

Tissue-specific glucocorticoid resistance-hypersensitivity syndromes: Multifactorial states of clinical importance

Glucocorticoids are crucial for maintenance of behavioral, cardiovascular, metabolic, and immune homeostasis. Importantly, glucocorticoids are potent immunosuppressive agents that are extremely valuable in the therapy of many atopic and inflammatory diseases and lymphoproliferative disorders.

The actions of glucocorticoids are mediated by an ubiquitous intracellular receptor protein, the glucocorticoid receptor (GR), which functions as a hormone-activated transcription factor of glucocorticoid target genes (Fig 1, A). These genes probably represent up to 10% of the human genome and can be influenced by the ligand-activated GR directly or indirectly.¹ The GR consists of 3 domains, the N-terminal or immunogenic domain, the central DNA-binding domain (DBD), and the C-terminal ligand-binding domain (LBD; Fig 1, B).² The GR in its nonligand but ligand-friendly state is located primarily in the cytoplasm as part of hetero-oligomeric complexes containing heat shock proteins 90, 70, and 50 and possibly other proteins as well (Fig 1, A).³ After binding to its agonist ligand, the GR undergoes conformational changes, dissociates from the heat shock proteins, homodimerizes, and translocates into the nucleus through the nuclear pore by means of an active process. There, the ligand-activated GR directly interacts with DNA sequences (the glucocorticoid-responsive elements) in the promoter regions of target genes or with other transcription factors through protein-protein interactions, indirectly influencing the activity of the latter on their target genes.

Because glucocorticoids have a broad array of life-sustaining functions and play an important role in therapeutic interventions, changes of tissue sensitivity to glucocorticoids might be associated with and influence the course of many pathologic states (Table I). Such changes might present on either side of an optimal range as glucocorticoid resistance or hypersensitivity and might be a generalized, tissue-specific, or both. Primary generalized

Abbreviations used

AF:	Activation function
AP-1:	Activator protein 1
C/EBP:	CAAT/enhancer binding protein
CBP:	CREB-binding protein
CREB:	Cyclic AMP-responsive element-binding protein
DBD:	DNA-binding domain
DRIP:	Vitamin D receptor-interacting protein
GR:	Glucocorticoid receptor
LBD:	Ligand-binding domain
MAPK:	Mitogen-activated protein kinase
NF- κ B:	Nuclear factor κ B
STATs:	Signal transducers and activators of transcription
TRAP:	Thyroid hormone receptor-associated protein

glucocorticoid resistance has been described as a rare familial or sporadic syndrome caused mostly by inactivating mutations of the gene encoding GR, whereas several autoimmune-inflammatory states, such as rheumatoid arthritis, osteoarthritis, Crohn's disease, ulcerative colitis, and asthma are often associated with resistance of the inflamed tissues to glucocorticoids.²⁻⁴ In addition, septic shock and respiratory distress syndrome have been associated with systemic glucocorticoid resistance.⁵ On the other hand, glucocorticoid hypersensitivity has been suggested in visceral obesity-related insulin resistance associated with components of the metabolic syndrome and in AIDS, which is caused by HIV-1 infection.^{2,6,7}

In this issue of the Journal, Irusen et al⁸ report on their studies on the potential cause of treatment resistance in glucocorticoid-resistant asthma. Generally, bronchial asthma responds well to inhaled glucocorticoids, which are devoid of major systemic effects.⁹⁻¹¹ However, a small percentage of patients are refractory to this therapeutic intervention, and thus administration of high doses of oral glucocorticoids, alternative agents, or both might be necessary.¹² The glucocorticoid resistance of these patients appears to be localized primarily in the lung and certain circulating immune cells. Indeed, these patients might experience undesired side effects of chronically administered excessive doses of glucocorticoids, such as Cushingoid features, manifestations of the metabolic syndrome, osteopenia-osteoporosis, and growth retardation and short final stature in children.^{6,10,11}

The resistance in the majority of patients with glucocorticoid-resistant asthma appears to be associated with several changes in the glucocorticoid signaling system in certain immune cells, which can be induced in naive white

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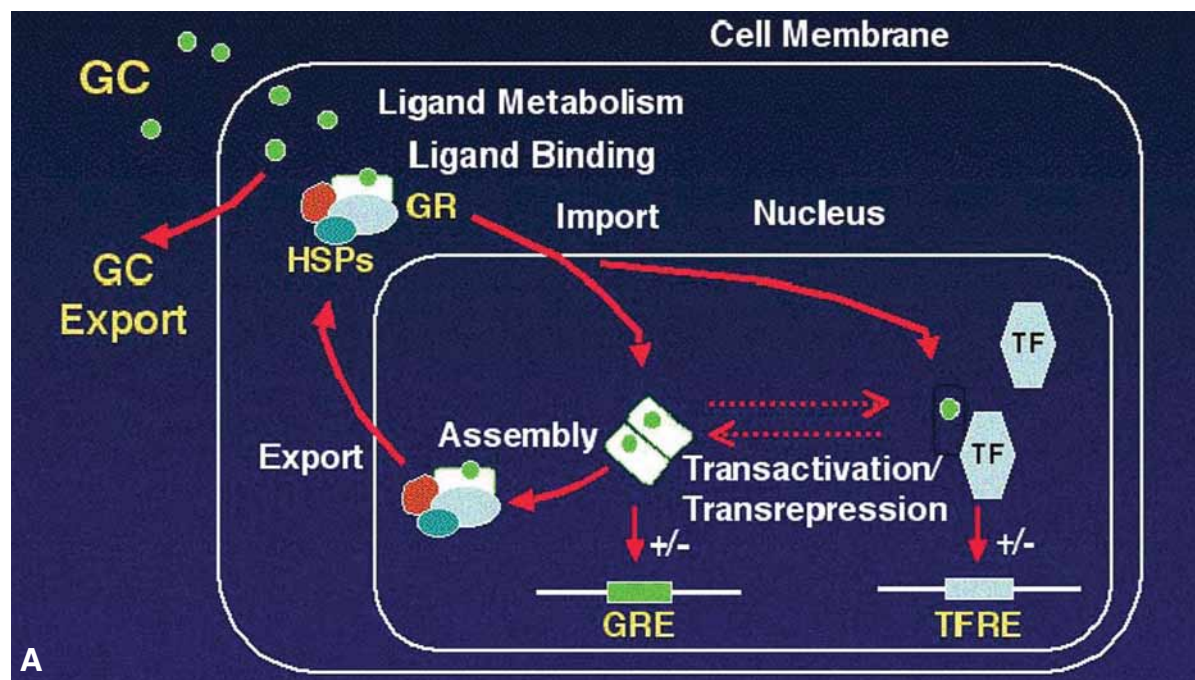


FIG 1. A, Circulation of the GR between the cytoplasm and the nucleus and its transactivating or transrepressive activities. *GRE*, Glucocorticoid-responsive elements; *TF*, transcription factor; *TFRE*, transcription factor–responsive element; *HSP*, heat shock protein.

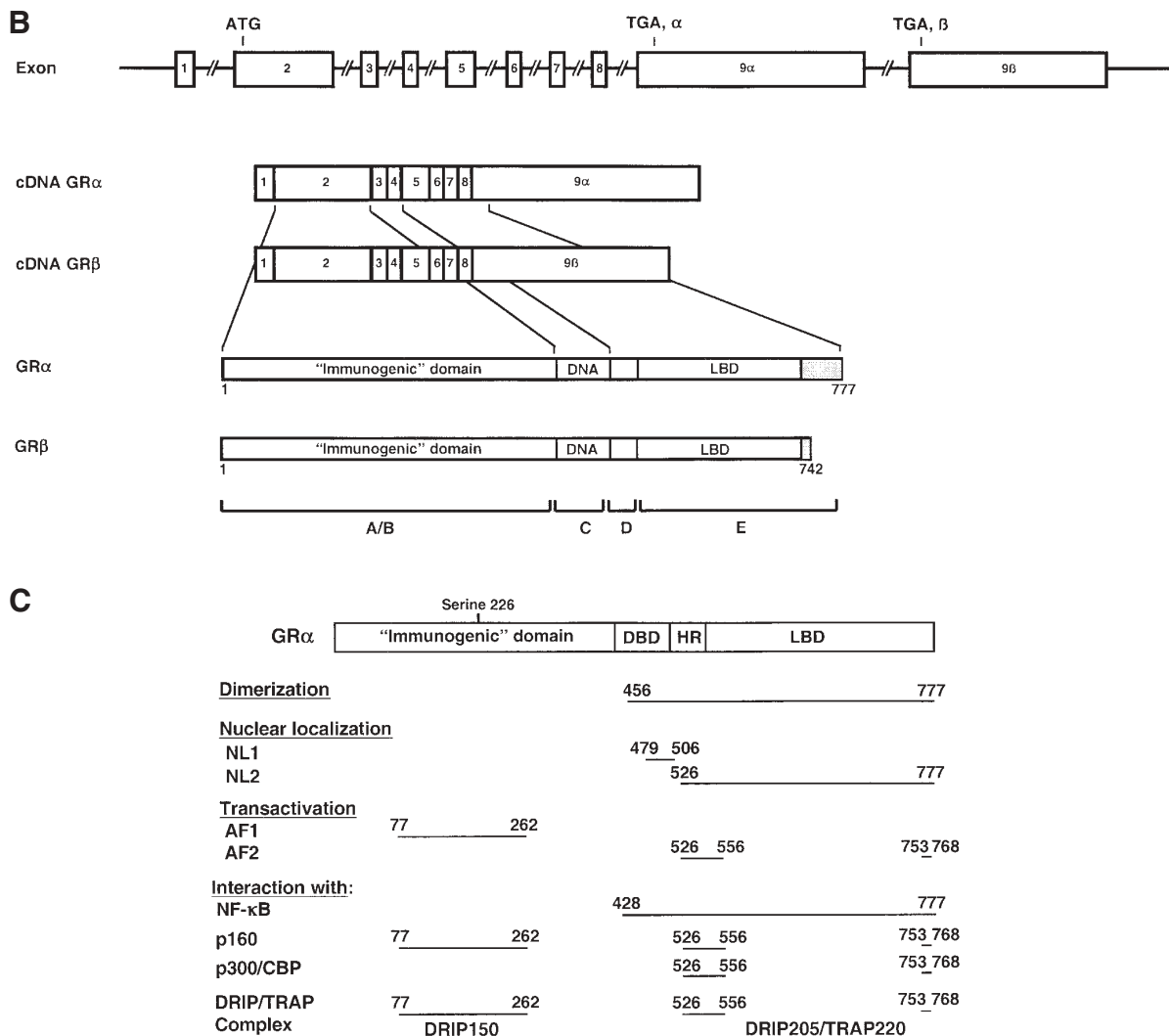
cells by means of incubation with IL-2 and IL-4 combined or IL-13 alone. These changes include decreases in the affinity of the nuclear fraction of the GR for its ligand and resistance of the cells to glucocorticoids *in vitro*.^{13,14}

Irusen et al⁸ suggest that the p38 mitogen-activated protein kinase (MAPK), stimulated by simultaneous administration of IL-2 and IL-4, might cause reduction in the affinity of the GR to its ligand through direct phosphorylation of the GR at serine 226 (Fig 1, C). Several other protein kinases involved in different signaling pathways, such as the cyclin-dependent kinases and c-Jun N-terminal kinase, might modulate GR and other steroid receptor activities, either directly through phosphorylation of the GR or indirectly through phosphorylation of cofactor molecules.^{15,16} Although these authors did not directly show that p38 MAPK phosphorylation of the GR is responsible for its reduced affinity to dexamethasone and its defective transduction of the signal, their hypothesis is plausible and worth testing.⁸ It could be pursued by examining the affinity and transcriptional activity of GR molecules harboring mutations at position 226.

If p38 MAPK indeed phosphorylates the GR at serine 226, how would such an action affect the GR's ligand-binding activity? Serine 226 is located in the N-terminal domain, whereas the ligand-binding pocket resides in the C-terminal portion of the molecule. Also, the LBD of the GR interacts with ligands in the absence of both the immunogenic domain and the DBD and is functional in a chimeric form with DBDs from other nuclear receptors. Yet there are pieces of evidence from other nuclear receptor systems, where posttranslational changes in the

immunogenic domain might cause alterations in the activities of the LBD. For instance, the androgen receptor shows functional and physical interactions between its immunogenic domain and the LBD, called N/C interaction, through which the former affects the ligand-binding activity of the latter.¹⁷ Although the specific motif of the androgen receptor immunogenic domain that supports the N/C interaction is not present in the GR, such interaction might occur through some other sequence. Also, 2 transactivation domains, activation functions (AF) 1 and 2, which are located in the immunogenic domain and the LBD, respectively, cooperatively contribute to the full transcriptional activity of the GR molecule on its responsive promoters; deletion of either of them dramatically reduces the transactivational activity of the GR (Fig 1, C).

There are several steps in the signaling cascade of glucocorticoids in which their actions could be interfered with to result in diminution or enhancement of their effect (Fig 1 and Table II). For instance, several nuclear proteins and protein complexes, including histone acetyltransferase coactivators, such as members of the p160 family and the coactivators p300/cyclic AMP-responsive element-binding protein (CREB)-binding protein (CRP), the components of the vitamin D receptor-interacting protein (DRIP)/thyroid hormone receptor-associated protein (TRAP), and the SWI/SNF chromatin modulators, interact with both AF1 and AF2 of the GR to support synergistic target gene transactivation of these activation functions (Fig 1, C).^{18,19} There are several reports showing that nuclear receptor coregulator molecules, namely coactivators and corepressors, can shift the



glucocorticoid-titration curve of GR transactivation of its responsive promoters to the left or right, respectively, indicating that they might change also the ligand-binding activity of the nuclear GR LBD.²⁰ Interestingly, serine 226 residue is located in the second α -helix of the core domain of AF1. Thus it is possible that phosphorylation at this serine residue might change the interaction of GR with coregulators, components, or both of the transcription machinery that interact with AF1.

In addition to the coregulators and transcription machinery components, there are several transcription factors that can modulate GR activity and thus might potentially contribute to changes in tissue sensitivity to

glucocorticoids. Thus the GR on the one side and transcription factors, such as nuclear factor κ B (NF- κ B), activator protein 1 (AP-1), signal transducers and activators of transcription (STATs), CREB, CAAT/enhancer binding protein (C/EBP), p53, and GATA-1, on the other might reciprocally modulate each others transactivational activities on their responsive promoters through protein-protein interactions, competition for common coregulators, or both.³ These interactions of the GR appear to be particularly important in the glucocorticoid-induced suppression of immune function and inflammation.²¹ Indeed, a substantial proportion of glucocorticoid effects on the immune system might be explained by means of

TABLE 1. Reported pathologic states associated with changes in tissue sensitivity to glucocorticoids

Resistance	Hypersensitivity
Familial-sporadic glucocorticoid resistance syndrome	Visceral-type obesity-related hypertension and insulin resistance
Rheumatoid arthritis	AIDS (HIV-1 infection)
Osteoarthritis	
Systemic lupus erythematosus	
Crohn's disease	
Ulcerative colitis	
Septic shock, respiratory distress syndrome	
Bronchial asthma	

TABLE 2. Factors that might change the sensitivity of tissues to glucocorticoids

Cellular factors
Ligand export system
Ligand activation-inactivation (eg, 11 β -HSD and 5 α -reductase)
Chaperones-cochaperones
Transcription factors (eg, NF- κ B, AP-1, CREB, STATs, C/EBP β , p53, and GATA-1)
Receptor isoform (GR β)
Coregulators
Coactivators (+)
Corepressors (-)
Viral coactivators (HIV-1 Vpr, Tat)
Chromatin modifiers (SWI/SNF)
DRIP/TRAP components (DRIP150 and DRIP205/TRAP220)
Phosphorylation status
Nitrosylation status
Thioredoxin
Extracellular factors
Small molecules (UDCA)
Inflammatory cytokines
Drugs (RU 486)

11 β -HSD, 11 β Hydroxysteroid dehydrogenase; AP-1, activator protein 1; CREB, cyclic AMP-responsive element-binding protein; STATs, signal transducers and activators of transcription; C/EBP β , CAAT/enhancer binding protein β ; DRIP150, vitamin D receptor-interacting protein 150; TRAP220, thyroid hormone receptor-associated protein 220; UDCA, ursodeoxycholic acid.

interaction of the GR with NF- κ B, AP-1, and probably several STATs and modulation of the latter's activities.^{5,21} Because glucocorticoid resistance has been reported in autoimmune-inflammatory diseases, some of these transcription factors might contribute to their pathogenesis.

There is accumulating clinical evidence suggesting that change in the expression levels of GR β , a splicing variant of the GR (also termed GR α), correlates with glucocorticoid insensitivity in several pathologic conditions.^{3,22} GR β cannot bind glucocorticoids and functions as a natural dominant negative isoform for GR α transactivation in vitro. It is constitutively present in the nucleus and loosely interacts with several heat shock proteins.^{3,23} The degree of contribution of this isoform to tissue glucocorticoid sensitivity and the mechanisms of its effects in physiologic and pathologic situations remain to be elucidated.

Exogenous viral proteins also might modulate GR activity. One of the HIV-1 accessory proteins, Vpr, a 96-amino-acid virion-associated protein with multiple functions, was shown to enhance GR transactivation by functioning as a coactivator in vitro.²⁴ Another HIV-1 accessory protein, Tat, which functions as a major transactivator of the HIV-1 long terminal repeat promoter, also potentiates GR activity, possibly by helping the accumulation of the positive transcription elongation factor b.²⁵ Through these 2 proteins, HIV-1 might enhance its replication by directly stimulating its long terminal repeat promoter, as well as indirectly by suppressing the host's immune functions through potentiation of ligand-activated GR in the absence of elevated levels of cortisol.

GR accessory proteins also modulate its activity. Chaperones and cochaperones, such as heat shock proteins and nuclear receptor-associating protein of approximately 46 kd (RAP46), affect the function of the GR.²⁶ Also, members of the 14-3-3 protein family, which bind to phosphorylated serine or threonine residues in several signal amino acid sequences and regulate the activity of their partner proteins by changing their intracellular localization, activity, and/or stability, also interact with the GR and modulate its transactivation and transrepression activity.²⁷

Endogenous small molecules other than glucocorticoids can also change GR activity. For instance, ursodeoxycholic acid, one of the hydrophilic bile acids that acts as a bile secretagogue, cytoprotective agent, and immunomodulator, induces translocation of the GR into the nucleus and causes GR-mediated inhibition of NF- κ B transactivation in a glucocorticoid-independent fashion.²⁸ On the other hand, thioredoxin, a compound accumulated in oxidative stress, enhances GR transactivation most possibly by functional replenishment of the GR.²⁹ Furthermore, membrane-associated glucocorticoid transporters might modulate the cellular sensitivity to glucocorticoids by changing the intracellular levels of glucocorticoids through actively exporting them into the extracellular space, whereas 11 β -hydroxysteroid dehydrogenase type 1 reduces the inactive cortisone to the active cortisol in several glucocorticoid target tissues, increasing glucocorticoid activity.³

Similarly to the findings of Irusen et al,⁸ Franchimont et al³⁰ have demonstrated that TNF- α causes glucocorticoid resistance in human mononuclear leukocytes, and this might be through activation of p38 MAPK in addition to activation of NF- κ B, whereas Meduri et al⁵ have shown that plasma from patients with glucocorticoid-resistant respiratory distress syndrome induces glucocorticoid resistance of naive human mononuclear leukocytes through profound activation of NF- κ B and suppression of I κ B. Also, nitrosylation of the GR as a result of increased nitric oxide synthase activity leads to its inactivation.³¹ These studies suggest that inflammation per se, by means of inflammatory cytokines, nitric oxide, and other mediators, might be a glucocorticoid resistance-inducing state and that intervention in the inflammatory cascade might improve the efficacy of glucocorticoid therapy.

It appears quite likely that several mechanisms might

contribute to the development of changes in tissue sensitivity to glucocorticoids seen in autoimmune-inflammatory diseases (Table II). Glucocorticoid resistance probably modifies the clinical phenotype and course of such diseases, as well as their responsiveness to glucocorticoid therapy. Thus it is critically important to elucidate these mechanisms, not only to better understand the pathophysiology of these devastating diseases but also to improve our therapeutic interventions.

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