PHYSIOLOGY IN MEDICINE: A SERIES OF ARTICLES LINKING MEDICINE WITH SCIENCE
Physiology in Medicine
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Annals of Internal Medicine
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REVIEW

How Do Corticosteroids Work in Asthma?

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Clinical Principles

Asthma is the most common chronic disease in westernized countries.

Patients with asthma have an underlying chronic inflammation of the airways characterized by activated mast cells, eosinophils, and T-helper 2 lymphocytes. This results in increased responsiveness of the airways to such triggers as exercise, allergens, and air pollutants.

This chronic inflammation underlies the typical symptoms of asthma, which include intermittent wheezing, coughing, shortness of breath, and chest tightness.

Corticosteroids are the most effective treatment for asthma, and inhaled corticosteroids have become first-line treatment for children and adults with persistent symptoms.

Corticosteroids suppress the chronic airway inflammation in patients with asthma, and the molecular mechanisms involved are now being elucidated.

Physiologic Principles

Inflammation in asthma is characterized by the increased expression of multiple inflammatory genes, including those encoding for cytokines, chemokines, adhesion molecules, and inflammatory enzymes and receptors.

Increased expression of inflammatory genes is regulated by proinflammatory transcription factors, such as nuclear factor-κB and activator protein-1. These bind to and activate coactivator molecules, which then acetylate core histones and switch on gene transcription.

Corticosteroids suppress the multiple inflammatory genes that are activated in asthmatic airways by reversing histone acetylation of the activated inflammatory genes.

This mechanism acts by binding of the activated glucocorticoid receptors to coactivators and recruitment of histone deacetylases to the activated transcription complex.

Understanding how corticosteroids work in patients with asthma may help in designing novel corticosteroids with less systemic effects, as well as novel anti-inflammatory approaches.

These molecular mechanisms of action of corticosteroids may also help elucidate the molecular basis of chronic inflammation and why corticosteroids are ineffective in patients with steroid-resistant asthma and with chronic obstructive pulmonary disease.

Corticosteroids (or glucocorticosteroids) are widely used to treat various inflammatory and immune diseases. The most common use of corticosteroids today is in the treatment of asthma, and inhaled corticosteroids have become established as first-line treatment in adults and children with persistent asthma, the most common chronic inflammatory disease. Recent developments in understanding the fundamental mechanisms of gene transcription (see Glossary) have led to major advances in understanding the molecular mechanisms by which corticosteroids suppress inflammation. This may have important clinical implications, as it will lead to a better understanding of the inflammatory mechanisms of many diseases and may signal the future development of new anti-inflammatory treatments. The new understanding of these new molecular mechanisms also helps explain how corticosteroids switch off multiple inflammatory pathways; in addition, it provides insights into why corticosteroids fail to work in patients with steroid-resistant asthma and in patients with chronic obstructive pulmonary disease (COPD).

The Molecular Basis of Inflammation in Asthma

All patients with asthma have a specific pattern of inflammation in the airways that is characterized by degranulated mast cells, an infiltration of eosinophils, and an increased number of activated T-helper 2 cells (see Glossary) (1). It is believed that this specific pattern of inflammation underlies the clinical features of asthma, including intermittent wheezing, dyspnea, cough, and chest tightness. Suppression of this inflammation by corticosteroids con-

Ann Intern Med. 2003;139:359-370.
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Glossary

Activator protein-1 (AP-1): A transcription factor that is activated by inflammatory stimuli and that increases the expression of multiple inflammatory genes.

CREB-binding protein (CBP): A coactivator that regulates the expression of inflammatory and other genes. It was first discovered as a binding protein for the transcription factor CREB (cyclic adenosine monophosphate response element–binding protein) but has subsequently been shown to bind several other transcription factors, including activator protein-1 and nuclear factor-κB.

Chromatin: The material of chromosomes. It is a complex of DNA, histones, and nonhistone proteins found in the nucleus of a cell.

Coactivator: Nuclear protein that activates gene transcription via intrinsic histone acetyltransferase activity.

Co-repressor: Nuclear protein that suppresses gene transcription and has histone deacetylase activity.

Glucocorticoid receptor α: The normal form of the glucocorticoid receptor that binds corticosteroids and translocates to the nucleus to bind to DNA.

Glucocorticoid receptor β: An alternatively spliced form of the glucocorticoid receptor that can bind to DNA (at glucocorticoid response element sites) but that does not bind corticosteroids; therefore, theoretically it may prevent activated glucocorticoid receptors from binding to DNA and other transcription factors.

Glucocorticoid response element (GRE): A specific sequence of DNA in the promoter region of a gene, where glucocorticoid receptors bind and confer steroid responsiveness on the gene.

Histone: The basic protein that forms the core of the chromosome around which DNA is wound. Modification of histones by acetylation or methylation changes their charge, and this affects DNA winding.

Histone acetyltransferases (HATs): Enzymes that acetylate lysine residues on core histones. Coactivator molecules have intrinsic histone acetyltransferase activity.

Histone deacetylases (HDACs): Enzymes that deacetylate acetylated core histones. About 12 such enzymes are now identified. Co-repressors have intrinsic histone deacetylase activity.

IKK2: Inhibitor of nuclear factor-κB (NF-κB) kinase-2 is the key enzyme that activates the NF-κB in the cytoplasm to prevent it from translocating to the nucleus to regulate inflammatory gene expression.

Messenger RNA (mRNA): Produced from DNA by action of RNA polymerase II.

Mitogen-activated protein (MAP) kinases: Enzymes that regulate signal transduction pathways that are involved in inflammatory and immune gene expression and cell proliferation.

Nuclear factor-κB (NF-κB): A transcription factor that is activated by inflammatory stimuli; it increases the expression of multiple inflammatory genes.

p300/CBP-associated factor (PCAF): A coactivator that interacts with other coactivators, such as CBP; similar to other coactivators, it also has histone acetyltransferase activity.

RNA polymerase II: The key enzyme that catalyzes the formation of messenger RNA from DNA and therefore transcription.

TATA box: DNA sequence that marks the start site of gene transcription from the coding region of the gene.

TATA box–binding protein (TBP): Proteins that interact with the TATA box and also bind coactivator and related molecules.

T-helper 2 cells: A subtype of T-helper ($CD4^+$) lymphocyte that predominates in allergic diseases and that is characterized by secretion of the cytokines interleukin-4, interleukin-5, and interleukin-13, which result in IgE formation and eosinophilic inflammation.

Transcription: Gene expression resulting in formation of messenger RNA.

Transcription factor: Protein that binds to specific sequences in the regulatory region of genes to switch on transcription.

Transfection: Transfer of DNA sequences that may contain transcription factor–binding sequences to a cell that is used to study the regulation of transcription by these transcription factors.

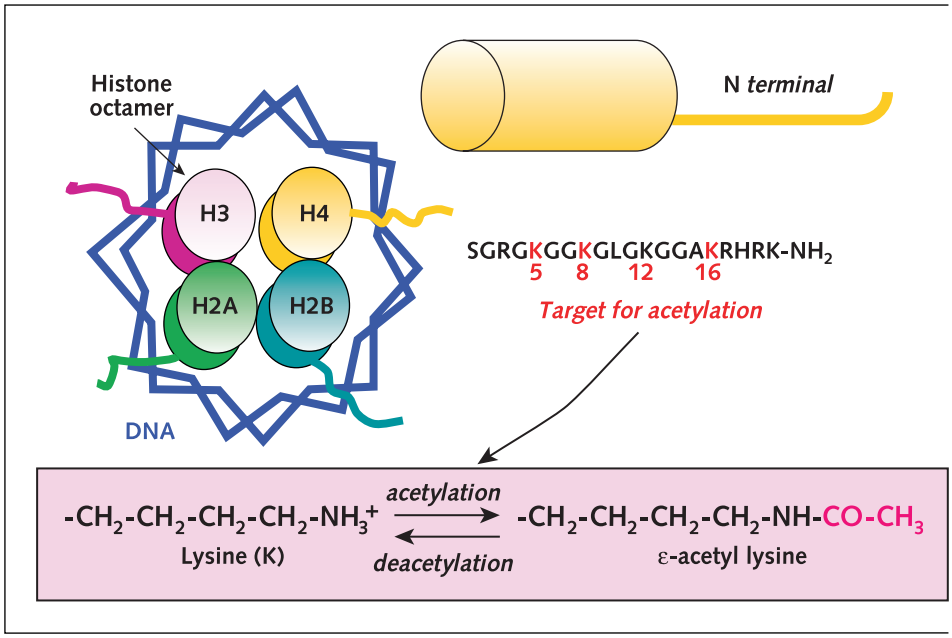
trols and prevents these symptoms in most patients. Multiple mediators are produced in asthma, and the approximately 100 known inflammatory mediators that are increased in patients with asthma include lipid mediators, inflammatory peptides, chemokines, cytokines, and growth factors (2). Increasing evidence suggests that structural cells of the airways, such as epithelial cells, airway smooth-muscle cells, endothelial cells, and fibroblasts, are a major source of inflammatory mediators in asthma. Epithelial cells may play a particularly important role because they may be activated by environmental signals and may release multiple inflammatory proteins, including cytokines, chemokines, lipid mediators, and growth factors.

Inflammation is mediated by the increased expression of multiple inflammatory proteins, including cytokines, chemokines, adhesion molecules, and inflammatory enzymes and receptors. Most of these inflammatory proteins are regulated by increased gene transcription, which is controlled by proinflammatory transcription factors, such as nuclear factor-κB (NF-κB) and activator protein-1 (AP-1), that are activated in asthmatic airways (see Glossary) (3). For example, NF-κB is markedly activated in epithelial cells of asthmatic patients (4), and this transcription factor regulates many of the inflammatory genes that are abnormally expressed in asthma (5). Nuclear factor-κB may be activated by rhinovirus infection and allergen exposure, both of which exacerbate asthmatic inflammation (6).

CHROMATIN REMODELING

The molecular mechanisms by which inflammatory genes are switched on by transcription factors are now much better understood. Alteration in the structure of chromatin (see Glossary) is critical to the regulation of gene expression. Chromatin is made up of nucleosomes, which are particles consisting of DNA associated with an octomer of two molecules each of the core histone proteins (see Glossary) (H2A, H2B, H3, and H4) (**Figure 1**). Expression and repression of genes are associated with remodeling of this chromatic structure by enzymatic modification

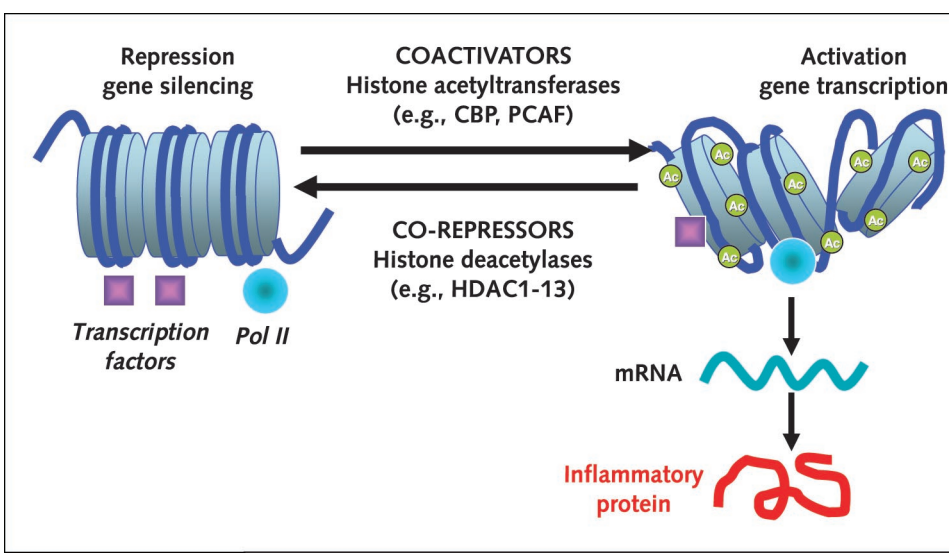
Figure 1. **Structure of chromatin.**



DNA is wound around an 8-histone molecule with two copies of two histones 2A, 2B, 3, and 4. Each histone molecule has a long tail rich in lysine residues (K) that are the sites of enzymatic modification, such as acetylation, thus changing the charge of the molecule and leading to DNA unwinding.

of core histones. Each core histone has a long terminal that is rich in lysine residues that may be acetylated, thus changing the electrical charge of the core histone. In the resting cell, DNA is wound tightly around these basic core histones, excluding the binding of the enzyme RNA polymerase II (see Glossary), which activates the formation of messenger RNA (mRNA) (see Glossary). This conformation of the chromatin structure is described as closed and is associated with suppression of gene expression. Gene transcription occurs only when the chromatin structure is opened up, with unwinding of DNA so that RNA polymerase II and basal transcription complexes can now bind to DNA to initiate transcription. When proinflammatory transcription factors, such as NF-κB, are activated, they bind to specific recognition sequences in DNA and subsequently interact with large coactivator molecules, such as p300/CREB (cyclic adenosine monophosphate response element–binding protein)–binding protein (CBP) and

Figure 2. **Gene activation and repression are regulated by acetylation of core histones.**



Histone acetylation is mediated by coactivators, which have intrinsic histone acetyltransferase activity, whereas repression is induced by histone deacetylases (*HDACs*), which reverse this acetylation. CBP = CREB (cyclic adenosine monophosphate response element–binding protein)-binding protein; mRNA = messenger RNA; PCAF = p300/CBP-associated factor.

p300/CBP-associated factor (PCAF) (see Glossary). These coactivator molecules act as the molecular switches that control gene transcription. All have intrinsic histone acetyltransferase (HAT) (see Glossary) activity (7, 8), which results in acetylation of core histones, thereby reducing their charge. Acetylation allows the chromatin structure to transform from the resting closed conformation to an activated open form (8). This results in unwinding of DNA, binding of TATA box–binding protein (TBP) (see Glossary), TBP-associated factors, and RNA polymerase II, which initiates gene transcription. This molecular mechanism is common to all genes, including those involved in differentiation, proliferation, and activation of cells. An important step forward has been the discovery of the enzymes that regulate histone acetylation. Core histones are characterized by long *N*-terminal tails rich in lysine residues that are the target for acetylation. In general, HATs act as coactivators that switch genes on; histone deacetylases (HDACs), which act as co-repressors (see Glossary), switch genes off (**Figure 2**).

Recently, these fundamental mechanisms have been applied to understanding the regulation of inflammatory genes that become activated in inflammatory diseases. In humans, epithelial cell line activation of NF-κB (by exposing the cell to inflammatory signals, such as interleukin-1β, tumor necrosis factor-α, or endotoxin) results in acetylation of specific lysine residues on histone-4 (the other histones do not seem to be so markedly acetylated), and this is correlated with increased expression of inflammatory genes, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) (9). The acetylation of histone that is associated with increased expression of inflammatory genes is counteracted by the activity of HDACs (more than 12 that are associated with gene suppression have been characterized [10]). In biopsy samples from patients with asthma, HAT activity is increased and HDAC activity is decreased, thus favoring increased inflammatory gene expression (11). Improved understanding of the molecular basis of asthma has helped to explain how corticosteroids are so effective in suppressing this complex inflammation that involves many cells, mediators, and inflammatory effects.

Cellular Effects of Corticosteroids

Corticosteroids are the only therapy that suppresses the inflammation in asthmatic airways; this action underlies the clinical improvement in asthma symptoms and prevention of exacerbations (12, 13). At a cellular level, corticosteroids reduce the number of inflammatory cells in the airways, including eosinophils, T lymphocytes, mast cells, and dendritic cells (**Figure 3**). These remarkable effects of corticosteroids are produced through inhibiting the recruitment of inflammatory cells into the airway by suppressing the production of chemotactic mediators and adhesion molecules and by inhibiting the survival in the airways of inflammatory cells, such as eosinophils, T lymphocytes, and mast cells. Epithelial cells may be a major cellular target for inhaled corticosteroids, which are the mainstay of modern asthma management (14). Thus, corticosteroids have a broad spectrum of anti-inflammatory effects in asthma, with inhibition of multiple inflammatory mediators and inflammatory and structural cells. Endogenous corticosteroids secreted by the adrenal cortex may also exert some anti-inflammatory action, and inhibition of endogenous cortisol enhances allergic inflammation in the skin (15). The broad anti-inflammatory profile of corticosteroids probably accounts for their marked clinical effectiveness in asthma. Attempts to find alternative treatments that are more specific, such as inhibitors of single mediators, have usually been unsuccessful, emphasizing the importance of simultaneously inhibiting many inflammatory targets (16). Any explanation of the anti-inflammatory effects of corticosteroids needs to account for this broad spectrum of anti-inflammatory effects.

Glucocorticoid Receptors

Corticosteroids diffuse across the cell membrane and bind to glucocorticoid receptors in the cytoplasm. Cytoplasmic glucocorticoid receptors are normally bound to proteins, known as molecular chaperones, that protect the receptor and prevent its nuclear localization by covering the sites on the receptor that are needed for transport across the nuclear membrane into the nucleus. A single gene encodes glucocorticoid receptors, but several variants are now recognized (17). Glucocorticoid receptor α binds corticosteroids, whereas glucocorticoid receptor β is an alternatively spliced form that binds to DNA but is not activated by corticosteroids (see Glossary). Glucocorticoid receptor β has been implicated in steroid resistance in asthma (18), although whether glucocorticoid receptor β has any functional significance has been questioned (19). Glucocorticoid receptors may also be modified by phosphorylation and other modifications, which may alter the response to corticosteroids. For example, several serines or threonines are in the *N*-terminal domain, where glucocorticoid receptors may be phosphorylated by various kinases; this may change corticosteroid-binding affinity, nuclear import and export, receptor stability, and *trans*activating efficacy (20).

After corticosteroids have bound to glucocorticoid receptors, changes in the receptor structure result in dissociation of molecular chaperone proteins, thereby exposing nuclear localization signals on glucocorticoid receptors. This results in rapid transport of the activated glucocorticoid receptor–corticosteroid complex into the nucleus, where it binds to DNA at specific sequences in the promoter region of steroid-responsive genes known as glucocorticoid response elements (GRE) (see Glossary). Two glucocorticoid receptor molecules bind together as a homodimer and bind to GRE, leading to changes in gene transcription.

CORTICOSTEROID-INDUCED GENE TRANSCRIPTION

Corticosteroids produce their effect on responsive cells by activating glucocorticoid receptors to directly or indirectly regulate the transcription of target genes (21). The number of genes per cell directly regulated by corticosteroids is estimated to be between 10 and 100, but many genes are indirectly regulated through an interaction with other transcription factors and coactivators. Glucocorticoid receptor dimers bind to DNA at GRE sites in the promoter region of steroid-responsive genes. Interaction of the activated glucocorticoid receptor dimer with GRE usually increases transcription, resulting in increased protein synthesis. Glucocorticoid receptor may increase transcription by interacting with coactivator molecules, such as CBP and PCAF, thus switching on histone acetylation and gene transcription. For example, relatively high concentrations of corticosteroids increase the secretion of the antiprotease secretory leukoprotease inhibitor from epithelial cells (9).

The activation of genes by corticosteroids is associated with a selective acetylation of lysine residues 5 and 16 on histone-4, resulting in increased gene transcription (9, 22) (**Figure 4**). Activated glucocorticoid receptors may bind to coactivator molecules, such as CBP or PCAF, as well as steroid-receptor coactivator-1, which itself has HAT activity (23, 24). However, steroid-receptor activator-1 does not seem to be involved in NF-κB–activated HAT activity (9), but other similar coactivator molecules are probably involved. Corticosteroids may suppress inflammation by increasing the synthesis of anti-inflammatory proteins, such as annexin-1, secretory leukoprotease inhibitor, interleukin-10, and the inhibitor of NF-κB, IκB-α. However, therapeutic doses of inhaled corticosteroids have not been shown to increase annexin-1 concentrations in bronchoalveolar lavage fluid (25), and an increase in IκB-α has not been shown in most cell types, including epithelial cells (26, 27). It seems highly unlikely that the widespread anti-inflammatory actions of corticosteroids could be explained by increased transcription of small numbers of anti-inflammatory genes, particularly because high concentrations of corticosteroids are usually required for this response, whereas in clinical practice, corticosteroids can suppress inflammation at much lower concentrations.

Little is known about the molecular mechanisms of corticosteroid side effects, such as osteoporosis, growth retardation in children, skin fragility, and metabolic effects. These actions of corticosteroids are related to their endocrine effects. The systemic side effects of corticosteroids may be due to gene activation. Some insight into this has been provided by mutant glucocorticoid receptors, which do not dimerize and therefore cannot bind to GRE to switch on genes. Transgenic mice that express these mutant glucocorticoid receptor corticosteroids show no loss of anti-inflammatory effect and can suppress NF-κB–activated genes in the normal way (28).

SWITCHING OFF INFLAMMATORY GENES

In controlling inflammation, the major effect of corticosteroids is to inhibit the synthesis of many inflammatory proteins through suppression of the genes that encode them. This effect was originally believed to occur through interaction of glucocorticoid receptors with GRE sites that

Figure 3. **Cellular effect of corticosteroids.**

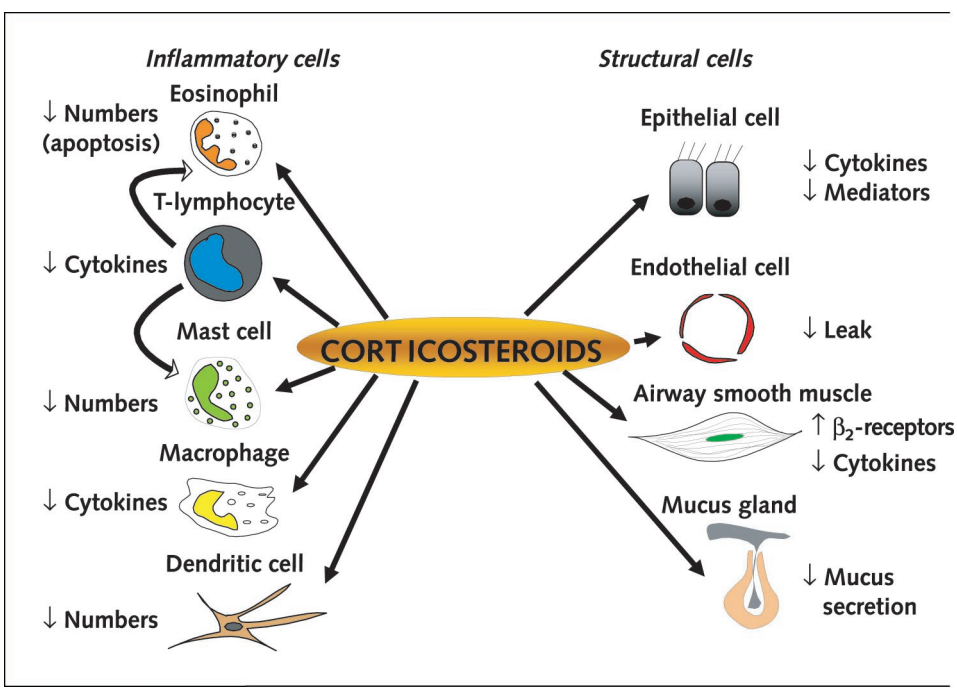
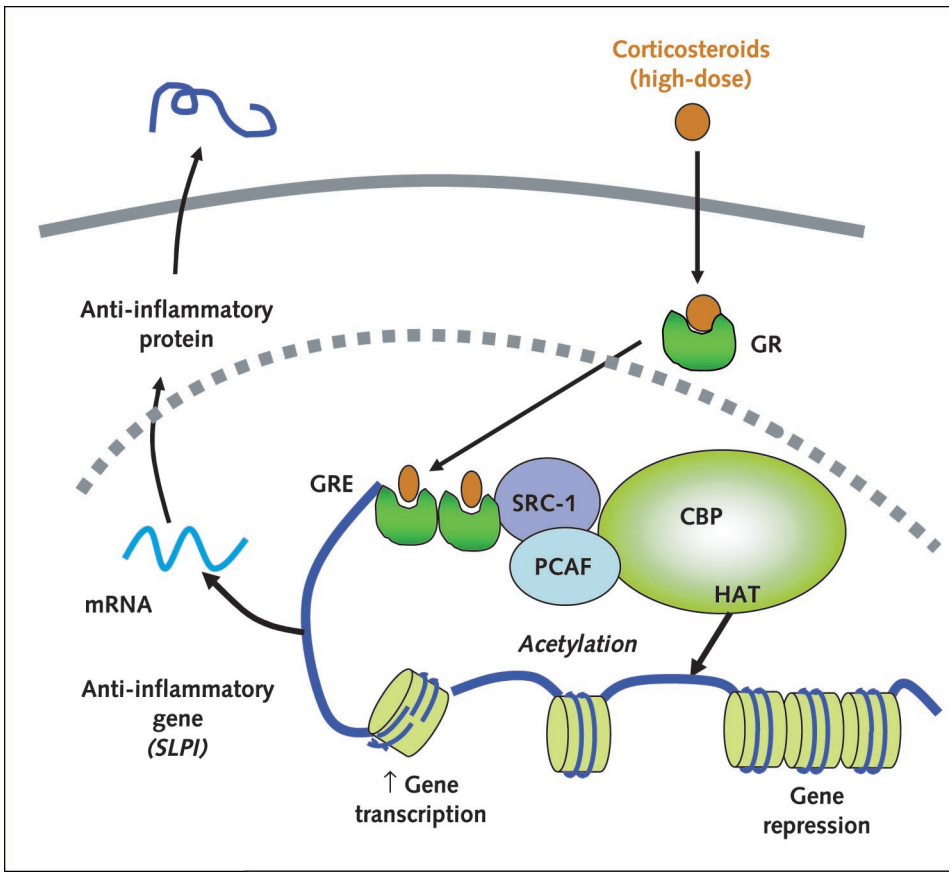


Figure 4. **How corticosteroids switch on anti-inflammatory gene expression.**



Corticosteroids bind to cytoplasmic glucocorticoid receptors (*GRs*), which translocate to the nucleus where they bind to glucocorticoid response elements (*GREs*) in the promoter region of steroid-sensitive genes. Corticosteroids also directly or indirectly bind to coactivator molecules, such as CREB (cyclic adenosine monophosphate response element–binding protein)-binding protein (*CBP*), p300/CBP-associated factor (*PCAF*), or steroid receptor coactivator-1 (*SRC-1*), which have intrinsic histone acetyltransferase (*HAT*) activity. This binding causes acetylation of lysines on histone-4, which leads to activation of genes encoding anti-inflammatory proteins, such as secretory leukoprotease inhibitor (*SLPI*). mRNA = messenger RNA.

switched off transcription and are termed *negative GREs* (in contrast to the usual type of GRE that is associated with increased transcription). However, these negative GREs have only rarely been demonstrated and are not a feature of the promoter region of the inflammatory genes that are suppressed by steroids in the treatment of asthma. Patients with asthma have increased expression of many inflammatory genes, including those encoding cytokines, chemokines, adhesion molecules, inflammatory enzymes, and inflammatory receptors (**Table**).

Interaction with Transcription Factors

Activated glucocorticoid receptors interact functionally with other activated transcription factors. Most of the inflammatory genes that are activated in asthma do not have GREs in their promoter regions yet are potently repressed by corticosteroids. The evidence is persuasive that corticosteroids inhibit the effects of proinflammatory transcription factors, such as AP-1 and NF-κB, that regulate the expression of genes that code for many inflammatory proteins, such as cytokines, inflammatory enzymes, adhesion molecules, and inflammatory receptors (3, 5). The activated glucocorticoid receptors can interact directly with activated transcription factors by protein–protein interaction, but this may be a feature of cells in which these genes are artificially overexpressed rather than a property of normal cells. Treatment of asthmatic patients with high doses of inhaled corticosteroids that suppress airway inflammation does not reduce NF-κB binding to DNA (29). This suggests that corticosteroids are more likely to be acting downstream of the binding of proinflammatory transcription factors to DNA, and attention has now focused on their effects on chromatin structure and histone acetylation.

Effects on Histone Acetylation

Repression of genes occurs through reversal of the histone acetylation that switches on inflammatory genes (30). Activated glucocorticoid receptors may bind to CBP or other coactivators directly to inhibit their HAT activity (9), thus reversing the unwinding of DNA around core histones and thereby repressing inflammatory genes. More important, particularly at low concentrations that are likely to be relevant therapeutically in asthma treatment, acti-

vated glucocorticoid receptors recruit HDACs to the activated transcriptional complex, resulting in deacetylation of histones and thus a decrease in inflammatory gene transcription (9) (**Figure 5**). At least 12 HDACs have now been identified, and these are differentially expressed and regulated in different cell types (10). Evidence now shows that the different HDACs target different patterns of acetylation (31). These differences in HDACs may contribute to differences in responsiveness to corticosteroids among different genes and cells.

An important question is why corticosteroids switch off only inflammatory genes; they clearly do not suppress all activated genes and are well tolerated as a therapy. Glucocorticoid receptors probably bind only to coactivators that are activated by proinflammatory transcription factors, such as NF-κB and AP-1, although we do not understand how this specific recognition occurs. It is likely that several specific coactivators interact with glucocorticoid receptors. Activator protein-1 and NF-κB repression is normal in mice that express a form of glucocorticoid receptors that does not dimerize ($dim^{-/-}$), indicating that glucocorticoid receptor monomers can mediate the anti-inflammatory effects of corticosteroids, whereas dimerization is needed for gene activation (21, 28).

Other Histone Modifications

It has recently become apparent that core histones may also be modified not only by acetylation but also by methylation, phosphorylation, and ubiquitination and that these modifications may regulate gene transcription (32). Methylation of histones, particularly histone-3, by histone methyltransferases, results in gene suppression (33). The anti-inflammatory effects of corticosteroids are reduced by a methyltransferase inhibitor, 5-aza-2′-deoxycytidine, suggesting that this may be an additional mechanism by which corticosteroids suppress genes (34). Indeed, there may be an interaction between acetylation, methylation, and phosphorylation of histones, so that the sequence of chromatin modifications may give specificity to expression of particular genes (35).

Nontranscriptional Effects

Although most of the actions of corticosteroids are mediated by changes in transcription through chromatin remodeling, it is increasingly recognized that they may also affect protein synthesis by reducing the stability of mRNA so that less protein is synthesized. Some inflammatory genes, such as the gene encoding GM-CSF, produce mRNA that is particularly susceptible to the action of ribonucleases that break down mRNA, thus switching off protein synthesis. Corticosteroids may have inhibitory effects on the proteins that stabilize mRNA, leading to more rapid breakdown and thus a reduction in protein expression (36).

Effects on Mitogen-Activated Protein Kinases

Mitogen-activated protein (MAP) (see Glossary) kinases play an important role in inflammatory gene expression through the regulation of proinflammatory transcription factors. Increasing evidence shows that corticosteroids may exert an inhibitory effect on these pathways. Corticosteroids may inhibit AP-1 and NF-κB via an inhibitory effect on c-Jun *N*-terminal kinases, which activate these transcription factors (37, 38). Corticosteroids reduce the stability of mRNA for some inflammatory genes, such as cyclooxygenase-2, through an inhibitory action on another MAP kinase, p38 MAP kinase (39). This inhibitory effect is mediated via the induction of a potent endogenous inhibitor of p38 MAP kinase called MAP kinase phosphatase-1 (40).

Table. **Effect of Corticosteroids on Gene Transcription***

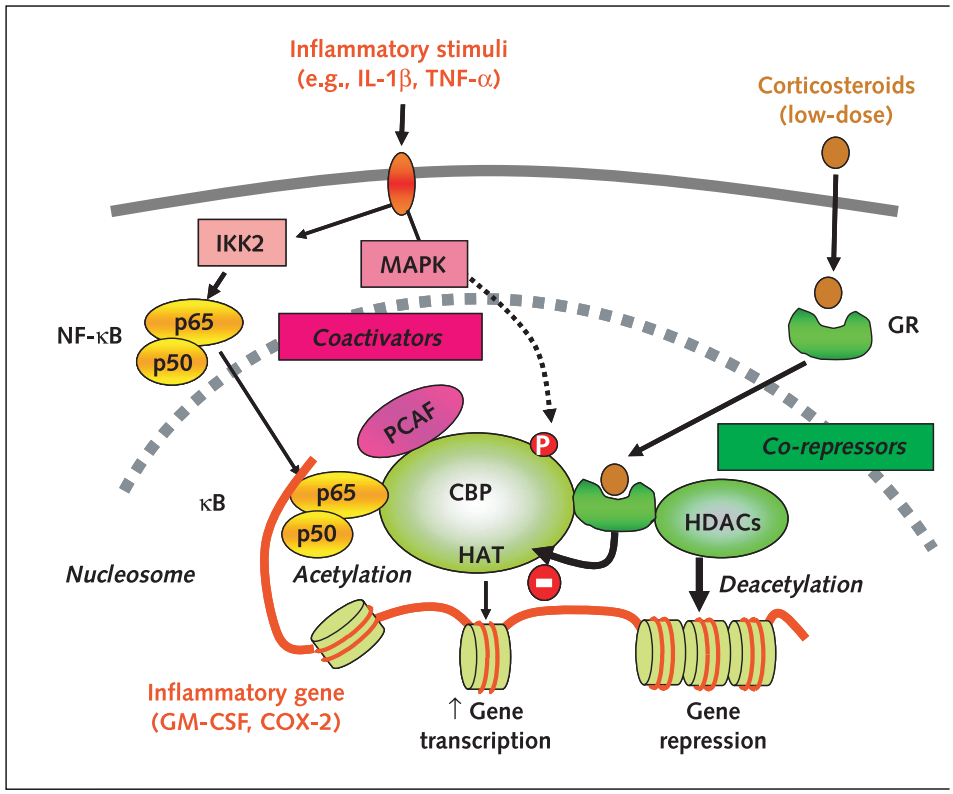
Increased transcription
Annexin-1 (lipocortin-1, phospholipase A_2 inhibitor)
β_2-adrenergic receptor
Secretory leukocyte inhibitory protein
Clara cell protein (CC10, phospholipase A_2 inhibitor)
IL-1 receptor antagonist
IL-1R2 (decoy receptor)
IκBα (inhibitor of NF-κB)
IL-10 (indirectly)
Decreased transcription
Cytokines
IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-11, IL-12, IL-13, IL-16, IL-17, IL-18, TNF-α, GM-CSF, SCF
Chemokines
IL-8, RANTES, MIP-1α, MCP-1, MCP-3, MCP-4, eotaxin
Adhesion molecules
ICAM-1, VCAM-1, E-selectin
Inflammatory enzymes
Inducible nitric oxide synthase
Inducible cyclooxygenase
Cytoplasmic phospholipase A_2
Inflammatory receptors
Tachykinin NK_1-receptors, NK_2-receptors
Bradykinin B_2-receptors
Peptides
Endothelin-1

* GM-CSF = granulocyte-macrophage colony-stimulating hormone; ICAM = intercellular adhesion molecule-1; IL = interleukin; MCP = monocyte chemoattractant protein; MIP = macrophage inflammatory protein; NF-κB = nuclear factor-κB; RANTES = regulated upon activation, normal cell expressed and secreted; SCF = stem-cell factor; TNF-α = tumor necrosis factor-α; VCAM-1 = vascular cell adhesion molecule-1.

Interactions between Corticosteroids and Other Drugs

Patients with asthma are usually treated with inhaled β_2-agonists as bronchodilators and inhaled corticosteroids as anti-inflammatory treatment. Indeed, fixed combination inhalers of long-acting β_2-agonists and corticosteroids are now available and seem to be the most effective way to control asthma because these two classes of drug have complementary and synergistic effects (41). Corticosteroids increase the expression of β_2-adrenergic receptors in the lung and prevent their downregulation and uncoupling in response to β_2-agonists (42–44). Recent studies also show that β_2-agonists enhance the action of corticosteroids, with an increase in nuclear translocation of glucocorticoid receptors in vitro (45) and enhanced suppression of inflam-

Figure 5. **Processes by which corticosteroids switch off inflammatory genes.**



Inflammatory genes are activated by inflammatory stimuli, such as interleukin-1β (*IL-1β*) or tumor necrosis factor-α (*TNF-α*), resulting in activation of NF-κB kinase 2 (*IKK2*), 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 (cyclic adenosine monophosphate response element–binding protein)-binding protein (*CBP*) or p300/CBP-activating factor (*PCAF*), which have intrinsic histone acetyltransferase (*HAT*) activity. This results in acetylation of lysines in core histone-4, resulting in increased expression of genes encoding inflammatory proteins, such as granulocyte-macrophage colony-stimulating factor (*GM-CSF*). Glucocorticoid receptors (*GR*s), after activation by corticosteroids, translocate to the nucleus and bind to coactivators to inhibit HAT activity directly. They also recruit histone deacetylases (*HDAC*s), which reverses histone acetylation leading in suppression of inflammatory genes. COX-2 = cyclooxygenase-2; MAPK = mitogen-activated protein kinase.

matory genes (46, 47). Nuclear localization of glucocorticoid receptors is also enhanced after treatment of asthmatic patients with a combination inhaler compared with the same dose of inhaled steroid given alone (48). The molecular mechanisms that result in increased nuclear localization of glucocorticoid receptors are not yet known but may involve phosphorylation of glucocorticoid receptors or an effect on nuclear transport proteins.

Theophylline has been used to treat asthma for many years, but its mechanism of action has been difficult to elucidate. Originally, theophylline was used as a bronchodilator; it relaxes airway smooth muscle by inhibiting phosphodiesterases. Accumulating evidence indicates that at lower doses, theophylline has anti-inflammatory effects, but these are probably not mediated by phosphodiesterase inhibition because the inhibition of these enzymes is trivial at low plasma concentrations that are clinically effective (49). We have recently shown that the anti-inflammatory effects of theophylline may be mediated via activation of HDAC and that this effect is independent of phosphodiesterase inhibition (50). Low doses of theophylline significantly increase HDAC activity in bronchial biopsy specimens from asthmatic patients, and the increase in HDAC activity is correlated with the reduction in airway eosinophils (50). Because corticosteroids also activate HDAC, but via a different mechanism, theophylline should enhance the anti-inflammatory actions of corticosteroids; this enhancement occurs because the HDAC recruited to the inflammatory gene will be more effective at switching off the gene. Indeed, therapeutic concentrations of theophylline markedly potentiate the anti-inflammatory effects of corticosteroids in vitro (50). This effect may explain why adding a low dose of theophylline is more effective than increasing the dose of inhaled corticosteroids in patients whose asthma is not adequately controlled (51–53).

Corticosteroid Resistance

Although corticosteroids are highly effective in the control of asthma and other chronic inflammatory or immune diseases, a small proportion of patients with asthma do not respond even to high doses of oral corticosteroids (54, 55). Resistance to the therapeutic effects of corticosteroids is also recognized in other inflammatory and immune diseases, including rheumatoid arthritis and inflammatory

bowel disease. Corticosteroid-resistant patients, although uncommon, present considerable management problems. The new insights into the mechanisms by which corticosteroids suppress chronic inflammation have shed light on the molecular basis for corticosteroid resistance in asthma. There is probably a spectrum of steroid responsiveness, with steroid resistance at one end; however, relative resistance is seen in patients who require high doses of inhaled and oral steroids (steroid-dependent asthma). Biopsy studies have demonstrated the typical eosinophilic inflammation of asthma in these patients (54).

Molecular Mechanisms of Corticosteroid Resistance

There may be several mechanisms for resistance to the effects of corticosteroids, and these may differ among patients. Certain cytokines (particularly interleukin-2, interleukin-4, and interleukin-13, which show increased expression in bronchial biopsy samples from patients with steroid-resistant asthma) may induce a reduction in affinity of glucocorticoid receptors in inflammatory cells, such as T lymphocytes, resulting in local resistance to the anti-inflammatory actions of corticosteroids (54, 56). We have recently demonstrated that the combination of interleukin-2 and interleukin-4 induces steroid resistance in vitro through activation of p38 MAP kinase, which phosphorylates glucocorticoid receptors and reduces corticosteroid-binding affinity and steroid-induced nuclear translocation of glucocorticoid receptors (57). The therapeutic implication is that p38 MAP kinase inhibitors now in clinical development might reverse this steroid resistance.

Another proposed mechanism for steroid resistance in asthma is increased expression of glucocorticoid receptor β, which may theoretically act as an inhibitor by competing with glucocorticoid receptor α for binding to GRE sites or from interacting with coactivator molecules (58). However, expression of glucocorticoid receptor β is not increased in the mononuclear cells of patients with steroid-dependent asthma (who have a reduced responsiveness to corticosteroids in vitro), and glucocorticoid receptor α greatly predominates over glucocorticoid receptor β, making it unlikely that it could have any functional inhibitory effect (59).

In patients with steroid-resistant and steroid-dependent asthma, the inhibitory effect of corticosteroids on cytokine release is reduced in peripheral blood mononuclear cells (60, 61). In one group of patients, nuclear localization of glucocorticoid receptors in response to a high concentration of corticosteroids was impaired, and this may be due to such abnormalities as the increased activation of p38 MAP kinase described earlier. However, in another group of patients, nuclear localization of glucocorticoid receptors was normal, and there was a defect in acetylation of histone-4 (62). In this group of patients, specific acetylation of lysine 5 was defective; presumably, corticosteroids cannot activate certain genes that are critical to the anti-inflammatory action of high doses of corticosteroids. Whether this is a genetic defect is not yet known.

Corticosteroid Resistance in COPD

Although inhaled corticosteroids are highly effective in asthma, they provide little benefit in COPD even though airway and lung inflammation is present. In COPD, inflammation is not suppressed by corticosteroids and there is no reduction in inflammatory cells, cytokines, or proteases in induced sputum, even with oral corticosteroids (63, 64). Corticosteroids do not suppress neutrophilic inflammation in the airways, and corticosteroids may prolong the survival of neutrophils (65). Some evidence shows that an active steroid resistance mechanism exists in COPD. For instance, in patients with COPD, corticosteroids do not inhibit cytokines that they normally suppress. In vitro studies show that cytokine release from alveolar macrophages is markedly resistant to the anti-inflammatory effects of corticosteroids compared with cells from normal smokers; these, in turn, are more resistant than alveolar macrophages from nonsmokers (66). This lack of response to corticosteroids may be explained, at least in part, by an inhibitory effect of cigarette smoking and oxidative stress on HDACs, thus interfering with the critical anti-inflammatory action of corticosteroids (67). There is a striking reduction in the activity and expression of HDACs in the peripheral lung of patients with COPD (68). Even in patients with COPD who have stopped smoking, the steroid resistance persists (63, 64), and these patients are known to have continuing oxidative stress (69).

THERAPEUTIC IMPLICATIONS

Because inhaled corticosteroids are the most effective currently available treatment for asthma, they are now used as first-line therapy for persistent asthma in adults and children in many countries (70). However, at high doses, systemic absorption of inhaled corticosteroids may have deleterious effects; therefore, investigators have searched for safer steroids for inhalation and even for oral administration.

Dissociated Corticosteroids

All currently available inhaled corticosteroids are absorbed from the lungs into the systemic circulation; therefore, inevitably they have some systemic component. Understanding the molecular mechanisms of action of corticosteroids has led to the development of a new generation of corticosteroids. The major task in developing these drugs is to dissociate the anti-inflammatory effects from the endocrine actions that are associated with side effects. As discussed earlier, a major mechanism of the anti-inflammatory effect of corticosteroids seems to be inhibition of the effects of proinflammatory transcription factors, such as NF-κB and AP-1, which are activated by proinflammatory cytokines (*trans*repression) via an inhibitory action on histone acetylation and stimulation of histone deacetylation. By contrast, the endocrine and metabolic effects of steroids that are

responsible for the systemic side effects of corticosteroids are likely to be mediated predominantly via DNA binding (*trans*activation). This speculation has led to a search for novel corticosteroids that selectively *trans*repress without significant *trans*activation, thus reducing the potential risk for systemic side effects. Because corticosteroids bind to the same glucocorticoid receptors, this seems at first to be an unlikely possibility, but while DNA binding involved a glucocorticoid receptor homodimer, interaction with transcription factors AP-1 and NF-κB and coactivators involves only a single glucocorticoid receptor (22). A separation of *trans*activation and *trans*repression has been demonstrated by using reporter gene constructs in transfected (see Glossary) cells using selective mutations of the glucocorticoid receptor (71). In addition, in mice with glucocorticoid receptors that do not dimerize, there is no *trans*activation, but *trans*repression seems to be normal (21, 28). Furthermore, some steroids, such as the antagonist RU486, have a greater *trans*repression than *trans*activation effect. Indeed, the topical steroids used in asthma therapy today, such as fluticasone propionate and budesonide, seem to have more potent *trans*repression than *trans*activation effects, which may account for their selection as potent anti-inflammatory agents (72). Recently, a novel class of steroids with potent *trans*repression and relatively little *trans*activation has been described. These "dissociated" steroids, including RU24858 and RU40066, have anti-inflammatory effects in vitro (73), although there is little separation of anti-inflammatory effects and systemic side effects in vivo (74). Several dissociated corticosteroids are now in clinical development and show good separation between *trans*repression and *trans*activation actions. This suggests that the development of steroids with a greater margin of safety is possible and may even lead to the development of oral steroids that do not have significant adverse effects. The recent resolution of the crystal structure of glucocorticoid receptors may help to better design dissociated steroids (75).

Other Approaches

Now that the molecular mechanisms of corticosteroids have been elucidated, the possibility exists that novel nonsteroidal anti-inflammatory treatments that mimic the actions of corticosteroids on inflammatory gene regulation might be developed. Other means of activating HDACs may have therapeutic potential, and theophylline is the first drug that has been shown to have this property; the result is a marked potentiation of the anti-inflammatory effects of corticosteroids. This action of theophylline is not mediated via phosphodiesterase inhibition or adenosine receptor antagonism and, therefore, seems to be a novel action of theophylline (50). It may be possible to discover other drugs in this class, and they could form the basis of a new class of anti-inflammatory drugs without the side effects that limit the use of theophylline (49).

Many of the anti-inflammatory effects of corticosteroids seem to be mediated via inhibition of the transcriptional effects of NF-κB, and small-molecule inhibitors of IκB kinase-2 (IKK2) (see Glossary), which activate NF-κB, are in development. However, because corticosteroids have additional effects, it is not certain whether IKK2 inhibitors will parallel the clinical effectiveness of corticosteroids; they may have side effects, such as increased susceptibility to infections.

Treatments that bypass or reverse steroid resistance are also needed. p38 MAP kinase inhibitors might reduce steroid resistance and act as anti-inflammatory treatments in patients with some forms of steroid-resistant asthma; however, these inhibitors would not be expected to benefit patients with the form of steroid resistance associated with a defect in acetylation of lysine 5 on histone-4. In patients with COPD, there is an urgent need to develop novel anti-inflammatory treatments or to reverse corticosteroid resistance (76). Because oxidative stress seems to inhibit HDAC activity and mimic the defect in HDAC seen in patients with COPD, antioxidants might be expected to be effective. Similarly, low-dose theophylline, by increasing HDAC activity, may also reverse corticosteroid resistance in patients with COPD (77).

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Potential Financial Conflicts of Interest: *Grants received:* P.J. Barnes, I.M. Adcock (GlaxoSmithKline and AstraZeneca); *Grants pending:* P.J. Barnes, I.M. Adcock (GlaxoSmithKline and AstraZeneca).

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