

Mechanisms of allergic diseases

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Corticosteroid resistance in patients with asthma and chronic obstructive pulmonary disease

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Reduced responsiveness to the anti-inflammatory effects of corticosteroids is a major barrier to effective management of asthma in smokers and patients with severe asthma and in the majority of patients with chronic obstructive pulmonary disease (COPD). The molecular mechanisms leading to steroid resistance are now better understood, and this has identified new targets for therapy. In patients with severe asthma, several molecular mechanisms have been identified that might account for reduced steroid responsiveness, including reduced nuclear translocation of glucocorticoid receptor (GR) α after binding corticosteroids. This might be due to modification of the GR by means of phosphorylation as a result of activation of several kinases (p38 mitogen-activated protein kinase α , p38 mitogen-activated protein kinase γ , and c-Jun N-terminal kinase 1), which in turn might be due to reduced activity and expression of phosphatases, such as mitogen-activated protein kinase phosphatase 1 and protein phosphatase A2. Other mechanisms proposed include increased expression of GR β , which competes with and thus inhibits activated GR α ; increased secretion of macrophage migration inhibitory factor; competition with the transcription factor activator protein 1; and reduced expression of histone deacetylase (HDAC) 2. HDAC2 appears to mediate the action of steroids to switch off activated inflammatory genes, but in patients with COPD, patients with severe asthma, and smokers with asthma, HDAC2 activity and expression are reduced by oxidative stress through activation of phosphoinositide 3-kinase δ . Strategies for managing steroid resistance include alternative anti-inflammatory drugs, but a novel approach is to reverse steroid resistance by increasing HDAC2 expression, which can be achieved with theophylline and phosphoinositide 3-kinase δ inhibitors. Long-acting β_2 -agonists can also increase steroid responsiveness by reversing GR α phosphorylation. Identifying the molecular mechanisms of steroid resistance in asthmatic patients and patients with COPD can thus lead to more effective

anti-inflammatory treatments. (J Allergy Clin Immunol 2013;■■■:■■■-■■■.)

Key words: Glucocorticoid receptor; histone deacetylase, p38 mitogen-activated protein kinase, protein phosphatase, theophylline, phosphoinositide 3-kinase

The symptoms of most patients with asthma are well controlled with low doses of inhaled **corticosteroids** (ICSs), providing they take the medication on a regular basis. However, some patients require higher doses of ICSs or even oral corticosteroids to achieve optimal control, indicating that their asthma might be relatively resistant to the anti-inflammatory actions of corticosteroids (steroid-insensitive asthma). Very rarely, asthmatic patients are completely resistant to corticosteroids, even in high oral doses (steroid-resistant asthma). In patients with chronic obstructive pulmonary disease (COPD), although there are some patients with “overlap syndrome” who have features of asthma and show a therapeutic response to corticosteroids, the majority of patients are resistant to even high doses of inhaled or oral steroids, indicating steroid resistance of the underlying inflammatory response. Indeed, there are some similarities between the airway inflammation seen in patients with severe asthma and those with COPD, which might indicate that there are several common mechanisms of steroid resistance between these diseases. Steroid resistance is a feature of several severe immune and inflammatory diseases, including rheumatoid arthritis, inflammatory bowel disease, and systemic lupus erythematosus.¹ Now that the anti-inflammatory mechanisms of actions of corticosteroids are better understood, it has been possible to elucidate the molecular basis of steroid resistance.^{2,3} It is clear that there are several molecular pathways that lead to steroid resistance, implying that there might be several therapeutic approaches to overcoming steroid resistance to more effectively treat patients.

MECHANISM OF ACTION OF CORTICOSTEROIDS

It is necessary to understand the molecular mechanisms whereby corticosteroids suppress inflammation to understand the various mechanisms of steroid resistance in asthmatic patients and patients with COPD.^{3,4} Corticosteroids activate several anti-inflammatory genes and suppress many inflammatory genes but can also modulate inflammation through additional posttranscriptional mechanisms.

Gene activation

Corticosteroids diffuse across the cell membrane and bind to the glucocorticoid receptor (GR) in the cytoplasm.⁴ On ligand

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Terms in boldface and italics are defined in the glossary on page ■■■.

Abbreviations used

AP-1: Activator protein 1
COPD: Chronic obstructive pulmonary disease
GR: Glucocorticoid receptor
GRE: Glucocorticoid response element
HDAC: Histone deacetylase
ICS: Inhaled corticosteroid
JNK: c-Jun N-terminal kinase
LABA: Long-acting β_2 -agonist
MAPK: Mitogen-activated protein kinase
MKP: Mitogen-activated protein kinase phosphatase
NF- κ B: Nuclear factor κ B
NO: Nitric oxide
Nrf2: Nuclear factor erythroid-derived-2 like 2
PDE: Phosphodiesterase
PI3K: Phosphoinositide 3-kinase
PP2A: Protein phosphatase 2A

binding, the GR changes conformation and is released from various chaperone proteins, such as *heat shock protein 90*, and crosses into the nucleus after binding to nuclear import proteins, such as importin α . Corticosteroids bind to GR α , which has a ligand-binding domain. GR β is an alternatively spliced form of GR that interacts with DNA but not with corticosteroids, so that interfering with the binding of the GR to DNA can act as an inhibitor of steroid action.⁵ The GR homodimerizes and binds to a DNA recognition site known as a glucocorticoid response element (GRE) in the *promoter region* of steroid-responsive genes. Binding of the GR to a GRE switches on (or occasionally switches off) gene transcription through interaction with a transcriptional coactivator molecules, such as CREB (cyclic AMP response

element binding protein)-binding protein, which has intrinsic *histone* acetyltransferase activity, resulting in the *acetylation* of core histones that are physically associated with DNA. This tags histones to recruit chromatin remodeling engines, such as SWI/SNF, and subsequent recruitment of RNA polymerase II, which results in gene activation.⁶ Genes that are switched on by corticosteroids include genes encoding β_2 -adrenergic receptors and the anti-inflammatory proteins glucocorticoid-induced leucine zipper and mitogen-activated protein kinase phosphatase 1 (MKP-1), which inhibits mitogen-activated protein kinase (MAPK) pathways. For example, in asthmatic patients inhaled budesonide increases the expression of glucocorticoid-induced leucine zipper.⁷ These effects might contribute to the anti-inflammatory actions of corticosteroids. GR interaction with negative GREs or GREs crossing the transcriptional start site might suppress gene transcription, and this mechanism could be important in mediating the side effects of steroids, such as inhibition of *osteocalcin*, which is involved in bone synthesis.⁸

Gene suppression

The major action of corticosteroids in suppression of inflammation is to switch off multiple activated inflammatory genes that encode for cytokines, chemokines, adhesion molecules, inflammatory enzymes, and receptors.³ These inflammatory genes are activated by proinflammatory transcription factors, such as nuclear factor κ B (NF- κ B) and activator protein 1 (AP-1), both of which are usually activated in the airways of patients with asthma and patients with COPD. Inflammatory genes are activated through interactions with transcription coactivator molecules in a similar manner to that described above for GR-mediated gene activation.

GLOSSARY

ACETYLATION: The placement of the acetyl radical (CH_3CO) into a compound.

CORTICOSTEROIDS: Adrenal cortex steroids that are further divided into glucocorticoids and mineralocorticoid. Structural changes in analogs of glucocorticoid hormones in drugs, such as prednisolone, prednisone, methylprednisolone, and dexamethasone, increase the glucocorticoid activity relative to the effects of mineralocorticoid. Research on the use of corticosteroids (cortisone for rheumatoid arthritis) was first published in 1949.

DEPOT CORTICOSTEROID: Preparations of corticosteroids delivered subcutaneously that release corticosteroids into the circulation over a prolonged period.

HEAT SHOCK PROTEIN 90 (HSP90): A protein that is increased when a cell encounters environmental stress, especially heat stress. HSP90 is a chaperone protein that helps prevent the unraveling of other proteins. Heat shock proteins were first isolated from bacteria exposed to heat.

HISTONE: Water-soluble proteins that are rich in the basic amino acids lysine and arginine and are complexed with DNA in the nucleosomes of chromatin.

IL-2: Produced principally by activated T cells. It is an autocrine growth factor for T cells. It is also a growth factor for B cells and induces class-switching. IL-2 induces IFN- γ secretion and activates macrophages.

OSTEOCALCIN: A protein produced by osteoblasts that is found in the extracellular matrix of bone. Increased serum levels of osteocalcin are seen in patients with Paget disease and other disorders of bone metabolism.

OXIDATIVE STRESS: Damage done to cell structures by reactive oxygen species (ROS). ROS levels increase during times of environmental stress and are capable of exerting cellular damage by reacting with intracellular constituents, such as DNA and membrane lipids.

PHA: A proteinaceous hemagglutinin of plant origin that induces nonspecific lymphocyte mitosis.

PLASMID: An extrachromosomal ring of DNA that replicates autonomously and is found especially in bacteria.

PROMOTER REGION: The site in a DNA chain at which RNA polymerase binds to initiate transcription of mRNA from nearby genes.

SUPERANTIGENS: A class of T-cell receptor ligands that have the ability to activate large fractions of the T-cell population. They do not require processing to bind to MHC class II molecules. They bind to polymorphic residues on the periphery of the class II molecule and interact with the T-cell receptor through the V β domain.

THEOPHYLLINE: A methylxanthine present in small amounts in tea. It is similar in structure to the xanthine caffeine. Theophylline has been used in asthma therapy for more than 70 years.

UBIQUITINATED: The conjugation of ubiquitin (a 76-amino-acid peptide) to a lysine residue of a target protein or another ubiquitin molecule, thereby forming a branching structure. This conjugation serves to modulate protein signaling.

ZINC FINGER PROTEINS: Small protein domains that use zinc ion binding to help stabilize the protein's folds.

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Activated GRs interact with corepressor molecules to attenuate NF- κ B-associated coactivator activity, thus reversing histone acetylation and chromatin remodeling actions.⁶ Reduction of histone acetylation more importantly occurs through the recruitment of histone deacetylase (HDAC) 2 to the activated inflammatory gene complex by activated GR, thereby resulting in suppression of multiple activated inflammatory genes. This probably accounts for how corticosteroids are so effective in controlling inflammation in asthmatic patients when many (probably >100) inflammatory genes are activated but also why they are relatively safe because other genes, such as genes regulating structure, ion channels, and receptors, are not influenced. The GR is acetylated on binding of corticosteroids, and this appears to be necessary for binding GREs, whereas it needs to be deacetylated by HDAC2 to associate with the NF- κ B complex and thus suppress inflammatory genes.⁹

Other mechanisms

Additional mechanisms can contribute to the anti-inflammatory actions of corticosteroids. Corticosteroids have potent inhibitory effects on MAPK signaling pathways through the induction of MKP-1, and this might inhibit the expression of multiple inflammatory genes in patients with asthma and those with COPD.^{10,11} Some inflammatory genes, such as *TNFA*, have unstable mRNA that is rapidly degraded by specific RNases but are stabilized when cells are stimulated by inflammatory mediators. Corticosteroids reverse this effect so that there is rapid degradation of mRNA and reduced inflammatory protein secretion. This can 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' adenylate-uridyate-rich untranslated region of mRNAs.¹²

STEROID RESISTANCE IN ASTHMATIC PATIENTS

The symptoms of most asthmatic patients can be controlled with low doses of ICSs, which have revolutionized management and become first-line treatment for the majority of patients. However, approximately 10% of patients require the maximal ICS dose, and approximately 1% require regular oral corticosteroids (corticosteroid-dependent asthma), whereas a small number of patients appear to be completely corticosteroid resistant, as defined by no clinical improvement after high doses of an oral corticosteroid (40 mg/d prednisone or prednisolone for 2 weeks). It now seems likely that corticosteroid resistance represents the end of a spectrum of corticosteroid responsiveness. However, it is difficult to quantify the degree of corticosteroid responsiveness in individual subjects because there is no well-defined way to quantify clinical steroid responsiveness, although a trial of oral corticosteroids or a single injection of a *depot corticosteroid* (triamcinolone acetonide) is useful in identifying patients with complete steroid resistance.¹³

Steroid-resistant asthma was described more than 40 years ago in 6 patients who did not respond clinically or have reduced blood eosinophilia with high doses of oral corticosteroids.¹⁴ A larger group of 58 patients with chronic asthma whose lung function did not improve (FEV₁ <15%) after oral prednisolone (20 mg/d for at least 7 days) was subsequently described.¹⁵ These steroid-resistant patients differed clinically from the usual responsive patients in having a longer duration of symptoms, lower morning lung function, a greater degree of airway

hyperresponsiveness, and a more frequent family history of asthma. Steroid-resistant patients are not cortisol deficient (Addisonian) and do not have the abnormalities in sex hormones described in the very rare familial glucocorticoid resistance.¹⁶ Plasma cortisol and adrenal suppression in response to exogenous corticosteroids are normal in patients with steroid-resistant asthma, and they usually have typical steroid side effects with systemic corticosteroids. The lack of response to oral steroids cannot be explained by reduced gastrointestinal absorption or other pharmacokinetic mechanisms.¹⁵ This suggests that there is a selective defect on the anti-inflammatory effects of corticosteroids rather than their metabolic or endocrine actions. Bronchial biopsy specimens from patients with corticosteroid-resistant asthma show a similar pattern of inflammation, with increased eosinophil and lymphocyte counts compared with those seen in patients with sensitive asthma, despite treatment with high doses of corticosteroids, and there is a failure to suppress the T_H2 cytokines IL-4 and IL-5.¹⁷

Patients with severe asthma are less responsive to corticosteroids than those with mild asthma, and therefore steroid resistance might be a mechanism contributing to asthma severity.¹⁸ Indeed, asthma severity is largely defined on the basis of the dose of corticosteroids that might be needed to control symptoms. Asthmatic patients who smoke cigarettes also have a reduced response to ICSs and oral corticosteroids, as well as having more severe asthma, a more rapid decrease in lung function with time, and increased mortality.¹⁹ The prevalence of smoking in asthmatic patients is surprisingly no different from that in the general population, and therefore this is a large clinical problem.¹⁹

Fortunately, circulating cells from patients with steroid-resistant asthma show reduced responses to corticosteroids *in vitro*, thus making it possible to explore molecular mechanisms. Proliferation of PBMCs stimulated by *PHA* and complement receptors on monocytes were not inhibited by steroids *in vitro* in patients with steroid-resistant asthma, whereas in patients with sensitive asthma, there was complete suppression, suggesting that circulating T lymphocytes and monocytes from these patients are corticosteroid resistant.^{20,21} Subsequent studies revealed that corticosteroids do not inhibit *IL-2* and IFN- γ secretion in these patients.²² Patients with severe asthma whose symptoms are not controlled with high doses of ICSs also show less inhibitory effect of corticosteroids on secretion of cytokines and chemokines of peripheral monocytes and alveolar macrophages than seen in patients with sensitive asthma.^{23,24} In addition, patients with steroid-resistant asthma also show a reduced skin-blanching response to topical corticosteroids, indicating that there might be a generalized abnormality in anti-inflammatory responsiveness to corticosteroids in these patients.²⁵

STEROID RESISTANCE IN PATIENTS WITH COPD

In sharp contrast to asthmatic patients, most patients with COPD respond very poorly to even high doses of ICSs or oral corticosteroids. ICSs have no effect on disease progression or mortality, but there is a small reduction in exacerbations,²⁶ although even this has been questioned.²⁷ ICSs do not reduce inflammatory cell counts and mediator levels in the sputum or airways of patients with COPD,²⁸⁻³⁰ nor do they inhibit cytokine release from alveolar macrophages *in vitro*.³¹ Approximately 10% of patients with COPD show some clinical response to ICSs, and these patients are characterized by increased numbers of

TABLE I. Molecular mechanisms of steroid resistance in asthmatic patients and patients with COPD

• Familial glucocorticoid resistance
• GR modification
↑ Phosphorylation: ↓ nuclear translocation
↑ p38MAPK α caused by IL-2 plus IL-4 or IL-13 in patients with severe asthma or caused by MIF in patients with severe asthma
↑ p38MAPK γ in patients with severe asthma
↑ JNK1 caused by proinflammatory cytokines in patients with severe asthma
↑ ERK caused by microbial superantigens in patients with severe nonallergic asthma
↓ MKP-1 in patients with severe asthma
↓ PP2A in patients with severe asthma
Nitrosylation: ↑ NO from inducible NO synthase
Ubiquitination: ↑ degradation by proteasome
• Increased GR β expression
• Increased proinflammatory transcription factors
AP-1, JNK
• Immune mechanisms
↓ Treg cells (↓ IL-19, ↓ vitamin D ₃)
↑ T _H 17 (IL-17)
• Defective histone acetylation
↓ Acetylation of lysine-5 on histone 4 in patients with severe asthma
↓ HDAC-2 in patients with COPD and patients with severe asthma and smokers with asthma
↑ Oxidative stress
↑ PI3K δ activation

ERK, Extracellular signal-regulated kinase; MAP, mitogen-activated protein; MIF, macrophage migration inhibitory factor; Treg, CD4⁺ regulatory T cell.

eosinophils in the airways and more bronchodilator reversibility,^{32,33} suggesting that these patients with COPD also have concomitant asthma.³⁴

MOLECULAR MECHANISMS OF STEROID RESISTANCE

Several molecular mechanisms contributing to decreased anti-inflammatory effects of corticosteroids have been identified in asthmatic patients, many of which have also been described in patients with other inflammatory diseases, including COPD (Table I).¹

Genetic susceptibility

Steroid resistance in asthmatic patients appears to be more common within families, suggesting that genetic factors can determine corticosteroid responsiveness.¹⁵ Microarray studies of PBMCs from patients with corticosteroid-sensitive asthma and those with corticosteroid-resistant asthma identified 11 genes that discriminated between these patients,³⁵ indicating that it might be possible to have a genomic test for steroid resistance. In healthy subjects differential gene expression between the 10% with the greatest and least steroid responsiveness identified 24 genes, of which the most discriminating was bone morphogenetic protein receptor type II (*BMPRII*), which enhanced steroid responsiveness when transfected into cells.³⁶ A genome-wide association study has identified an association between responsiveness to ICSs in asthmatic patients and a functional polymorphism of the gene glucocorticoid-induced transcript 1 (*GLCCI1*), which has been linked to corticosteroid-induced apoptosis.³⁷ In patients with steroid-resistant asthma, mutational analysis has shown no obvious abnormality in GR structure.³⁸ A polymorphism of GR β (GR-9 β) is associated with a reduced transrepressional response to corticosteroids,³⁹ but none of these polymorphisms have been linked to steroid resistance in patients with asthma or those with COPD.

Defective GR binding and nuclear translocation

IL-2 and IL-4 are overexpressed in the airways of patients with corticosteroid-resistant asthma,¹⁷ and *in vitro* these cytokines in combination reduce GR nuclear translocation and binding affinity within the nuclei of T cells.^{40–42} IL-13 alone mimics this effect in monocytes.^{41,43} The mechanism whereby these cytokines reduce GR function appears to be mediated through phosphorylation of the GR through p38MAPK, and their effect is blocked by a p38MAPK inhibitor.⁴¹ The GR can be phosphorylated by several kinases that can alter its binding, stability, translocation to the nucleus, binding to DNA, and interaction with other proteins, such as transcription factors and molecular chaperones (Fig 1).⁴⁴ A large proportion of patients with steroid-insensitive or severe asthma show reduced nuclear translocation of GR and reduced GRE binding in PBMCs after exposure to corticosteroids, which might be explained by GR phosphorylation.^{42,45} p38MAPK shows a greater degree of activation in alveolar macrophages from asthmatic patients with a poor response to corticosteroids than in those from patients who show a normal response.²⁴ Selective p38MAPK inhibitors increase the responsiveness to corticosteroids in alveolar macrophages and PBMCs from patients with severe asthma.⁴⁶ The effect of a p38MAPK inhibitor is most marked in PBMCs from patients with severe asthma who show the greatest defect in GR translocation.⁴⁷ Furthermore, the patients with severe asthma with the greatest defect in GR translocation are those with the lowest FEV₁ and the highest use of oral corticosteroids. The p38MAPK inhibitor was shown to inhibit phosphorylation of serine 226 (Ser²²⁶) on the GR, which is induced by exposure to IL-2 plus IL-4. The currently available p38MAPK inhibitors selectively inhibit the α - and β -isoforms, but a recent study suggests that the p38MAPK γ isoform also plays a role in phosphorylation of the GR and steroid resistance.⁴⁸ p38MAPK γ shows increased expression in PBMCs from patients with severe asthma, and knockdown of p38MAPK γ by interference RNA prevents the development of steroid resistance in response to IL-2 plus IL-4.

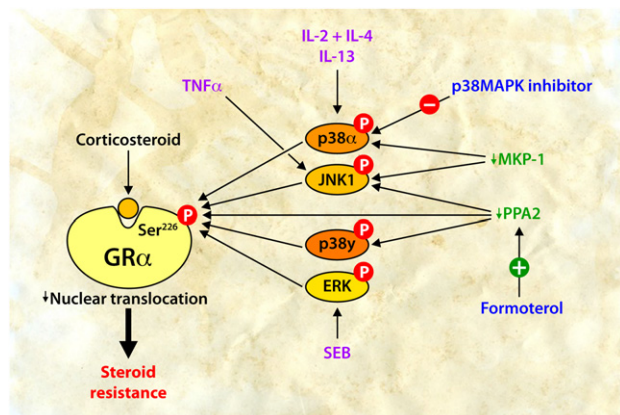


FIG 1. GR α phosphorylation at serine 226 (Ser²²⁶) impedes nuclear translocation, leading to steroid resistance. The GR can be phosphorylated by several kinases: p38MAPK α (p38 α), which is activated by IL-2 and IL-4 or IL-13 and inhibited by p38MAPK inhibitors; JNK, which is activated by TNF- α ; p38MAPK- γ (p38 γ), which is also activated by IL-2 plus IL-4; and extracellular signal-regulated kinase (ERK), which is activated by staphylococcal enterotoxin B (SEB). These kinases are dephosphorylated by the phosphatases MKP-1 and PP2A, both of which are defective in cells from patients with severe asthma. PP2A is increased by the LABA formoterol.

Another MAPK, c-Jun N-terminal kinase (JNK), which is activated by TNF- α and other proinflammatory cytokines, also directly phosphorylates GR at Ser²²⁶ and inhibits GRE binding.⁴⁹ JNK1 is also involved in GR phosphorylation in PBMCs from patients with severe asthma.^{48,50} Microbial *superantigens*, such as staphylococcal enterotoxin B, induce steroid resistance in T cells *in vitro* through activation of extracellular signal-regulated kinase pathways, resulting in GR phosphorylation.⁵¹

MKP-1, an endogenous inhibitor of the p38MAPK and JNK pathways, is activated by corticosteroids, as discussed above. Macrophages from *MKP1* gene knockout mice show reduced anti-inflammatory responses to corticosteroids *in vitro*.⁵² In alveolar macrophages from patients with severe asthma with reduced steroid responsiveness, there is a significant reduction in MKP-1 expression after corticosteroid treatment, which is correlated with increased p38MAPK activity.²⁴ The serine/threonine phosphatase protein phosphatase 2A (PP2A) is also involved in dephosphorylation of phosphorylated GR.⁵⁰ PP2A expression and activity are reduced in PBMCs from patients with steroid resistance, and knockdown of PP2A or an inhibitor, okadaic acid, reduces steroid responsiveness with reduced GR Ser²²⁶ phosphorylation and nuclear translocation, as well as an increase in JNK1 phosphorylation. Coprecipitation studies show that the GR, PP2A, and JNK1 are all physically associated. Furthermore, overexpression of PP2A in a macrophage cell line (U937) results in increased steroid responsiveness, indicating the important role of this phosphatase in maintaining steroid responsiveness.

In vitro the GR can be nitrosylated by nitric oxide (NO) donors, resulting in reduced binding affinity for corticosteroids.⁵³ In asthmatic patients there is increased expression of inducible NO synthase, which produces large amounts of NO that could reduce steroid responsiveness. Whether this mechanism is relevant in patients with steroid-resistant asthma has not yet been evaluated by using inducible NO synthase inhibitors, for example. The GR can also be *ubiquitinated* and tagged for proteasomal degradation, implying that proteasome inhibitors might increase steroid responsiveness.⁵⁴

Increased GR β expression

Increased expression of GR β has been described in patients with steroid-resistant asthma,^{55,56} although this has not been confirmed in other studies.^{57,58} GR β is induced by proinflammatory cytokines and has the capacity to compete for the binding of GR α to GREs, thus acting as a dominant negative inhibitor.^{59,60} GR β expression is also increased by microbial superantigens, such as staphylococcal enterotoxins, which might account for steroid resistance in some patients with severe nonallergic asthma.⁶¹ However, in most cell types, apart from neutrophils, the expression of GR β is considerably less than GR α , making this mechanism unlikely.⁵⁷ Another mechanism might be through interference with GR α nuclear translocation because knockdown of GR β in alveolar macrophages from patients with steroid-resistant asthma results in increased GR α nuclear localization and increased steroid responsiveness.⁶² Although corticosteroids do not bind to GR β , it is transcriptionally active, and the GR antagonist mifepristone (RU-486) binds to GR β , making it translocate to the nucleus, although the endogenous ligand of GR β has not yet been identified.⁶³ To date, there are no reported studies of GR β expression in patients with COPD.

Transcription factor activation

Excessive activation of AP-1 might be a mechanism of steroid resistance in asthmatic patients (and possibly patients with COPD) because AP-1 physically interacts with the GR, thereby preventing its binding to GREs and other transcription factors.^{64,65} AP-1 is a heterodimer of Fos and Jun proteins and might be activated by proinflammatory cytokines, such as TNF- α , through the JNK pathway. JNK is activated to a greater extent, and there is increased expression of c-Fos in PBMCs and bronchial biopsy specimens of steroid-resistant compared with steroid-sensitive asthma, with no reduction of JNK activity or c-Jun after high doses of oral corticosteroids.⁶⁶ Although JNK is activated in patients with COPD, whether this contributes to corticosteroid resistance has not been investigated.

Abnormal histone acetylation

As discussed above, histone acetylation plays a critical role in the regulation of inflammatory genes and the mechanism of action of steroids. Corticosteroids switch on steroid-responsive genes, such as *MKP1*, through acetylation of specific lysine residues (K5 and K16) on histone 4.⁶ In a small proportion of patients with steroid-resistant asthma, the GR translocates normally to the nucleus after dexamethasone exposure but does not acetylate K5 so that transactivation of genes does not occur.⁴² These patients show a poor response to high-dose ICSs, but unlike most patients with steroid-resistant asthma, they seem to have fewer side effects because many of these are mediated through GRE binding.⁸

Recruitment of HDAC2 to activated inflammatory genes is a major mechanism of inflammatory gene repression by corticosteroids and reduced HDAC2 activity, and expression is reduced in some diseases in which patients respond poorly.⁶⁷ HDAC2 expression is markedly reduced in alveolar macrophages, airways, and the peripheral lungs in patients with COPD,⁶⁸ and similar changes are found in PBMCs and alveolar macrophages of patients with refractory asthma²³ and in the airways of smoking asthmatic patients.⁶⁹ The steroid resistance of bronchoalveolar macrophages from patients with COPD is reversed by overexpressing HDAC2

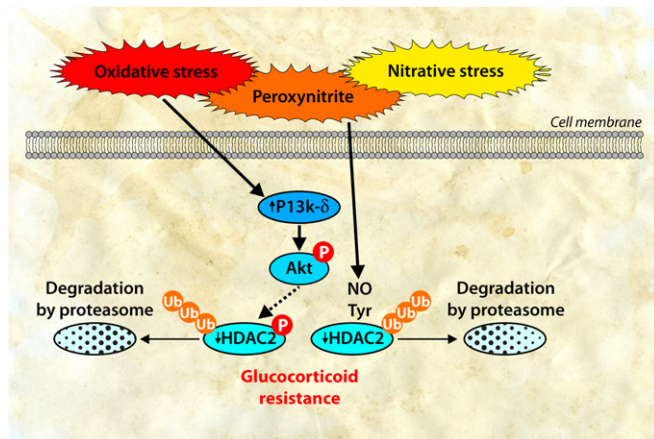


FIG 2. Mechanism of reduced HDAC2 expression in patients with COPD, patients with severe asthma, and smokers with asthma. Oxidative stress activates PI3K δ , which phosphorylates downstream kinases, such as Akt, resulting in the phosphorylation and inactivation of HDAC2. In addition, oxidative and nitritive stress generate peroxynitrite, which nitrates (NO) tyrosine residues (Tyr) on HDAC2 to inhibit its activity. These modifications of HDAC2 result in its ubiquitination (Ub), targeting the enzyme for degradation by the proteasome and leading to reduced expression and steroid resistance.

to the level seen in control subjects (by using a *plasmid* vector).⁹ Molecular mechanisms for the reduction in HDAC2 expression have now been elucidated.⁷⁰ Oxidative and nitritive stress result in the formation of peroxynitrite, which nitrates tyrosine residues on HDAC2, resulting in its inactivation, ubiquitination, and degradation (Fig 2).^{71,72} Oxidative stress also activates phosphoinositide 3-kinase (PI3K) δ , which leads to phosphorylation and inactivation of HDAC2.⁷³ This suggests that oxidative stress might be an important mechanism of steroid resistance in patients with COPD and those with severe asthma, in whom there is an increase in oxidative stress and peroxynitrite levels in the lungs.⁷⁴

Immune mechanisms

IL-10 is an anti-inflammatory and immunoregulatory cytokine that is secreted by regulatory T (Treg) cells in response to corticosteroids.⁷⁵ In patients with steroid-resistant asthma, there appears to be a failure of Treg cells to secrete IL-10, but this is restored by 25-hydroxy vitamin D3 (calcitriol), *in vitro*.^{76,77}

Numbers of T_H17 cells, which secrete IL-17 and related cytokines, appear to be increased in patients with severe asthma and those with COPD and might orchestrate neutrophilic inflammation.⁷⁸ In mice T_H17 cells appear to be steroid resistant,⁷⁹ but it is not certain that this is the case in the setting of airway disease. IL-17 increases the expression of GR β in airway epithelial cells to a greater extent than in normal cells and is not suppressed by corticosteroids *in vitro*.⁶⁰

Macrophage migration inhibitory factor

Macrophage migration inhibitory factor is a proinflammatory cytokine that has potent antisteroid effects.⁸⁰ It is induced by corticosteroids and appears to inhibit their anti-inflammatory effects, mainly through inhibiting the induction of MKP-1.⁸¹ Macrophage migration inhibitory factor has also been implicated in the pathogenesis of allergic inflammation in mouse models of asthma⁸² and

in the steroid resistance of asthmatic patients,⁸³ but its role in patients with COPD is not yet explored.

THERAPEUTIC IMPLICATIONS

Resistance to the anti-inflammatory effects of steroids is a major barrier to the effective treatment of COPD and severe asthma. Better understanding of the molecular mechanisms involved in steroid resistance has identified several potential therapeutic strategies for better treating COPD and severe asthma.

A novel strategy for the treatment of steroid-resistant/severe asthma and COPD is reversal of steroid resistance by interfering with the pathways that cause it.^{84,85} Understanding these pathways has highlighted several therapeutic targets, such as p38MAPK, as discussed above. Perhaps the most attractive target is HDAC2 because restoration of HDAC2 with a plasmid vector has been shown to restore steroid responsiveness in macrophages from patients with COPD that are normally resistant.⁹

Alternative broad-spectrum anti-inflammatory treatments

A popular strategy for the treatment of severe asthma and COPD is the development of broad-spectrum anti-inflammatory treatments as an alternative to corticosteroids. It has proved very difficult to develop these drugs because side effects after oral administration are common, leading to the search for drugs that are effective through inhaled delivery. Phosphodiesterase (PDE) 4 inhibitors are now in clinical development for the treatment of COPD and severe asthma, and oral roflumilast is already marketed as the first anti-inflammatory drug for COPD.⁸⁶ However, the oral dose is limited by side effects that are also due to PDE4 inhibition, including nausea, diarrhea, and headaches. Inhaled PDE4 inhibitors have also been developed but have thus far proved ineffective.

Several p38MAPK inhibitors have been in clinical development and theoretically could be particularly effective because p38MAPK activation appears to reduce steroid responsiveness in patients with asthma and those with COPD.⁸⁷ Selective p38MAPK inhibitors are now in clinical development for COPD and severe asthma, but the dose is limited by side effects after oral administration. In patients with rheumatoid arthritis, a loss of efficacy (tolerance) has been seen after prolonged administration, suggesting that escape pathways can develop.⁸⁸ Inhaled p38MAPK inhibitors are also in development.⁸⁹

Blocking NF- κ B with selective inhibitors of inhibitor of NF- κ B kinase (IKK β) is another way of treating corticosteroid-resistant inflammation, but it is likely that these drugs will have toxicity and side effects and therefore might only be suitable for topical application.⁹⁰

Restoring HDAC2 activity to reverse corticosteroid resistance

Perhaps the most attractive target for reversing steroid resistance is HDAC2 because restoration of HDAC2 with a plasmid vector has been shown to restore steroid responsiveness in macrophages from patients with COPD who are normally steroid resistant.⁹ There are several potential drugs that can increase HDAC2 expression that has been reduced as a consequence of oxidative stress, suggesting that these therapies might reverse the

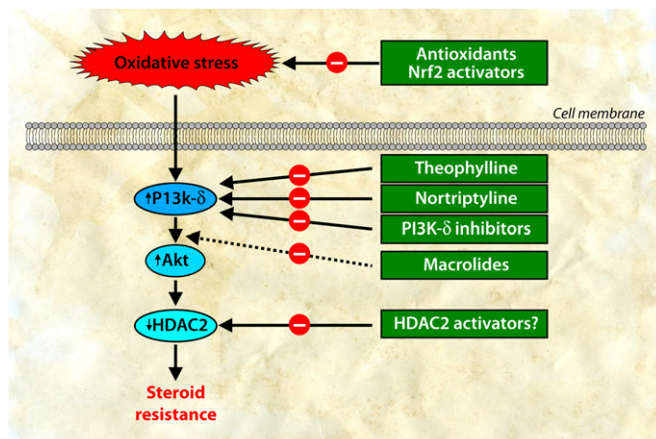


FIG 3. Steroid resistance as a result of oxidative stress can be reversed by several drugs that increase HDAC2 expression. HDAC2 activity is increased by theophylline and nortriptyline, which inhibit PI3K δ ; by antioxidants, including Nrf2 activators; and by macrolides, which target the PI3K pathway. In the future, drugs that directly activate HDAC2 might be discovered.

steroid resistance associated with oxidative stress in patients with COPD, patients with severe asthma, and smokers with asthma (Fig 3). Selective activation of HDAC2 can be achieved with low therapeutic concentrations of *theophylline*, which restores HDAC2 activity in macrophages from patients with COPD back to normal and reverses corticosteroid resistance.⁹¹ In cigarette smoke-exposed mice, which have steroid-resistant inflammation, oral theophylline is also effective in reversing steroid resistance.⁷³ In patients with COPD, a low dose of oral theophylline combined with an ICS is more effective in reducing inflammation in sputum than either drug alone.⁹² This action of theophylline is independent of PDE inhibition and appears to be mediated by direct inhibition of oxidant stress-activated PI3K δ .⁷³ Larger clinical trials with low-dose theophylline combined with oral corticosteroids and ICSs are now underway.

The tricyclic antidepressant nortriptyline also increases HDAC2 expression and reverses steroid resistance by directly inhibiting PI3K δ .⁹³ Selective PI3K δ inhibitors, such as IC87114, are similarly effective,⁷³ and inhaled PI3K δ inhibitors are now in clinical development for the treatment of COPD and severe asthma.

Oxidative stress is a major mechanism leading to steroid resistance in patients with COPD, patients with severe asthma, and smokers with asthma through reduced HDAC2 expression, suggesting that antioxidants should also reverse steroid resistance. Currently available antioxidants are poorly effective because they are inactivated by oxidative stress, but there is now considerable interest in activators of the transcription factor nuclear factor erythroid-derived-2 like 2 (Nrf2), which regulates the expression of several antioxidant genes. Nrf2 activity is markedly reduced in cells from patients with COPD, and this is linked to reduced HDAC2 expression, which normally keeps this protein deacetylated and active.^{94,95} Sulforaphane is an activator of Nrf2 and has been shown to increase HDAC2 expression and reverse steroid resistance in mice exposed to cigarette smoke and in macrophages from patients with COPD.⁹⁶ Curcumin (diferuloylmethane), which is found in turmeric spice used in curry, also increases HDAC2 expression after it has been reduced by oxidative stress and at concentrations lower than required for its

antioxidant effects.⁹⁷ Macrolides, including nonantibiotic macrolides, also reverse corticosteroid resistance through inhibition of PI3K pathways but might act more distally in the pathway.⁹⁸

Inhaled long-acting β_2 -agonists

It is now well established that adding long-acting β_2 -agonists (LABAs) to ICSs improves asthma control to a greater degree than increasing the dose of ICS. Accumulating evidence suggests that LABAs might enhance the function of corticosteroids through increasing nuclear translocation of the GR, which increases the anti-inflammatory effects of corticosteroids.⁹⁹⁻¹⁰¹ This suggests that LABAs might be able to overcome steroid resistance in asthmatic patients when this is due to reduced nuclear translocation of the GR, as discussed above. LABAs reverse the increased GR phosphorylation that is found in PBMCs of some patients with severe asthma.⁴⁸ The GR phosphorylation at Ser²²⁶ induced in PBMCs by IL-2 plus IL-4 stimulation is reversed by the LABA formoterol though inhibition of JNK1 and p38MAPK γ .⁴⁸ The effects of formoterol might be mediated by activation of the phosphatase protein phosphatase A2, which reverses the phosphorylation of the GR and JNK1.¹⁰² However, this effect of formoterol is not inhibited by β_2 -receptor antagonists and is seen in cell-free systems, suggesting a receptor-independent effect. LABAs might also reverse steroid resistance induced by oxidative stress through a different mechanism involving inhibition of PI3K δ .¹⁰³ Interestingly, both formoterol and salmeterol restore steroid responsiveness in PBMCs from patients with severe asthma, whereas only formoterol has this effect in cells from patients with COPD, suggesting that the full agonist effect of formoterol compared with the partial agonist effect of salmeterol might be necessary to reverse steroid resistance when there is a greater degree of oxidative stress. LABAs also enhance the steroid-induced increase in MKP-1 expression, thereby more effectively inhibiting p38MAPK and JNK, an effect that is mediated through protein kinase A.¹⁰⁴

CONCLUSIONS AND FUTURE DIRECTIONS

Steroid resistance in patients with severe asthma or smokers with asthma and in patients with COPD is a major barrier to effective therapy of these diseases. Considerable progress has recently been made in understanding the molecular basis for steroid resistance in both patients with severe asthma and those with COPD. In asthmatic patients there appear to be several different molecular mechanisms that can contribute to reduced responsiveness to corticosteroids, suggesting that it might be necessary to phenotype patients carefully to elucidate the predominant mechanism or mechanisms and thus to indicate the most appropriate therapy. For example, PBMCs of patients with severe asthma might show a reduced response to corticosteroids because of activation of several different kinases, including p38MAPK α , p38MAPK γ , JNK, or PI3K δ , which could indicate the need for different therapeutic strategies in different phenotypes of patients. In patients with COPD, PI3K δ activation through increased oxidative stress appears to be a predominant mechanism, although additional mechanisms involving other kinase pathways have yet to be investigated fully. In the future, it might be possible to develop biomarkers that indicate the predominant mechanism of steroid resistance to stratify therapy appropriately.

Reversal of steroid resistance is a new strategy to address steroid-resistant inflammation in asthmatic patients and patients with COPD. There are already existing treatments, such as theophylline, nortriptyline, and macrolides, that are effective through targeting the PI3K δ pathway, but undertaking long-term studies in patients with severe asthma and those with COPD is expensive. However, such long-term studies with low-dose theophylline are now underway. Novel drugs, such as inhaled PI3K δ inhibitors or Nrf2 activators, are already in clinical development and therefore might be combined with corticosteroid therapy in the future. Broad-spectrum anti-inflammatory treatments, such as PDE4 and p38MAPK inhibitors, have side effects when given systemically that limit the oral dose and thereby reduce efficacy. Developing inhaled drugs to circumvent this problem has proved difficult. By contrast, ICSs are well tolerated, especially in low doses, and therefore would be a preferable anti-inflammatory therapy if only corticosteroid responsiveness could be increased. Furthermore, they could be combined with ICSs in a single inhaler. Because steroid resistance pathways are better understood and suitable biomarkers are developed to demonstrate their relevance in individual patients, it is likely that these approaches will be incorporated into the management of severe asthma and COPD, which still remains a major clinical challenge. Because similar mechanisms of steroid resistance have also been described in patients with other chronic inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel disease,¹ this area of research might lead to the development of new treatments that could be useful in the treatment of many other chronic diseases.

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