

Molecular mechanisms of steroid action in asthma

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Glucocorticosteroids are highly effective in the control of many inflammatory and immune diseases, and recently there has been an enormous increase in our understanding of the molecular mechanisms involved.¹ Recently important advances have occurred in understanding the molecular mechanisms of steroid action, largely through the application of molecular biology techniques.^{2,3}

GLUCOCORTICOID RECEPTORS

Glucocorticoids exert their effects by binding to a single glucocorticoid receptor (GR), which is predominantly localized to the cytoplasm of target cells, and only on binding of the glucocorticoid does it move into the nuclear compartment. The affinity of cortisol binding to GR is approximately 30 mmol/L, which falls within the normal range for plasma concentrations of free hormone. There is a single class of GRs, with no evidence of subtypes of differing affinity in different tissues, and its structure has been elucidated with the use of site-directed mutagenesis, which has revealed distinct domains.⁴ The glucocorticoid-binding domain is at the C-terminal end of the molecule, and two finger-like projections that interact with DNA are in the middle of the molecule. Each of these "zinc fingers" is formed by a zinc molecule bound to four cysteine residues. An N-terminal domain (τ_1) is involved in transcriptional *trans*-activation of genes once binding to DNA occurs, and this region may also be involved in binding to other transcription factors.⁵ This is the least conserved part of the molecule. Deletion analysis has demonstrated a 41-amino acid core at the C-terminal end of the τ_1 domain that is critical for *trans*-activation. In human GRs there is another *trans*-acting domain (τ_2) adjacent to the steroid-binding domain, and this region is also important for the nuclear translocation

Abbreviations used

AMP:	Adenosine monophosphate
AP-1:	Activator protein 1
CREB:	Cyclic AMP response element binding protein
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
GR:	Glucocorticoid receptor
GRE:	Glucocorticoid-response element
ICAM-1:	Intracellular adhesion molecule-1
IL:	Interleukin
iNOS:	Inducible form of NOS
mRNA:	Messenger RNA
NF-AT:	Nuclear factor of activated T cells
NF- κ B:	Nuclear factor- κ B
nGRE:	Negative glucocorticoid-response element
NO:	Nitric oxide
NOS:	Nitric oxide synthase
PLA ₂ :	Inducible form of phospholipase A ₂
RANTES:	Released by activated normal T cells expressed and secreted
SLPI:	Secretory leukocyte protease inhibitor
TNF- α :	Tumor necrosis factor- α
TPA:	Tetradecanoylphorbol 13-acetate
TRE:	TPA response element

tion of the receptor. GR is phosphorylated (predominantly on serine residues at the N-terminal), but the role of phosphorylation in steroid actions is not yet certain.⁴

The inactivated GR is bound to a protein complex (≈ 300 kd) that includes two molecules of 90 kd heat shock protein (hsp 90) and a 59 kd immunophilin protein and various other inhibitory proteins. The hsp 90 molecules act as a "molecular chaperone," preventing the unoccupied GR localizing to the nuclear compartment. Once the glucocorticoid bound to GR, hsp 90 dissociates, nuclear localization of the activated GR-steroid complex and its binding to DNA occur (Fig. 1).

EFFECTS ON GENE TRANSCRIPTION

Glucocorticoids produce their effect on responsive cells by activating GRs to directly or indirectly

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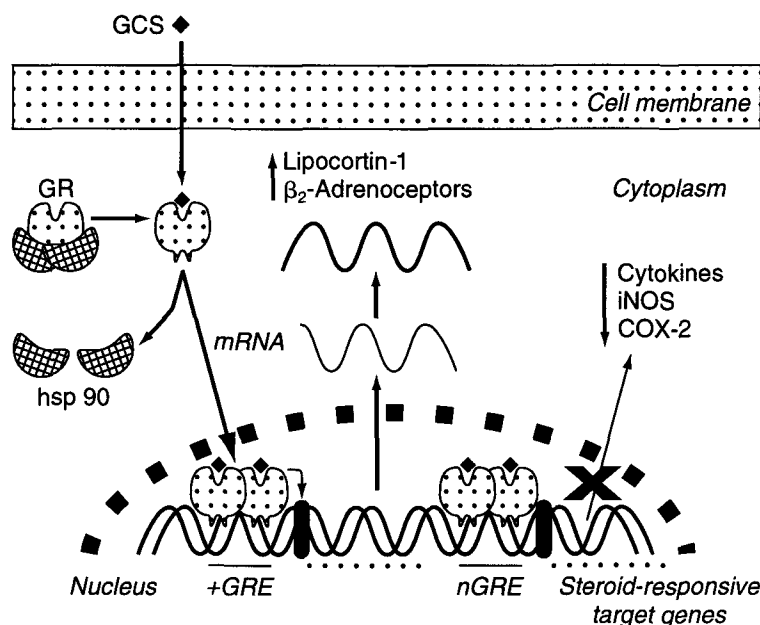


FIG. 1. Classic model of glucocorticoid action. Glucocorticoid (GCS) enters the cell and binds to a cytoplasmic GR that is complexed with two molecules of a 90 kd heat shock protein (hsp 90). GR translocates to the nucleus where, as a dimer, it binds to a GRE on the 5'-upstream promoter sequence of steroid-responsive genes. GREs may increase transcription, and nGREs may decrease transcription, resulting in increased or decreased mRNA and protein synthesis.

regulate the transcription of certain target genes.^{2,3} The number of genes per cell directly regulated by steroids is estimated to be between 10 and 100, but many genes are indirectly regulated through an interaction with other transcription factors. On activation, GR forms a dimer, which binds to DNA at consensus sites termed *glucocorticoid response elements* (GREs) in the 5'-upstream promoter region of steroid-responsive genes. This interaction changes the rate of transcription and results in either induction or repression of the gene. The consensus sequence for GRE binding is the palindromic 15-base pair sequence GGTA-CAnnnTGTTCT (where n is any nucleotide), although for repression of transcription, the negative GRE (nGRE) has a more variable sequence (ATYACnnTnTGATCn). Crystallographic studies indicate that the zinc finger binding to DNA occurs within the major groove of DNA, with each finger interacting with one half of the palindrome. In contrast to these simple GREs, there are "composite" GREs that do not share these GRE sequences but depend on the presence of other transcription factors binding to DNA.⁶ Interaction with other transcription factors may also be important to determine differential steroid responsiveness in different cell types. Other transcription factors binding in the vicinity of GRE may have a powerful

influence on steroid inducibility, and the relative abundance of different transcription factors may contribute to the steroid responsiveness of a particular cell type. GR may also inhibit protein synthesis by reducing the stability of messenger RNA (mRNA) via enhanced transcription of specific ribonucleases that break down mRNA-containing constitutive AU-rich sequences in the untranslated 3'-region, thus shortening the turnover time of mRNA.

INTERACTION OF GR WITH TRANSCRIPTION FACTORS

GR may interact directly with other transcription factors, which bind to each other via so-called leucine zipper interactions.⁷ This could be an important determinant of steroid responsiveness and is a key mechanism whereby glucocorticoids exert their antiinflammatory actions.¹ This interaction was first demonstrated for the collagenase gene induced by the transcription factor activator protein-1 (AP-1), which is a heterodimer of Fos and Jun oncoproteins. AP-1 binds to a specific DNA binding site (TPA response element [TRE] or tetradecanoylphorbol 13-acetate [TPA] response element, TGACTCA). Steroids are potent inhibitors of collagenase gene transcription induced by tumor necrosis factor- α (TNF- α) and

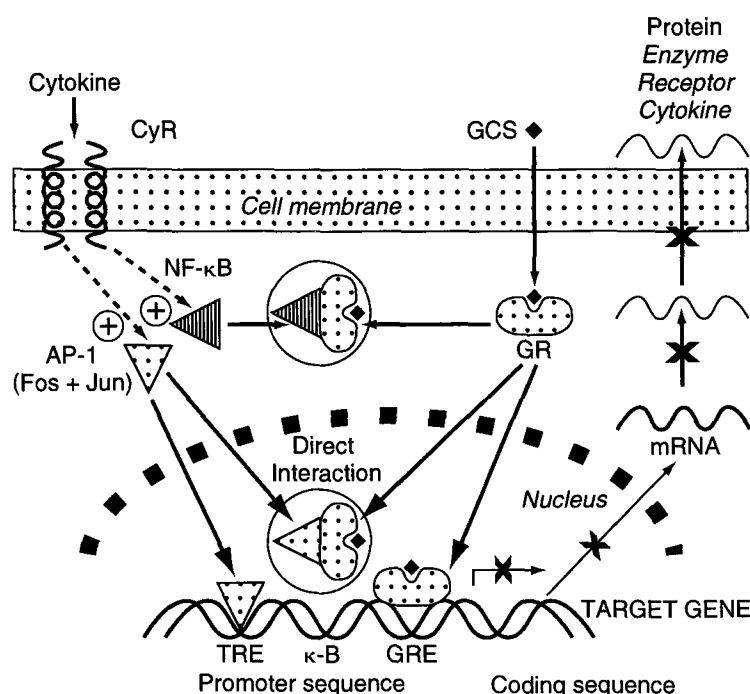


FIG. 2. Direct interaction between transcription factors AP-1 and NF- κ B, and GR may result in mutual repression. In this way steroids (GCS) may counteract chronic inflammatory effects of cytokines that activate these transcription factors.

phorbol esters than activate AP-1. AP-1 forms a protein-protein complex with activated GR, and this prevents GR interacting with DNA and thereby reduces steroid responsiveness.

In human lung, TNF- α and phorbol esters increase AP-1 binding to DNA, and this is inhibited by glucocorticoids.^{8,9} GRs may interact with other transcription factors that are activated by inflammatory signals, including nuclear factor- κ B (NF- κ B) in a similar manner (Fig. 2).⁸⁻¹⁰ There is evidence that β_2 -agonists, via cyclic adenosine monophosphate (AMP) formation and activation of protein kinase A, result in the activation of the transcription factor (cyclic AMP response element binding protein [CREB]) that binds to a cyclic AMP-responsive element on genes. A direct interaction between CREB and GR has been demonstrated.¹¹ β -Agonists increase cyclic AMP response element binding in human lung in vitro and at the same time reduce GRE binding, which suggests that there may be a protein-protein interaction between CREB and GR within the nucleus.^{12,13} Similar interactions have also been described in human pulmonary epithelial cells.¹⁴ These interactions between activated GR and transcription factors occur within the nucleus, but recent observations suggest that these protein-protein interactions may also occur in the cytoplasm.¹⁵

TARGET GENES IN INFLAMMATION CONTROL

Corticosteroids may control inflammation by inhibiting several aspects of the inflammatory process through increasing or decreasing gene transcription (Table I).¹

Cytokines

Although it is not yet possible to be certain of the most critical aspects of steroid action in asthma, it is likely that their inhibitory effects on cytokine synthesis are of particular relevance. Steroids inhibit the transcription of several cytokines that are relevant in asthma, including interleukin (IL)-1 β , TNF- α , granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-2, through IL-6, and the chemokines IL-8, RANTES, and macrophage inflammatory protein-1 α (MIP-1 α). These effects were thought to be mediated directly via interaction of GR with an nGRE in the upstream promoter sequence of the cytokine genes, resulting in reduced gene transcription. Surprisingly, there is no apparent nGRE consensus sequence in the upstream promoter region of many cytokines, which suggests that glucocorticoids inhibit transcription indirectly. Thus the 5'-promoter sequence of the human IL-2 gene has no GRE consensus sequences, and yet glucocorticoids are

TABLE I. Effect of glucocorticoids on transcription of genes relevant to asthma

Increased transcription
Lipocortin-1
β_2 -Adrenoreceptor
Endonucleases
Secretory leukocyte inhibitory protein
Decreased transcription
Cytokines
(IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-11, IL-12, IL-13, TNF- α , GM-CSF, RANTES, MIP-1 α , SCF)
iNOS
Inducible cyclooxygenase
Inducible phospholipase A ₂
Endothelin-1
N κ_1 receptors
Adhesion molecules (ICAM-1)

potent inhibitors of IL-2 gene transcription in T lymphocytes. Transcription of the IL-2 gene is predominantly regulated by a cell-specific transcription factor, nuclear factor of activated T cells (NF-AT), which is activated in the cytoplasm on T-cell receptor stimulation via calcineurin. A nuclear factor is also necessary for increased activation, and this factor appears to be AP-1, which binds directly to NF-AT to form a transcriptional complex.¹⁶ Therefore glucocorticoids inhibit IL-2 gene transcription indirectly by binding to AP-1, thus preventing increased transcription caused by NF-AT.¹⁷ Other examples of cytokine genes negatively regulated by glucocorticoids that do not have a GRE in their promoter region include IL-8, which is regulated predominantly via NF- κ B,¹⁸ and RANTES, which is regulated by NF- κ B and AP-1.¹⁸ There may be marked differences in the response of different cells and different cytokines to the inhibitory action of glucocorticoids, and this may be dependent on the relative abundance of transcription factors. Thus in alveolar macrophages and peripheral blood monocytes, GM-CSF secretion is more potently inhibited by glucocorticoids than is IL-1 β or IL-6 secretion.

Enzymes

Nitric oxide synthase (NOS) may be induced by various cytokines, which result in increased nitric oxide (NO) production. NO may increase airway blood flow and plasma exudation and may amplify the proliferation of Th2-lymphocytes, which orchestrate eosinophilic inflammation in the airways.²⁰ The induction of the inducible form of NOS (iNOS) is potently inhibited by glucocorti-

coids. In cultured human pulmonary epithelial cells, proinflammatory cytokines result in increased expression of iNOS and increased NO formation.²¹ This is due to increased transcription of the iNOS gene and is inhibited by glucocorticoids. There is no obvious nGRE in the promoter sequence of the iNOS gene, but NF- κ B appears to be the most important transcription factor in regulating iNOS gene transcription.²² Because TNF- α and oxidants may activate NF- κ B in airway epithelial cells, this may account for their activation of iNOS expression.²³ Glucocorticoids may therefore prevent induction of iNOS by inactivating NF- κ B, thereby inhibiting transcription.

Glucocorticoids inhibit the synthesis of several inflammatory mediators implicated in asthma through an inhibitory effect on enzyme induction. Glucocorticoids inhibit the induction of the gene coding for inducible cyclooxygenase in monocytes and epithelial cells, and this also appears to be via NF- κ B activation.^{24, 25} Glucocorticoids also inhibit the gene transcription of a form of phospholipase A₂ (PLA₂) induced by cytokines. Whether steroids also modulate expression of the 5'-lipoxygenase has not yet been established, but studies of cysteinyl-leukotriene formation in asthmatic patients *in vivo* indicate that doses of oral glucocorticoids that are effective clinically do not significantly reduce the excretion of leukotriene E₄, the major metabolite of leukotriene D₄.²⁶

Steroids also inhibit the synthesis of endothelin-1 in lung and airway epithelial cells, and this effect may also be via inhibition of transcription factors.²⁷

Inhibitory proteins

Glucocorticoids may have an inhibitory effect on inflammation by increasing the synthesis of antiinflammatory proteins. Steroids increase the synthesis of lipocortin-1, a 37 kd protein that has an inhibitory effect on PLA₂ and therefore may inhibit the production of lipid mediators. Steroids induce the formation of lipocortin-1 in several cells, and recombinant lipocortin-1 has short-term anti-inflammatory properties.²⁸ However, glucocorticoids do not induce lipocortin-1 expression in all cells, and this may be only one of many genes regulated by glucocorticoids. Glucocorticoids also increase the synthesis of secretory leukocyte protease inhibitor (SLPI) in human airway epithelial cells by increasing gene transcription.²⁹ SLPI is the predominant antiprotease in conducting airways and may be important in reducing airway inflammation by counteracting inflammatory enzymes, such as tryptase.

Receptors

Glucocorticoids also decrease the transcription of genes coding for certain receptors. Thus the NK₁ receptor that mediates the inflammatory effects of substance P in the airways may show increased gene expression in asthma.³⁰ This may be inhibited by steroids through an interaction with AP-1, because the NK₁ receptor gene promoter region has no GRE but has an AP-1 response element.

Steroids increase the expression of β_2 -adrenoreceptors by increasing the rate of transcription, and the human β_2 -receptor gene has three potential GREs. Steroids increase β_2 -receptor gene transcription in human lung *in vitro*.³¹ This may be relevant in asthma because it may prevent down-regulation in response to prolonged treatment with β_2 -agonists. In rats glucocorticoids prevent the down-regulation and reduced transcription of β_2 -receptors in response to long-term β -agonist exposure.³²

IL-1 acts on two types of surface receptor, designated IL-1 RI and IL-1 RII. The inflammatory effects of IL-1 β are mediated exclusively via IL-1 RI. IL-1 RII has no signaling activity, but binds IL-1 and therefore acts as a molecular trap that interferes with the actions of IL-1. Glucocorticoids are potent inducers of this decoy IL-1 receptor and result in release of a soluble form of the receptor, thus reducing the functional activity of IL-1.³³

Cell survival

Steroids markedly reduce the survival of certain inflammatory cells, such as eosinophils. Eosinophil survival is dependent on the presence of certain cytokines, such as IL-5 and GM-CSF. Exposure to steroids blocks the effects of these cytokines and leads to programmed cell death or apoptosis.³⁴ This may involve the increased expression of specific endonucleases. Steroids may increase the transcription of specific endonucleases, and this may be relevant to the action of steroids on eosinophil and mast cell survival in the airways of asthmatic patients.

Adhesion molecules

Adhesion molecules play a key role in the trafficking of inflammatory cells to sites of inflammation. The expression of many adhesion molecules on endothelial cells is induced by cytokines, and steroids may lead indirectly to a reduced expression via their inhibitory effects on cytokines such as IL-1 β and TNF- α . Steroids may also have a direct inhibitory effect on the expression of adhesion molecules, such as ICAM-1 and E-selectin at the level of gene transcription.³⁵ ICAM-1 expression in

bronchial epithelial cell lines and monocytes is inhibited by glucocorticoids.³⁶

Other effects

Steroids exert several other antiinflammatory effects that are not yet understood at a molecular level. Steroids have a direct inhibitory effect on plasma exudation from postcapillary venules at inflammatory sites. The onset of effect is delayed, which suggests that gene transcription and protein synthesis are involved. The mechanism for this antipermeability effect has not been fully elucidated, but there is evidence that synthesis of a 100 kd protein distinct from lipocortin-1, called vasocortin, may be involved.³⁷

EFFECT ON CELL FUNCTION

Steroids may have direct inhibitory actions on several inflammatory cells implicated in asthma.³⁸ Steroids inhibit the release of inflammatory mediators and cytokines from alveolar macrophages *in vitro*. Oral prednisone inhibits the increased gene expression of IL-1 β in alveolar macrophages obtained by bronchoalveolar lavage from asthmatic patients. Steroids have a direct inhibitory effect on mediator release from eosinophils, although they are only weakly effective in inhibiting secretion of reactive oxygen species and eosinophil basic proteins. Steroids appear to inhibit the permissive action of cytokines such as GM-CSF and IL-5 on eosinophil survival, presumably by activation of an endonuclease that leads to programmed cell death. One of the best described actions of steroids in asthma is a reduction in circulating eosinophils, which may reflect an action on eosinophil production in the bone marrow. After inhaled steroids there is a marked reduction in the number of low-density eosinophils, presumably reflecting inhibition of cytokine production in the airways. An important target cell in asthma may be the T lymphocyte, because steroids are very effective in inhibition of activation of these cells and in blocking the release of cytokines, which are likely to play an important role in the recruitment and survival of several inflammatory cells involved in asthmatic inflammation.

Although steroids do not appear to have a direct inhibitory effect on mediator release from lung mast cells,³⁸ long-term steroid treatment is associated with a marked reduction in mucosal mast cell numbers. This may be linked to a reduction in IL-3 or stem cell factor production, which appears to be necessary for mast cell expression in tissues. Mast cells also secrete various cytokines, but whether this is inhibited by steroids is not yet certain.

Steroids do not appear to directly inhibit the expression of adhesion molecules, although they may inhibit cell adhesion indirectly by suppression of cytokines involved in the regulation of adhesion molecule expression.³⁸ Steroids may have an inhibitory action on airway microvascular leakage induced by inflammatory mediators.³⁹ This appears to be a direct effect on postcapillary venular epithelial cells. The mechanism for this antipermeability effect has not been fully elucidated, but induction of vasocortin may be involved.³⁷ Although there have been no direct measurements of the effects of steroids on airway microvascular leakage in asthmatic airways, regular treatment with inhaled steroids decreases the elevated plasma proteins found in bronchoalveolar lavage fluid of patients with stable asthma.

Epithelial cells may be one of the most important targets for inhaled glucocorticoids in asthma. Steroids inhibit the increased transcription of the IL-8 gene induced by TNF- α in cultured human airway epithelial cells *in vitro*⁴⁰ and the transcription of the RANTES gene in an epithelial cell line.⁴¹ Inhaled steroids inhibit the increased expression of GM-CSF and the chemokine RANTES (chemotactic for eosinophils) in the epithelium of asthmatic patients. iNOS is expressed in the airway epithelium of patients with asthma that is not observed in normal individuals.⁴² This may account for the increase in NO in the exhaled air of patients with asthma compared with that of normal subjects.⁴³ Asthmatic patients who take regular inhaled steroid therapy, however, do not show such an increase in exhaled NO⁴³; this suggests that glucocorticoids have suppressed epithelial iNOS expression. Furthermore, double-blind, randomized studies show that oral and inhaled glucocorticoids reduce the elevated exhaled NO in asthmatic patients to normal values.^{44, 45}

STEROID RESISTANCE

Although most patients with asthma respond to steroids, there is a small minority of patients who appear to be resistant to steroid therapy. Steroid resistance is defined by a lack of response to oral steroids (usually 40 mg of prednisolone daily for 2 weeks).^{46, 47} Some patients require very high doses of oral steroids for control, and these patients should be considered as part of the steroid-resistant asthma spectrum.⁴⁸ It is important before steroid-resistant asthma is diagnosed to ensure that the patient has asthma, that there are no problems with compliance, and that absorption of the oral steroid is normal.⁴⁸ There are no epide-

miologic surveys of true steroid-resistant asthma, but as strictly defined above this may only occur in 1:1000 to 1:10,000 asthmatic persons. There is a tendency for these patients to have more severe asthma of a longer duration and nocturnal exacerbations. There is often a family history of asthma. Steroid resistance does not appear to be explained by pharmacokinetic problems, such as impaired absorption or rapid elimination of oral steroids.⁴⁹⁻⁵¹ The inhibitory effect of steroids on phytohemagglutinin-stimulated growth of cultured blood monocytes is reduced in steroid-resistant patients compared with steroid-sensitive control subjects,^{51, 52} suggesting that there is a cellular defect in steroid responsiveness. Steroids also fail to suppress the release of cytokines from monocytes of resistant patients.⁵³ This defect is not confined to monocytes but is also seen in peripheral T lymphocytes,⁴⁹ and a defect in the skin-blanching response to topical steroids has also been described in such patients.⁵⁴

Molecular mechanisms

The precise mechanism of steroid resistance is not certain. There is no significant defect in binding of steroids to GR in monocytes or lymphocytes from these patients, and there is no defect in nuclear transcription of GR.^{49, 50} Chemical mutational analysis shows that there is no apparent abnormality in the structure of GR.⁵⁶ This is in contrast to the rare patients with familial glucocorticoid resistance who may have marked abnormalities in steroid-receptor binding parameters and an abnormality in GR sequence.^{56, 57} There is no evidence for lipocortin-1 auto-antibodies in patients with steroid-resistant asthma,^{58, 59} as has been described in patients with steroid-resistant arthritis.^{60, 61}

A recent study has suggested impaired binding of GR to GRE and also a defect in the inhibitory effect of AP-1 activation on GRE binding in peripheral blood mononuclear cells of resistant compared with sensitive patients.^{62, 63} There is also an increase in basal AP-1 activity in these cells, and this may result in a reduced number of GRs available for DNA binding or for binding to other activated transcription factors. This would explain why resistance to the antiinflammatory effects of steroids is seen in these patients and yet circulating cortisol levels, adrenal suppression in response to steroids, and metabolic actions of steroids are apparently normal.

Resistance to steroids is not confined to asthma but is also described in other conditions in which steroids are required for disease control, including

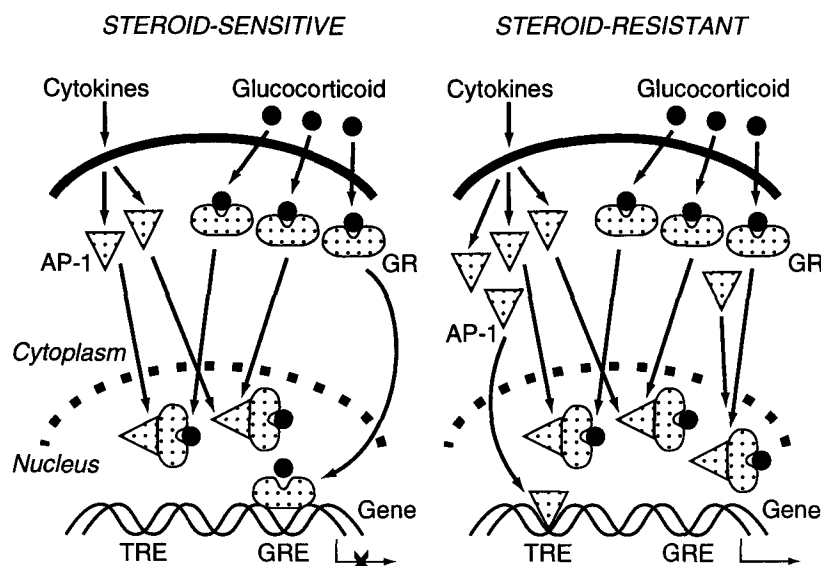


FIG. 3. Possible mechanism of primary steroid resistance in asthma. Increased activation of AP-1 results in complexing of glucocorticoid GRs, thus preventing antiinflammatory action of steroids.

rheumatoid arthritis, leukemias, and transplant rejection.⁴⁶ Although patients with resistance to steroids may be an extreme, there may be a spectrum of steroid responsiveness. Down-regulation of steroid receptors after exposure to steroids *in vitro* is well described,^{64, 65} and there is some evidence that this can occur in circulating lymphocytes *in vivo* after oral steroid treatment. Reduction in GR mRNA has been described in human lung *in vitro* after exposure to high-dose steroids,⁶⁶ but whether this is important in asthmatic patients exposed to high-dose inhaled steroids is not known. Another possibility is that inflammation itself may counteract the antiinflammatory effects of steroids through the direct interaction between GR and AP-1 (activated by exposure to proinflammatory cytokines)^{1, 67} or with the transcription factor CREB (cyclic AMP response element binding protein) activated by exposure to high-dose β_2 -agonists.¹³

Secondary steroid resistance

Although complete steroid resistance is uncommon, there may be a spectrum of steroid responsiveness in inflammatory diseases. This may reflect several mechanisms that are secondary either to disease activity or the effects of therapy (Fig. 3).

Several proinflammatory cytokines, including IL-1 β , IL-6, and TNF- α , activate AP-1 and NF- κ B in human lung.^{8, 68} Because all these cytokines are known to be secreted in asthmatic inflammation,

this suggests that these transcription factors will be activated in the cells of asthmatic airways. These activated transcription factors may then form protein-protein complexes with activated GR both in the cytoplasm and within the nucleus, thus reducing the number of effective GRs and thereby decreasing steroid responsiveness.¹ In a model *in vitro* system increased expression of c-fos or c-jun oncoproteins prevents the activation of mouse mammary tumor virus promoter by GRs, thus creating a model of steroid resistance.⁶⁹ The addition of recombinant c-jun or c-fos proteins to partially purified GR results in inhibition of DNA binding.⁶⁹ Phorbol esters, which activate AP-1, result in attenuation of glucocorticoid-mediated gene activation.⁷⁰ Any reduction in glucocorticoid responsiveness would be greater as the intensity of asthmatic inflammation increases; this may contribute, for example, to the failure of oral or intravenous glucocorticoids to control acute exacerbations of asthma. Once the inflammation is brought under control with large doses of oral glucocorticoids, steroid responsiveness increases again so that lower doses of inhaled or oral glucocorticoids are needed to control asthmatic inflammation.

Increased resistance may also be the result of the effects of cytokines on GR-receptor function, because high concentrations of IL-2 and IL-4 reduce GR affinity in T lymphocytes *in vitro*.⁷¹ This effect would only be seen in mucosal T cells of patients with

severe asthma, and therefore it is difficult to obtain evidence to support this possibility.

High concentrations of β_2 -agonists activate CREB in rat and human lung and in inflammatory cells via an increase in cyclic AMP concentration.^{9, 13, 14} This results in reduced GRE binding because of the formation of GR-CREB complexes.¹² This predicts that high concentrations of β_2 -agonists induce steroid resistance. A recent study in asthmatic patients found that although 3 weeks of treatment with an inhaled steroid blocked the airway response to inhaled allergen, concomitant treatment with inhaled steroid and a relatively large dose of inhaled β_2 -agonist provided no significant protection against allergen challenge.⁷² This suggests that high doses of an inhaled β_2 -agonist may interfere with the anti-asthma effect of inhaled glucocorticoids. It is possible that some patients who use very high doses of inhaled β_2 -agonists (more than two canisters per month of metered-dose inhalers or regular nebulized doses) may develop a degree of steroid resistance that is overcome by increasing the dose of inhaled or oral glucocorticoid. This may account for some of the deleterious effects of high-dose β_2 -agonists on asthma mortality and morbidity.⁷³ The use of high doses of nebulized β_2 -agonists in the treatment of acute exacerbations of asthma may result in resistance to the effects of high-dose intravenous glucocorticoids in the treatment of these exacerbations. Steroid responsiveness might be restored by reducing the dose of inhaled β_2 -agonists. In an uncontrolled study in steroid-dependent patients with severe asthma, gradual withdrawal of nebulized β_2 -agonists resulted in a reduced requirement for oral prednisolone.⁷⁴

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