisms of Glucocorticoids

There have been major advances in understanding the molecular mechanisms whereby glucocorticoids suppress inflammation in asthma (Kadmiel and Cidlowski 2013; Barnes 2010b). Glucocorticoids activate many anti-inflammatory genes, and repress many proinflammatory genes that have been activated in inflammation (Table 1), as well as having several post-transcriptional effects. Understanding the molecular mechanisms of glucocorticoid action has also provided new insights into understanding molecular mechanisms involved in glucocorticoid resistance (Barnes and Adcock 2009; Barnes 2010b).

### 4.1 Glucocorticoid Receptors

Glucocorticoids diffuse across the cell membrane and bind to glucocorticoid receptors (GR) in the cytoplasm (Nicolaides et al. 2010). 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 proteins importin-α (karyopherin-β), importin-7 and importin-13 (Goldfarb et al. 2004; Hakim et al. 2013; Tao et al. 2006). 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 glucocorticoid action by interfering with the binding of GR to DNA (Kino et al. 2009). In addition, there is evidence that multiple GR isoforms are generated by alternative splicing and alternative translation initiation (Kadmiel and Cidlowski 2013). These isoforms have unique tissue distribution patterns and transcriptional regulatory profiles. Furthermore, each is subject to

**Table 1** Effect of glucocorticoids on transcription of genes relevant to asthma

| *Increased transcription* (*trans-activation*) |
|---|
| • Lipocortin-1 |
| • $\beta_2$-Adrenoceptor |
| • Secretory leukocyte inhibitory protein |
| • IkB-α (inhibitor of NF-kB) |
| • MKP-1 (inhibits MAP kinase pathways) |
| • Glucocorticoid inducible leucine zipper (GILZ) |
| • Anti-inflammatory or inhibitory cytokines |
| *IL-10, IL-12, IL-1 receptor antagonist* |
| *Decreased transcription* (*trans-repression*) |
| • Inflammatory cytokines |
| *IL-2, IL-3, IL-4, IL-5, IL-6, IL-13, IL-15, TNFα, GM-CSF, SCF, TSLP* |
| • Chemokines |
| *CCL1, CCL5, CCL11, CXCL8* |
| • Inflammatory enzymes |
| *Inducible nitric oxide synthase (iNOS), inducible cyclo-oxygenase (COX-2)* |
| *Inducible phospholipase $A_2$ ($cPLA_2$)* |
| • Inflammatory peptides |
| *Endothelin-1* |
| • Inflammatory mediator receptors |
| *Neurokinin ($NK_1$)-, bradykinin ($B_2$)-receptors* |
| • Adhesion molecules |
| *ICAM-1,VCAM-1* |

various post-translational modifications that may affect receptor function, which determine the cell-specific response to glucocorticoids.

## 4.2 Gene Activation

GR homodimerise and bind to glucocorticoid response elements (GRE) usually 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) (Fig. 1). This tags histones to recruit chromatin remodelling engines such as SWI/SNF and subsequent association of RNA polymerase II resulting in gene activation (Ito et al. 2000; John et al. 2008). Genes that are switched on by glucocorticoids include genes encoding $\beta_2$-adrenergic receptors and the anti-inflammatory proteins secretory leukoprotease inhibitor and mitogen-activated protein kinase phosphatase-1 (MKP-1), also known as dual specificity phosphatase-1 (DUSP-1) which inhibits MAP kinase pathways. These effects may contribute to the anti-inflammatory actions of glucocorticoids. GR interaction with negative GREs, or to GREs that cross the transcriptional start

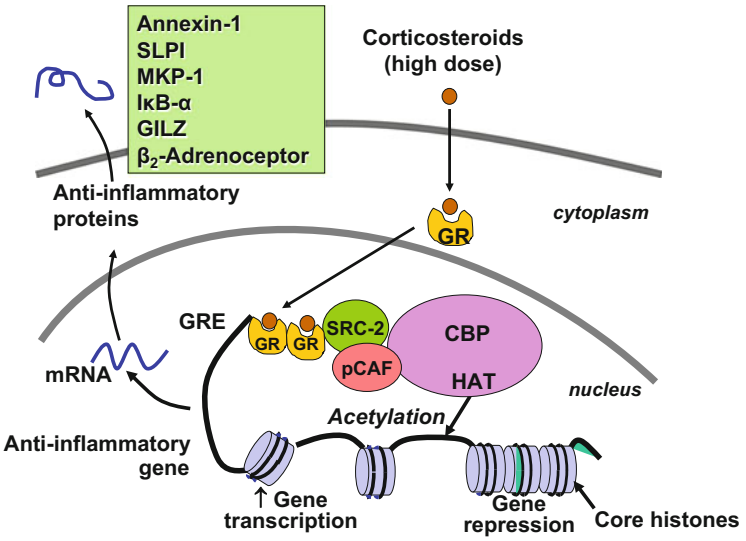


**Fig. 1** Glucocorticoid activation of anti-inflammatory gene expression. Glucocorticoids bind to cytoplasmic glucocorticoid receptors (GR) which translocate to the nucleus where they bind to glucocorticoid response elements (GRE) in the promoter region of steroid-sensitive genes and also directly or 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-kB (IkB-α) and glucocorticoid-induced leucine zipper protein (GILZ)

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 (Dostert and Heinzel 2004) (Fig. 2).

## 4.3 Switching Off Activated 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 (Barnes 2011). These genes are switched on in the airways by proinflammatory transcription factors, such as nuclear factor-kB (NF-kB) and activator protein-1 (AP-1), both of which are usually activated at sites of inflammation in asthma and COPD, 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.

Activated GR interact with co-repressor molecules to attenuate NF-kB-associated coactivator activity, thus reducing histone acetylation, chromatin

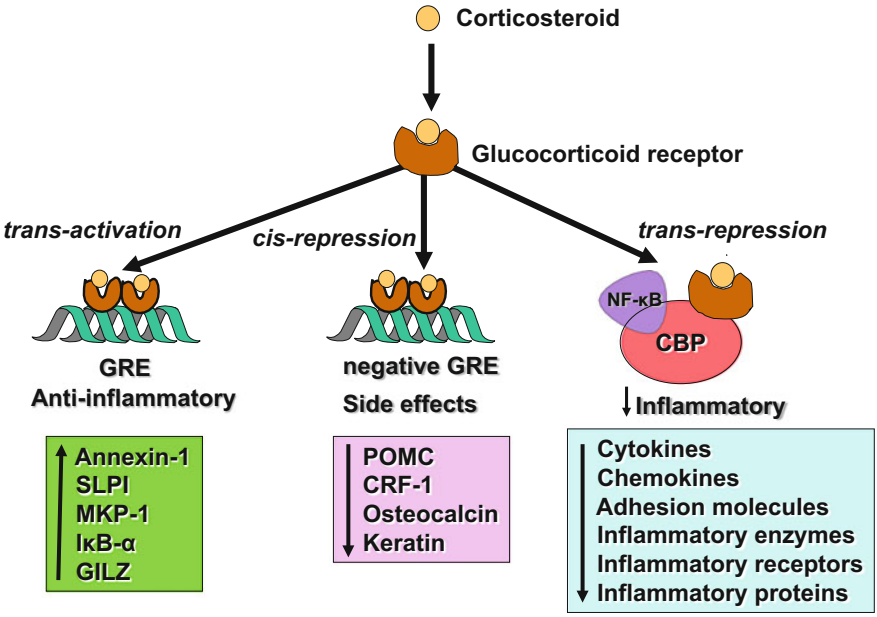


**Fig. 2** Glucocorticoids regulate gene expression in several ways. Glucocorticoids enter the cell to bind to glucocorticoid receptors (GR) in the cytoplasm that translocate to the nucleus. GR homodimers bind to glucocorticoid response elements (GRE) in the promoter region of steroid-sensitive genes, 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-kB (NF-kB), 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, *IkB-α* inhibitor of NF-kB, *GILZ* glucocorticoid-induced leucine zipper protein, *POMC* proopiomelanocortin, *CRH* corticotrophin releasing factor

remodelling and RNA polymerase II actions. 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 (Ito et al. 2000) (Fig. 3). This may account for why glucocorticoids are so effective in the control of inflammation, but also why they are relatively safe, since genes other than those that encode inflammatory proteins 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-kB complex (Ito et al. 2006). 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 glucocorticoids, whereas repression of NF-kB is unaffected.

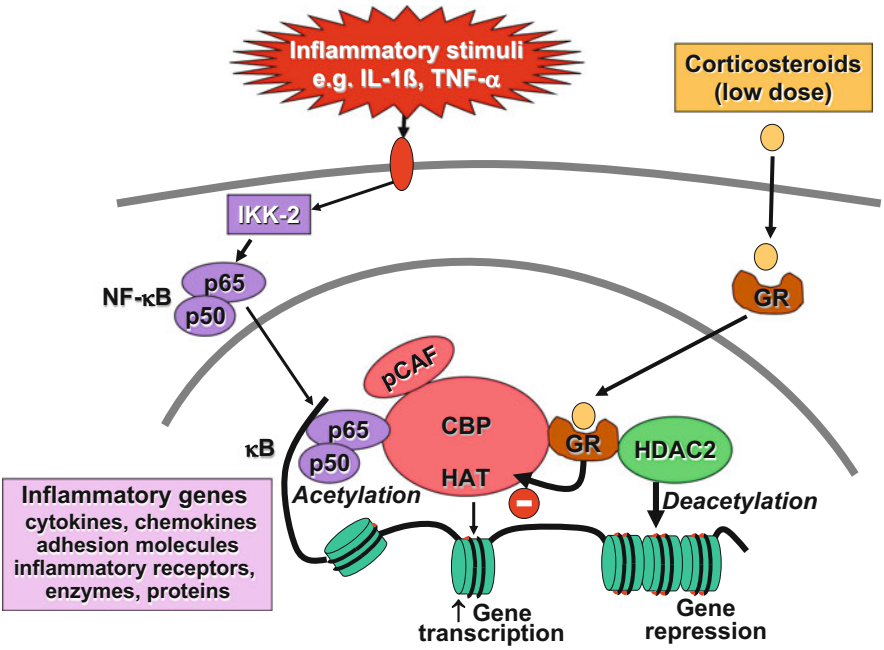


**Fig. 3** Corticosteroid suppression of activated inflammatory genes. Inflammatory genes are activated by inflammatory stimuli, such as interleukin-1β (IL-1β) or tumour necrosis factor-α (TNF-α), resulting in activation of IKK2 (inhibitor of I-kB kinase-2), which activates the transcription factor nuclear factor kB (NF-kB). A dimer of p50 and p65 NF-kB proteins translocates to the nucleus and binds to specific kB 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 glucocorticoids 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

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/DUSP-1 as discussed above. An important effect of glucocorticoids in the treatment of allergic diseases is through suppression of Th2 cells and Th2 cytokines (IL4, IL-5, and IL-13) and this may be mediated via inhibition of the transcription factor GATA3 which regulates the transcription of Th2 cytokine genes. This is controlled by translocation of GATA3 from the cytoplasm to the nucleus via importin-α after phosphorylation by p38 MAPK. Glucocorticoids potently inhibit GATA3 nuclear translocation as GR competes for nuclear import via importin-α and also induces MKP-1 to reverse the phosphorylation of GATA3 by p38 MAPK (Maneechotesuwan et al. 2009). A further immunosuppressive effect of glucocorticoids is through enhanced activity and expression of indoleamine-2,3-

dioxygenase (IDO), a tryptophan-degrading enzyme that plays a key role in the regulation of T-lymphocyte function in allergic diseases through increased secretion of the anti-inflammatory cytokine IL-10 (Maneechotesuwan et al. 2008).

### 4.4 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 degradation of mRNA and reduced inflammatory protein secretion (Bergmann et al. 2004). 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 (Prabhala and Ammit 2015).

## 5 Cellular Effects in Asthma and COPD

At a cellular level glucocorticoids reduce the numbers of inflammatory cells in the airways of asthmatic patients, including eosinophils, T-lymphocytes, mast cells and dendritic cells (Barnes et al 1998) (Fig. 4). These effects of glucocorticoids are produced through inhibiting the recruitment of inflammatory cells into the airway by suppressing the production of chemotactic mediators and adhesion molecules

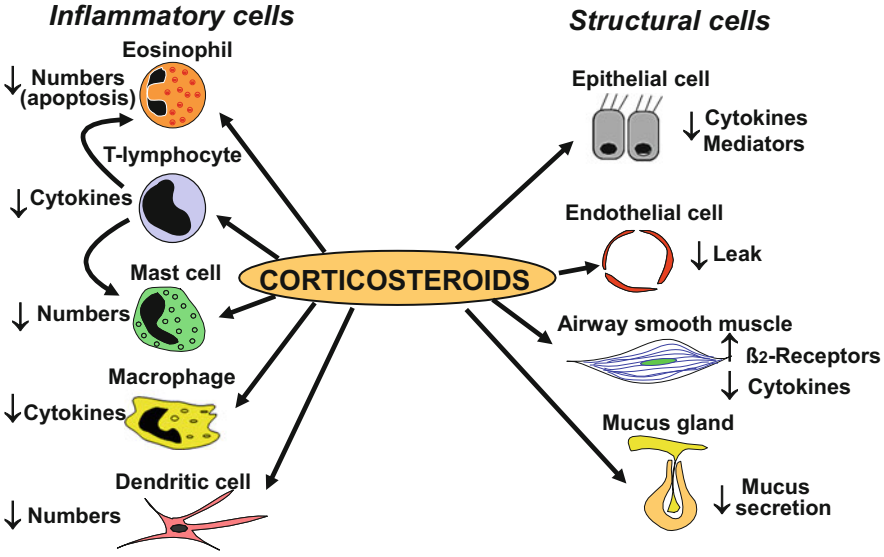


**Fig. 4** Cellular effect of corticosteroids

and by inhibiting the survival in the airways of inflammatory cells, such as eosinophils, T-lymphocytes, mast cells and dendritic cells. Epithelial cells may be the major cellular target for ICS, which are the mainstay of modern asthma management. ICS suppress many activated inflammatory genes in airway epithelial cells. Epithelial integrity is restored by regular ICS. The suppression of mucosal inflammation is relatively rapid with a significant reduction in eosinophils detectable within 3 h and associated with reduced airway hyperresponsiveness (Erin et al. 2008). This almost certainly accounts for the clinical benefits seen with inhalation of budesonide and formoterol combination inhaler as a rescue therapy in asthma, as this is likely to stop the evolution of an exacerbation (Barnes 2007). However, reversal of airway hyperresponsiveness may take several months to reach a plateau, probably reflecting recovery of structural changes in the airway.

In COPD patients even high doses of ICS fail to reduce airway inflammation. This glucocorticoid resistance has been demonstrated by the failure of high doses of ICS to reduce inflammatory markers in sputum or bronchial biopsies of COPD patients (Keatings et al. 1997; Culpitt et al. 1999). The reason why ICS fail to suppress inflammation cannot be explained by impaired access of the inhaled drug to sites of inflammation as an oral glucocorticoid is equally ineffective.

## 6 Interaction with $\beta_2$-Adrenergic Agonists

Inhaled $\beta_2$-agonists and glucocorticoids are frequently used together, usually as a fixed combination inhaler containing a glucocorticoid with a long-acting $\beta_2$-agonist (LABA) in the control of asthma and it is now recognized that there are important molecular interactions between these two classes of drug (Barnes 2002; Black et al. 2009) (Fig. 5). Glucocorticoids increase the transcription of the $\beta_2$-receptor gene, resulting in increased expression of cell surface receptors. This has been demonstrated in human lung in vitro and nasal mucosa in vivo after topical application of a glucocorticoid. In this way glucocorticoids protect against the down-regulation of $\beta_2$-receptors after long-term administration. This may be important for the non-bronchodilator effects of $\beta_2$-agonists, such as mast cell stabilisation. Glucocorticoids may also enhance the coupling of $\beta_2$-receptors to G-protein ($G_s$), thus enhancing $\beta_2$-agonist effects and reversing the uncoupling of $\beta_2$-receptors that may occur in response to inflammatory mediators, such as IL-1β through a stimulatory effect on a G-protein coupled receptor kinase (Mak et al. 1995). Glucocorticoids may increase responses to β2-agonists in COPD patients and this may account for the effects of adding ICS to LABA in COPD patients (Nannini et al. 2013).

There is now increasing evidence that $\beta_2$-agonists may affect GR function and thus enhance the anti-inflammatory effects of glucocorticoids. LABA increase the translocation of GR from cytoplasm to the nucleus after activation by glucocorticoids (Roth et al. 2002). This effect has now been demonstrated in sputum macrophages of asthmatic patients after an ICS and inhaled LABA in asthma and COPD (Usmani et al. 2005; Haque et al. 2013). This suggests that

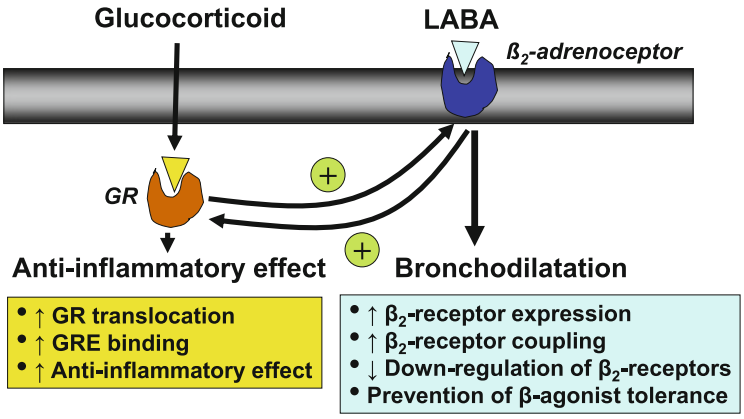


**Fig. 5** Interaction between glucocorticosteroids and long-acting $\beta_2$-agonists (LABA). Glucocorticoids increase the numbers of $\beta_2$-receptors, whereas $\beta_2$-agonists, as well as inducing direct bronchodilatation, act on glucocorticoid receptors (GR) to increase the anti-inflammatory effects of glucocorticoids

LABA and glucocorticoids enhance each other's beneficial effects in asthma therapy and this may contribute to the greater efficacy of combination inhalers compared to increased doses of ICS in clinical trials in asthma. LABA also enhance GR translocation in COPD macrophages and to reduce the corticosteroid resistance found in these cells in COPD patients (Haque et al. 2013).

## 7 Glucocorticoid Resistance

Patients with severe asthma have a poor response to glucocorticoids, which necessitates the need for high doses and a very small number of patients are completely resistant. These patients are difficult to manage as they get side effects from high doses of glucocorticoids, despite their lack of clinical benefit. All patients with COPD show a degree of glucocorticoid resistance (Barnes 2010a). Asthmatics who smoke are also relatively glucocorticoid-resistant and require increased doses of glucocorticoids for asthma control (Polosa and Thomson 2012). Several molecular mechanisms have now been identified to account for glucocorticoid resistance in severe asthma and COPD (Table 2) (Barnes 2013a).

### 7.1 Genetic Susceptibility

Glucocorticoid-resistant asthma suggested that it was more commonly found within families, indicating that there may genetic factors may determine glucocorticoid responsiveness. Microarray studies of peripheral blood mononuclear cells (PBMC) from glucocorticoid-sensitive and glucocorticoid-insensitive asthma patients have

**Table 2** Molecular mechanisms of steroid resistance in asthma and COPD

| |
|---|
| • Familial glucocorticoid resistance |
| • Glucocorticoid receptor modification<br>↑ Phosphorylation: ↓ nuclear translocation<br>↑ p38MAPKα due to IL-2 + IL-4 or IL-13 in severe asthma due to MIF in severe asthma<br>↑ p38MAPKγ in severe asthma<br>↑ JNK1 due to proinflammatory cytokines in severe asthma<br>↑ ERK due to microbial superantigens in severe non-allergic asthma<br>↓ MKP-1 in severe asthma<br>↓ PP2A in severe asthm<br>Nitrosylation: ↑ NO from inducible NO synthase<br>Ubiquitination: ↑ degradation by proteasome |
| • Increased GRβ expression |
| • Increased proinflammatory transcription factors<br>Activator protein-1, JNK |
| • Defective histone acetylation<br>↓ Acetylation of lysine-5 on histone 4 in severe asthma<br>↓ Histone deacetylase-2 in COPD, severe asthma. smoking asthma<br>↑ Oxidative stress<br>↑ Phosphoinositide-3-kinase-δ activation |

*GR* glucocorticoid receptor, *IL* interleukin, *MAP* mitogen-activated protein, *MIF* macrophage migration inhibitory factor, *MKP* MAP kinase phosphatase, *JNK* c-Jun N-terminal kinase, *ERK* extracellular signal-regulated kinase, *NO* nitric oxide, *PP* protein phosphatase

identified several genes that discriminated between these patients (Hakonarson et al. 2005), 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 identified 24 genes of which the most discriminant was bone morphogenetic protein receptor type II (BMPR2), which enhanced glucocorticoid responsiveness when transfected into cells (Donn et al. 2007).

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 (Charmandari et al. 2013). Clinical manifestations are due to an excess of non-glucocorticoid adrenal steroids, stimulated by high adrenocorticotropin levels, resulting in hypertension with hypokalaemia and/or signs of androgen excess. Inheritance appears to be dominant with variable expression, but only about a few cases have so far been reported. Sporadic cases have also been described. Several mutations in GR have been described in FGR, with impaired GR function in PBMC or fibroblasts, including decreased binding for cortisol, reduced numbers and abnormal binding to DNA. 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 (Lane et al. 1994).

## 7.2 Defective GR Binding and Translocation

There is increased expression of interleukin(IL)-2 and IL-4 in the airways of patients with glucocorticoid-resistant asthma and in vitro these cytokines in combination reduce GR nuclear translocation and binding affinity within the nucleus of T-cells IL-13 alone mimics this effect in monocytes (Sher et al. 1994; Irusen et al. 2002). The mechanism whereby these cytokines reduce GR function may be mediated via phosphorylation of GR by p38 MAPK and their effect is blocked by a p38 MAPK-α inhibitor (Irusen et al. 2002). In support of this p38 MAPK shows a greater degree of activation in alveolar macrophages from asthmatics with a poor response to glucocorticoids than patients who show a normal response (Bhavsar et al. 2008) and a p38 MAPK inhibitor increases glucocorticoid sensitivity in PBMC from patients with severe asthma (Mercado et al. 2012). The γ-isoform of p38 MAPK is also involved in phosphorylation of GR and reduced GR nuclear translocation (Mercado et al. 2011). 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 (Weigel and Moore 2007). In patients with 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 (Matthews et al. 2004). 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 (Ismaili and Garabedian 2004). Activation of PI3 kinase signalling through oxidative stress leads to activation of mTOR and which in turn activates JNK, resulting in corticosteroid resistance (Mitani et al. 2016). Microbial superantigens induce glucocorticoid resistance in T cells in vitro via activation of extracellular receptor kinase (ERK) pathways, resulting in GR phosphorylation (Li et al. 2004). Macrophages from MKP-1 gene knock-down mice show reduced anti-inflammatory responses to glucocorticoids in vitro due to increased MAPK activation (Abraham et al. 2006). In asthmatic patients with glucocorticoid insensitivity there is a significant reduction in MKP-1 expression in alveolar macrophages after glucocorticoid exposure and this is correlated with increased p38 MAPK activity (Bhavsar et al. 2008). Another phosphatase PP2A plays an important role in dephosphorylating phosphorylated GR and thus reversing corticosteroid resistance and there is a defect in PP2A expression in patients with severe asthma (Kobayashi et al. 2011) (Fig. 6).

In vitro GR may be nitrosylated by NO donors resulting in reduced binding affinity for glucocorticoids (Galigniana et al. 1999). In severe asthma and COPD there is increased expression of inducible NO synthase (iNOS) which produces large amounts of NO that could reduce glucocorticoid responsiveness, although this mechanism has not yet been demonstrated in asthma and COPD.

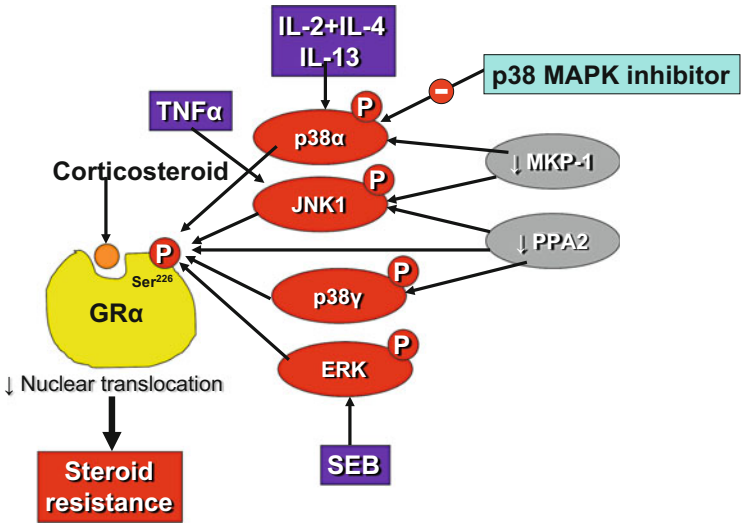


**Fig. 6** Glucocorticoid receptor phosphorylation. GRα phosphorylation at serine-226 ($Ser^{226}$) impedes nuclear translocation, leading to steroid resistance. GR may be phosphorylated by several kinases: p38 mitogen-activated kinase (MAPK)-α (p38α), which is activated by interleukin(IL)-2 and IL-4 or IL-13 and inhibited by p38MAPK inhibitors; C-terminal N-terminal kinase (JNK), which is activated by tumour necrosis factor(TNF)-α; p38MAPK-γ (p38γ), which is also activated by IL-2 + IL-4; extracellular signal-regulated kinase (ERK), which is activated by staphylococcal enterotoxin B (SEB). These kinases are dephosphorylated by the phosphatases MAP kinase phosphatase-1 (MKP-1) and protein phosphatase(PP)2A, both of which are defective in cells from severe asthma patients

## 7.3 Increased GRβ

Increased expression of GRβ has been reported in glucocorticoid-resistant patients of several diseases, including asthma but this has not been confirmed in several other studies (Pujols et al. 2007). 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. GRβ expression is also increased by microbial superantigens, such as staphylococcal enterotoxins, which may account for glucocorticoid resistance in atopic dermatitis (Fakhri et al. 2004). However, in most cell types the expression of GRβ is much lower than GRα, making this mechanism unlikely. 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 (Goleva et al. 2006).

## 7.4 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 (Adcock et al. 1995). 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.

## 7.5 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 (Ito et al. 2000). 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 (Matthews et al. 2004). 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 GRE binding.

## 7.6 Decreased HDAC2

Recruitment of HDAC2 to activated inflammatory genes is a major mechanism of inflammatory gene repression by glucocorticoids and reduced HDAC2 activity and expression is reduced in some diseases where patients respond poorly (Barnes 2009) (Fig. 7). For example, HDAC2 is markedly reduced in alveolar macrophages, airways and peripheral lung in patients with COPD (Ito et al. 2005), and similar changes are found in PBMCs and alveolar macrophages of patients with refractory asthma and in the airways of smoking asthmatics (Hew et al. 2006). The glucocorticoid resistance of COPD bronchoalveolar macrophages is reversed by overexpressing HDAC2 (using a plasmid vector) to the level seen in control subjects (Ito et al. 2006). The mechanisms for HDAC2 reduction in COPD involve nitrates tyrosine residues on HDAC2 resulting in its inactivation, ubiquitination and degradation (Osoata et al. 2009). Oxidative stress also activates PI3Kδ, which leads to phosphorylation and inactivation of HDAC2 (To et al. 2010). This is confirmed in mice exposed to cigarette smoke that develop glucocorticoid-resistant pulmonary inflammation. This glucocorticoid-resistance is completely absent in mice where the PI3Kδ gene is inactivated (Marwick et al. 2009). This suggests that oxidative stress may be an important mechanism of glucocorticoid resistance and is increased in most severe and glucocorticoid-resistant inflammatory diseases.

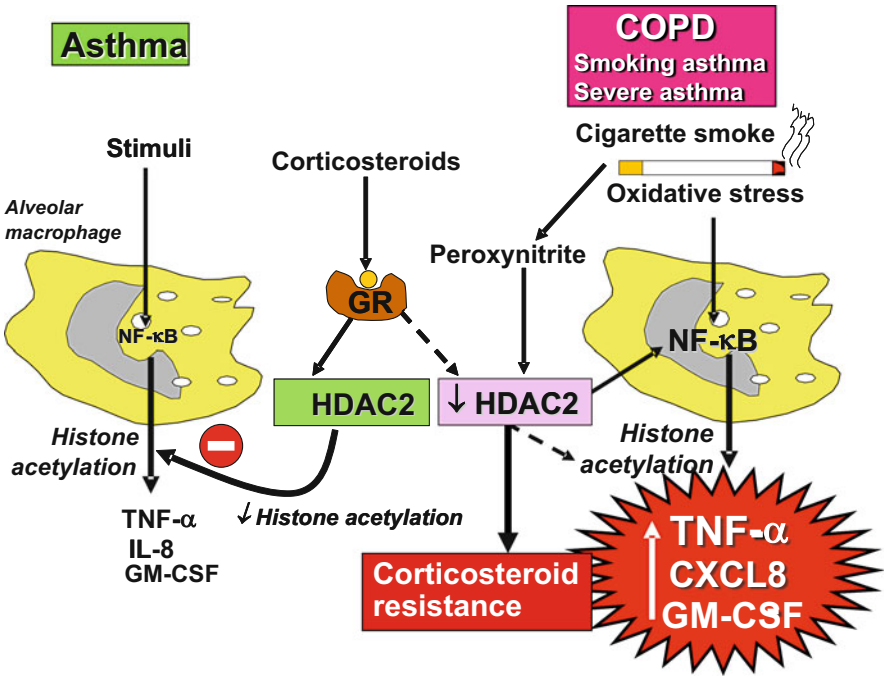


**Fig. 7** Mechanism of corticosteroid resistance in COPD, smoking asthma and severe asthma. Stimulation of mild asthmatic alveolar macrophages activates nuclear factor-kB (NF-kB) and other transcription factors to switch on histone acetyltransferase leading to histone acetylation and subsequently to transcription of genes encoding inflammatory proteins, such as tumour necrosis factor-α (TNF-α), interleukin-8 (IL-8) and granulocyte-macrophage colony stimulating factor (GM-CSF). Corticosteroids reverse this by binding to glucocorticoid receptors (GR) and recruiting histone deacetylase-2 (HDAC2). This reverses the histone acetylation induced by NF-kB and switches off the activated inflammatory genes. In COPD patients and smoking asthmatics cigarette smoke generates oxidative stress (acting through the formation of peroxynitrite) and in severe asthma and COPD intense inflammation generates oxidative stress to impair the activity of HDAC2. This amplifies the inflammatory response to NF-kB activation, but also reduces the anti-inflammatory effect of corticosteroids, as HDAC2 is now unable to reverse histone acetylation

## 8 Therapeutic Implications

ICS are highly effective in treating most patients with asthma. Patients with severe asthma may require high doses and this has a risk of systemic side effects, which has led to a search for ICS with even greater therapeutic ratios. A few patients with asthma and most patients with COPD are poorly responsive to glucocorticoids and are at risk of side effects, so that alternative anti-inflammatory treatments are needed, or the mechanisms of glucocorticoid resistance need to be reversed. 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.

## 8.1 Dissociated Steroids

There has been a major effort to develop glucocorticoids that have reduced side effects, while retaining anti-inflammatory efficacy. Selective glucocorticoid receptor agonists (SEGRAs or dissociated steroids) are more effective in trans-repression than trans-activation so theoretically have less side effects (Belvisi et al. 2001). Several dissociated steroids have now been developed, including non-glucocorticoid GR modulators, 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 which may reflect the anti-inflammatory effects of glucocorticoid mediated through trans-activation of genes, such as MKP-1 (Kleiman and Tuckermann 2007). While several inhaled non-steroidal GR modulators are currently in clinical development for asthma, there are no studies demonstrating any clinical advantage. For example, inhaled GW870086X is effective in asthma but no safety advantage has been demonstrated (Leaker et al. 2015).

## 8.2 Alternative Anti-Inflammatory Treatments

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. Several non-steroidal 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 inflammation, but have not been found to be very effective in glucocorticoid-resistant asthma (Evans et al. 2001). This has led to a search for novel anti-inflammatory treatments, particularly for diseases with marked glucocorticoid resistance, such as COPD, where no effective and safe anti-inflammatory treatments are available (Barnes 2013b).

Phosphodiesterase-4 inhibitors are broad-spectrum anti-inflammatory treatments that are now in clinical development for several inflammatory diseases, such as COPD (Hatzelmann et al. 2010). However, systemic doses have been limited by side effects, such as nausea, diarrhoea and headaches. Roflumilast is the first PDE4 inhibitor licensed for the treatment of inflammation in COPD patients and reduces neutrophilic inflammation with some improvement in lung function and reduction in exacerbations (Garnock-Jones 2015).

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. 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 (Medicherla et al. 2007). However these drugs have had problems with toxicity and side effects. Blocking NF-kB by selective inhibitors of inhibitor of NF-kB 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.

### 8.3 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 and might be possible for some patients with glucocorticoid-resistant asthma with p38 MAPK or JNK inhibitors in the future. Selective activation of HDAC2 can be achieved with theophylline, which restores HDAC2 activity in COPD macrophages back to normal and reverses glucocorticoid resistance (Cosio et al. 2004). In COPD patients the combination of theophylline and ICS is more effective in reducing airway inflammation than either drug alone (Ford et al. 2010). There are now therapeutic trials in COPD with low doses of theophylline (Devereux et al. 2015). Low dose theophylline also improves asthma control in smoking asthmatic patients who show no response to ICS alone (Spears et al. 2009). The molecular mechanism of action of theophylline in restoring HDAC2 is through selective inhibition of PI3Kδ, which is activated by oxidative stress in COPD patients (To et al. 2010). This suggests that selective PI3Kδ inhibitors may also be effective and these drugs are currently in clinical trials in COPD and severe asthma. 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 (Barnes 2005).

## 9 Conclusions

Glucocorticoids remain by far the most effective therapy for controlling asthma and suppress airway inflammation mainly through repression of activated inflammatory genes, but also by increasing the transcription of anti-inflammatory genes, such as MKP-1 (Barnes 2006). It is unlikely that it will be possible to develop more effective anti-inflammatory treatments for asthma in the future as glucocorticoids

have such a broad-spectrum of anti-inflammatory actions, reflecting their ability to switch off all activated inflammatory genes. ICS are now amongst the most widely used drugs in the world and there has been considerable effort expended in trying to improve their therapeutic ratio. Addition of LABA in the form of fixed combination inhalers improves asthma control to a greater extent than increasing the dose of ICS and this has become the standard approach for controlling patients with moderate to severe asthma. This is, at least in part, explained by the favourable molecular interactions between glucocorticoids and $\beta_2$-agonists. Selective GR modulators which favour trans-repression over trans-activation mechanisms were designed to reduce side effects that are largely due to gene activation, but so far have proved difficult to develop clinically. Furthermore, it is now clear that some anti-inflammatory effects of corticosteroids are due to trans-activation of anti-inflammatory genes, whereas some adverse effects may be due to trans-repression.

The major area of research interest is now focussed on understanding glucocorticoid resistance as it is a major barrier to the effective treatment of COPD patients and asthmatic patients with severe disease or who smoke. The recognition that there are different molecular mechanisms of glucocorticoid resistance has identified several new therapeutic targets. A major mechanism for reduced glucocorticoid responsiveness in COPD, severe and smoking asthma is reduction in HDAC2 activity and expression as a result of oxidative stress via activation of PI3Kδ. This pathway may be blocked by low concentrations of theophylline as well as selective PI3Kδ inhibitors, suggesting new therapeutic approaches to the treatment of severe asthma and COPD in the future. Other drugs that target this pathway are also in development and may lead to a new therapeutic strategy whereby drugs are able to reverse glucocorticoid resistance in airway diseases and perhaps other glucocorticoid-resistant inflammatory diseases, such as atherosclerosis and multiple sclerosis.

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