REVIEW ARTICLE

MECHANISMS OF DISEASE

Antiinflammatory Action of Glucocorticoids — New Mechanisms for Old Drugs

Turk Rhen, Ph.D., and John A. Cidlowski, Ph.D.

I NFLAMMATION IS A REFLEXIVE RESPONSE TO INFECTION, THE BINDING of antibodies to antigens within the body, mechanical irritation, or injury.[1] Microbes that breach epithelial barriers, for instance, directly activate complement and toll-like receptors, two principal components of the innate immune system. The activation of these sentinels triggers the synthesis and release of inflammatory mediators with acute effects on the vasculature. Localized vasodilation, increased vascular permeability, extravasation of plasma (and humoral) proteins, and migration of leukocytes into the affected tissue produce the classic signs of inflammation: calor, dolor, rubor, tumor, and functio laesa. A positive feedback loop initiates the production of additional inflammatory cytokines once infiltrating leukocytes become activated. Antiinflammatory homeostatic mechanisms reverse these processes as the infectious agent is cleared by the innate and adaptive immune systems. The hypothalamic–pituitary–adrenal axis and glucocorticoids in particular are essential in limiting and resolving the inflammatory process.[2]

Whereas restricted inflammation is beneficial, excessive or persistent inflammation incites tissue destruction and disease. Together, inflammatory disorders such as allergies, asthma, autoimmune diseases, and sepsis are a major cause of illness and death. Asthma affects approximately 21.9 million adults and 8.9 million children in the United States alone. The prevalence of autoimmune diseases, which affect 8.5 million Americans,[3,4] is also noteworthy. Rheumatoid arthritis, Graves' disease, glomerulonephritis, type 1 diabetes mellitus, multiple sclerosis, thyroiditis, pernicious anemia, systemic lupus erythematosus, psoriasis, and vitiligo account for most of these autoimmune diseases. Sepsis is fatal for roughly 30 percent of the 700,000 patients affected annually in the United States.[5-7] Glucocorticoids are indicated for the treatment of many of these diverse conditions. The efficacy of glucocorticoids in alleviating inflammatory disorders results from the pleiotropic effects of the glucocorticoid receptor on multiple signaling pathways. Pleiotropy can, however, also have adverse effects: growth retardation in children, immunosuppression, hypertension, inhibition of wound repair, osteoporosis, and metabolic disturbances. All these harmful properties contraindicate prolonged glucocorticoid therapy. Here, we review mechanisms whereby glucocorticoids inhibit inflammation and the therapeutic limitations of these hormones. We then provide a prospectus for research on drugs that dissociate the beneficial and detrimental effects of glucocorticoids.

BASIC ACTIONS OF ENDOGENOUS GLUCOCORTICOIDS

The hypothalamic–pituitary–adrenal axis plays a central role in regulating signaling by the glucocorticoid receptor, which is expressed in virtually all cells. In brief, neural, endocrine, and cytokine signals converge at the level of the periventricular nucleus of the

hypothalamus to control the secretion of corticotropin-releasing hormone into the hypophyseal portal system (Fig. 1).[2,8] In turn, corticotropin-releasing hormone stimulates the release of corticotropin from the anterior pituitary. Corticotropin induces the synthesis and secretion of cortisol by the adrenal cortex. Most of the secreted cortisol (approximately 90 percent) is bound to corticosteroid-binding globulins in the blood.[9] Free cortisol is the biologically active form of the hormone and is converted to cortisone by type 2 11β-hydroxysteroid dehydrogenase.[10] Conversely, type 1 11β-hydroxysteroid dehydrogenase converts cortisone into cortisol.[10]

The glucocorticoid receptor is a member of the steroid-hormone–receptor family of proteins.[11,12] It binds with high affinity to cortisol; the bound cortisol promotes the dissociation of molecular chaperones, including heat–shock proteins, from the receptor (Fig. 2). Within the cell, cortisol acts in three ways. First, the cortisol–glucocorticoid receptor complex moves to the nucleus, where it binds as a homodimer (see the Glossary) to DNA sequences called glucocorticoid-responsive elements. The resulting complex recruits either coactivator or corepressor proteins that modify the structure of chromatin, thereby facilitating or inhibiting assembly of the basal transcription machinery and the initiation of transcription by RNA polymerase II.[13] This process is highly dynamic in cell culture and is presumably so in vivo.[14] Second, regulation of other glucocorticoid-responsive genes involves interactions between the cortisol–glucocorticoid receptor complex and other transcription factors, such as nuclear factor-κB (NF-κB) (Fig. 2).[15,16] These latter actions seem to occur at lower cortisol levels than the cortisol–glucocorticoid receptor–glucocorticoid-responsive element complex needs to change transcription. The third mechanism is glucocorticoid signaling through membrane-associated receptors and second messengers (so-called nongenomic pathways) (Fig. 2).[17,18] Evidence indicates that the glucocorticoid receptor inhibits inflammation through all three mechanisms: direct and indirect genomic effects and nongenomic mechanisms.
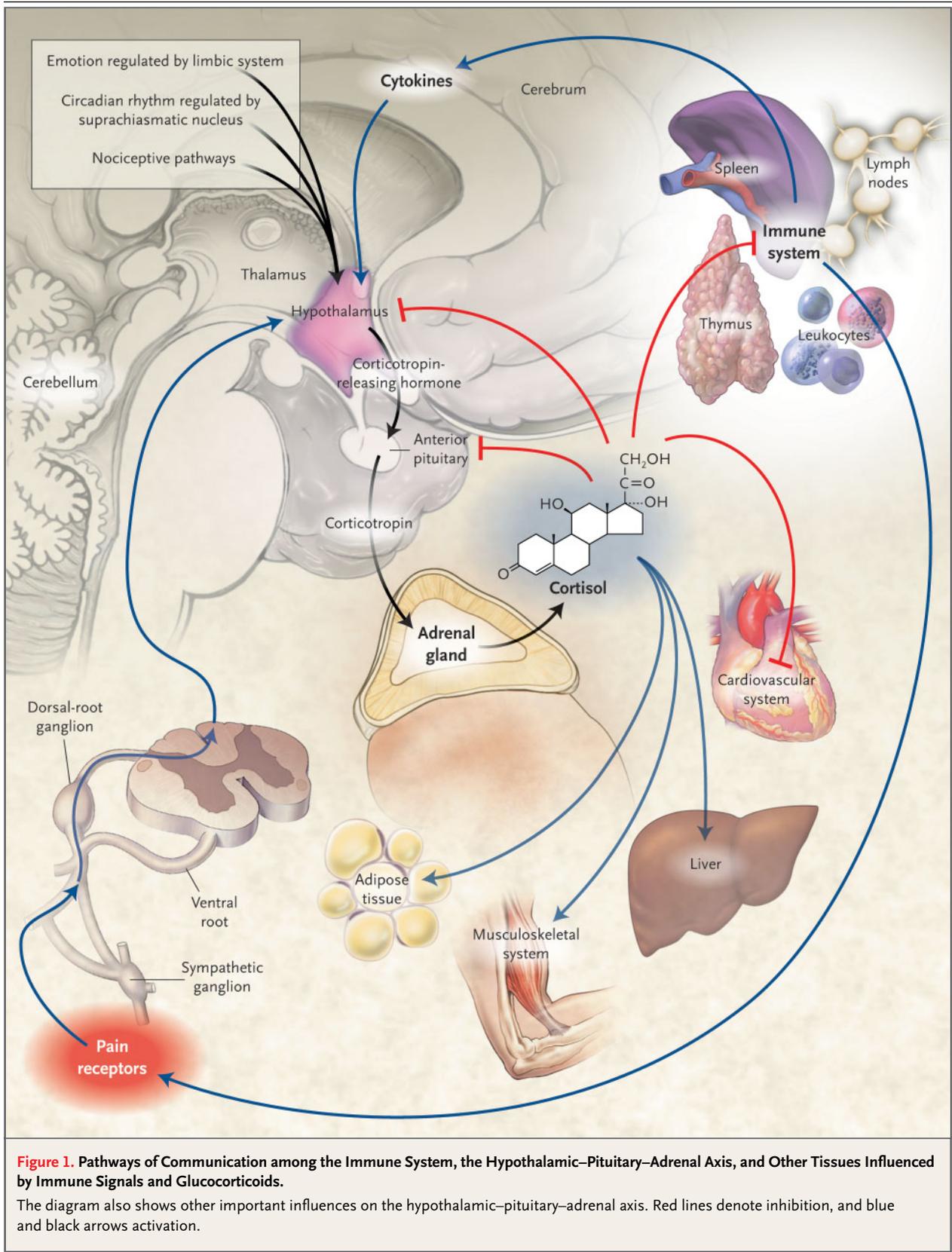
STRUCTURE OF THE GLUCOCORTICOID RECEPTOR

The human glucocorticoid receptor (*GR*) gene is one locus on chromosome 5q31–32 (Fig. 3). Even so, variation in the structure and the expression of the gene generates diversity in glucocorticoid signaling.[19] The genomic structure includes three transcription-initiation sites; each produces an alternative first exon that is spliced to a common exon 2 (Fig. 3). Though the first exon is not translated, there is a potential for functional differences among exons 1A, 1B, and 1C because dexamethasone up-regulates all three *GR* transcripts to a similar degree in acute lymphoblastic leukemia T cells but depresses these transcripts to different degrees in a B-cell line.[20,21] These observations highlight the importance of understanding the regulation of the expression of the glucocorticoid receptor in health and disease.

Human *GR* messenger RNA (mRNA) has alternative splice variants.[19] Whereas exons 2 through 8 are constant components of *GR* mRNA, there are two exon 9 isoforms that can be spliced to produce mature mRNA. Splicing of exon 9α produces *GRα* mRNA, which is translated into a protein with a unique sequence of 50 amino acids at its carboxy end (Fig. 3). The glucocorticoid receptor α isoform binds cortisol, DNA, and other transcription factors, thereby modifying transcriptional activity of target genes. Limited evidence suggests glucocorticoid receptor α may act through nongenomic pathways. Splicing of exon 9β produces *GRβ* mRNA, which is translated into a protein with 15 distinct amino acids at its carboxy end (Fig. 3).[19] Although glucocorticoid receptor β protein forms homodimers that bind DNA, it does not bind any ligands examined so far and fails to activate transcription. Glucocorticoid receptor β can also form heterodimers with glucocorticoid receptor α and interfere with the function of this protein. The relative levels of glucocorticoid receptor α and β in a cell influence the cell's sensitivity to glucocorticoid, with higher levels of glucocorticoid receptor β leading to glucocorticoid resistance.[22] The inflammatory cytokines tumor necrosis factor α (TNF-α) and interleukin-1 can selectively up-regulate the levels of glucocorticoid receptor β, suggesting its role in inflammation.[23-25]

Alternative translation-initiation sites within exon 2 produce additional isoforms of the glucocorticoid receptor[19] (Fig. 3). Translation from the first methionine codon in *GRα* and *GRβ* mRNA produces proteins that consist of 777 amino acids (glucocorticoid receptor α-A) and 742 amino acids (glucocorticoid receptor β-A). Translation from a second methionine produces proteins with 751 ami-

Figure 1. Pathways of Communication among the Immune System, the Hypothalamic–Pituitary–Adrenal Axis, and Other Tissues Influenced by Immune Signals and Glucocorticoids.

The diagram also shows other important influences on the hypothalamic–pituitary–adrenal axis. Red lines denote inhibition, and blue and black arrows activation.

no acids (glucocorticoid receptor α-B) and 716 amino acids (glucocorticoid receptor β-B), respectively. There are important functional differences between the two isoforms: glucocorticoid receptor α-B has roughly twice the biologic activity of glucocorticoid receptor α-A in gene-expression studies in vitro.[26] The finding that the two isoforms are expressed at different ratios in various types of cells and tissues also suggests that they may have distinct functions in vivo.[27]

POST-TRANSLATIONAL MODIFICATIONS OF THE GLUCOCORTICOID RECEPTOR

The human glucocorticoid receptor has five serine residues that are phosphorylated under different conditions by cyclin-dependent kinases and mitogen-activated protein kinases (MAPKs) (Fig. 3).[28] The phosphorylation of several of the serines is dependent on the binding of ligands such as cortisol to the glucocorticoid receptor, whereas other serines are phosphorylated in a ligand-independent manner. The specific combination of serines that are phosphorylated has distinct effects on its transcriptional activity. For example, the glucocorticoid receptor is found primarily in the cytoplasm and is inactive when phosphorylated at serine 203, but it actively transcribes DNA when phosphorylated at serine 211.[28] Another important modification of the glucocorticoid receptor that cortisol binding induces is the covalent attachment of ubiquitin to the receptor (Fig. 3), thus marking it for degradation by the proteasome[29]; however, this process can be cell-type specific.[30] Recent studies show that sumoylation (the attachment of small, ubiquitin-related modifiers) of the glucocorticoid receptor potentiates its transcriptional activity.[31,32] Little is known about the effect of post-translational modifications on the repression of gene transcription, interactions with other transcription factors, or nongenomic signaling pathways.

## NEUROENDOCRINE REGULATION OF INFLAMMATION

Interactions among the nervous system, the hypothalamic–pituitary–adrenal axis, and components of the innate and adaptive immune system play a key role in the regulation of inflammation and immunity (Fig. 1).[2,8] For instance, cytokines and inflammatory mediators activate peripheral pain receptors whose axons project to the dorsal horn and synapse with the lemniscal tract, which in turn carries pain signals to the thalamus and the somatosensory cortex. Activation of this nociceptive pathway ultimately stimulates hypothalamic–pituitary–adrenal activity. Glucocorticoids inhibit the synthesis of cytokines and inflammatory mediators, thus forming a negative feedback loop. Cytokines can also act directly on the brain to activate the hypothalamic–pituitary–adrenal axis. Dysregulation of this neuroendocrine loop by hyperactivity or hypoactivity of the hypothalamic–pituitary–adrenal axis causes systemic changes in inflammation and immunity.[2,8]

Hyperactivity of the hypothalamic–pituitary–adrenal axis in the absence of inflammation, as in Cushing's syndrome, causes immunosuppression and increased susceptibility to infection.[33] Physical pain, emotional trauma, and caloric restriction also activate the hypothalamic–pituitary–adrenal axis and cause immunosuppression.[34,35] In contrast, decreased activity of the axis and low levels of glucocorticoids increase susceptibility to and the severity of inflammation. Patients with Addison's disease, for example, require supplemental glucocorticoids during infection and inflammation to prevent the toxic effects of cytokines.[36] Dysregulation of the hypothalamic–pituitary–adrenal axis by inflammation is associated with adverse outcomes among patients with the acute respiratory distress syndrome.[37] Likewise, acquired glucocorticoid resistance is a common occurrence in patients with severe rheumatoid arthritis.[38] Glucocorticoid resistance is a common finding and can be due to decreased expression of glucocorticoid receptor α, increased expression of glucocorticoid receptor β, or activation of MAPK, which phosphorylates the glucocorticoid receptor and thereby inhibits glucocorticoid signaling.[39,40]
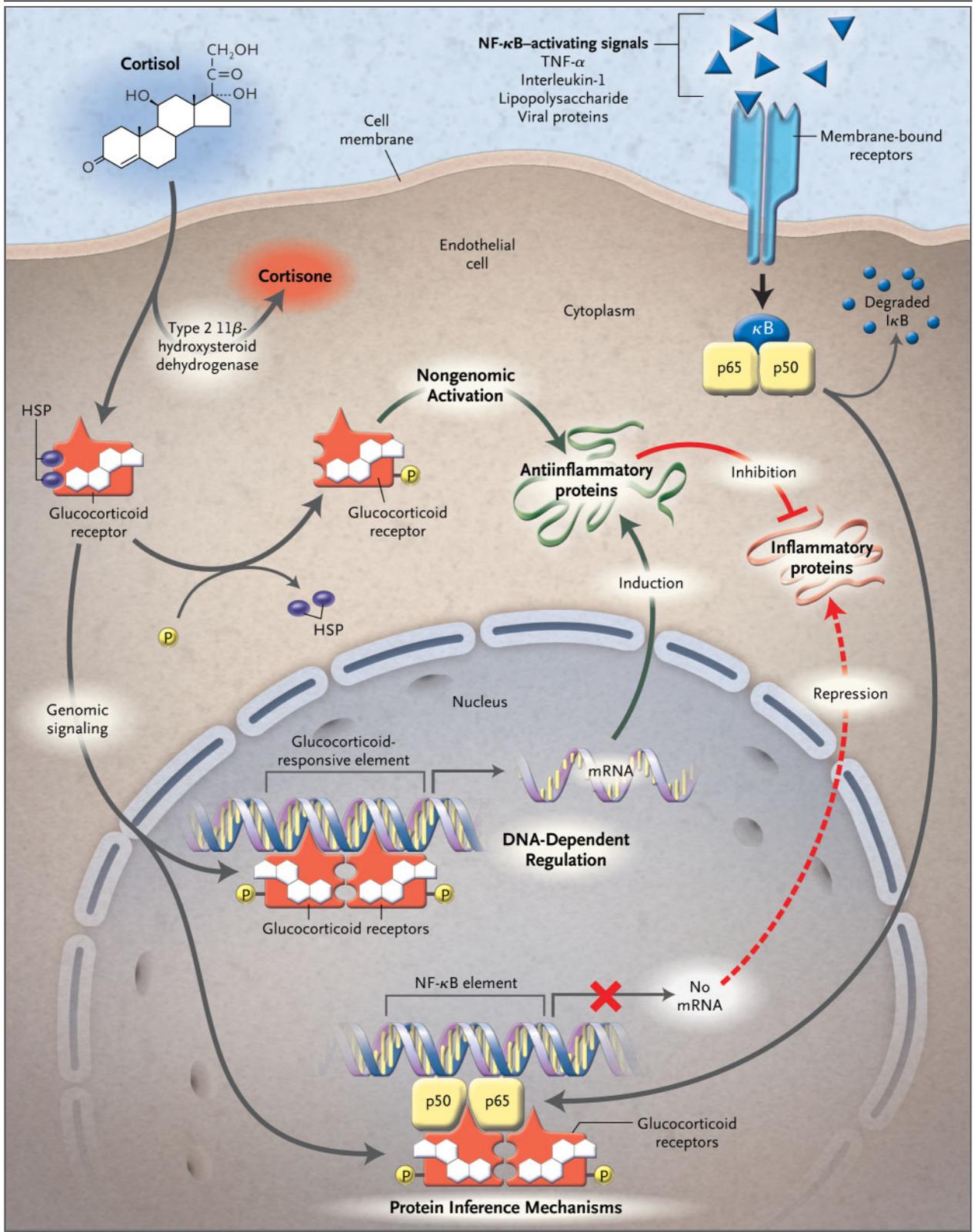
Glossary

Activator protein 1: An inflammatory transcription factor composed of two polypeptide subunits (c-Jun homodimers or c-Jun–Fos heterodimers)

Coactivator : A class of eukaryotic proteins that modulate the structure of chromatin or serve as a bridge between transcription factors and RNA polymerase II to facilitate the transcription of target genes

Corepressor: A class of eukaryotic proteins that modulate the structure of chromatin or inhibit RNA polymerase II to suppress transcription of target genes

Glucocorticoid-responsive element: A DNA motif usually found in the promoter of glucocorticoid-responsive genes that binds glucocorticoid receptor and enhances transcription

Heterodimer: A complex of two different protein chains held together by noncovalent bonds

Homodimer: A complex of two identical protein chains held together by noncovalent bonds

Negative glucocorticoid-responsive element: A DNA motif usually found in the promoter of glucocorticoid-responsive genes that binds glucocorticoid receptor and represses transcription

Nuclear factor-κB (NF-κB): An inflammatory transcription factor composed of two polypeptides from the Rel family of proteins

Pleiotropy: The ability of one gene to have multiple phenotypic effects

Transcription: The synthesis of RNA from a DNA template

Transactivation: The stimulation of transcription by a specific protein (i.e., transcription factor)

Transrepression: The inhibition of transcription by a specific protein (i.e., transcription factor)

ANTIINFLAMMATORY SIGNALING MECHANISMS

Glucocorticoids and the glucocorticoid receptor reside at the apex of a regulatory network that blocks several inflammatory pathways (Fig. 4). For example, glucocorticoids can inhibit prostaglandin production through three independent mechanisms: the induction and activation of annexin I, the induction of MAPK phosphatase 1, and the repression of transcription of cyclooxygenase 2. Annexin I (also called lipocortin-1) is an antiinflammatory protein that physically interacts with and inhibits cytosolic phospholipase $A_2\alpha$ ($cPLA_2\alpha$).[41-44] This calcium-binding protein requires elevated calcium levels and phosphorylation by the protein kinases MAPK, calcium/calmodulin–dependent kinase II, and MAPK interacting kinase to exert its enzymat-

ic activity.[45] The activation of $cPLA_2\alpha$ by inflammatory stimuli begins with the movement of the phospholipase from the cytosol to the perinuclear membrane, where it hydrolyzes phospholipids containing arachidonic acid. Glucocorticoids induce annexin I, which by inhibiting $cPLA_2\alpha$, blocks the release of arachidonic acid and its subsequent conversion to eicosanoids (i.e., prostaglandins, thromboxanes, prostacyclins, and leukotrienes). Mice lacking annexin I have elevated levels of $cPLA_2\alpha$, an exaggerated inflammatory response, and partial resistance to the antiinflammatory action of glucocorticoids.[46-48] A strong correlation exists between basal and corticotropin-stimulated cortisol levels and the expression of annexin I in neutrophils in humans, but the clinical importance of annexin I as an antiinflammatory protein is unknown.[49]

A second antiinflammatory protein induced by glucocorticoids is MAPK phosphatase 1 (Fig. 4).[50-52] Cytokines, bacterial and viral infections, and ultraviolet radiation are but a few of the inflammatory signals that activate MAPK cascades.[16] Ultraviolet light triggers a kinase cascade that phosphorylates and activates Jun N-terminal kinase, which in turn phosphorylates the transcription factor c-Jun. Phosphorylated c-Jun homodimers and c-Jun–Fos heterodimers bind DNA sequences called activator protein 1 response elements and induce the transcription of inflammatory and immune genes.[16] Glucocorticoid-induced MAPK phosphatase 1 dephosphorylates and inactivates Jun N-terminal kinase, thereby inhibiting c-Jun–mediated transcription. MAPK phosphatase 1 also dephosphorylates and inactivates all members of the MAPK family of proteins, including Jun N-terminal kinase, extracellular-signal–related kinase 1 and 2, and p38 kinase. Consequently, MAPK phosphatase 1 may also inhibit $cPLA_2\alpha$ activity by blocking its phosphorylation by MAPKs and MAPK-interacting kinase. In addition to blocking an essential upstream component of the c-Jun pathway, glucocorticoids and the glucocorticoid receptor directly interfere with c-Jun–mediated transcription (Fig. 4). Transcriptional interference between the glucocorticoid receptor and c-Jun homodimers (and c-Jun–Fos heterodimers) results from protein–protein interactions and has proved to be a major antiinflammatory mechanism.[16]

The cortisol–glucocorticoid receptor complex also physically interacts with NF-κB to block its transcriptional activity.[15,16] In its inactive state,
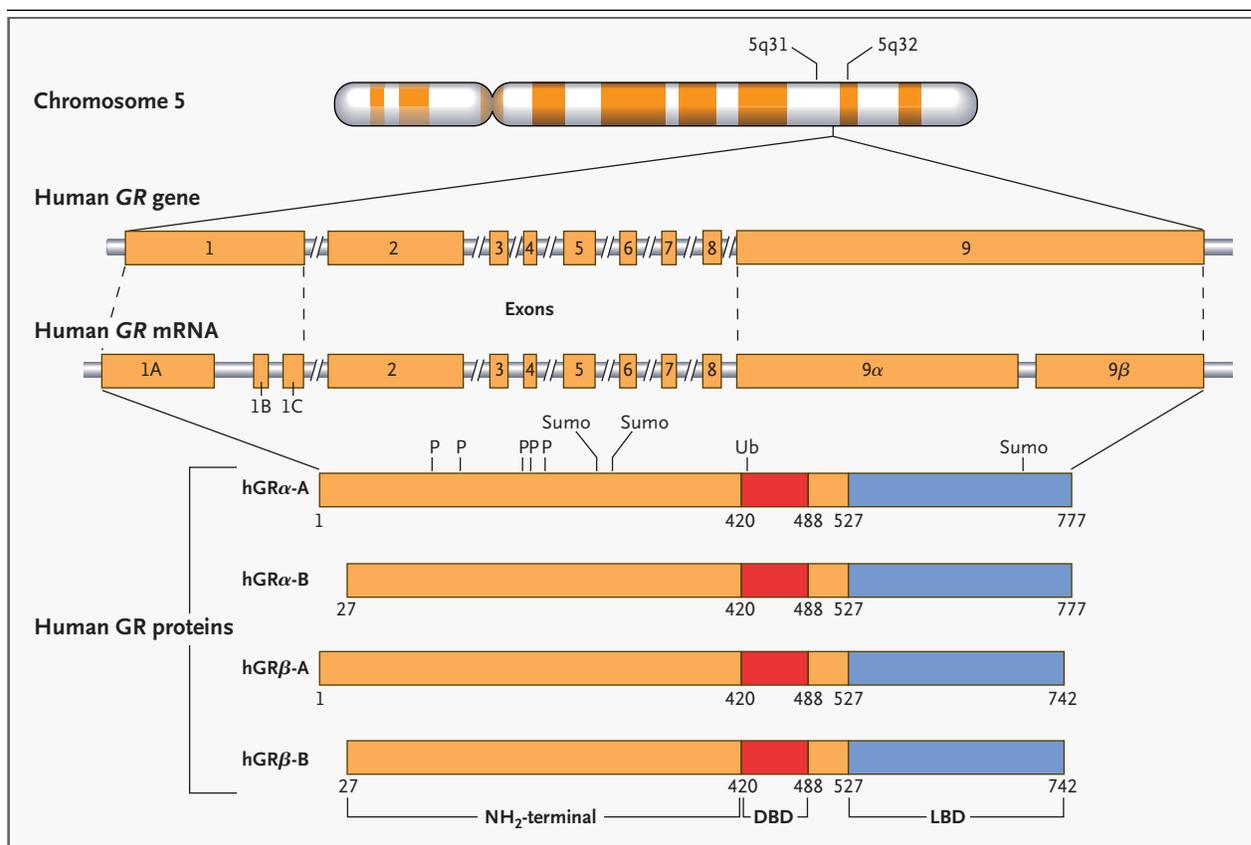
Figure 3. Genomic Location and Organization of the Human Glucocorticoid Receptor (GR).

Diversity in the expression and function of the glucocorticoid receptor results from alternative sites for the initiation of transcription (exon 1A, 1B, or 1C), as well as alternative splicing of pre–messenger RNA (mRNA) at exon 9α or 9β. Additional variation in the structure and function of the protein results from alternative sites for the initiation of translation within exon 2 and post-translational modifications in the form of phosphorylation (P), ubiquination (Ub), and sumoylation (Sumo). DBD denotes DNA-binding domain, LBD ligand-binding domain, and hGR human GR.

NF-κB is sequestered in the cytoplasm by an inhibitory protein named IκB. TNF-α, interleukin-1, microbial pathogens, viral infections, and other inflammatory signals trigger signaling cascades that activate IκB kinases (Fig. 2).[53] Phosphorylation of IκB leads to its ubiquination and degradation by the proteasome, unmasking a nuclear localization signal on NF-κB. In the nucleus, NF-κB binds DNA sequences called NF-κB elements and stimulates the transcription of cytokines, chemokines, cell-adhesion molecules, complement factors, and receptors for these molecules. NF-κB also induces the transcription of cyclooxygenase 2, an enzyme essential for prostaglandin production.[54] Thus, glucocorticoid-induced antagonism of NF-κB and repression of cyclooxygenase 2 is the third mechanism for the inhibition of prostaglandin synthesis after the induction of the antagonists of cPLA$_2\alpha$, annexin I, and MAPK phosphatase 1 (Fig. 4). Direct interactions between the glucocorticoid receptor and NF-κB probably account for most of the inhibitory effects of glucocorticoids on NF-κB signaling.[15,55] Despite the analogous nature of glucocorticoid receptor–mediated repression of activator protein 1 and NF-κB, different parts of the surface of the glucocorticoid receptor contact each transcription factor.[56] Glucocorticoids and the glucocorticoid receptor also modulate the activity of other transcription factors.[57]

Recent work suggests that glucocorticoids can have rapid effects on inflammation that are not mediated by changes in gene expression. The best-described nongenomic mechanism involves the activation of endothelial nitric oxide synthetase
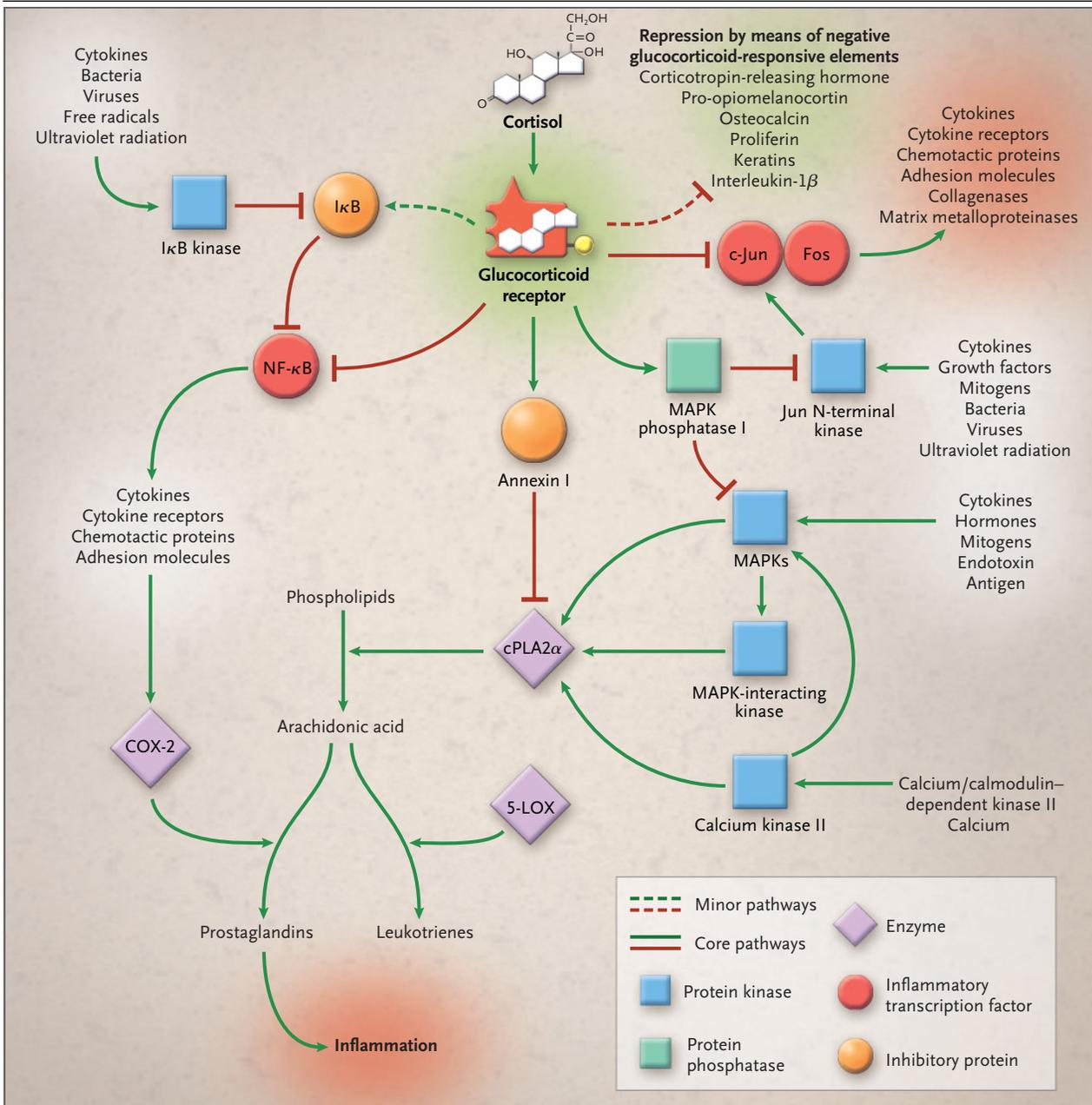
Figure 4. Partial Molecular Architecture Underlying the Glucocorticoid-Induced Antagonism of Inflammation.

Inflammatory pathways are characterized by positive feedback loops (i.e., cytokines activate NF-κB, which in turn stimulates the synthesis of more cytokines) and by redundancy (i.e., cytokines also activate c-Jun–Fos). The glucocorticoid receptor inhibits these pathways at multiple points by directly blocking the transcription of inflammatory proteins by NF-κB and activator protein 1 and by inducing the expression of antiinflammatory proteins such as IκB, annexin I, and MAPK phosphatase I. 5-LOX denotes 5-lipoxygenase, and COX-2 cyclooxygenase 2. Red lines denote inhibition, and black arrows activation. An interactive version of this figure is available with the full text of the article at www.nejm.org.

(eNOS).[17] Glucocorticoids stimulate the activity of phosphatidylinositol-3-hydroxykinase (PI3K) in a glucocorticoid receptor–dependent, but transcription-independent, manner in human endothelial cells. Activation of PI3K leads to phosphorylation of Akt. Phosphorylated Akt then phosphorylates and activates eNOS, resulting in the production of nitric oxide. In mice, glucocorticoid-induced acti-

vation of the PI3K–Akt–eNOS pathway protects against ischemia- or reperfusion-induced injury in the heart and the cremaster muscle. This finding is surprising because the production of nitric oxide is generally associated with vasodilation and inflammation.[58] More research is needed to clarify the role of nontranscriptional mechanisms in the inhibition of vasodilation, vascular permeability, and migration of leukocytes across endothelium.[59] Another mechanism of the glucocorticoid-induced inhibition of inflammation involves decreased stability of mRNA for genes for inflammatory proteins such as vascular endothelial growth factor and cyclooxygenase 2.[51,60,61] Glucocorticoids clearly act on diverse targets through multiple mechanisms to control inflammation.

LIMITATIONS OF GLUCOCORTICOID THERAPY

Although the benefits of glucocorticoid therapy are derived from short-term vascular changes and limited immunosuppression, prolonged or high-dose glucocorticoid therapy has multiple side effects (Table 1).[62] Here, we discuss specific mechanisms involved in a few of these side effects. For instance, extended glucocorticoid treatment can cause hypertension by two distinct mechanisms: one involves renal sodium retention and the ensuing increase in blood volume; a second results from potentiation of vasopressor responses to angiotensin II and catecholamines.[63] Enhanced responses to angiotensin II are due to the induction of angiotensin II receptors by glucocorticoids. Glucocorticoids do not affect the numbers or affinity of α_1-adrenergic receptors but, rather, potentiate downstream α_1-adrenergic signaling.[63]

Although the systemic vascular resistance induced by glucocorticoids is detrimental, localized changes in vasoreactivity may actually contribute to the beneficial effects of combined treatment with glucocorticoids and β_2-agonists in patients with asthma. Specifically, glucocorticoid-enhanced α_1-adrenergic signaling (i.e., pulmonary vasoconstriction) could counteract the unfavorable effects of β_2-agonists (i.e., pulmonary vasodilation). Patients with asthma have a normal response to β_2-agonists in terms of the relaxation of bronchial smooth muscles and increased flow rates, but they have an elevated baseline level of mucosal blood flow that is hypersensitive to vasoconstriction by α_1-agonists and insensitive to further vasodilation

Table 1. Tissue-Specific Side Effects of High-Dose or Prolonged Glucocorticoid Therapy.

Tissue	Side Effects
Adrenal gland	Adrenal atrophy, Cushing's syndrome
Cardiovascular system	Dyslipidemia, hypertension, thrombosis, vasculitis
Central nervous system	Changes in behavior, cognition, memory, and mood (i.e., glucocorticoid-induced psychoses), cerebral atrophy
Gastrointestinal tract	Gastrointestinal bleeding, pancreatitis, peptic ulcer
Immune system	Broad immunosuppression, activation of latent viruses
Integument	Atrophy, delayed wound healing, erythema, hypertrichosis, perioral dermatitis, petechiae, glucocorticoid-induced acne, striae rubrae distensae, telangiectasia
Musculoskeletal system	Bone necrosis, muscle atrophy, osteoporosis, retardation of longitudinal bone growth
Eyes	Cataracts, glaucoma
Kidney	Increased sodium retention and potassium excretion
Reproductive system	Delayed puberty, fetal growth retardation, hypogonadism

by β_2-agonists.[64] A two-week course of inhaled glucocorticoids decreased baseline perfusion of pulmonary mucosa and restored vascular responsiveness to β_2-agonists in patients with asthma.[65] In this study, glucocorticoid modulation of the action of α_1-agonists was not determined; thus, it is unclear whether catecholamine signaling was completely restored to normal. This work illustrates one of the potential advantages of inhaled glucocorticoids, which were developed to target lung tissue and thus decrease the adverse effects of systemic delivery.[66,67] Unfortunately, inhaled glucocorticoids are absorbed by the circulatory system and still cause side effects such as a decreased growth rate in children.[67,68]

Longitudinal growth in children is a result of the organized proliferation and differentiation of chondrocytes and the subsequent ossification of the extracellular matrix laid down in the growth plates of long bones.[69] Stem cells reside at the epiphyseal end of the growth plate and give rise to proliferating chondrocytes. Moving toward the metaphyseal bone, chondrocytes slow their rate of proliferation, begin to hypertrophy, and produce extracellular matrix proteins and matrix metalloproteinases. As chondrocytes synthesize this scaffolding, they take up calcium and secrete calcium phosphate and hy-

droxyapatite. Chondrocytes ultimately undergo apoptosis, leaving behind mineralized bone. Glucocorticoids slow longitudinal growth by reducing the proliferation of chondrocytes and inducing apoptosis of these cells. Inhibition of insulin-like growth factor I signaling is one mechanism underlying decreased chondrocyte proliferation.[69] In contrast to their effects in the circulatory system, glucocorticoid-induced apoptosis in chondrocytes involves the suppression of signaling through the Akt pathway.[70] Interestingly, insulin-like growth factor I increases the phosphorylation of Akt and acts as a survival factor in glucocorticoid-treated chondrocytes. Although there is generally a period of catch-up growth once glucocorticoid therapy is stopped, sustained treatment with substantive amounts of glucocorticoids during childhood is often associated with decreased adult stature.[69]

Glucocorticoids also have damaging effects on bone in adults. Osteoporosis and an increased risk of fractures are the main side effects of glucocorticoid therapy.[62] Osteoporosis is mediated in part by the binding of glucocorticoid receptors to negative glucocorticoid-responsive elements that inhibit transcription of osteocalcin in osteoblasts; osteocalcin is an important extracellular matrix protein that promotes bone mineralization.[71,72] Several other side effects of glucocorticoids, including the inhibition of corticotropin-releasing hormone and the expression of pro-opiomelanocortin, are also mediated by negative glucocorticoid-responsive elements (Fig. 4). Glucocorticoids exacerbate osteoporosis by inducing apoptosis in osteoblasts and by increasing the activity of osteoclasts. Some of these effects are directly mediated by glucocorticoid receptors in bone cells, whereas indirect effects are mediated by interactions with other endocrine signals.[73]

The repair of aseptic wounds is also inhibited by glucocorticoids. For example, fractures trigger inflammation and the production of cytokines crucial for the healing and remodeling of bone.[74-76] In addition to blocking cytokine signaling, glucocorticoids inhibit the synthesis of matrix metalloproteinases and collagen, which are important factors in wound repair.[77-80] Glucocorticoids also promote gluconeogenesis in the liver, the degradation of proteins to free amino acids in muscle (and muscle atrophy), and lipolysis,[81-83] ultimately producing hyperglycemia. There are currently no means of ameliorating the side effects of prolonged glucocorticoid therapy that function at the level of

the glucocorticoid receptor or the glucocorticoid-responsive elements; rather, treatments such as insulin (or its analogues) for glucocorticoid-induced diabetes, bisphosphonates for osteoporosis, and standard lipid regulators for dyslipidemia are often used.[84] This problem has led to research that has identified potentially selective glucocorticoids.

SELECTIVE GLUCOCORTICOIDS AND FUTURE THERAPY

The pleiotropic effects of glucocorticoids lie between two theoretical extremes. On the one hand, their manifold effects could be inseparable. Alternatively, each effect could be fully dissociated. It has been posited that the antiinflammatory effects of glucocorticoids are primarily mediated by the inhibition of NF-κB and activator protein 1, whereas their side effects result from the activation of transcription. Although this hypothesis is overly simplistic, a recent study described a novel glucocorticoid (ZK216348) with a pattern of repression and activation of transcription that was dramatically different from that of known glucocorticoids.[85] The level of glucocorticoid required to repress interleukin-8 in monocytes was 8 to 12 times as high as that required to induce tyrosine aminotransferase in liver cells. In contrast, the ratio of ZK216348 required to repress interleukin-8 to the level required to activate tyrosine aminotransferase was just 0.4, which was reflected in a better therapeutic index in vivo. Thus, it is possible to develop ligands that inhibit NF-κB–induced expression of inflammatory genes and activate transcription by means of glucocorticoid-responsive elements much more selectively than do currently available glucocorticoids.

Mechanistically, ligands with different structures induce different receptor conformations; for example, the position of helix 12 differs between glucocorticoid receptors bound to agonists and receptors bound to antagonists. Helix 12 closes behind dexamethasone as it sits in the hormone-binding pocket.[86,87] In this position, helix 12 recruits coactivators required for ligand-dependent transcription.[86] The antagonist mifepristone resides in the same pocket as dexamethasone but causes helix 12 to assume a position that precludes coactivator binding[87] and results in the recruitment of transcriptional corepressors.[88] Further comparison of glucocorticoid analogues reveals that they have divergent transcriptional activities.[89] It is also important that subtle mutations in the *GR* gene

can be used to differentiate the repression of transcription by activator protein 1 from repression by NF-κB[56] and that different glucocorticoids vary in their capacity to activate genomic and nongenomic mechanisms.[90] These observations highlight the potential for the development of selective glucocorticoids with improved therapeutic profiles.

The rational development of compounds that dissociate the effects of glucocorticoids will require intricate knowledge of the structure of receptors bound to various ligands and an understanding of the way different isoforms of the glucocorticoid receptor activate each signaling pathway. Several commercial entities are actively pursuing these goals.[91] Despite our optimism, it would be naive to suggest that therapeutic effects and side effects are mediated by separate mechanisms or that one could develop ligands that exclusively activate one molecular mechanism. Consequently, it will also be important to optimize the pharmacokinetic and pharmacodynamic properties of new drugs and to develop novel ways to target these drugs to inflamed tissues, as is the case with inhaled glucocorticoids.[67,92]

CONCLUSIONS

The potency of glucocorticoids as inhibitors of diverse inflammatory disorders guarantees their continued use as therapeutic agents. The antiinflammatory and immunosuppressive effects of glucocorticoids rely on several molecular mechanisms, which have been elucidated by basic research. Three main mechanisms include direct effects on gene expression by the binding of glucocorticoid receptors to glucocorticoid-responsive elements (i.e., the induction of annexin I and MAPK phosphatase 1), indirect effects on gene expression through the interactions of glucocorticoid receptors with other transcription factors (i.e., NF-κB and activator protein 1), and glucocorticoid receptor–mediated effects on second-messenger cascades (i.e., the PI3K–Akt–eNOS pathway). Unfortunately, because some of these mechanisms are also involved in physiologic signaling rather than inflammatory signaling, the therapeutic effects of glucocorticoids in inflammation are often accompanied by clinically significant side effects. It is unclear whether isoforms of the glucocorticoid receptor are differentially involved in signaling through each of these mechanisms. Similarly, we do not know whether glucocorticoid-induced activation of certain mechanisms alleviates specific diseases or causes particular side effects. If this sort of signaling specificity exists in vivo, there will be tremendous potential for the development of synthetic ligands that activate antiinflammatory mechanisms but do not affect other pathways. Such drugs would in essence mimic the beneficial effects of natural glucocorticoids without their detrimental side effects.

REFERENCES

1. Gallin JI, Goldstein IM, Snyderman R. Overview. In: Gallin JI, Goldstein IM, Snyderman R, eds. Inflammation: basic principles and clinical correlates. 2nd ed. New York: Raven Press, 1992:1-4.

2. Webster JI, Tonelli L, Sternberg EM. Neuroendocrine regulation of immunity. Annu Rev Immunol 2002;20:125-63.

3. Jacobson DL, Gange SJ, Rose NR, Graham NM. Epidemiology and estimated population burden of selected autoimmune diseases in the United States. Clin Immunol Immunopathol 1997;84:223-43.

4. Cooper GS, Stroehla BC. The epidemiology of autoimmune diseases. Autoimmun Rev 2003;2:119-25.

5. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med 2001;29:1303-10.

6. Riedemann NC, Guo RF, Ward PA. The enigma of sepsis. J Clin Invest 2003;112:460-7.

7. Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. N Engl J Med 2003;348:1546-54.

8. Rivest S. How circulating cytokines trigger the neural circuits that control the hypothalamic-pituitary-adrenal axis. Psychoneuroendocrinology 2001;26:761-88.

9. Breuner CW, Orchinik M. Plasma binding proteins as mediators of corticosteroid action in vertebrates. J Endocrinol 2002;175:99-112.

10. Yang S, Zhang L. Glucocorticoids and vascular reactivity. Curr Vasc Pharmacol 2004;2:1-12.

11. Laudet V, Hanni C, Coll J, Catzeflis F, Stehelin D. Evolution of the nuclear receptor gene superfamily. EMBO J 1992;11:1003-13.

12. Rhen T, Cidlowski JA. Steroid hormone action. In: Strauss JF III, Barbieri RL, eds. Yen and Jaffe's reproductive endocrinology. 5th ed. Philadelphia: Elsevier Saunders, 2004:155-74.

13. Hebbar PB, Archer TK. Chromatin remodeling by nuclear receptors. Chromosoma 2003;111:495-504.

14. Nagaich AK, Rayasam GV, Martinez ED, et al. Subnuclear trafficking and gene targeting by steroid receptors. Ann N Y Acad Sci 2004;1024:213-20.

15. McKay LI, Cidlowski JA. Molecular control of immune/inflammatory responses: interactions between nuclear factor-kappa B and steroid receptor-signaling pathways. Endocr Rev 1999;20:435-59.

16. De Bosscher K, Vanden Berghe W, Haegeman G. Interplay between the glucocorticoid receptor and nuclear factor-κB or activator protein-1: molecular mechanisms for gene repression. Endocr Rev 2003;24:488-522.

17. Hafezi-Moghadam A, Simoncini T, Yang Z, et al. Acute cardiovascular protective effects of corticosteroids are mediated by non-transcriptional activation of endothelial nitric oxide synthase. Nat Med 2002;8:473-9.

18. Cato AC, Nestl A, Mink S. Rapid actions of steroid receptors in cellular signaling pathways. Sci STKE 2002;2002(138):RE9.

19. Lu NZ, Cidlowski JA. The origin and functions of multiple human glucocorticoid receptor isoforms. Ann N Y Acad Sci 2004; 1024:102-23.

20. Zhang T, Haws P, Wu Q. Multiple variable first exons: a mechanism for cell- and tissue-specific gene regulation. Genome Res 2004;14:79-89.

21. Pedersen KB, Vedeckis WV. Quantification and glucocorticoid regulation of glucocorticoid receptor transcripts in two human leukemic cell lines. Biochemistry 2003;42: 10978-90.

22. Pujols L, Mullol J, Perez M, et al. Expression of the human glucocorticoid receptor alpha and beta isoforms in human respiratory epithelial cells and their regulation by dexamethasone. Am J Respir Cell Mol Biol 2001;24:49-57.

23. Webster JC, Oakley RH, Jewell CM, Cidlowski JA. Proinflammatory cytokines regulate human glucocorticoid receptor gene expression and lead to the accumulation of the dominant negative beta isoform: a mechanism for the generation of glucocorticoid resistance. Proc Natl Acad Sci U S A 2001;98: 6865-70.

24. Gagliardo R, Vignola AM, Mathieu M. Is there a role for glucocorticoid receptor beta in asthma? Respir Res 2001;2:1-4.

25. Torrego A, Pujols L, Roca-Ferrer J, Mullol J, Xaubet A, Picado C. Glucocorticoid receptor isoforms alpha and beta in in vitro cytokine-induced glucocorticoid insensitivity. Am J Respir Crit Care Med 2004;170:420-5.

26. Yudt MR, Cidlowski JA. Molecular identification and characterization of A and B forms of the glucocorticoid receptor. Mol Endocrinol 2001;15:1093-103.

27. Lu NZ, Cidlowski JA. Translational regulatory mechanisms generate N-terminal glucocorticoid receptor isoforms with unique transcriptional target genes. Mol Cell 2005; 18:331-42.

28. Ismaili N, Garabedian MJ. Modulation of glucocorticoid receptor function via phosphorylation. Ann N Y Acad Sci 2004;1024: 86-101.

29. Wallace AD, Cidlowski JA. Proteasome-mediated glucocorticoid receptor degradation restricts transcriptional signaling by glucocorticoids. J Biol Chem 2001;276: 42714-21.

30. Wang X, Pongrac JL, DeFranco DB. Glucocorticoid receptors in hippocampal neurons that do not engage proteasomes escape from hormone-dependent down-regulation but maintain transactivation activity. Mol Endocrinol 2002;16:1987-98.

31. Le Drean Y, Mincheneau N, Le Goff P, Michel D. Potentiation of glucocorticoid receptor transcriptional activity by sumoylation. Endocrinology 2002;143:3482-9.

32. Holmstrom S, Van Antwerp ME, Iniguez-Lluhi JA. Direct and distinguishable inhibitory roles for SUMO isoforms in the control of transcriptional synergy. Proc Natl Acad Sci U S A 2003;100:15758-63.

33. Lionakis MS, Kontoyiannis DP. Glucocorticoids and invasive fungal infections. Lancet 2003;362:1828-38.

34. Delbende C, Delarue C, Lefebvre H, et al. Glucocorticoids, transmitters, and stress. Br J Psychiatry 1992;Suppl 15:24-35.

35. Miller DB, O'Callaghan JP. Neuroendocrine aspects of the response to stress. Metabolism 2002;51:Suppl 1:5-10.

36. Kapcala LP, Chautard T, Eskay RL. The protective role of the hypothalamic-pituitary-adrenal axis against lethality produced by immune, infectious, and inflammatory stress. Ann N Y Acad Sci 1995;771:419-37.

37. Meduri GU, Yates CR. Systemic inflammation-associated glucocorticoid resistance and outcome of ARDS. Ann N Y Acad Sci 2004;1024:24-53.

38. Chikanza IC. Mechanisms of corticosteroid resistance in rheumatoid arthritis: a putative role for the corticosteroid receptor beta isoform. Ann N Y Acad Sci 2002;966: 39-48.

39. Tsitoura DC, Rothman PB. Enhancement of MEK/ERK signaling promotes glucocorticoid resistance in CD4+ T cells. J Clin Invest 2004;113:619-27.

40. Li LB, Goleva E, Hall CF, Ou LS, Leung DY. Superantigen-induced corticosteroid resistance of human T cells occurs through activation of the mitogen-activated protein kinase kinase/extracellular signal-regulated kinase (MEK-ERK) pathway. J Allergy Clin Immunol 2004;114:1059-69.

41. Solito E, de Coupade C, Parente L, Flower RJ, Russo-Marie F. IL-6 stimulates annexin 1 expression and translocation and suggests a new biological role as class II acute phase protein. Cytokine 1998;10:514-21.

42. Mizuno H, Uemura K, Moriyama A, et al. Glucocorticoid induced the expression of mRNA and the secretion of lipocortin 1 in rat astrocytoma cells. Brain Res 1997;746: 256-64.

43. Antonicelli F, De Coupade C, Russo-Marie F, Le Garrec Y. CREB is involved in mouse annexin A1 regulation by cAMP and glucocorticoids. Eur J Biochem 2001;268: 62-9.

44. Kim SW, Rhee HJ, Ko J, et al. Inhibition of cytosolic phospholipase A2 by annexin I: specific interaction model and mapping of the interaction site. J Biol Chem 2001;276: 15712-9.

45. Hirabayashi T, Murayama T, Shimizu T. Regulatory mechanism and physiological role of cytosolic phospholipase A2. Biol Pharm Bull 2004;27:1168-73.

46. Roviezzo F, Getting SJ, Paul-Clark MJ, et al. The annexin-1 knockout mouse: what it tells us about the inflammatory response. J Physiol Pharmacol 2002;53:541-53.

47. Lim LH, Solito E, Russo-Marie F, Flower RJ, Perretti M. Promoting detachment of neutrophils adherent to murine postcapillary venules to control inflammation: effect of lipocortin 1. Proc Natl Acad Sci U S A 1998; 95:14535-9.

48. Pitzalis C, Pipitone N, Perretti M. Regulation of leukocyte-endothelial interactions by glucocorticoids. Ann N Y Acad Sci 2002; 966:108-18.

49. Mulla A, Leroux C, Solito E, Buckingham JC. Correlation between the anti-inflammatory protein annexin 1 (lipocortin 1) and serum cortisol in subjects with normal and dysregulated adrenal function. J Clin Endocrinol Metab 2005;90:557-62.

50. Kassel O, Sancono A, Kratzschmar J, Kreft B, Stassen M, Cato AC. Glucocorticoids inhibit MAP kinase via increased expression and decreased degradation of MKP-1. EMBO J 2001;20:7108-16.

51. Lasa M, Abraham SM, Boucheron C, Saklatvala J, Clark AR. Dexamethasone causes sustained expression of mitogen-activated protein kinase (MAPK) phosphatase 1 and phosphatase-mediated inhibition of MAPK p38. Mol Cell Biol 2002;22:7802-11.

52. Toh ML, Yang Y, Leech M, Santos L, Morand EF. Expression of mitogen-activated protein kinase phosphatase 1, a negative regulator of the mitogen-activated protein kinases, in rheumatoid arthritis: up-regulation by interleukin-1beta and glucocorticoids. Arthritis Rheum 2004;50:3118-28.

53. Rhen T, Cidlowski JA. Nuclear factor-κB and glucocorticoid receptors. In: Martini L, ed. Encyclopedia of endocrine diseases. Vol. 3. Boston: Elsevier Academic Press, 2004:391-8.

54. Tanabe T, Tohnai N. Cyclooxygenase isozymes and their gene structures and expression. Prostaglandins Other Lipid Mediat 2002;68-69:95-114.

55. Nissen RM, Yamamoto KR. The glucocorticoid receptor inhibits NFkappaB by interfering with serine-2 phosphorylation of the RNA polymerase II carboxy-terminal domain. Genes Dev 2000;14:2314-29.

56. Bladh LG, Liden J, Dahlman-Wright K, Reimers M, Nilsson S, Okret S. Identification of endogenous glucocorticoid repressed genes differentially regulated by a glucocorticoid receptor mutant able to separate between nuclear factor-kappaB and activator protein-1 repression. Mol Pharmacol 2005;67:815-26.

57. Barnes PJ, Adcock IM. Transcription factors and asthma. Eur Respir J 1998;12:221-34.

58. Ortiz PA, Garvin JL. Cardiovascular and renal control in NOS-deficient mouse models. Am J Physiol Regul Integr Comp Physiol 2003;284:R628-R638.

59. Perretti M, Ahluwalia A. The microcirculation and inflammation: site of action for glucocorticoids. Microcirculation 2000;7: 147-61.

60. Saklatvala J, Dean J, Clark A. Control of the expression of inflammatory response genes. Biochem Soc Symp 2003;70:95-106.

61. Gille J, Reisinger K, Westphal-Varghese B, Kaufmann R. Decreased mRNA stability as a mechanism of glucocorticoid-mediated inhibition of vascular endothelial growth factor gene expression by cultured keratinocytes. J Invest Dermatol 2001;117:1581-7.

62. Schacke H, Docke WD, Asadullah K.

Mechanisms involved in the side effects of glucocorticoids. Pharmacol Ther 2002;96:23-43.

63. Ullian ME. The role of corticosteroids in the regulation of vascular tone. Cardiovasc Res 1999;41:55-64.

64. Brieva J, Wanner A. Adrenergic airway vascular smooth muscle responsiveness in healthy and asthmatic subjects. J Appl Physiol 2001;90:665-9.

65. Brieva JL, Danta I, Wanner A. Effect of an inhaled glucocorticosteroid on airway mucosal blood flow in mild asthma. Am J Respir Crit Care Med 2000;161:293-6.

66. Umland SP, Schleimer RP, Johnston SL. Review of the molecular and cellular mechanisms of action of glucocorticoids for use in asthma. Pulm Pharmacol Ther 2002;15:35-50.

67. Allen DB, Bielory L, Derendorf H, Dluhy R, Colice GL, Szefler SJ. Inhaled corticosteroids: past lessons and future issues. J Allergy Clin Immunol 2003;112:Suppl 3:S1-S40.

68. Lipworth BJ. Systemic adverse effects of inhaled corticosteroid therapy: a systematic review and meta-analysis. Arch Intern Med 1999;159:941-55.

69. van der Eerden BC, Karperien M, Wit JM. Systemic and local regulation of the growth plate. Endocr Rev 2003;24:782-801.

70. Chrysis D, Zaman F, Chagin AS, Takigawa M, Savendahl L. Dexamethasone induces apoptosis in proliferative chondrocytes through activation of caspases and suppression of the Akt-phosphatidylinositol 3′-kinase signaling pathway. Endocrinology 2005;146:1391-7.

71. Dostert A, Heinzel T. Negative glucocorticoid receptor response elements and their role in glucocorticoid action. Curr Pharm Des 2004;10:2807-16.

72. Iwamoto J, Takeda T, Sato Y. Effects of vitamin K2 on osteoporosis. Curr Pharm Des 2004;10:2557-76.

73. Canalis E, Delany AM. Mechanisms of glucocorticoid action in bone. Ann N Y Acad Sci 2002;966:73-81.

74. Sato S, Kim T, Arai T, Maruyama S, Tajima M, Utsumi N. Comparison between the effects of dexamethasone and indomethacin on bone wound healing. Jpn J Pharmacol 1986;42:71-8.

75. Seidenberg AB, An YH. Is there an inhibitory effect of COX-2 inhibitors on bone healing? Pharmacol Res 2004;50:151-6.

76. Beer HD, Fassler R, Werner S. Glucocorticoid-regulated gene expression during cutaneous wound repair. Vitam Horm 2000;59:217-39.

77. Chakraborti S, Mandal M, Das S, Mandal A, Chakraborti T. Regulation of matrix metalloproteinases: an overview. Mol Cell Biochem 2003;253:269-85.

78. Richardson DW, Dodge GR. Dose-dependent effects of corticosteroids on the expression of matrix-related genes in normal and cytokine-treated articular chondrocytes. Inflamm Res 2003;52:39-49.

79. Cutroneo KR. How is Type I procollagen synthesis regulated at the gene level during tissue fibrosis. J Cell Biochem 2003;90:1-5.

80. Nuutinen P, Riekki R, Parikka M, et al. Modulation of collagen synthesis and mRNA by continuous and intermittent use of topical hydrocortisone in human skin. Br J Dermatol 2003;148:39-45.

81. Dallman MF, Strack AM, Akana SF, et al. Feast and famine: critical role of glucocorticoids with insulin in daily energy flow. Front Neuroendocrinol 1993;14:303-47.

82. Mitch WE. Mechanisms accelerating muscle atrophy in catabolic diseases. Trans Am Clin Climatol Assoc 2000;111:258-69.

83. Leal-Cerro A, Soto A, Martinez MA, Dieguez C, Casanueva FF. Influence of cortisol status on leptin secretion. Pituitary 2001;4:111-6.

84. Trence DL. Management of patients on chronic glucocorticoid therapy: an endocrine perspective. Prim Care 2003;30:593-605.

85. Schacke H, Schottelius A, Docke WD, et al. Dissociation of transactivation from transrepression by a selective glucocorticoid receptor agonist leads to separation of therapeutic effects from side effects. Proc Natl Acad Sci U S A 2004;101:227-32.

86. Bledsoe RK, Montana VG, Stanley TB, et al. Crystal structure of the glucocorticoid receptor ligand binding domain reveals a novel mode of receptor dimerization and coactivator recognition. Cell 2002;110:93-105.

87. Kauppi B, Jakob C, Farnegardh M, et al. The three-dimensional structures of antagonistic and agonistic forms of the glucocorticoid receptor ligand-binding domain: RU-486 induces a transconformation that leads to active antagonism. J Biol Chem 2003;278:22748-54.

88. Garside H, Stevens A, Farrow S, et al. Glucocorticoid ligands specify different interactions with NF-kappaB by allosteric effects on the glucocorticoid receptor DNA binding domain. J Biol Chem 2004;279:50050-9.

89. Coghlan MJ, Elmore SW, Kym PR, Kort ME. The pursuit of differentiated ligands for the glucocorticoid receptor. Curr Top Med Chem 2003;3:1617-35.

90. Croxtall JD, van Hal PT, Choudhury Q, Gilroy DW, Flower RJ. Different glucocorticoids vary in their genomic and nongenomic mechanism of action in A549 cells. Br J Pharmacol 2002;135:511-9.

91. Einstein M, Greenlee M, Rouen G, et al. Selective glucocorticoid receptor nonsteroidal ligands completely antagonize the dexamethasone mediated induction of enzymes involved in gluconeogenesis and glutamine metabolism. J Steroid Biochem Mol Biol 2004;92:345-56.

92. Kemp JE. Expected characteristics of an ideal, all-purpose inhaled corticosteroid for the treatment of asthma. Clin Ther 2003;25:Suppl C:C15-C27.

Copyright © 2005 Massachusetts Medical Society.