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Overall Purpose/Goal: To provide excellent reviews on key aspects of allergic disease to those who research, treat, or manage allergic disease.

Target Audience: Physicians and researchers within the field of allergic disease.

Activity Objectives

- (a) To understand the molecular mechanisms of glucocorticoid action.
- (b) To recognize potential mechanisms of glucocorticoid resistance.
- (c) To review evaluation and management of patients with glucocorticoid resistance.

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Update on glucocorticoid action and resistance

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Glucocorticoids (GCs) are the most common group of medications used in the treatment of allergic and autoimmune disorders. They produce potent anti-inflammatory effects by inducing or repressing the expression of target genes. Although most patients with allergic diseases and autoimmune disorders respond to GC therapy, a small subset of patients demonstrate persistent tissue inflammation despite treatment with high doses of GCs. This condition results from an interaction

between susceptibility genes, the host's environment, and immunologic factors. The treatment of these patients requires a systematic approach to rule out underlying conditions that lead to steroid resistance or treatment failure, as well as the use of alternative strategies to inhibit tissue inflammation. (*J Allergy Clin Immunol* 2003;111:3-22.)

Key words: Asthma, steroid-resistant asthma, glucocorticoid, T cells

Tissue inflammation and immune activation play a key role in the pathogenesis of chronic allergic and autoimmune diseases.¹⁻³ Glucocorticoids (GCs) are currently the most efficacious agents used for the treatment of allergic diseases.^{4,5} Although most patients with allergic diseases respond to GC therapy, a small subset of patients demonstrate persistent tissue inflammation despite treatment with high doses of GCs. GC resistance complicates the management of these patients, and knowledge of the molecular mechanisms responsible for GC insensitivity is crucial for the development of effective therapies.

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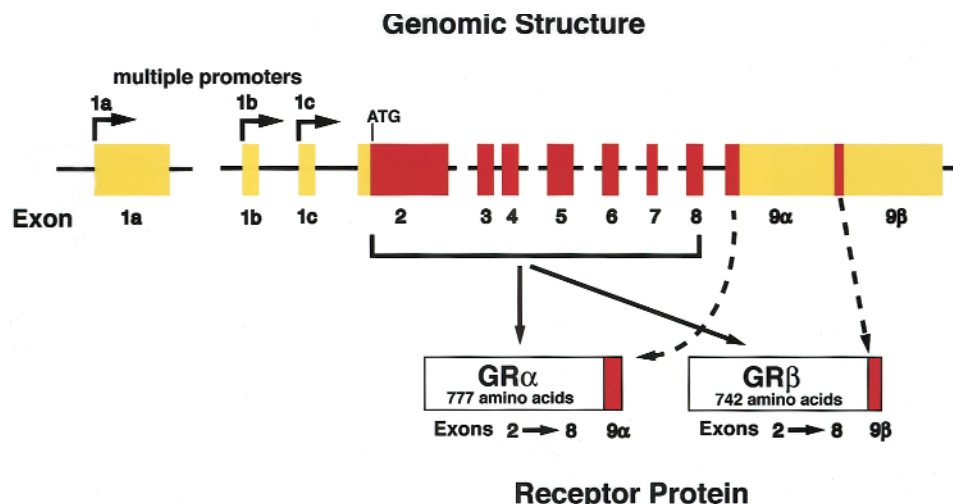


FIG 1. Genomic organization of the *GR* gene encoding and the protein structure of its receptor isoforms. Translated sequences in each of the 9 exons are shown in *black areas*, and the 5'-untranslated and 3'-untranslated sequences in exons 1 and 2 and in exon 9, respectively, are shown in *shaded areas*. Introns are shown as a *single line*. There is evidence for 3 promoters with unique 5'-untranslated regions for exon 1. The protein structures for the GR and GR isoforms are identical for amino acids 1 to 727 and differ only in the carboxyl terminal region as a result of alternative splicing in exon 9.

Abbreviations used

AP-1:	Activator protein 1
BAL:	Bronchoalveolar lavage
CBP:	CREB-binding protein
GC:	Glucocorticoid
GR:	Glucocorticoid receptor
GRE:	Glucocorticoid response element
HAT:	Histone acetyltransferase
HDAC:	Histone deacetylase
hsp 90:	Heat shock protein 90
ICAM-1:	Intercellular adhesion molecule 1
IkB:	Inhibitory κ B
MAP:	Mitogen-activated protein
NF κ B:	Nuclear factor κ B
NL:	Nuclear localization signal
NO:	Nitric oxide
NP:	Nasal polyp
SEB:	Staphylococcal enterotoxin B
SRC:	Steroid receptor coactivator
STAT:	Signal transducer and activator of transcription
Swi/Snf:	Switch/sucrose nonfermentable
UC:	Ulcerative colitis
VCAM-1:	Vascular cell adhesion molecule 1

The anti-inflammatory effects of GCs are mediated through receptors that modulate inflammatory gene expression. GC receptors (GRs) are members of the large steroid-nuclear receptor family that also includes receptors for mineralocorticoids, androgens, estrogens, progesterone, vitamin D, thyroid hormone, and retinoic acid. This article will review the cellular and molecular mechanisms of GC action and factors that contribute to GC resistance. GC resistance results from an interaction between susceptibility genes, the host's environment, and immunologic factors. The management of these patients requires a

systematic approach to rule out underlying conditions that lead to GC resistance or treatment failure, as well as the use of alternative strategies to inhibit tissue inflammation.

GC ACTION

Organization of the *GR* gene

The *GR* gene is located on chromosome 5 in the 5q31-q32 region.⁶ The genomic structure of the *GR* gene (Fig 1) consists of 9 exons spanning more than 80 kb of the human genome, with protein coding beginning in exon 2.⁷ There is evidence for 3 distinct promoters for the *GR* gene.⁸ The presence of multiple promoters leads to several *GR* mRNAs with unique 5'-untranslated regions in exon 1, but these different sequences in exon 1 do not affect the open reading frame beginning in exon 2. Although, at present, the use of the different GR promoters in various cell types remains unclear, it is possible that promoter heterogeneity might produce cell type-specific regulation of GR expression.

A GR receptor isoform, GR β , has been described that is distinct from the classical ligand-activated GR (GR α).⁹⁻¹² Alternative splicing of exon 9 of the *GR* gene results in the synthesis of 2 homologous mRNAs and protein isoforms. GR β differs from GR α only in the carboxyl terminus, with replacement of the last 50 amino acids of GR α with a unique 15-amino-acid sequence (Fig 1). The region of the variation between GR α and GR β is located in the hormone-binding domain (Fig 2). In contrast to GR α , GR β does not bind ligand.

Structure of the GR

The GR has a modular structure in which discrete functions are performed by different domains within the receptor protein. The major domains are a carboxyl ter-

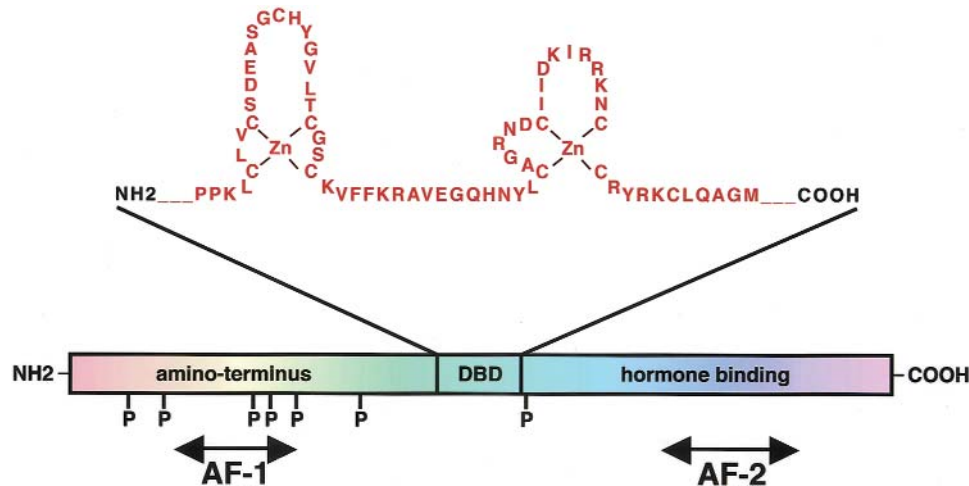


FIG 2. Structure-function map of the GR. The receptor protein consists of 3 domains termed the amino-terminus, the DNA-binding domain, and the hormone-binding domain. For the DNA-binding domain, the amino acid sequence and zinc finger structures are depicted. The locations of the ligand-independent (*AF-1*) and ligand-dependent (*AF-2*) transcriptional activation functions, as well as sites of phosphorylation, are shown.

minimal ligand-binding domain, an amino-terminal transcriptional activation domain, and a small central DNA-binding domain that comprises 2 repeats of a protein motif termed a zinc finger (Fig 2). In the hormone-binding domain a core region of approximately 135 amino acids has been shown to interact with ligand during hormone binding.¹³ Studies of this region suggest a model of a ligand-binding cavity for the receptor in which multiple amino acids are necessary for proper folding of the cavity. Crystallographic analysis demonstrates that the high-affinity binding of dexamethasone is explained by extensive hydrophobic and hydrophilic interactions between dexamethasone and the GR protein.¹⁴ Heat shock protein 90 (hsp 90) also binds sequences in the hormone-binding domain, and this interaction with hsp 90 might mask nuclear localization signals (NLs) located in this region. Nuclear import of liganded GR is mediated through a sequence situated adjacent to the receptor DNA-binding domain, NL1, and a second motif, NL2, that overlaps with the ligand-binding domain.¹⁵ The classical notion is that GR translocation to the nucleus is only possible after ligand binding and the resulting dissociation of heat shock proteins from the GR. There is also new evidence to suggest that nucleocytoplasmic trafficking of unliganded GR also occurs and is mediated by the NL1 sequence.¹⁶

A conserved motif within the hormone-binding domain, referred to as AF-2, appears to be important in GR transcriptional function. Several lines of evidence suggest that GR interacts with additional factors through an intact AF-2 domain to mediate transcriptional activities.¹⁷ The AF-2 domain appears to be the interaction site for the GR with coactivators (described below), and hormone binding to the GR greatly stimulates this interaction. The AF-2 function in the GR hormone-binding domain has been mapped to 4 putative α -helical regions that make direct contacts with coactivator proteins.¹⁸

The interaction of the GR with DNA is mediated by the DNA-binding domain, which is a 66-amino-acid segment in the central domain of the GR (Fig 2). The GR regulates gene transcription by binding as a homodimer to specific GC response elements (GREs) in GR target genes. The consensus GRE is a partial, palindromic 15-bp sequence, GGTACAnnnTGTTCT.¹⁹ In the DNA-binding domain 8 cysteine residues tetrahedrally coordinate 2 zinc atoms in 2 zinc fingers. The amino-terminal zinc finger is the main determinant of binding specificity, but the carboxyl terminal finger is necessary for dimerization and overall binding affinity (Fig 2).

The domain located at the amino terminus of the GR contains a 200-amino-acid region, termed AF-1, that includes a hormone-independent transcriptional activation function.²⁰ The transcriptional activity of AF-1 appears to lie in a core region of 41 negatively charged acidic amino acids. This region has been shown to interact with general transcription factors²¹ and a number of coactivator proteins and complexes.²² There is also evidence that the AF-1 region might be important in mediating transcriptional repression by the GR.²³

Mechanism of GC-mediated transcriptional activation

The GR functions primarily as a ligand-activated transcription factor. The classical notion of how GCs activate gene transcription is shown in Fig 3. GCs are extremely lipophilic and enter the cell cytoplasm from the extracellular space, primarily by means of passive diffusion. The GR is localized predominantly in the cytoplasm and enters the nucleus of the cell after activation by hormone. In the cytoplasm the unliganded GR (approximately 90 kd) exists as a component of a large heteromeric complex (approximately 330 kd) that includes a dimer of hsp 90 molecules, a subunit p23 protein, and any one of several

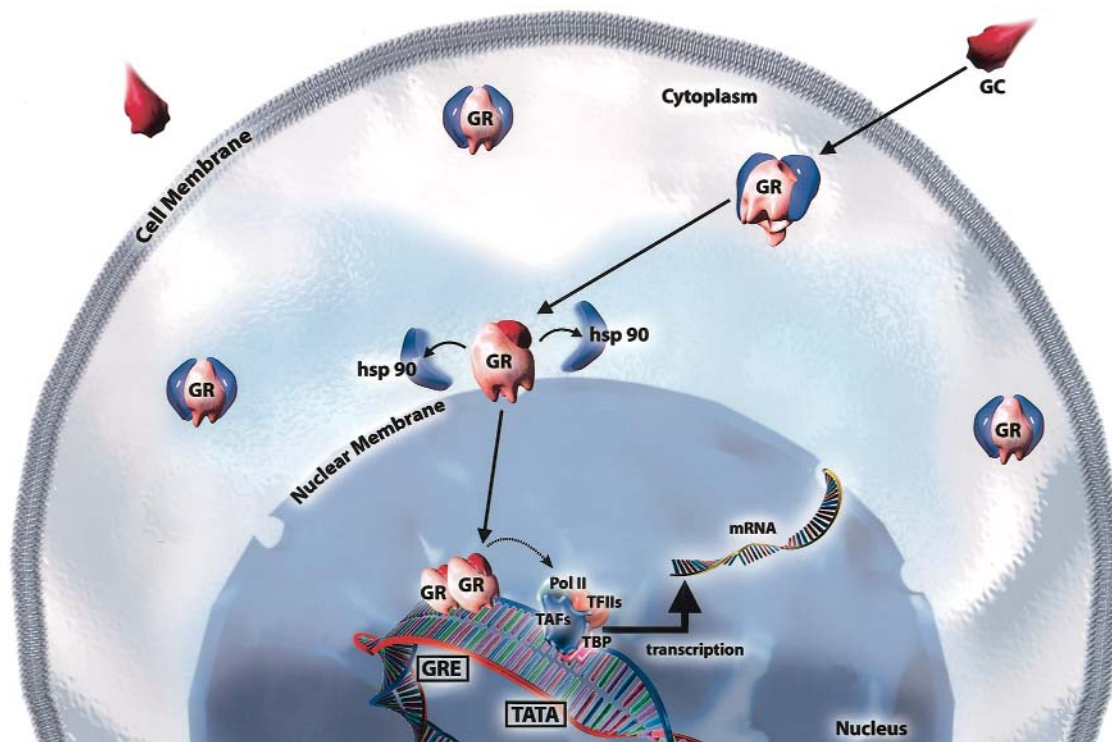


FIG 3. GC-mediated transcriptional activation. Before GC binding, the GR exists as a large multiunit complex in the cytoplasm, which includes 2 molecules of hsp 90. After activation by binding of GC hormone (GC), the GR dissociates from the chaperone proteins and translocates to the nucleus. In the nucleus the GR binds as a homodimer to a specific palindromic DNA sequence, termed a GRE, located in the regulatory regions of target genes. The bound GR homodimer interacts with the basal transcriptional machinery shown bound to the TATA box. The basal transcription complex includes TATA-binding protein, associated transcription factors (TAFs and TFIIIs), and RNA polymerase II (*pol II*). The interaction between GR and the basal transcription complex enhances transcription of the GR target gene.

immunophilin-related proteins in the complex.²⁴ The heat shock proteins associated with GR appear to be important in maintaining GR in a conformation that is appropriate for ligand binding and for inhibition of GR translocation to the nucleus.

After ligand activation and nuclear localization, the GR binds as a homodimer at GREs to the specific palindromic DNA sequences found in and around genes that are transcriptionally activated by the GR. The mechanism by which the GR activates transcription was not well characterized until recently. The established concept was that binding of the GR homodimer stabilized the basal transcription machinery through interacting proteins, but the identity of these proteins was unknown. Several distinct groups of coactivator proteins have been identified that appear to participate in GR-mediated transcriptional activation.²⁵

CREB-binding protein (CBP) is an ubiquitous nuclear coactivator that functions, at least in part, by uniting transcriptional activators, such as the GR, with the basal transcription initiation complex. CBP was first discovered as a coactivator of CREB.²⁶ A highly related protein, p300, shares extensive homology with CBP.²⁷ The CBP and p300 make protein-protein interactions not only with CREB but also with steroid-nuclear receptors, including the GR, and

multiple other transcription factors. In addition, the CBP and p300 have been shown to form protein-protein interactions with members of the basal transcription machinery, including RNA polymerase II and the general transcription factors TFIIB and TATA-binding protein.

Recently, other coactivator proteins that interact with the GR have been identified. These coactivator proteins modulate GR activity and might play a role in the cell specificity of GR effects. The steroid receptor coactivator 1 (SRC-1) interacts with the AF-2 region of the hormone-binding domain of the GR.²⁸ This SRC family of 160-kd proteins binds to the steroid-nuclear receptor in a hormone-dependent manner through 3 leucine-rich motifs (LXXLL, where L denotes leucine and X is any amino acid) clustered in the central region of the SRC protein.^{29,30} Thus the CBP-p300 and SRC family proteins that form interactions with both steroid-nuclear receptors and the basal transcription machinery can serve as a link between the DNA-bound GR and the general transcription factors to enhance transcription (Fig 4). Additional GR coactivators include another p160 coactivator, termed GR interacting protein 1, and a 152-kd protein, p/CIP.³¹

Linking the GR and the basal transcription machinery appears to be only one of the transcriptional activities of the coactivator proteins. In addition, the coactivators

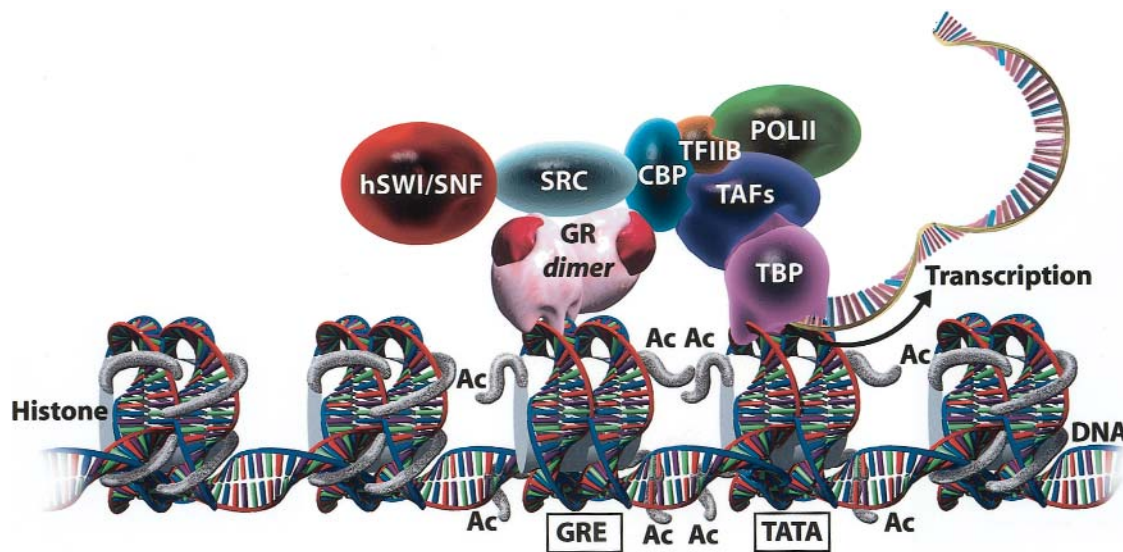


FIG 4. Interactions of the GR with coactivators and chromatin. The basal transcription complex consisting of TATA-binding protein, TAFs, TFIIB, and RNA polymerase II (*pol II*) is labeled as noted in Fig 3. The interaction of the GR with the basal transcription complex through the coactivator proteins SRC and CBP is depicted. The ATP-dependent chromatin remodeling factor (*hSWI/SNF*) is also shown as part of the complex. DNA is shown packaged into chromatin by histones. SRC and CBP, as well as some proteins in the basal transcription complex, have intrinsic HAT activity and are able to acetylate histones (see text). Acetylation of histone tails produces an allosteric change in the nucleosome conformation, destabilizes the interaction between the histone tails and DNA, and allows the nucleosomal DNA to become more accessible to transcription factors. Ac, Hyperacetylation of the histone tails.

might play an even more intriguing role in gene transcription that involves modulation of chromatin structure. CBP-p300 and SRC-1 have histone acetyltransferase (HAT) activity that might influence promoter accessibility to transcription factors.³² Over 30 years ago, an association between histone acetylation and enhanced transcriptional activity was identified.³³ Histones package all chromosomal DNA into chromatin, and the packaging of DNA into chromatin performs a critical role in regulating gene expression. The basic structural unit of chromatin is the nucleosome, which consists of approximately 146 bp of DNA wrapped approximately 2 turns around an octamer of core histone proteins (Fig 4).³⁴ The functional consequence of chromatin packaging is to limit access of transcription factors to the DNA.

For transcriptional activators, such as the GR, to regulate gene transcription from the RNA polymerase II transcription initiation complex, the necessary transcription factors must gain access to the gene promoter that is folded into chromatin fibers. Nucleosome assembly into chromatin eliminates the ability of the general transcription factors, such as the RNA polymerase II holoenzyme, to interact with promoter sequences. Thus one of the key roles of gene-specific transcriptional activators is to target the gene promoter for unfolding or remodeling of chromatin. Transcriptional activators, such as the GR, must recruit not only the required basal transcription machinery but also the specialized enzymes required to acetylate and remodel chromatin.

Acetylation of histones occurs at lysine residues located on the amino-terminal tails of the histone, thereby neutralizing the positive charge of the histone tails and decreasing their affinity for the DNA. As a consequence of acetylation of the histone tails, the nucleosomal conformation is more open, and the DNA becomes accessible to GR binding (Fig 4). Thus in addition to providing a bridge between the DNA-bound GR and the basal transcription machinery, the coactivators, such as CBP-p300 and SRC family proteins, also supply the essential histone acetylation that alters the conformation of nucleosomes in the region and allows binding of factors necessary to enhance transcription.³⁵

In addition to proteins with histone acetylation, a group of corepressor proteins with histone deacetylase (HDAC) activity has been identified.^{36,37} HDACs function to decrease the acetylation of the histone lysine tails and thus restore the dense chromatin structure that inhibits transcription-factor binding. Deacetylation of histones correlates with reduced transcription and gene silencing. A requirement for the recruitment of HDAC activity has been described as a potential mechanism for GC suppression of inflammatory gene expression.³⁸ Alterations in HAT and HDAC activity might play a role in chronic inflammatory disease states. In recent studies bronchial biopsy specimens from asthmatic patients expressed higher levels of HAT activity and lower HDAC activity than those from control subjects, suggesting the possibility that HAT-HDAC dysregulation might underlie pro-inflammatory gene expression.³⁹

An emerging view indicates that transcriptional activators, such as the GR, must also target a second type of chromatin-remodeling enzyme in addition to coactivators with HAT activity. These are ATP-dependent chromatin-remodeling complexes referred to as switch/sucrose non-fermentable (Swi/Snf). The Swi/Snf remodeling complex hydrolyzes ATP and uses the energy of ATP hydrolysis to disrupt histone-DNA interactions and remodel chromatin, allowing transcription factors to bind at the promoter.⁴⁰ Although the exact mechanism by which Swi/Snf alters chromatin structure is not clear, there is evidence that Swi/Snf recruitment is essential for the regulation of gene transcription, including that mediated by the GR.⁴¹

Exactly how this complex transcriptional machine consisting of multiple activators and coactivators can detect and respond quickly to changes in GC hormone levels has been a mystery. Recent studies demonstrate that despite their size, GR transcriptional complexes get on and off DNA very quickly.⁴² There is now evidence suggesting that the rapid disassembly is mediated either by chaperone proteins, such as p23 and hsp 90, or ATP-dependent complexes, such as Swi/Snf.^{43,44} These findings are consistent with a dynamic process of gene regulation in which the GR occupies its target promoter sites only transiently, relying on a hit-and-run mechanism to alter gene expression. After release from chromatin, the GR can disassociate from ligand and be recycled in the nucleus, exported to the cytoplasm, and reassociated with chaperone proteins or undergo degradation after ubiquitination.⁴⁵

As described above, the classical mechanism of GR-mediated transcriptional activation requires GR binding to DNA at a consensus GRE. Recently, transcriptional effects independent of DNA binding have been described for the GR with the transcription factor signal transducer and activator of transcription 5 (STAT5).⁴⁵⁻⁴⁷ The GR acts as a transcriptional coactivator for STAT5 at the gene encoding β -casein, and the STAT5/GR complex binds DNA independently of a GRE. Conversely, STAT5 diminishes the induction of GC-responsive genes.^{45,48} Functional synergy for gene activation by the GR is not limited to interaction with STAT5. The GR has been shown to interact with a number of other signaling pathways, including STAT3, Ets, Oct, and CCAAT/enhancer-binding protein.⁴⁹⁻⁵²

Phosphorylation of the GR

Although hormone binding is necessary for the activation of the GR, the receptor is also regulated by means of posttranslational modification through phosphorylation. It has been suggested that phosphorylation of the GR might determine target promoter specificity, cofactor interaction, subcellular localization, and receptor stability.⁵³ The GR is a phosphoprotein and is phosphorylated in the absence of ligand, but further phosphorylation occurs with GC binding.⁵⁴ Initial studies of phosphorylation involving mouse and rat GRs demonstrated several potential phosphorylation sites in the amino terminal region of the GR. Three of these sites are conserved in the human GR (serines 203, 211, and 226; Fig 2), and there is evi-

dence that they are phosphorylated by means of cyclin-dependent and mitogen-activated protein (MAP) kinases.⁵⁵ Phosphorylation sites at serines 203 and 211 in the human GR play a critical role in GR function.⁵⁴ In the absence of GC, the GR is phosphorylated predominantly at the serine 203 site. Treatment with the GR agonist dexamethasone results in increased phosphorylation at serine 211 relative to serine 203. Interestingly, localization studies showed that after dexamethasone treatment, the serine 203-phosphorylated form is predominantly in the cytoplasm, whereas the serine 211-phosphorylated GR is found in the nucleus. Of particular importance, the transcriptional activity of the GR correlated with the amount of phosphorylation at serine 211.⁵⁴

Serine 226 can be phosphorylated by MAP kinases, and phosphorylation at this site has been shown to inhibit GR-mediated transcriptional activation.⁵⁶ Thus MAP kinase-mediated pro-inflammatory signals might inhibit GR-dependent gene expression. A potential mechanism for the inhibitory effect of serine 226 phosphorylation on GR-mediated transcription has been demonstrated. Phosphorylation of the GR at serine 226 by the MAP kinase c-Jun N-terminal kinase enhances GR nuclear export and likely contributes to termination of GR-mediated transcription.⁵⁷ Thus posttranslational modification of the GR by means of phosphorylation induces a distinct conformation, influences the association with coregulatory proteins that modulate the GR subcellular location and transcriptional activation, or both.

Targets of GC action

GCs produce anti-inflammatory effects by controlling expression of specific target genes. The exact target genes that are critical for GC action are unknown, but the anti-inflammatory action of GCs involves the regulation of a number of genes. GCs enhance transcription of several genes that have significant inhibitory effects on inflammation. An early proposal for the inhibitory mechanism of GCs was an increase in lipocortin 1 synthesis.⁵⁸ Lipocortins are specific inhibitors of phospholipase A2, a presumed regulator in the production of prostaglandins, leukotrienes, and platelet-activating factor, but the importance of the anti-inflammatory effects of lipocortin is uncertain. GCs induce the expression of the type II IL-1 receptor, a decoy molecule for IL-1,⁵⁹ and increased levels of this IL-1 decoy receptor could produce anti-inflammatory activity by blocking the effects of IL-1. Transcription of the secretory leukocyte proteinase inhibitor is also increased by GCs.⁶⁰ Secretory leukocyte proteinase inhibitor might block nuclear factor (NF) κ B activation in addition to its anti-inflammatory effects on neutrophils.⁶¹ Because GCs increase expression of only a limited number of anti-inflammatory genes, enhanced gene expression by GCs might not be the dominant mechanism for GR-mediated anti-inflammatory effects.

The inhibition of gene expression might be particularly important in producing the anti-inflammatory effects of GCs. There is evidence that GCs limit expression of multiple inflammatory cytokines, including IL-1 through

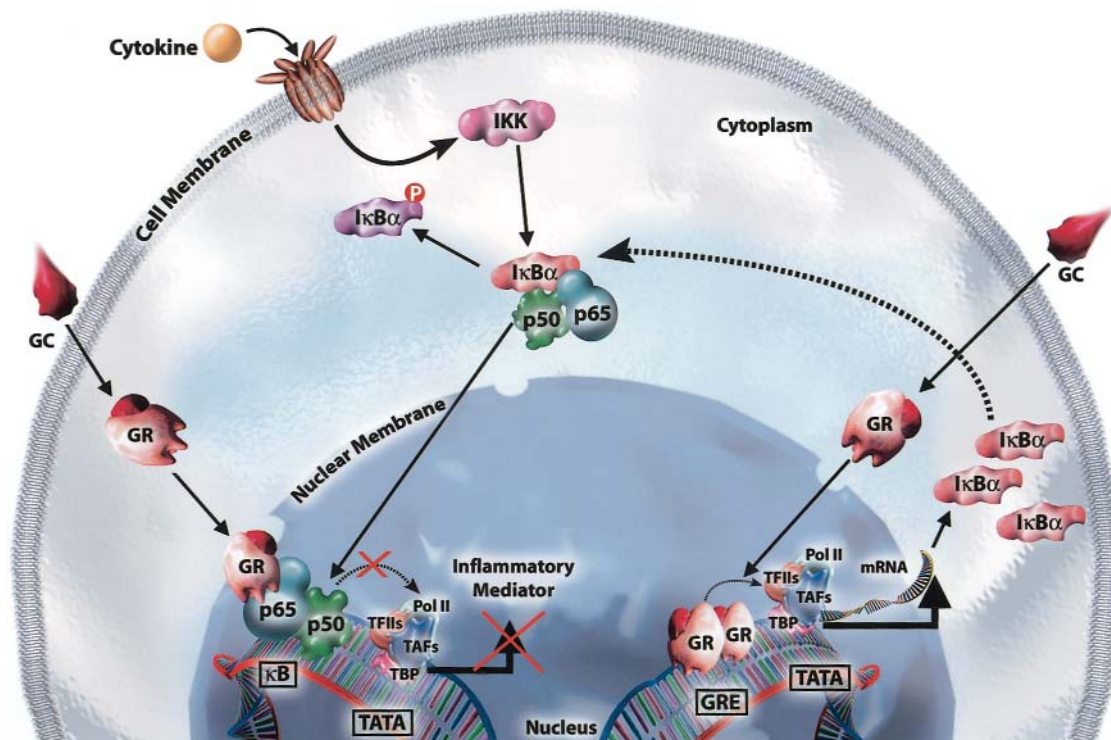


FIG 5. GR-mediated repression of NFκB activity. In the inactive state NFκB (heterodimer of p65 and p50) is anchored in the cytoplasm by IκBα. Activation signals through cell-surface receptors result in activation of IκB kinase, which phosphorylates IκBα. After phosphorylation, IκBα undergoes proteolytic degradation, and the NFκB heterodimer (p65/p50) is free to pass into the nucleus, where it binds to B sites in the promoter regions of inflammatory mediator genes and enhances transcription. The GR might block NFκB activity by either of 2 mechanisms. Inhibition might occur through protein-protein interactions between the ligand-activated GR and NFκB (see text). A second less plausible mechanism for GR-mediated inhibition of NFκB is activation of the *IKBA* gene by the GR. The enhanced synthesis of IκBα replaces the degraded IκB and neutralizes the free NFκB.

IL-6, IL-11, IL-13, IL-16, GM-CSF, TNF-α, matrix metalloproteinase 9, and the chemokines IL-8, RANTES, eotaxin, macrophage inflammatory protein 1α, and monocyte chemoattractant protein 1.⁶² GCs also inhibit the expression of adhesion molecules, such as intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1).^{63,64} This effect could decrease inflammatory cell migration into tissues. In addition, anti-inflammatory effects of GCs might be mediated by inhibiting expression of various enzymes, as well as expression of the IL-4 receptor and the high-affinity IgE receptor.^{65,66} Nitric oxide (NO) synthase can be induced by cytokines released during inflammation, leading to increased NO production. Although the exact role NO plays in inflammatory disease is unclear, it is possible that NO might increase blood flow and inflammatory plasma exudation. The inducible form of NO synthase is potentially inhibited by GCs, resulting in diminished NO generation. Potential anti-inflammatory effects of GCs in epithelial cells include inhibition of the inducible isoform of cyclo-oxygenase, as well as reduced mucin gene expression.^{67,68}

Repression of gene expression by GCs

Most evidence suggests that GC regulation of target genes occurs at the level of gene transcription, but regulation of gene expression for some cytokine genes is, at least partially, posttranscriptional.^{69,70} Repression of the genes encoding IL-11, GM-CSF, and cyclo-oxygenase in lung epithelial cells and fibroblasts is mediated by both transcriptional and posttranscriptional mechanisms.⁷¹⁻⁷³ Nevertheless, the primary mechanism of GC-mediated gene repression appears to be an effect on gene transcription.

At the transcriptional level, the molecular basis of GC action is due to either GR-mediated induction of specific target genes as a result of sequence-specific DNA binding (Fig 3) or GR-dependent repression of expression.⁷⁴ Repression of target gene expression by GCs might occur by means of transcriptional interference (ie, when a transcription factor is prevented from successfully interacting with the transcription initiation complex by means of direct or indirect interaction with another transcription factor). There is evidence that expression of multiple cytokine genes is mediated, at least in part, by the tran-

scription factors NF κ B and activator protein 1 (AP-1).⁷⁵ Therefore NF κ B and AP-1 might be important targets for GC-mediated repression cytokine gene expression.

Transcriptional repression through interaction with AP-1

Analyses of the promoters for many of the genes involved in inflammation have not demonstrated GRE or negative GRE sequences essential for binding of the activated GR. Examples of transcriptional repression that require GR binding to the DNA appear to be the exception rather than the rule.⁷⁶⁻⁷⁸ A protein-protein interaction model of repression has been developed from data derived from multiple studies. An example is GR-mediated repression of AP-1 induction of the collagenase gene.^{79,80} Studies have clearly demonstrated that GR represses collagenase gene transcription but does not bind directly to the collagenase gene promoter. AP-1 functions as a transcriptional activator of the collagenase gene. DNA-independent protein-protein interactions between the GR and the AP-1 components Jun and Fos are responsible for GR inhibition of AP-1-regulated target genes. Conversely, functional antagonism can also affect GR action, thus supporting the model of AP-1/GR protein-protein interaction.

The model of GR-mediated transcriptional repression independent of DNA binding has recently been corroborated by *in vivo* studies in mice. Reichardt et al⁸¹ knocked in a point mutation in the GR gene DNA-binding domain, leading to substitution of alanine by threonine at amino acid 458 in the second zinc finger. This mutation results in a dimerization-defective GR that is unable to bind cooperatively as a homodimer to GREs.⁸² In these mice (termed GR^{dim/dim} for dimerization defective) DNA-binding dependent transcriptional activation of genes is defective.⁸¹ In addition to the defect in GRE-mediated gene induction, the mice are deficient in repression of genes that are regulated through negative GREs, the prolactin and pro-opiomelanocortin genes. In contrast, transcriptional repression through protein-protein interactions is clearly intact in GR^{dim/dim} mice. Interference with AP-1 and repression of AP-1-regulated genes by the GR are intact in cells isolated from GR^{dim/dim} mice.

GR blockade of NF κ B signaling

AP-1 regulates only a portion of the cytokine genes that are important in inflammation. The transcription factor NF κ B is ubiquitous and is associated with the induction of multiple genes, including genes encoding cytokines, chemokines, and adhesion molecules, such as TNF- α , IL-1 β , IL-6, IL-8, RANTES, GM-CSF, ICAM-1, and VCAM-1. NF κ B is a member of the NF κ B/Rel multigene family of transcription factors, the activity of which is regulated by subcellular localization. NF κ B is a dimer most frequently composed of 2 subunits, p65 (RelA) and p50.⁸³ NF κ B transcriptional activity occurs when the p65/p50 dimer binds DNA at specific decameric DNA sequences, termed B sites (Fig 5). NF κ B resides in the cytoplasm of unstimulated cells, and DNA binding and transcriptional

activities of NF κ B are tightly controlled by accessory proteins termed inhibitory κ B (I κ B) subunits. The I κ B family of inhibitory molecules consists of several proteins, but I κ B α appears to play a central role in regulation of NF κ B in most cells.⁸⁴ I κ B proteins bind to NF κ B in the cytoplasm and block translocation to the nucleus and DNA binding by NF κ B. NF κ B is activated by various signals, including TNF- α , IL-1 β , stress, UV irradiation, oxidants, LPS, and viral agents. Activation signals lead to phosphorylation and degradation of I κ B with subsequent activation of NF κ B. I κ B α synthesis is transcriptionally regulated by NF κ B, such that after NF κ B activation, I κ B α is resynthesized quickly and inactivates NF κ B in the cytoplasm, thus terminating NF κ B activity.

Chemical cross-linking and overexpression studies have shown that the GR can interact directly with the p65 subunit of NF κ B,⁸⁵ thus blocking NF κ B binding to DNA and transcriptional activity (Fig 5). These data suggest a mechanism of NF κ B inhibition caused by the GR through protein-protein interaction that is similar to the mechanism of GR interference with AP-1 activity. Subsequent studies have demonstrated that the GR represses NF κ B-mediated activation of pro-inflammatory genes, such as *IL8* and *ICAM1*, by associating through protein-protein interaction with NF κ B bound at the gene promoter, but the GR did not disrupt DNA binding by NF κ B.⁸⁶ Instead, the mechanism of NF κ B inhibition by the GR appeared to be interference with phosphorylation of RNA polymerase II that is required for transcriptional activity. Other investigators have demonstrated that GR/NF κ B protein-protein interaction might interfere with the link between NF κ B and the basal transcription machinery.⁸⁷

Recent studies have examined the potential role of HATs and HDACs in repression of NF κ B-mediated gene expression by the GR. Ito et al⁸⁸ investigated the mechanism by which GCs repress IL-1 β -stimulated GM-CSF expression in epithelial cells and found that the site of cross-talk between the GR and NF κ B occurred at the level of regulation of histone H4 acetylation. IL-1 β -stimulated CBP associated histone acetylation at the GM-CSF promoter and the activated GR inhibited the acetylation through direct inhibition of CBP-associated HAT activity, but not that of CBP itself, and through recruitment of the corepressor HDAC2 to the NF κ B activation complex.

Although considerable data support protein-protein interaction between the GR and NF κ B as the mechanism of GR blockade of NF κ B signaling, 2 groups of investigators have reported that GCs are able to inhibit NF κ B activity by means of transcriptional activation of the *IKBA* gene (Fig 5).⁸⁸ As described above, the activation of NF κ B involves the targeted degradation of cytoplasmic I κ B inhibitor and translocation of NF κ B to the nucleus, where it binds to κ B response elements of inducible genes. Induction of the *IKBA* gene by GCs leads to an increased rate of I κ B α protein synthesis. It is proposed that the increased expression of I κ B α by GCs could block translocation of NF κ B to the nucleus. In several cell types it was demonstrated that after stimulation

by TNF- α in the presence of GC, NF κ B quickly reassociates with newly synthesized I κ B α , thus markedly decreasing the amount of NF κ B that translocates to the nucleus.⁸⁹ This mechanism of inhibition of NF κ B has not been supported by other studies in different cell types.^{90,91} In addition, recent studies in the GR^{dim/dim} mice (described above) established that inhibition of NF κ B signaling by the GR is intact.⁹² This demonstrates that the mechanism of I κ B α induction by GCs, if mediated by GR binding to a GRE at the I κ B α promoter, is not an important pathway of GR-mediated inhibition of NF κ B activity. Overall, the different mechanisms for GC action in various cell types likely contribute to the diversity of GC responses in different individuals.

GC RESISTANCE

Identification of GC resistance

Although GCs are highly effective anti-inflammatory agents, patients vary greatly in their responses to GCs. GC resistance has been most extensively studied in chronic asthma because the failure here to respond to GCs is more readily demonstrated than in other diseases (ie, by lack of improvement in pulmonary function after GC therapy). For research purposes, we have defined GC-resistant asthma as the failure to improve baseline morning prebronchodilator FEV₁ values by greater than 15% of predicted value after 7 to 14 days of 20 mg of twice daily oral prednisone.⁹³ GC-resistant asthma generally represents a relative insensitivity to GC therapy, and although some patients might respond to higher doses of oral prednisone or its equivalent administered for longer periods of time, such doses would be undesirable because of the marked adverse effects associated with prolonged courses of high-dose prednisone. In a clinical practice setting any patient not responding to 40 to 60 mg of daily prednisone after 3 weeks should be suspected of having GC-resistant asthma. Importantly, the diagnosis of GC-resistant asthma should only be made after an extensive evaluation to rule out other potential causes of wheezing or factors, which contribute to the severity of asthma. Patients with GC-resistant asthma should fulfill the American Thoracic Society criteria for diagnosis of asthma. Furthermore, they should have a significant bronchodilator response that distinguishes them from fixed airflow obstruction caused by chronic obstructive pulmonary disease or airway remodeling from severe asthma.

By using this definition of GC-resistant asthma, in one report nearly 25% of patients with difficult-to-control asthma had GC-resistant asthma.⁹⁴ Interestingly, a recent study found that nearly one third of asthmatic patients failed to show significant improvement in pulmonary function after treatment with inhaled corticosteroids.⁹⁵ GC resistance has also been reported in up to 30% of patients with rheumatoid arthritis,⁹⁶ systemic lupus erythematosus,⁹⁷ and severe ulcerative colitis (UC).^{98,99} Chronic sinusitis and atopic dermatitis have also been associated with GC insensitivity.^{100,101} Hearing et al¹⁰² recently studied the inhibitory effects of dexamethasone

TABLE I. Features of acquired type I GC-resistant asthma

Suppressed cortisol levels and cushingoid features on prednisone therapy
Increased levels of T-cell activation
Increased <i>IL2</i> and <i>IL4</i> gene expression in the airways
Failure of GCs to:
inhibit PHA-induced T-cell proliferation in vitro
decrease production of airway IL-2, IL-4, and IL-5 after GC therapy
reduce eosinophilia
suppress monocyte-macrophage secretion of monokines (eg, IL-8)
inhibit cutaneous tuberculin delayed skin responses
Decreased GR DNA- and ligand-binding affinity of mononuclear cells
Enhanced AP-1 transcriptional activity in PBMCs
Increased GR β expression in PBMCs and airway cells

on proliferation by peripheral blood lymphocytes from healthy volunteers and found that 30% of subjects had evidence of in vitro GC resistance, suggesting that up to 30% of the healthy population could fail to respond to steroid therapy for severe inflammatory conditions. These studies indicate that GC resistance is likely to be disease independent and might be more common than previously appreciated.

Mechanisms underlying GC resistance

The potential mechanisms for corticosteroid resistance have been studied in greatest detail in patients with GC-resistant asthma. We will therefore focus on studies carried out on GC-resistant asthma and examine their potential applicability to other illnesses.

Immune activation. There is a defective response of PBMCs from patients with GC-resistant asthma to corticosteroids, which correlates with clinical resistance to GC therapy.¹⁰³⁻¹⁰⁵ An increased expression of the CD25 and HLA-DR activation antigens is found on peripheral blood T cells of patients with GC-resistant asthma, even when they are receiving treatment with GCs.¹⁰³ A defect in GC-induced production of the anti-inflammatory cytokine IL-10 has also been observed in patients with GC-resistant asthma.¹⁰⁶ Furthermore, dexamethasone inhibits IL-2 production in cells from patients with GC-sensitive asthma but not in cells from patients with GC-resistant asthma. GC resistance in these individuals, however, is generally not absolute but reflects a shift in the dose-response curve, such that several-fold higher concentrations of steroids are required to inhibit T-cell proliferation of PBMCs from patients with GC-resistant asthma compared with patients with GC-sensitive asthma.¹⁰⁷

Leung et al¹⁰⁸ examined bronchoalveolar lavage (BAL) cells from patients with GC-sensitive and GC-resistant asthma. Before prednisone therapy, patients with GC-resistant and GC-sensitive asthma had similar numbers of BAL fluid total eosinophils and activated T cells. After 1 week of daily high-dose prednisone therapy, there was a significant decrease in BAL eosinophil counts and BAL-activated T cells in the GC-sensitive

asthma group. Prednisone therapy, however, was not accompanied by a decrease in BAL eosinophil counts or numbers of BAL-activated T cells in the GC-resistant asthma group. At baseline, BAL cells from patients with GC-resistant asthma had a significantly higher number of cells expressing mRNA for IL-2 and IL-4 than cells from patients with GC-sensitive asthma (Table I). However, no significant differences between these 2 patient populations were observed in the expression of IL-5 mRNA at baseline. Prednisone therapy was also found to decrease the number of BAL cells expressing IL-4 mRNA and IL-5 mRNA in patients with GC-sensitive but not in patients with GC-resistant asthma.

GR abnormalities. The GC resistance found in allergic and autoimmune diseases must be distinguished from the rare, primary, generalized GC resistance syndrome, which is caused by inactivating mutations of the GR gene.¹⁰⁹ DNA sequencing studies have not demonstrated any structural differences in the GR gene between patients with GC-resistant versus those with GC-sensitive asthma.¹¹⁰ Allergic and autoimmune diseases are associated with resistance of the inflamed tissues to GCs. However, because there is only a single GR gene, other noninflamed tissues are susceptible to GCs, and chronic treatment with high doses of systemic GCs to overcome the GC resistance-insensitivity of inflamed tissues can lead to significant GC side effects.

There are 2 major types of GC-resistant asthma.⁹³ Type I GC-resistant asthma is cytokine-induced or acquired. This grouping can further be divided into 2 subtypes: primary GC resistance involving immune responses that might be associated with genetic polymorphisms, leading to overproduction of certain cytokines (eg, IL-4) or various key molecules that can induce GC resistance. Acquired GC resistance might result from allergen- or infection-induced cell activation or chronic exposure to medications, such as β -agonists or corticosteroids (see below). Thus this type of GC resistance is likely to contain multiple subtypes that depend on the trigger or genetic background of the host.

Clinically, patients with this first type of GC resistance present as patients with GC-resistant asthma who have severe side effects, including adrenal gland suppression and cushingoid features from pharmacologic doses of systemic steroids (Table I). This is because there is only one GR gene, and the GC resistance of these patients is only at the level of their immune-inflammatory cells (eg, T cells). The rest of the tissues in their body are unfortunately quite sensitive to the deleterious effects of systemic steroids. Patients with this type I form of GC resistance have poor GR binding to GCs and DNA GRE, primarily in their mononuclear cells. This defect is reversible in vitro, when their cells are cultured in the absence of cytokines.

The combination of IL-2 and IL-4 induces T-cell steroid resistance.¹¹¹ Of note, monocyte GR binding affinity can be decreased by IL-13.¹¹² The difficulty in separating this group of patients into distinct subtypes is that overproduction of cytokines can occur as the result of both genetic and environmental exposures, leading to a common final

pathway for inducing decreased GR binding affinity. More studies are needed to determine whether there is overlap in these various groups or whether distinct phenotypes arise that result in reduced clinical responses to GCs.

Type II GC resistance involves generalized primary cortisol resistance, which affects all tissues and is likely associated with a mutation in the GR gene or in genes that modulate GR function. This form of GC-resistant asthma is not associated with the development of steroid-induced side effects, and the GR defect includes a low number of GRs. It is analogous to patients with primary familial cortisol resistance with genetic basis. Type II GC-resistant asthma has an irreversible GR defect that involves all cell types. In our experience most patients with a provisional diagnosis of primary GC-resistant asthma are simply non-adherent and not taking their medications. When patients present with a history of primary GC-resistant asthma, it is important to confirm they are taking the oral prednisone by checking for suppression of morning serum cortisol levels after a course of therapy under strict supervision, including mouth checks after administration of oral GCs. The cytokine-induced form (type I) accounts for greater than 95% of GC-resistant asthma.

Molecular mechanisms. The precise mechanism by which cytokines, such as the combination of IL-2 and IL-4, decrease GC responsiveness in patients with type I GC-resistant asthma is unknown. Several postulated mechanisms that are not mutually exclusive have been reported. The first is based on in vitro observations that cytokines induce the activation of transcription factors and that overexpression of AP-1 or other transcription factors interferes with GR binding to GRE DNA recognition sites. In this regard increased AP-1 expression has been reported in the PBMCs of patients with GC-resistant asthma, suggesting that abnormal GR/AP-1 interactions might contribute to corticosteroid insensitivity in asthma.¹¹³ These investigators have also reported increased c-fos transcription rates in T cells and monocytes from patients with GC-resistant asthma.¹¹⁴ Furthermore, pretreatment of mononuclear cells from patients with GC-resistant asthma with c-fos antisense oligonucleotides enhanced GR-DNA-binding activity in GC-resistant cells. These data suggested that increased c-fos synthesis might act as a mechanism for the increased AP-1 and decreased GR-DNA binding. Activation of AP-1 in GC-resistant asthma might be due to increased phosphorylation of Jun N-terminal kinase and the failure of GCs to inhibit Jun N-terminal kinase phosphorylation.¹¹⁵ There is also evidence that phosphorylation of the GR by MAP kinases might play a crucial role in the development of GC resistance, and histone acetylation has been postulated to alter GC responsiveness.^{116,117}

Previous studies have demonstrated that IL-2 can induce steroid resistance in mouse T cells and therefore might provide insight into the mechanism by which cytokines induce GC resistance in human subjects. Gol-eval et al¹¹⁸ found that when mouse T cells are treated with IL-2, the GR no longer translocates to the cell nucleus after dexamethasone treatment. IL-2-induced

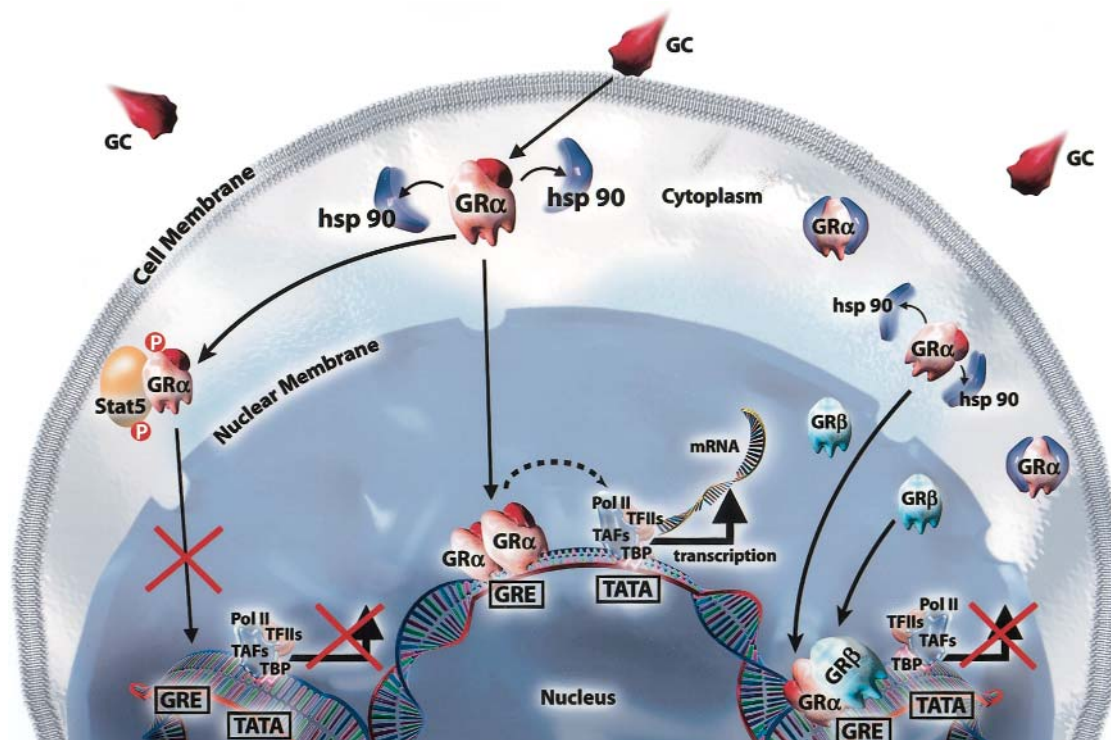


FIG 6. Mechanisms of GC resistance. The usual mechanism of transcriptional activation through the GR (center) is described in Fig 3. In IL-2-treated cells, the interaction of the GR with the STAT5 protein in the cytoplasm (left) prevents translocation of the GR to the nucleus, thus blocking transcriptional activation. The GR/STAT5 interaction appears to require phosphorylation of the GR and STAT5 (see text). An alternative, nonexclusive mechanism of resistance results from enhanced expression of the GR β isoform (right). GR β does not bind GC hormone, but in contrast to the GR, it is located in the nucleus of cells independent of hormone treatment. GR β can bind as a heterodimer with the GR at GREs and inhibit GR-mediated transcription by means of a dominant negative effect.

GC insensitivity in HT-2 cells appears to be a signaling event because the effects of IL-2 on nuclear translocation of the GR occur within 30 minutes, even in the presence of cycloheximide, and preincubation of cells with a Janus-associated kinase 3 inhibitor restored nuclear translocation of the GR, even in the presence of IL-2. Immunoprecipitation experiments revealed that phosphorylated STAT5 and the GR formed immune complexes. This association might lead to retardation of GR nuclear translocation because IL-2 was not able to induce steroid insensitivity in splenocytes from STAT5 knockout mice. This study demonstrates a novel role for STAT5 in IL-2-induced steroid insensitivity (Fig 6).

The most extensive investigation, however, has examined the potential role of GR β in GC resistance. Increased alternative splicing of exon 9 of the *GR* gene gives rise to increased levels of GR β , rather than GR α and could result in GC resistance. Unlike GR α , GR β is unable to bind GC hormones and is unable to activate GC-sensitive genes. Although GR β does not bind GC hormone, it is located in the nucleus of cells independent of hormone treatment. GR β has an intact DNA-binding domain and is able to bind to DNA at GREs.⁹⁻¹² GR β can inhibit GR α activity by binding with GR α as a heterodimer at GREs in the promoter of GR-regulated genes (Fig 6).¹¹⁹⁻¹²¹

This dominant negative activity of GR β makes it a potentially important mechanism of GC resistance. The combination of IL-2 and IL-4, IL-8, or TNF- α has been reported to induce the expression of GR β in different cell types.¹²¹⁻¹²³ The mechanism for induction of alternative splicing to GR β might be due to upregulation of trans-acting serine-arginine-rich proteins, which control alternative splicing by acting through specific cis-acting elements present in alternatively spliced exons.¹²⁴ Furthermore, a TNF-responsive NF κ B-binding site has been identified in the GR promoter that upregulates total GR expression.¹²³ Because the half-life of GR β protein is twice as long as that of GR α protein, TNF stimulation leads to a disproportionate accumulation of the GR β protein isoform over the GR α protein isoform accompanied by the development of GC resistance. Overall, these studies indicate several mechanisms through which pro-inflammatory cytokines lead to an increase in GR β levels and GC resistance.

The increased expression of GR β has now been reported in a number of diseases associated with GC resistance. In GC-resistant asthma we have found that airway cells and PBMCs express significantly higher levels of GR β than airway cells and PBMCs in patients with GC-sensitive asthma or healthy subjects.^{122,125} GR β expression

was significantly higher in airway T cells than in peripheral blood T cells, and the combination of IL-2 and IL-4 upregulated GR β expression, suggesting that the immune activation within the lung was driving the increased GR β expression. Of note, New World Monkeys, which have high-level systemic GC resistance, express 10-fold higher levels of GR β than GR α .¹²⁶ Interestingly, mice, which are known to be extremely GC-sensitive animals, do not have GR β .¹²⁷ When mouse cells are transduced with the *GRB* gene, they become steroid resistant.¹²⁰

Recently, Sousa et al¹²⁸ reported that GR β was increased in their cohort of patients with GC-resistant asthma who were previously described to have increased cFOS expression in their mononuclear cells as a potential cause of steroid resistance.¹¹⁴ These investigators studied the effects of oral corticosteroids on the tuberculin-mediated, cutaneous, delayed-typed hypersensitivity response in subjects with GC-sensitive and GC-resistant asthma. Interestingly, oral corticosteroids inhibited the delayed skin response in patients with GC-sensitive, but not in patients with GC-resistant, asthma, suggesting that steroid resistance affects the systemic immune response in GC-resistant asthma.

GR β as a general marker of GC resistance

If cytokine-driven expression of GR β is an important mechanism for GC resistance, it would be expected that increased expression of GR β could be found in various conditions associated with GC resistance or insensitivity. Indeed, there is accumulating evidence to support this concept.

Fatal asthma. Steroid resistance might be a major contributing factor to fatal asthma because many of these patients die in the emergency department despite receiving high doses of intravenous corticosteroids. Christodoulou et al¹²⁹ investigated the expression of GR β in the lungs of 7 patients who died of fatal asthma, 6 patients who died of emphysema, and 8 patients who died of nonpulmonary diseases. In tissue sections from both the large and small airways, there were significantly higher numbers of GR β -immunoreactive cells in patients with fatal asthma compared with that in patients with emphysema and control subjects. The expression of GR β was increased in the small and large airways of asthmatic patients. This study supports the association of GC resistance and fatal asthma.

Nocturnal asthma. Recently, it was found that PBMCs from patients with nocturnal asthma exhibit reduced steroid responsiveness at 4 AM compared with at 4 PM.¹³⁰ To further examine the mechanism for reduced GC responsiveness in nocturnal asthma, Kraft et al¹³¹ studied BAL lymphocytes and macrophages incubated in the presence and absence of dexamethasone. Dexamethasone suppressed proliferation of BAL lymphocytes similarly at 4 PM and 4 AM in asthmatic patients, regardless of the diagnosis of nocturnal asthma. However, BAL macrophages from only the nocturnal asthma group exhibited less suppression of IL-8 and TNF- α production by dexamethasone at 4 AM compared with 4 PM, whereas in the nonnocturnal asthma group dexamethasone suppressed IL-8 and TNF- α

production equally well at both time points. Of note, GR β expression was increased at night only in nocturnal asthma, primarily because of significantly increased IL-13 expression by BAL macrophages. IL-13 mRNA expression was increased at night but only in the nocturnal asthma group, and addition of neutralizing antibodies to IL-13 reduced GR β expression by BAL GR β macrophages. These data suggest that the airway macrophage might be the airway inflammatory cell driving the reduction in steroid responsiveness at night in nocturnal asthma, and this function is modulated by IL-13.

Chronic sinusitis. Chronic sinusitis with nasal polyposis is often associated with severe asthma. Nasal polyps (NPs) frequently demonstrate a poor response to treatment with intranasal steroids. Hamilos et al¹³² recently examined whether expression of GR β is increased in NPs and whether its level of expression might predict GC responsiveness. Biopsy specimens of the NPs were obtained 1 week before and 4 weeks after treatment with intranasal fluticasone. GR β expression was increased in NP inflammatory cells compared with that seen in control subjects. GR β expression in the initial NP biopsy specimens correlated inversely with steroid responsiveness in terms of reduction in eosinophils and reduction in immunostaining for endothelial VCAM-1 and RANTES in the pretreatment and posttreatment biopsy specimens. GR β expression also correlated with the number of activated CD4⁺ T lymphocytes in the posttreatment NP biopsy specimens. Thus GR β expression is a marker of steroid resistance in NPs.

Ulcerative colitis. Up to 30% of patients with UC do not respond to steroid treatment and require surgery or alternative anti-inflammatory medications, such as cyclosporine. Interestingly, these patients with GC-resistant UC have been found to have in vitro T-cell resistance to steroids.⁹⁸ Furthermore, GR β mRNA and protein levels in PBMCs of patients with GC-resistant UC was significantly higher than those of patients with GC-sensitive UC.⁹⁹ Overall, these data suggest that increased GR β expression predicts GC response in UC and supports the concept that a similar mechanism for GC resistance can be found in a number of different diseases.

Factors contributing to GC resistance

Because the majority of patients with GC-resistant asthma have an acquired form of GC resistance induced by immune activation, it is of interest to ascertain whether factors or conditions known to contribute to poorly controlled asthma and increased corticosteroid requirements have an effect on GR-binding affinity or response to corticosteroids.

Genetic predisposition. The topic of genetic predisposition remains largely unexplored. However, the importance of increased IL-4 production as a contributory factor to asthma severity and steroid resistance is supported by recent reports demonstrating an association between asthma severity and steroid resistance with genotypes known to enhance IL-4 production or action. In this regard Burchard et al¹³³ found an association between the IL-4 C-589→T sequence variant in the *IL4* gene promoter polymorphism

and decrement in pulmonary function among white asthmatic subjects. Of note, the IL-4 C-589→T sequence variant is associated with increased *IL4* gene transcription. Rosa-Rosa et al¹³⁴ has also found that the IL-4 receptor α sequence variant Q-576→R associated with enhanced response to IL-4 correlates with asthma severity.

The importance of IL-4 in the pathogenesis of GC resistance is further supported by a recent study done at National Jewish Medical and Research Center.¹³⁵ In these experiments we examined the potential association of GC-resistant asthma with a C-589→T sequence variant in the IL-4 promoter that results in enhanced *IL4* gene transcription and production. We examined 24 patients with GC-resistant asthma at National Jewish Medical and Research Center and compared them with 682 GC-sensitive asthmatic patients from other facilities. Overexpression of the T allele was statistically significant in GC-resistant asthma, with a *P* value of .009. These data provide a link between enhanced IL-4 production, as indicated by the presence of this DNA sequence variant, and GC resistance in asthmatic patients.

Interestingly, the frequency of this IL-4 C-589→T sequence variant is significantly greater among African American asthmatic patients than among white asthmatic patients.¹³³ This observation might account for the observations in a report by Spahn et al,¹³⁶ which demonstrated that T cells from African Americans display a diminished response to GCs in vitro. Chan et al⁹⁴ found that African Americans had a 38% prevalence of GC-resistant asthma compared with 12% in white patients.

Allergen exposure. Allergens can increase the amount of GCs required to control asthma. We have therefore examined whether exposure to allergens can alter GC response and GR-binding affinity in PBMCs from atopic asthmatic patients.¹³⁷ PBMC GR-binding affinity from asthmatic patients with ragweed allergy were found to be significantly decreased during ragweed pollen season compared with before and after the ragweed season. In vitro effects of allergen treatment on GR-binding affinity were also examined on PBMCs from asthmatic patients with cat allergy by incubating their cells with cat allergen. GR-binding affinity was significantly reduced after a 48-hour incubation with cat allergen. These effects were allergen specific because in PBMCs from the same subjects, *Candida albicans* had no effect on GR-binding affinity. Furthermore, allergen-induced reductions in GR-binding affinity were associated with significant decreases in the inhibitory effects of dexamethasone on T cells from these atopic asthmatic patients. The induction of GR-binding defects were reversible with antibodies that neutralized the effects of IL-2 and IL-4.

Microbial superantigens. To identify the potential stimulus for T-cell activation in patients with poorly controlled asthma, we analyzed the T-cell receptor repertoire of asthmatic patients whose symptoms were poorly controlled ($FEV_1 < 75\%$ predicted despite use of high doses of inhaled corticosteroids) compared with those of asthmatic patients whose symptoms were well controlled ($FEV_1 > 85\%$ predicted) and normal control subjects.¹³⁸

In patients with poorly controlled asthma, TCR-BV8+ (but not other TCR-BV+) T cells were significantly increased. These T-cell changes were not seen in the control groups. BV8+ BAL T cells were abnormally increased in both the CD4+ and CD8+ T-cell subsets of patients with poorly controlled asthma, suggesting activation by a microbial superantigen.¹³⁹

To determine whether microbial superantigens could alter GC sensitivity, we assessed the capacity of dexamethasone to inhibit the stimulation of normal PBMCs with a prototypic superantigen, staphylococcal enterotoxin B (SEB), versus PHA.¹⁴⁰ Dexamethasone caused a significant inhibition of PHA-induced PBMC proliferation but not of SEB-induced PBMC proliferation. These observations suggest that bacterial or viral agents secreting superantigens might contribute to poorly controlled asthma and reduced GC sensitivity. Superantigens have also been found to contribute to severe atopic dermatitis.^{141,142} Because poor response to GC therapy is often a criteria for severe atopic dermatitis, superantigen-induced steroid resistance in atopic dermatitis might be a contributing factor to disease severity. Interestingly, recent studies also indicate that SEB is a potent inducer of the GR β isoform in T cells.¹⁴⁰

Neutrophilia. Immunologic studies on GC-resistant asthma have generally involved patients with ongoing airway obstruction (ie, $FEV_1 < 75\%$ of predicted values) who were not taking chronic oral GCs. However, many patients with severe asthma and chronic airway obstruction are treated with prolonged courses of high-dose oral GCs. Wenzel et al¹⁴³ carried out bronchoscopic studies of the inflammatory cell infiltrate in patients with severe asthma who were dependent on high-dose oral corticosteroids and compared them with results for patients with moderate asthma (not requiring prednisone therapy) and healthy control subjects. The concentration of eosinophils in BAL fluid was highest in the patients with moderate asthma who were not taking prednisone, with little difference in eosinophils between healthy control subjects and patients with severe asthma. In contrast, the patients with severe asthma demonstrated a significantly higher numbers of airway neutrophils than either control group.

These studies indicate a distinct form of inflammation in patients with severe asthma despite treatment with high-dose oral corticosteroids. Importantly, it is well established that neutrophils are constitutively GC resistant. To determine the potential mechanism of GC resistance in neutrophils, we examined relative amounts of GR α and GR β in freshly isolated neutrophils.¹²¹ We observed increased GR β , but not GR α , protein and mRNA expression in neutrophils at baseline and after IL-8 exposure. Thus high constitutive expression of GR β by neutrophils might provide a mechanism by which these cells escape GC-induced cell death.

Management of GC resistance

The management of patients with GC resistance poses a considerable challenge to the clinician. These patients are often subjected to the unwanted side effects of prolonged

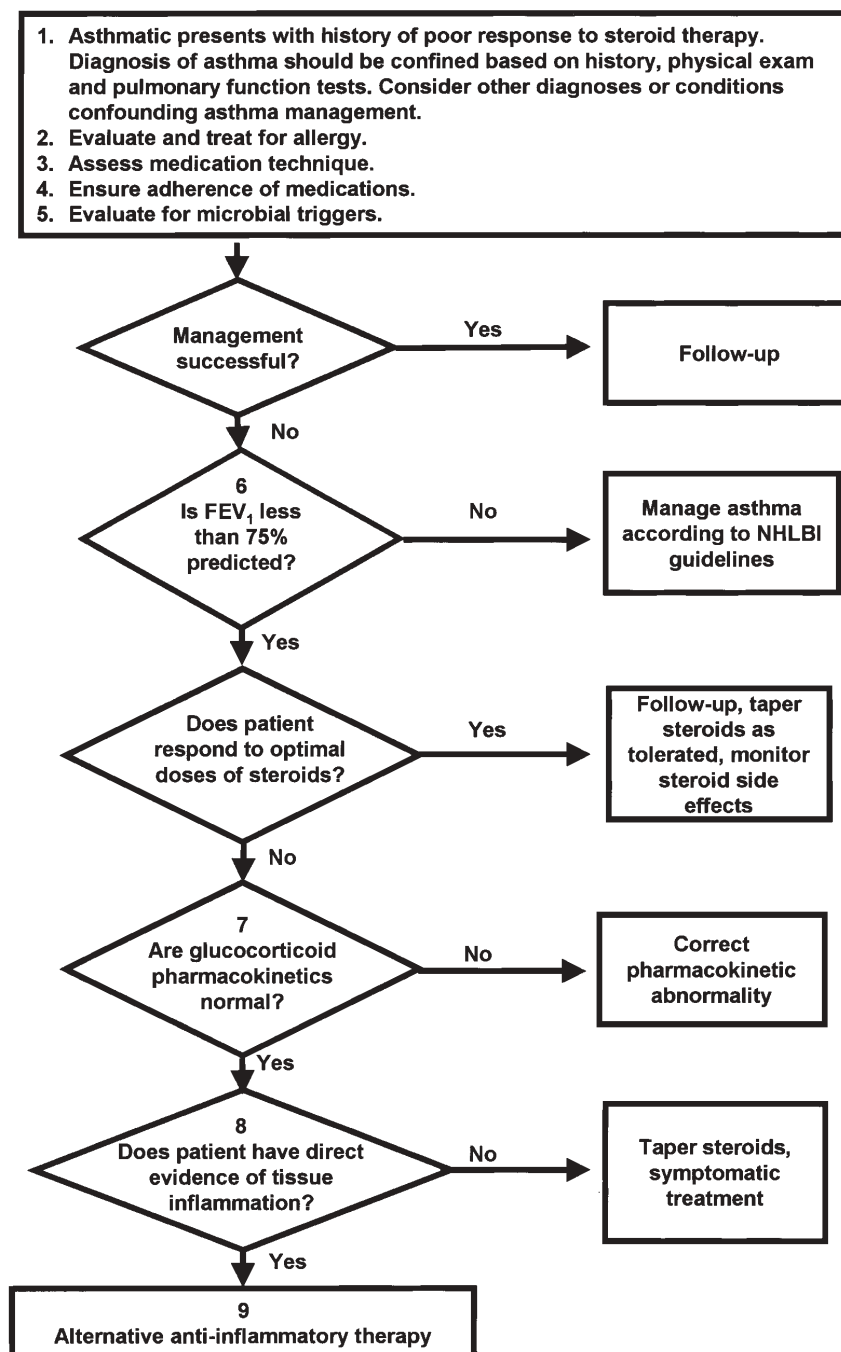


FIG 7. Algorithm for management of GC-resistant asthma.

systemic GC therapy in situations in which there is no evidence that it is exerting any appreciable benefit. Endogenous cortisol secretion has been found to be important in suppressing the magnitude of tissue allergic responses.¹⁴⁴ Therefore even a relative GC insensitivity might contribute to the severity of allergic and autoimmune diseases.

Fig 7 shows a potential algorithm for approaching GC-resistant asthmatic patients in a stepwise approach. A similar approach could be applied to the management of

other diseases associated with GC resistance. In a recent study we found that 25% of patients referred to the National Jewish Medical and Research Center in Denver for severe asthma had GC-resistant asthma.⁹⁴ Thus 75% of patients with severe asthma are GC sensitive and can be approached by optimizing management.

The first step is to obtain a thorough history, physical examination, and appropriate laboratory tests to confirm the diagnosis of asthma. In the case of patients presenting

with GC-resistant asthma, it is also critical to rule out concomitant medical disorders that can complicate the management of patients with chronic asthma but who are not GC responsive (eg, vocal cord dysfunction, gastroesophageal reflux, tracheomalacia, and chronic sinusitis).¹⁴⁵

The second step is to identify potential allergens that might trigger the patient's disease and institute appropriate environmental controls at home, in school, and at work. The focus should be on areas where the patient spends the greatest time. Patients with chronic asthma who are allergic to animals and live with these animals in the home require higher doses of steroids to maintain control of their asthma.¹⁴⁶ Several studies have also implicated schools as a major source of animal dander exposure.¹⁴⁷ As noted above, allergen exposure can induce GR insensitivity.¹³⁷

The third step is to review the patient's technique of medication administration. In the case of asthma, this should be incorporated as a routine part of the physical examination because patients often forget proper inhaler technique. This concept also applies to patients with other allergic diseases, such as atopic dermatitis and allergic rhinitis, where close attention must be paid to the application of skin medications, bathing-emollients, and nasal care. Patients should demonstrate their technique of medication delivery in the clinic to identify potential flaws in their technique.

The fourth step is to rule out psychosocial factors affecting the illness. A large proportion of patients presenting with a history of GC resistance have an inadequate response to therapy because of poor adherence with recommended therapy.¹⁴⁸ The basis for poor adherence is complex and can range from simple forgetfulness, in which case a medication diary or pill box is useful, to the inability to pay for the medications or severe psychologic problems, such as chronic depression, which impair the patient's ability to function and adhere to a medical regimen. Recognition of these underlying problems are important for the development of strategies to increase adherence to therapy. In addition, it is important to keep the medication regimen as simple as possible, to prioritize recommendations, to educate the patient regarding his or her asthma management, and to tailor the dosing to the patient's schedule. A written action plan for management of acute asthma exacerbations and routine prophylactic medications is often helpful for such patients.

The fifth step is to evaluate asthmatic patients for potential microbial infection of the airways. This is particularly important for patients taking high doses of inhaled steroids or chronic oral steroids because their local immune response might be compromised, thus predisposing them to colonization with opportunistic organisms, including *Mycoplasma* and *Chlamydia* species, which can trigger airway inflammation.^{149,150} Such individuals might respond to a long-term course of clarithromycin. In the case of atopic dermatitis, it has been found that staphylococci can produce superantigens that promote GC resistance, and treatment with antibiotics can improve the response of skin inflammation to topical steroids.¹⁵¹

The sixth step is to maximize combination therapy for control of disease symptoms. The recent introduction of

a combination inhaled steroid and long-acting β -agonist product can not only improve symptom control but also facilitate adherence. Inhaled salmeterol has been found to reduce corticosteroid requirements in asthma, leading to the recent release of combination salmeterol-fluticasone inhalation aerosols.^{152,153} Interestingly, salmeterol has been found to enhance nuclear translocation of the GR.¹⁵⁴ In patients with poorly controlled symptoms who have not had an adequate trial of leukotriene antagonists or theophylline, these medications should be considered because they can have steroid-sparing effects.^{155,156}

The seventh step is to evaluate systemic corticosteroid pharmacokinetics to maximize pulmonary function with oral corticosteroids.^{157,158} The purpose of these studies is to determine whether there is incomplete corticosteroid absorption, failure to convert to an active form, or rapid elimination. This evaluation is particularly important in a patient who fails to demonstrate the anticipated adverse effects of long-term, high-dose corticosteroid therapy. Measurements of plasma cortisol levels can also be used in an assessment of compliance. Patients with poor absorption of prednisone frequently respond well to oral liquid steroid preparations. In patients with rapid corticosteroid elimination, a split-dosing regimen, with the second dose of the day administered in the afternoon, should be considered. In such patients the morning dose should be titrated, followed by conversion of the afternoon dose to the morning dose and an attempt to reduce to alternate-day therapy.

The eighth step is to assess evidence for persistent tissue inflammation despite treatment with high-dose GCs. This could be approached by using markers of inflammation (eg, exhaled NO) or plasma eosinophilic cationic protein to examine medication response.¹⁵⁹ This is most useful before and after a 1- to 2-week course of oral prednisone therapy. However, in refractory patients it is more reliable to carry out bronchoscopy to directly examine the airways for evidence of airway inflammation in the BAL or bronchial biopsy specimens, although assessment of inflammatory cells in induced sputum is also becoming more common. Failure to respond to GCs with persistent increased levels of inflammation, despite treatment with high-dose prednisone, provides a strong basis for incorporating alternative therapies and the diagnosis of GC-resistant asthma.

The final step is to consider alternative anti-inflammatory and immunomodulator approaches. This is of particular importance in patients with the type II or primary form of GC-resistant asthma associated with a generalized primary GC resistance but also applies to patients with poorly controlled type I GC-resistant asthma. Unfortunately, there have been no well-controlled studies of alternative therapies in GC-resistant asthma. Treatment with intravenous immunoglobulin, cyclosporine, methotrexate, and gold and have been reported to have steroid-sparing effects and might be potentially useful in patients in whom steroid therapy fails.¹⁶⁰ Limited information from in vitro studies suggest that T cells from GC-resistant asthma will respond to the immunosuppressive actions of cyclosporine, thus providing a rationale for use of this agent in the management of these

patients.¹⁰⁵ In a recent study treatment of steroid-dependent asthma with intravenous immunoglobulin was associated with increased GR-binding affinity.¹⁶¹

To date, studies of these medications have not systematically incorporated bronchial biopsy and BAL fluid examination to verify resolution of inflammation, although several case reports have now demonstrated decreased airway inflammation in patients with GC-resistant asthma treated with intravenous immunoglobulin or cyclosporine.¹⁶²⁻¹⁶⁴ An organized program with carefully designed protocols and larger numbers of patients is needed to understand the role of these alternative anti-inflammatory therapies in the treatment of GC-resistant asthma and to identify a hierarchy of medication selection for patients with severe asthma.¹⁶⁵

In the future, more information is also needed on the pathology of severe asthma to determine whether there are ultrastructural abnormalities present that might be irreversible and might be GC resistant.¹⁶⁶ In this regard it is possible that aggressive courses of anti-inflammatory or immunomodulator therapy can suppress acute inflammation, but airway remodeling might predispose the patient to residual symptoms and the development of irreversible airway disease. Of greater concern is the possibility that the persistent symptoms in certain patients could be related to noninflammatory airways hyperresponsiveness. If this is indeed the case for certain individuals, then increasing the dose of GC therapy will predispose them to adverse effects with little gain in beneficial effect. In such patients their therapy should be directed to maximal bronchodilator therapy and symptomatic relief until new treatments are available to treat this component of chronic asthma. New therapies, such as anti-IgE or cytokine antagonists, might prove beneficial in the treatment of severe asthma. Anti-IgE could be useful in reducing the inflammatory response related to allergic inflammation.¹⁶⁷ Cytokine antagonism, such as therapy directed to antagonize IL-4 or IL-2, could restore steroid responsiveness.¹⁶⁸

There have been no systematic studies examining the long-term prognosis of patients with GC-resistant asthma. The major concern with this group of patients is that they might be at high risk for morbidity and mortality caused by asthma and the adverse effects of therapy, especially high-dose and long-term steroid therapy, that might alter their quality of life.¹⁶⁹ During acute exacerbations of their asthma, patients with type I GC-resistant asthma require much higher doses of intravenous corticosteroids than patients with GC-sensitive asthma to gain control of their inflammation. This places them at higher risk for steroid-induced side effects. Thus patients taking high-dose GCs must be monitored carefully for adverse effects related to GC therapy, and measures should be initiated to minimize their effect. For example, steroid-induced osteoporosis can be monitored with bone densitometry. Attention should be placed on providing adequate dietary calcium and vitamin D, as well as other therapeutic interventions, as indicated.^{170,171} It should be emphasized that patients with GC-resistant asthma do respond to bronchodilator therapy and that such medications should be instituted

early as rescue therapy. Finally, the presence of high-level persistent airway inflammation in this group of asthmatic patients predisposes them to the development of airway remodeling and long-term irreversible airways diseases. Thus it is of paramount importance to treat their inflammation early and effectively.¹⁷²

CONCLUSIONS

The pathways through which GC treatment leads to anti-inflammatory effects in disease are complex and require multiple steps, including expression of the GR by its gene, GR activation by steroid hormone, and regulation of anti-inflammatory and pro-inflammatory gene transcription. GC resistance might develop at any one of a number of points along this route. At the beginning of the pathway, alterations in the expression of GR protein could produce resistance. It is evident that changes, such as decreased expression of the functional GR or enhanced expression of the dominant negative GR β isoform, can diminish GR function. In regard to steroid activation of the cytoplasmic GR and subsequent translocation of GR to the nucleus, phosphorylation or other posttranslational alterations of the GR can affect GR functions. Phosphorylation of the GR can alter the interactions of the GR with other proteins in the cytoplasm, the localization of the GR to the nucleus, or the interactions of the GR with nuclear coactivator proteins. Any of these changes could affect GR-mediated transcriptional activation or repression of target genes. Finally, as described, GR-mediated effects on gene expression are complex and involve the recruitment of a large number of coactivator or repressor proteins by the GR into an extensive transcriptional complex that remodels chromatin and initiates transcription in both a gene-specific and cell type-specific manner. Obviously, this complicated process, which depends on the orchestrated interaction of so many factors in addition to the GR, could be interrupted at a number of points. In addition to direct effects on the GR, inflammatory mediators could produce GC resistance by altering levels or function of any one of the numerous coactivator or chromatin remodeling proteins, as well as directly affecting the chromatin structure through modification of histone proteins. This suggests the likelihood that inflammatory mediators might produce GC resistance by means of multiple distinct mechanisms that are potentially both cell type and gene specific.

Importantly, not all aspects of disease pathogenesis are inflammation driven. For example, the development of airway fibrosis or smooth muscle hyperplasia in severe asthma is not likely to respond to GC therapy. In the future, it will be important to develop reliable and easy-to-perform biomarkers of inflammation to monitor GC response and genetic markers of GC resistance so that such patients are started on alternative anti-inflammatory therapies earlier in the course of the illness.

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