Generalised Glucocorticoid Resistance

a report by

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Glucocorticoids are steroid hormones synthesised and secreted by the adrenal cortices under the regulation of the hypothalamic–pituitary–adrenal (HPA) axis. Glucocorticoids regulate a broad spectrum of physiological functions essential for life and play an important role in the maintenance of basal and stress-related homeostasis.[1–4] At the cellular level, the actions of glucocorticoids are mediated by the human glucocorticoid receptor (hGR), which belongs to the steroid/thyroid/retinoic acid nuclear receptor superfamily of transcription factor proteins and is expressed in virtually all cells.[5,6] The gene encoding hGRα (hGR gene) is one locus on the long arm of chromosome 5 (q31.3), and consists of nine exons spanning over 150kb. Expressed hGRα is a panel of eight amino terminal translational isoforms of varying lengths, each of which consists of three subdomains: the N-terminal (NTD), the DNA-binding (DBD) and the ligand-binding (LBD) domains. In our expression and functional studies referred to here we have employed as representative the longest GRα isoform, comprising 777 amino acids. The hGR gene also produces an equal number of hGRβ isoforms by the use of an alternative 3′ exon 9β, which cannot bind glucocorticoids and exert a dominant negative effect upon the transcriptional activity of hGRα.[1,5,6]

In the absence of ligand, hGRα resides mostly in the cytoplasm of cells as part of a hetero-oligomeric complex, which contains chaperon heat shock proteins (HSPs) 90, 70, 23 and FKBP51, as well as other proteins.[7] Upon ligand-induced activation, the receptor dissociates from this multiprotein complex and translocates into the nucleus through the nuclear pore with the energy-dependent mechanism that includes importin α and β. Inside the nucleus, hGRα binds as a homodimer to glucocorticoid response elements (GREs) in the promoter regions of target genes and regulates their expression positively or negatively, depending on GRE sequence and promoter context.[7–10] The ligand-activated hGRα can also modulate gene expression independently of DNA binding by interacting, possibly as a monomer, with other transcription factors such as nuclear factor-κB (NF-κB), activator protein-1 (AP-1), p53 and signal transducers and activators of transcription (STATs)[11–14] (see *Figure 1a*).

To initiate the transcription, hGRα uses its transcriptional activation domains, activation function (AF)-1 and AF-2, located in NTD and LBD, respectively, as surfaces to interact with co-activators or co-repressors.[15–19] The p160 co-activators such as the glucocorticoid receptor-interacting protein 1 (GRIP1) play an important role in the hGRα-mediated transactivation of glucocorticoid-responsive genes given that they interact directly with both the AF-1 of hGRα through their carboxyl-terminal domain and the AF-2 through multiple amphipathic LXXLL signature motifs located in their nuclear receptor-binding (NRB) domain.[20] They also have histone acetyltransferase (HAT) activity, which promotes chromatin decondensation and facilitates initiation of transcription[15–18] (see *Figure 1b*).

Alterations in the molecular mechanisms of hGRα action may lead to alterations in tissue sensitivity to glucocorticoids, which may take the form of resistance or hypersensitivity and may be associated with significant morbidity.[21–24] In this article we summarise the molecular mechanisms underlying primary generalised glucocorticoid resistance and secondary, inflammation-induced, generalised glucocorticoid resistance.

Primary Generalised Glucocorticoid Resistance

Primary generalised glucocorticoid resistance is a rare condition characterised by generalised, partial, target-tissue insensitivity to glucocorticoids.[25–30] This leads to activation of the HPA axis and compensatory elevations in circulating cortisol and adrenocorticotropic hormone (ACTH) concentrations, which maintain circadian rhythmicity and appropriate responsiveness to stressors. The excess ACTH secretion results in adrenal hyperplasia and increased production of adrenal steroids with mineralocorticoid and/or androgenic activity[25–30] (see *Figure 2*).

The clinical manifestations of primary generalised glucocorticoid resistance are summarised in *Table 1* and relate to the pathophysiological alterations

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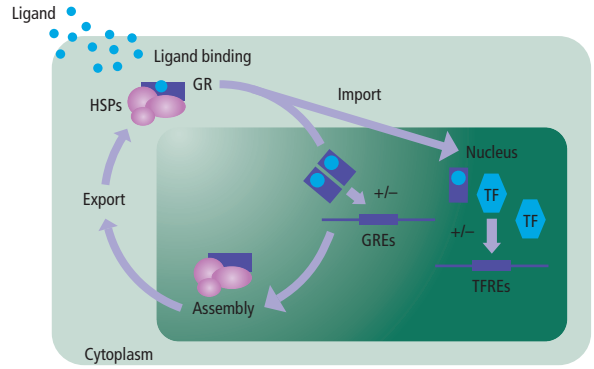
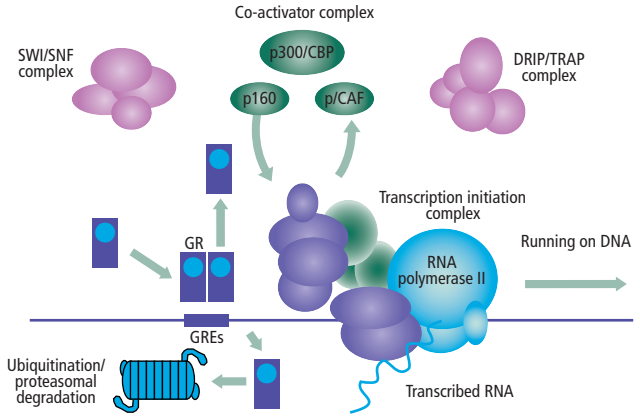
Figure 1a: Nucleocytoplasmic Shuttling of the Glucocorticoid Receptor



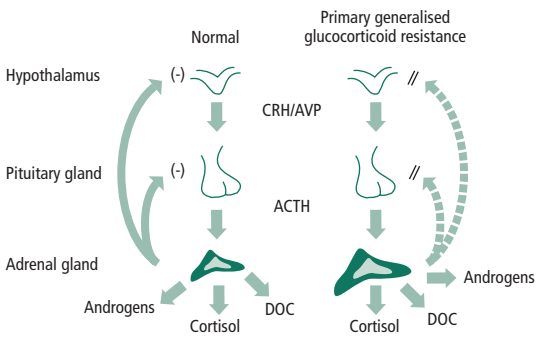
Upon ligand binding, the activated human glucocorticoid receptor (hGR)-α dissociates from heat shock proteins (HSPs) and translocates into the nucleus, where it binds as a homodimer to glucocorticoid response elements (GREs) in the promoter region of target genes or interacts as a monomer with other transcription factors.

Figure 1b: Schematic Representation of the Interaction of Activation Function-1 and -2 of Human Glucocorticoid Receptor-α with Co-activators



AF = activation function; DRIP/TRAP = vitamin D receptor-interacting protein/thyroid hormone receptor-associated protein; GR = glucocorticoid receptor; GREs = glucocorticoid response elements; HSP = heat shock protein; SWI/SNF = switching/sucrose non-fermenting; TF = transcription factor; TFREs = transcription factor response elements.

Figure 2: Alterations in the Hypothalamic–Pituitary–Adrenal Axis in Primary Generalised Glucocorticoid Resistance



The impaired glucocorticoid feedback inhibition at the hypothalamic and anterior pituitary levels results in increased secretion of corticotropin-releasing hormone (CRH) and adrenocorticotropin hormone (ACTH), adrenal hyperplasia and increased secretion of adrenal steroids with mineralocorticoid and/or androgenic activity.
AVP = arginine vasopressin; DOC = deoxycorticosterone.

depicted in *Figure 2*. The clinical spectrum of the condition is broad and ranges from most severe to mild forms, while a number of patients may be asymptomatic, displaying only biochemical alterations[25–42] (see *Table 1*). The variable clinical phenotype of primary generalised glucocorticoid resistance may be due to: variations in the glucocorticoid, mineralocorticoid or androgen receptor signalling pathways; variations in the degree of tissue sensitivity to glucocorticoids, mineralocorticoids and/or androgens; variations in the activity of key hormone-inactivating or -activating enzymes, such as the 11β-hydroxysteroid dehydrogenase[43] and 5α-reductase;[44] and other genetic or epigenetic factors, such as insulin resistance and visceral obesity.[27]

Molecular Mechanisms of Primary Generalised Glucocorticoid Resistance

The molecular basis of primary generalised glucocorticoid resistance has been ascribed to mutations in the hGR gene that impair the molecular mechanisms of hGR action and reduce tissue sensitivity to glucocorticoids. We have identified most hGR gene mutations associated with primary generalised glucocorticoid resistance and studied the molecular mechanisms through which the corresponding mutant receptors affect glucocorticoid signal transduction. More specifically, we determined: the transcriptional activity of the mutant receptors; the ability of the mutant receptors to exert a dominant negative effect upon the wild-type receptor; the affinity of the mutant receptors for the ligand; the subcellular localisation of the mutant receptors and their nuclear translocation following exposure to the ligand; the ability of the mutant receptors to bind to GREs; the interaction of the mutant receptors with the GRIP1 co-activator; and the motility of the mutant receptors within the nucleus of living cells.[25–42,45,46] The molecular defects elucidated in the reported cases are summarised in *Table 2*, while the corresponding mutations in the hGR gene are shown in *Table 2* and *Figure 3*.

All mutant receptors demonstrated variable reduction in their ability to transactivate the glucocorticoid-responsive mouse mammary tumour virus (MMTV) promoter in response to dexamethasone compared with the wild-type receptor, with the most severe impairment observed in the cases of R477H, I559N, V571A and D641V mutations.[31–42] The mutant receptors hGRαI559N, hGRαF737L, hGRαI747M and hGRαL773P exerted a dominant negative effect upon the wild-type receptor that might have contributed to manifestation of the disease at the heterozygote state.[31,35,38,40,42]

All mutant receptors in which the mutation is located in the LBD of the hGRα showed a variable reduction in their affinity for the ligand, with the most severe reduction observed in the cases of I559N, I747M and V571A mutations.[31–42] The decreased affinity of the mutant receptors for the ligand most likely reflects the location of the mutations in the LBD of hGRα. The mutant receptor hGRαR477H, in which the mutation is located at the DBD of the receptor, demonstrated normal affinity for the ligand[41] (see *Figure 2*).

In the absence of dexamethasone, the wild-type hGRα was primarily localised in the cytoplasm of cells. The pathological mutant receptors were also localised in the cytoplasm of cells in the absence of ligand, except for hGRαV729I and hGRαF737L, which were localised in both the cytoplasm and the nucleus of cells. The addition of dexamethasone (10^{-6}M) resulted in translocation of the wild-type receptor into the nucleus within 12 minutes, but a much slower translocation of the mutant receptors into the nucleus, which ranged from 20 minutes (R477H) to 180 minutes (I559N

and F737L).[31–42] These findings suggest that all hGR mutations affect the nucleocytoplasmic shuttling of hGRα, probably through impairment of the function of the nuclear localisation signals NL1 and/or NL2.[47–50] Defective mechanisms that may relate to delayed nuclear export, such as the calreticulin export pathway and certain motifs in the DBD that function as nuclear export signals, may account for the nuclear localisation of the unliganded hGRαV729I and hGRαF737L,[51,52] an effect that may be similar to the nuclear retention mechanism of hGRα.[35]

The wild-type and all mutant receptors in which the mutations are located in the LBD of hGRα preserved their ability to bind to DNA.[31–42] The only mutant receptor that failed to bind to DNA was hGRαR477H, in which the mutation is located at the C-terminal zinc finger of the DBD of the receptor.[41] A major function of the C-terminal zinc finger of the DBD of hGRα is to contribute to receptor homodimerisation, a prerequisite for potent receptor binding to GREs and efficient transactivation of glucocorticoid-responsive genes.[53,54] Point mutations in the DBD of the hGR may abolish DNA binding, resulting in silencing of transcriptional activation, although they may not affect the ability of the mutant receptors to transrepress AP-1-, NF-κB- and/or other target-gene-dependent transcription, possibly through protein–protein interactions and/or tethering of other co-factors to the transcriptional machinery.[54–57]

We next investigated the interaction between the mutant receptors and the GRIP1 co-activator *in vitro*. GRIP1 contains two sites that bind to steroid receptors: one site, the NRB site, is located at the central part of the protein and interacts with the AF-2 of hGRα in a ligand-dependent fashion, while the other site is located at the carboxyl-terminus of the protein and binds to the AF-1 of hGRα in a ligand-independent fashion.[58–60] The wild-type and most mutant receptors bound to full-length GRIP1 and the carboxyl-terminal fragment of GRIP1, but not to the NRB fragment of GRIP1, suggesting that they interact with the GRIP1 co-activator *in vitro* only through their AF-1. Exceptions were the receptors hGRαR477H, which interacted with both the AF-1 and AF-2 of hGRα, and hGRαI559N, which did not interact with either fragment of GRIP1.[31–42] These findings suggest that the hGR mutant receptors may form a defective complex with GRIP1, which is partially or completely ineffective. Furthermore, the mutant receptors may also display an abnormal interaction with other AF-2-associated proteins such as the p300/CBP co-integrators and components of the vitamin D-receptor interacting protein (DRIP)/thyroid-hormone-receptor-associated proteins (TRAP) complex.[15–18] Using fluorescence recovery after photobleaching (FRAP) analysis, we demonstrated that all hGR pathological mutant receptors had defective transcriptional activity and dynamic motility defects inside the nucleus of living cells.[46] In the presence of dexamethasone, these mutants displayed a curtailed 50% recovery time ($t^{1/2}$) after photobleaching and, hence, significantly increased intranuclear motility and decreased chromatin retention. The $t^{1/2}$ values of the mutants correlated positively with their transcriptional activities and depended on the hGR domain affected. Therefore, the motility defect of the mutant receptors is a good overall index of functionality.[46]

In summary, mutations in the hGR gene impair the molecular mechanisms of glucocorticoid action and lead to generalised tissue insensitivity to glucocorticoids. A consequent increase in the activity of the HPA axis compensates for the reduced sensitivity of peripheral tissues to glucocorticoids at the expense of ACTH hypersecretion-related pathology. The study of the molecular defects of natural hGR mutants enhances our understanding of hGR action and highlights the importance of integrated cellular and molecular signalling mechanisms in maintaining homeostasis and preserving normal physiology.

Table 1: Clinical Manifestations and Diagnostic Evaluation of Generalised Glucocorticoid Resistance*

Clinical Manifestations
Apparently normal glucocorticoid function
Asymptomatic
Chronic fatigue (glucocorticoid deficiency?)
Mineralocorticoid excess
Hypertension
Hypokalemic alkalosis
Androgen excess
Children: Ambiguous genitalia at birth,** premature adrenarche, precocious puberty
Females: Acne, hirsutism, male-pattern hair loss, menstrual irregularities, oligo-anovulation, infertility
Males: Acne, hirsutism, oligospermia, adrenal rests in the testes, infertility
Increased HPA axis activity (CRH/ACTH hypersecretion)
Anxiety
Adrenal rests
Diagnostic Evaluation
Absence of clinical features of Cushing syndrome
Normal or elevated plasma ACTH concentrations
Elevated plasma cortisol concentrations
Increased 24-hour urinary free cortisol excretion
Normal circadian and stress-induced pattern of cortisol and ACTH secretion
Resistance of the HPA axis to dexamethasone suppression
Thymidine incorporation assays: Increased resistance to dexamethasone-induced suppression of phytohemaglutinin-stimulated thymidine incorporation compared to control subjects
Dexamethasone-binding assays: Decreased affinity of the glucocorticoid receptor for the ligand compared to control subjects
Molecular studies: Mutations/deletions of the glucocorticoid receptor

**Modified from reference 27.*
***This is the only case of ambiguous genitalia documented in a child with 46,XX karyotype who also harboured a heterozygous mutation of the 21-hydroxylase gene.*

Secondary Generalised Glucocorticoid Resistance Associated with Systemic Inflammation and Chronic Inflammatory Diseases

Acute and chronic inflammatory processes and diseases such as sepsis, asthma, rheumatoid arthritis and inflammatory bowel disease are characterised by increased expression of multiple inflammatory genes. The latter are regulated by pro-inflammatory transcription factors such as NF-κB and AP-1, which play a critical role in amplifying and perpetuating the inflammatory process. Furthermore, inflammation-mediated activation of the nervous system and the HPA axis is an integral component in the regulation of the innate and adaptive immune response.[61] Cytokines and inflammatory mediators activate the HPA axis both directly and indirectly. Activation of the HPA axis has profound inhibitory effects on the immune/inflammatory response.[62] At the cellular level, the main anti-inflammatory and immunosuppressive effects of glucocorticoids include alterations in leukocyte traffic and function, decreases in production of cytokines and mediators of inflammation and inhibition of their action on target tissues by the latter. It is worth noting that hyperactivity or hypoactivity of the HPA axis leads to dysregulation of this neuroendocrine loop and may lead to systemic changes in inflammation and immunity.[61,62]

Glucocorticoids represent the most effective anti-inflammatory treatment for many of the above inflammatory conditions.[63,64] However, failure to

Table 2: Mutations of the Human Glucocorticoid Receptor Gene Causing Generalised Glucocorticoid Resistance

	Mutation Position				
Author (Reference)	cDNA	Amino acid	Molecular Mechanisms	Genotype	Phenotype
Chrousos et al.[27]	1922 (A→T)	641 (D→V)	Transactivation ↓	Homozygous	Hypertension
Hurley et al.[32]			Affinity for ligand ↓ (x 3) Nuclear translocation: 22 minutes Abnormal interaction with GRIP1		Hypokalemic alkalosis
Karl et al.[33]	4 bp deletion in exon-intron 6 Inactivation of the affected allele		hGRα number: 50% of control	Heterozygous	Hirsutism Male-pattern hair loss Menstrual irregularities
Malchoff et al.[34]	2185 (G→A)	729 (V→I)	Transactivation ↓ Affinity for ligand ↓ (x 2) Nuclear translocation: 120 minutes Abnormal interaction with GRIP1	Homozygous	Precocious puberty Hyperandrogenism
Karl et al.[31]	1676 (T→A)	559 (I→N)	Transactivation ↓	Heterozygous	Hypertension
Kino et al.[35]			Decrease in hGR binding sites Transdominance (+) Nuclear translocation: 180 minutes Abnormal interaction with GRIP1		Oligospermia Infertility
Ruiz et al.[36]	1430 (G→A)	477 (R→H)	Transactivation ↓	Heterozygous	Hirsutism
Charmandari et al.[41]			No DNA binding Nuclear translocation: 20 minutes		Hypertension
Ruiz et al.[36]	2035 (G→A)	679 (G→S)	Transactivation ↓	Heterozygous	Hirsutism
Charmandari et al.[41]			Affinity for ligand ↓ (x 2) Nuclear translocation: 30 minutes Abnormal interaction with GRIP1		Fatigue Hypertension
Mendonca et al.[37]	1712 (T→C)	571 (V→A)	Transactivation ↓ Affinity for ligand ↓ (x 6) Nuclear translocation: 25 minutes Abnormal interaction with GRIP1	Homozygous	Ambiguous genitalia Hypertension Hypokalemia Hyperandrogenism
Vottero et al.[38]	2241 (T→G)	747 (I→M)	Transactivation ↓ Transdominance (+) Affinity for ligand ↓ (x 2) Nuclear translocation ↓ Abnormal interaction with GRIP1	Heterozygous	Cystic acne Hirsutism Oligo-amenorrhea
Charmandari et al.[40]	2318 (T→C)	773 (L→P)	Transactivation ↓ Transdominance (+) Affinity for ligand ↓ (x 2.6) Nuclear translocation: 30 minutes Abnormal interaction with GRIP1	Heterozygous	Fatigue Anxiety Acne Hirsutism Hypertension
Charmandari et al.[42]	2209 (T→C)	737 (F→L)	Transactivation ↓ Transdominance (time-dependent) (+) Affinity for ligand ↓ (x 1.5) Nuclear translocation: 180 minutes	Heterozygous	Hypertension Hypokalaemia

cDNA = complementary DNA; GRIP1 = glucocorticoid receptor-interacting protein 1.

respond to glucocorticoid therapy (glucocorticoid resistance) or need for chronic glucocorticoid treatment to maintain disease remission (glucocorticoid dependence) is common among patients treated with glucocorticoids and may be associated with significant morbidity and/or mortality. This variable response to glucocorticoid therapy is likely to be due to the process of inflammation, the disease itself or the genetic background of the patient.[63,64]

Anti-inflammatory Glucocorticoid Signalling Mechanisms

Glucocorticoids are important in suppressing several inflammatory pathways. For example, glucocorticoids inhibit prostaglandin production through three independent mechanisms: the induction and activation of annexin I, the induction of mitogen-activated protein kinase (MAPK) phosphatase 1 and the repression of transcription of cyclo-oxygenase 2. Annexin I (also called lipocortin-1) is an anti-inflammatory protein that physically interacts with and inhibits cytosolic phospholipase A2 (cPLA2), which in turn blocks the release of arachidonic acid and its subsequent conversion to eicosanoids (i.e. prostaglandins, thromboxanes, prostacyclins and leukotrienes).[62–64] Glucocorticoids also induce MAPK phosphatase 1, an anti-inflammatory protein that dephosphorylates and inactivates Jun N-terminal kinase (JNK), thereby inhibiting c-Jun-mediated transcription of inflammatory and immune genes.[64] MAPK phosphatase 1 also dephosphorylates and inactivates all members of the MAPK family of proteins, including JNK, extracellular-signal-related kinase 1 and 2 and p38 kinase. Consequently, MAPK phosphatase 1 may also inhibit cPLA2 activity by blocking its phosphorylation by MAPKs and MAPK-interacting kinase. Furthermore, glucocorticoids and the hGR directly interfere with c-Jun-mediated transcription. The transcriptional interference between the hGR and c-Jun homodimers and/or c-Jun-Fos heterodimers occurs through protein–protein interactions and has proved to be a major anti-inflammatory mechanism of glucocorticoids.[64]

In addition to the above, the glucocorticoid–hGR complex physically interacts with NF-κB to block its transcriptional activity.[65] NF-κB is a heterogeneous collection of dimers, the most common form consisting of a p65 and a p50 subunit. In its inactive state, NF-κB is sequestered in the cytoplasm by an inhibitory protein, the IκB. Tumour necrosis factor (TNF)-α, interleukin (IL)-1, microbial pathogens, viral infections and other inflammatory signals trigger signalling cascades that activate IκB kinases. Phosphorylation of IκB leads to its ubiquitination and degradation by the proteasome, thereby unmasking a nuclear localisation signal on NF-κB. In the nucleus, NF-κB binds DNA sequences, the NF-κB response 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 cyclo-oxygenase 2, an enzyme essential for prostaglandin production (see *Figures 4a* and *4b*). A more detailed description of the interactions between the hGR and the NF-κB signalling systems are discussed in the following section.[64,65]

Glucocorticoids may also have rapid effects on inflammation that are not mediated by alterations in gene expression but, rather occur through membrane-associated receptors and second messengers (non-genomic actions)[66] (see *Figures 4a* and *4b*). Another mechanism of glucocorticoid-induced inhibition of inflammation involves decreased stability of messenger RNA (mRNA) for genes coding for inflammatory proteins, such as vascular endothelial growth factor and cyclo-oxygenase 2.[67] Therefore, glucocorticoids act on diverse target tissues through multiple mechanisms to control inflammation.

Molecular Mechanisms of Glucocorticoid Resistance Associated with Systemic Inflammation and Chronic Inflammatory Diseases

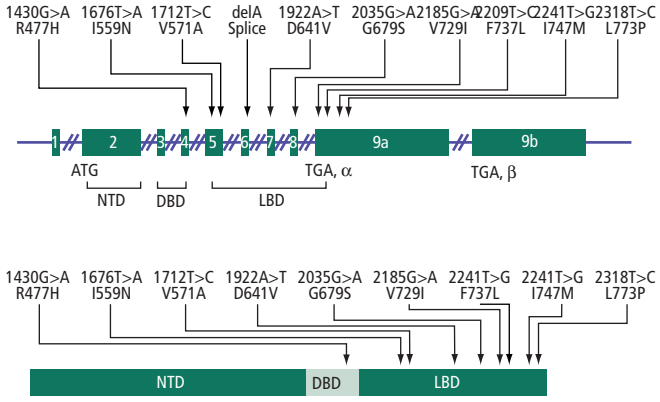
Abnormalities of the Human Glucocorticoid Receptor

Research studies on T-lymphocytes and other target inflammatory cells have demonstrated several hGR abnormalities as potential mechanisms influencing response to glucocorticoid treatment in inflammatory conditions. Several studies have demonstrated that cytokines modulate glucocorticoid sensitivity. IL-1β, IL-2, IL-4, IL-6, IL-13, TNF-α and interferon (IFN)-γ alter hGR numbers and binding affinity,[68–72] while IL-2 and IL-4 enhance the expression of hGRβ, a hGR splicing variant that acts as a dominant negative inhibitor of hGRα-mediated transactivation of glucocorticoid-responsive genes.[73,74] Patients with steroid-resistant asthma may display decreased hGRβ binding affinity,[69] abnormalities in hGR-AP-1 binding[75] and increased expression of hGRβ.[76] Increased hGRβ mRNA expression in peripheral lymphocytes has also been documented in patients with steroid-resistant ulcerative colitis (UC)[77] and steroid-resistant rheumatoid arthritis.[78]

Inflammation and NF-κB

A substantial part of the anti-inflammatory actions of hGRα are mediated by its interference with the potent transcription factor NF-κB, which results in inhibition of the synthesis of cytokines and other gene products of the inflammatory cascade, such as chemokines, cell adhesion molecules, complement factors and receptors for these molecules. Glucocorticoids (GCs) penetrate the cell membrane readily and exert their effects through activation of the hGR, which translocates into the nucleus and influences NF-κB activity in five major ways: by physically interacting with the p65 subunit with formation of

Figure 3: Known Mutations of the Human Glucocorticoid Receptor Gene (Upper Panel) and Protein (Lower Panel)



DBD = DNA-binding domain; NTD = amino terminal domain; LBD = ligand-binding domain.

Figure 4a: General Mechanisms of Action of Glucocorticoids and Glucocorticoid Receptor in the Inhibition of Inflammation

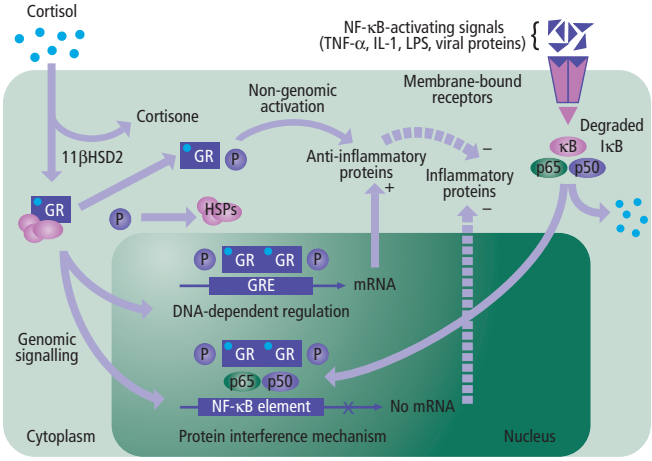
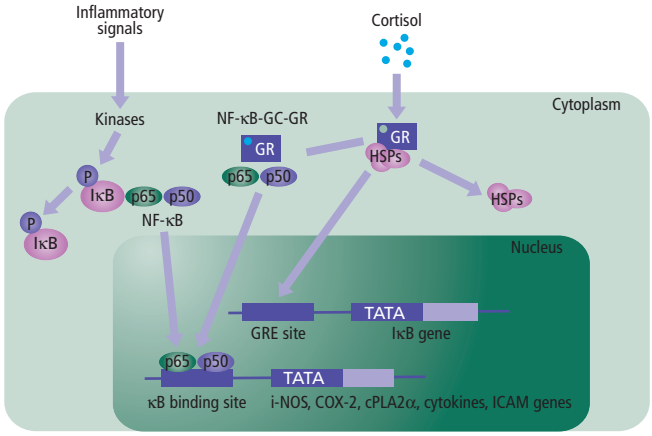


Figure 4b: Interaction Between NF-κB and Activated Glucocorticoid Receptor



When cells are stimulated by inflammatory signals, specific kinases phosphorylate the inhibitory protein IκB and cause its rapid degradation. The activated form of NF-κB then moves to the nucleus initiating the transcription of mRNA of inflammatory cytokines, chemokines, cell adhesion molecules, and inflammation-associated enzymes (cyclo-oxygenase, phospholipase A 2 and inducible nitric oxide). Cortisol or exogenous GCs freely cross into the cytoplasm and bind to their specific receptors and influence NF-κB activity. COX-2 = cyclooxygenase-2; cPLA2α = cytosolic phospholipase A2α; GC = glucocorticoid; GR = glucocorticoid receptor; GRE = glucocorticoid response element; HSP = heat shock protein; 11βHSD2 = 11β-hydroxy-steroid dehydrogenase type 2; ICAM = intercellular adhesion molecule; IL-1 = interleukin-1; iNOS = inducible nitric oxide synthase; LPS = lipopolysacharide; mRNA = messenger RNA; NF-κB = nuclear factor-κB; P = phosphate; TNF-α = tumor necrosis factor-α. Solid arrows denote activation, while dashed arrows denote inhibition and/or repression.

an inactive (GC-hGRα/NF-κB) complex;[79,80] by inducing the synthesis of the inhibitory protein IκBα via interaction with GREs in the promoter region of the IκB gene;[79,80] by blocking the degradation of IκBα via enhanced synthesis of IL-10;[79,80] by impairing TNF-α-induced degradation of IκBα;[79,80] and by competing for limited amounts of hGRα co-activators, such as CREB-binding protein and steroid receptor co-activator-1[79,80] IκBα, in addition to maintaining NF-κB in an inactive state in the cytoplasm of cells also translocates into the nucleus, where it binds activated NF-κB complexes to induce their export to the cytoplasm[65,79,80] (see *Figure 4b*).

On the other hand, NF-κB can repress the transcriptional activity of hGRα. In a regulated response, GC-hGRα activation is sufficient to maintain NF-κB levels in homeostasis and achieve a reduction in transcription of inflammatory mediators over time. Studies that examined the activities of GC-hGRα and NF-κB in patients with systemic inflammation and acute respiratory distress syndrome (ARDS) showed that those who improved on treatment with moderate doses of glucocorticoids had an excess activation of GC-hGRα compared to NF-κB, as evidenced by the increased GC-hGRα binding to NF-κB and increased nuclear GC-hGRα binding (GC-hGRα-driven response). On the other hand, non-improvers demonstrated an excess of NF-κB activation compared with GC-hGRα, leading to protracted transcription of inflammatory mediators over time. In non-improvers, GC-hGRα binding to NF-κB was modestly increased, while nuclear NF-κB binding to its respective response elements increased substantially over time and nuclear GC-hGRα and cytoplasmic IκBα levels declined (NF-κB-driven response).[79,80]

Further studies that assessed the activity of NF-κB in biopsy specimens from steroid-resistant and steroid-sensitive patients with severe Crohn's disease (CD) and UC showed that NF-κB activation was mainly noted in lamina propria macrophages in steroid-sensitive patients and in epithelial cells in steroid-resistant patients.[81] Similar were the findings related to the activation of AP-1 and the upstream kinases p38 and JNK.[82] The functional interference of these pro-inflammatory mediators with the glucocorticoid response was supported by reporter gene assays, which demonstrated that NF-κB, JNK and p38 inhibited the transcriptional activity of hGRα. These findings suggest that the generalised glucocorticoid resistance documented in patients with systemic inflammation, CD and UC is associated with constitutive activation of NF-κB and stress-activated protein kinases, which may inhibit the anti-inflammatory action of a limited number of hGRα molecules by reducing the transcriptional activity of hGRα.

The Multidrug Resistance Gene

The multidrug resistance gene (MDR1) codes for a drug efflux pump P-glycoprotein-170, which is expressed on the apical surface of lymphocytes and intestinal epithelial cells and actively transports glucocorticoids and other drugs out of target cells, thereby reducing their efficacy. Increased expression of MDR1 has been demonstrated in peripheral T-lymphocyte and intestinal epithelial cells of patients with CD and UC who did not respond to glucocorticoid therapy.[83] This suggests that a subset of patients with refractory CD and UC might escape effective immunosuppression by steroids and other immunosuppressive agents including cyclosporin because these drugs are MDR substrates and are effectively 'pumped out' of target cells. Increased T-lymphocyte MDR1 expression has also been demonstrated in patients with rheumatoid arthritis who require glucocorticoids,[84] renal graft recipients who undergo graft rejection on cyclosporin therapy[85] and patients with systemic lupus erythematosus.[86] Specific MDR pump inhibitors (e.g. PSC 833) can significantly increase intracellular human intestinal epithelial and T-lymphocyte levels of cortisol and cyclosporin,[87] and may have therapeutic applications in overcoming glucocorticoid resistance in many inflammatory conditions.

Histone Acetylation

The repression of genes occurs through the reversal of histone acetylation that activates inflammatory genes.[88] The ligand-bound hGRα may bind to CBP and other co-activators directly to inhibit their HAT activity,[89] thereby reversing the unwinding of DNA around core histones and suppressing inflammatory genes. Furthermore, the activated hGRα may also recruit histone deacetylase 2 (HDAC2) to the activated transcriptional complex, which results in deacetylation of hyperacetylated histones and a decrease in inflammatory gene transcription.[89] Several chronic inflammatory conditions such as asthma, rheumatoid arthritis, inflammatory bowel disease and chronic obstructive pulmonary disease (COPD) are characterised by a high degree of oxidative stress that decreases HDAC2 activity and leads to reduced glucocorticoid sensitivity.[90] Indeed, there is a correlation between HDAC activity and the suppressive effects of glucocorticoids on cytokine release.[90] The reduced HDAC2 expression in alveolar macrophages in patients with COPD was restored following overexpression of HDAC2 using a viral vector, and this effect was associated with restoration of glucocorticoid responsiveness in these cells. In contrast, transfection with an HDAC1 vector failed to restore corticosteroid responsiveness in COPD cells.[91]

Acetylation of Human Glucocorticoid Receptor

Non-histone proteins are also acetylated by histone acetyltransferases (HATs) and deacetylated by HDACs, and this may be an important mechanism of regulating their function.[92] Acetylation of several nuclear receptors including the oestrogen and androgen receptors may affect binding to their ligands.[93] Acetylation of the hGR occurs after ligand-binding and prior to nuclear translocation.[91] The acetylated hGR is deacetylated by HDAC2 and this deacetylation is necessary for the hGR to be able to inhibit NF-κB activation of inflammatory genes.[91] The site of acetylation of the hGR is the lysine-rich 'hinge' region 492–495 (sequence KKTK), which is analogous to the acetylation sites identified in other nuclear hormone receptors. Site-directed mutagenesis of the lysine residues K494 and K495 prevents hGR acetylation and reduces the activation of the SLPI gene by glucocorticoids, whereas repression of NF-κB is unaffected.[91]

A reduction in HDAC2 activity prevents deacetylation of acetylated hGR so that glucocorticoids are no longer able to repress NF-κB-activated inflammatory genes, which required deacetylation of the liganded receptor.[91] Moreover, this results in excessive acetylated hGR, which may then bind to GREs to induce genes responsible for the side effects of glucocorticoids. Therefore, a reduction in HDAC2 activity may not only lead to glucocorticoid resistance and poor disease control, but also to increased incidence of glucocorticoid side effects.

Post-translational Modifications

Cytokines may also impair hGR action through post-translational modifications. Phosphorylation and dephosphorylation of the hGR plays

an important role in ligand binding, recycling and turnover of the receptor. MAPKs, extracellular regulated kinases (ERKs), JNK and p38 MAPK phosphorylate the hGR and inhibit hGRβ-mediated transacriptional activation.[94,95] This may represent an early repression effect of mitogenic and pro-inflammatory signals on the expression of hGR-dependent genes.

Conclusion

In summary, glucocorticoid resistance associated with acute or chronic inflammation is characterised by insensitivity of the immune system to glucocorticoids. The underlying molecular mechanisms involve alterations in genomic and non-genomic glucocorticoid signalling mechanisms leading to impaired glucocorticoid signal transduction and inefficient suppression of the inflammatory process. The response to glucocorticoid treatment varies considerably among individuals depending on the type and severity of inflammation and the genetic background of the patient. Variations in the activity of the HPA axis and the stress response in general, as well as the presence of mutations or polymorphisms in the hGR gene may also account for the variable response to treatment. ■

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1. Kino T, Chrousos GP, In Handbook of Stress and the Brain, 2005;295–311.
2. Chrousos GP, Charmandari E, Kino T, *J Clin Endocrinol Metab*, 2004:89;563–4.
3. Chrousos GP, *Am J Med*, 2004:117;204–7.
4. Galon J, Franchimont D, Hiroi N, et al., *FASEB J*, 2002;16(1):61–71.
5. Zhou J, Cidlowski JA, *Steroids*, 2005;70(5-7):407–17.
6. Duma D, Jewell CM, Cidlowski JA, *J Steroid Biochem Mol Biol*, 2006;102(1–5):11–21.
7. Pratt WB, *J Biol Chem*, 1993;268(29):21455–8.
8. Terry LJ, Shows EB, Wente SR, *Science*, 2007;318(5855):1412–16.
9. Bamberger CM, Schulte HM, Chrousos GP, *Endocr Rev*, 1996;17(3):245–61.
10. Schaaf MJ, Cidlowski JA, *J Steroid Biochem Mol Biol*, 2002:83(1–5);37–48.
11. Jonat C, Rahmsdorf HJ, Park KK, et al., *Cell*, 1990;62(6):1189–204.
12. Scheinman RI, Gualberto A, Jewell CM, et al., *Mol Cell Biol*, 1995:15(2):943–53.
13. Chrousos GP, Kino T, *Sci STKE*, 2005;304:48.
14. Kino T, Chrousos G, *J Allergy Clin Immunol*, 2002:109(4);609–13.
15. McKenna NJ, Lanz RB, O'Malley BW, *Endocr Rev*, 1999:20(3);321–44.
16. McKenna NJ, Xu J, Nawaz Z, et al., *J Steroid Biochem Mol Biol*, 1999:69:3–12.
17. McKenna NJ, O'Malley BW, *Cell*, 2002:108;465–74.
18. Auboeuf D, Honig A, Berget SM, *Science*, 2002:298;416–19.
19. Hittelman AB, Burakov D, Iniguez-Lluhi JA, et al., *EMBO J*, 1999:18(19);5380–88.
20. Heery DM, Kalkhoven E, Hoare S, *Nature*, 1997;387(6634):733–6.
21. Kino T, Chrousos GP, *J Endocrinol*, 2001;169(3):437–45.
22. Chrousos G, In: Modern Endocrinology Series. In: Chrousos GP, Olefsky JM, Samols E (eds), Lippincott, Williams & Wilkins; Philadelphia, PA, 2002;542.
23. Kino T, De Martino MU, Charmandari E, et al., *J Steroid Biochem Mol Biol*, 2003;85(2–5):457–67.
24. Chrousos GP, Kino T, *Stress*, 2007;10(2):213–9.
25. Vingerhoeds AC, Thijssen JH, Schwarz F, *J Clin Endocrinol Metab*, 1976;43(5):1128–33.
26. Chrousos GP, Vingerhoeds A, Brandon D, et al., *J Clin Invest*, 1982;69(6):1261–9.
27. Chrousos GP, Detera-Wadleigh SD, Karl M, *Ann Intern Med*, 1993;119(11):1113–24.
28. Kino T, Vottero A, Charmandari E, et al., *Ann N Y Acad Sci*, 2002;970:101–11.
29. Charmandari E, Kino T, Chrousos GP, *Ann N Y Acad Sci*, 2004;1024:168–81.
30. Charmandari E, Kino T, Ichijo T, Chrousos GP, *J Clin Endocrinol Metab*, 2008;93(5):1563–72.
31. Karl M, Lamberts SW, Koper JW, et al., *Proc Assoc Am Physicians*, 1996:108(4):296–307.
32. Hurley DM, Accili D, Stratakis CA, et al., *J Clin Invest*, 1991;87(2):680–86.
33. Karl M, Lamberts SW, Detera-Wadleigh SD, et al., *J Clin Endocrinol Metab*, 1993;76(3):683–9.
34. Malchoff DM, Brufsky A, Reardon G, et al., *J Clin Invest*, 1993;91(5):1918–25.
35. Kino T, Stauber RH, Resau JH, et al., *J Clin Endocrinol Metab*, 2001;86(11):5600–8.
36. Ruiz M, Lind U, Gafvels M, et al., *Clin Endocrinol (Oxf)*, 2001;55(3):363–71.
37. Mendonca BB, Leite MV, de Castro M, Kino T, et al., *J Clin Endocrinol Metab*, 2002;87(4):1805–9.
38. Vottero A, Kino T, Combe H, et al., *J Clin Endocrinol Metab*, 2002;87(6):2658–67.
39. Charmandari E, Kino T, Vottero A, et al., *J Clin Endocrinol Metab*, 2004;89(4):1939–49.
40. Charmandari E, Raji A, Kino T, et al., *J Clin Endocrinol Metab*, 2005;90(6):3696–705.
41. Charmandari E, Kino T, Ichijo T, Zachman K, et al., *J Clin Endocrinol Metab*, 2006;91(4):1535–43.
42. Charmandari E, Kino T, Ichijo T, et al., *J Clin Endocrinol Metab*, 2007;92(10):3986–90.
43. Tomlinson JW, Walker EA, Bujalska IJ, et al., *Endocr Rev*, 2004;25(5):831–66.
44. Wilson JD, Griffin JE, Russell DW, *Endocr Rev*, 1993;14(5):577–93.
45. Huizenga NA, de Lange P, Koper JW, de Herder WW, et al., *J Clin Endocrinol Metab*, 2000;85(5):2076–81.
46. Kino T, Liou SH, Charmandari E, *Mol Med*, 2004;10(7–12):80–88.
47. Savory JG, Hsu B, Laquian IR, et al., *Mol Cell Biol*, 1999;19(2):1025–37.
48. Picard D, Yamamoto KR, *EMBO J*, 1987;6(11):3333–40.
49. Qi M, Hamilton BJ, DeFranco D, *Mol Endocrinol*, 1989;3(8):1279–88.
50. Wikstrom AC, Bakke O, Okret S, et al., *Endocrinology*, 1987;120(4):1232–42.
51. Holaska JM, Black BE, Rastinejad F, *Mol Cell Biol*, 2002;22(17):6286–97.
52. Black BE, Holaska JM, Rastinejad F, *Curr Biol*, 2001;11(22):1749–58.
53. Dahlman-Wright K, Wright A, Gustafsson JA, Carlstedt-Duke J, *J Biol Chem*, 1991;266(5):3107–12.
54. Umesono K, Evans RM, *Cell*, 1989;57(7):1139–46.
55. Liden J, Delaunay F, Rafter I, et al., *J Biol Chem*, 1997;272(34):21467–72.
56. Reichardt HM, Kaestner KH, Tuckermann J, et al., *Cell*, 1998;93(4):531–41.
57. Tao Y, Williams-Skipp C, Scheinman RI, *J Biol Chem*, 2001;276(4):2329–32.
58. Ding XF, Anderson CM, Ma H, et al., *Mol Endocrinol*, 1998;12(2):302–13.
59. Hong H, Kohli K, Garabedian MJ, Stallcup MR, GRIP1, *Mol Cell Biol*, 1997;17(5):2735–44.
60. Hong H, Kohli K, Trivedi A, et al., GRIP1, *Proc Natl Acad Sci USA*, 1996;93(10):4948–52.
61. Webster JI, Tonelli L, Sternberg EM, *Annu Rev Immunol*, 2002;20:125–63.
62. Chrousos GP, *N Engl J Med*, 1995;332:1351–62.
63. Franchimont D, Kino T, Galon J, *Neuroimmunomodulation*, 2002;10(5):247–60.
64. Rhen T, Cidlowski JA, *N Engl J Med*, 2005;353(16):1711–23.
65. McKay LI, Cidlowski JA, *Endocr Rev*, 1999;20:435–59.
66. Stellato C, *Proc Am Thorac Soc*, 2004;1(3):255–63.
67. Gille J, Reisinger K, Westphal-Varghese B, *J Invest Dermatol*, 2001;117:1581–7.
68. Franchimont D, Martens H, Hagelstein MT, et al., *J Clin Endocrinol Metab*, 1999;84(8):2834–9.
69. Kam JC, Szefler SJ, Surs W, et al., *J Immunol*, 1993;151(7):3460–66.
70. Rakasz E, Gal A, Biró J, et al., *Scand J Immunol*, 1993;37(6):684–9.
71. Salkowski CA, Vogel SN, *J Immunol*, 1992;148(9):2770–77.
72. Spahn JD, Szefler SJ, Surs W, et al., *J Immunol*, 1996;157(6):2654–9.
73. Oakley RH, Jewell CM, Yudt MR, et al., *J Biol Chem*, 1999;274(39):27857–66.
74. Charmandari E, Chrousos GP, Ichijo T, et al., *Mol Endocrinol*, 2005;19(1):52–64.
75. Adcock IM, Lane SJ, Brown CR, et al., *J Exp Med*, 1995;182(6):1951–8.
76. Leung DY, Hamid Q, Vottero A, et al., *J Exp Med*, 1997;186(9):1567–74.
77. Honda M, Orii F, Ayabe T, et al., *Gastroenterology*, 2000;118(5):859–66.
78. Chikanza IC, *Ann N Y Acad Sci*, 2002; 966:39–48.
79. Meduri GU, Muthiah MP, Carratu P, et al., *Neuroimmunomodulation*, 2005;12(6):321–38.
80. Meduri GU, Yates CR, *Ann N Y Acad Sci*, 2004;1024:24–53.
81. Bantel H, Domschke W, Schulze-Osthoff K, et al., *Am J Gastroenterol*, 2000;95(7):1845–6.
82. Bantel H, Schmitz ML, Raible A, et al., *FASEB J*, 2002;16(13):1832–4.
83. Farrell RJ, Murphy A, Long A, et al., *Gastroenterology*, 2000;118(2):279–88.
84. Maillefert JF, Maynadie M, Tebib JG, et al., *Br J Rheumatol*, 1996;35(5):430–35.
85. Zanker B, Barth C, Menges AV, et al., *Transplant Proc*, 1995;27(1):925–6.
86. Diaz-Borjon A, Richaud-Patin Y, Alvarado de la Barrera C, et al., *Joint Bone Spine*, 2000;67(1):40–48.
87. Farrell RJ, Menconi MJ, Keates AC, *Aliment Pharmacol Ther*, 2002;16(5):1021–31.
88. Imhof A, Wolffe AP, *Curr Biol*, 1998;8(12):R422–4.
89. Ito K, Barnes PJ, Adcock IM, *Mol Cell Biol*, 2000;20(18):6891–903.
90. Ito K, Hanazawa T, Tomita K, *Biochem Biophys Res Commun*, 2004;315(1):2405.
91. Ito K, Yamamura S, Essilfie-Quaye S, et al., *J Exp Med*, 2006;203(1):7–13.
92. Glozak MA, Sengupta N, Zhang X, *Gene*, 2005;363:15–23.
93. Fu M, Wang C, Zhang X, Pestell RG, *Biochem Pharmacol*, 2004;68(6):1199–208.
94. Rogatsky I, Logan SK, Garabedian MJ, *Proc Natl Acad Sci USA*, 1998;95(5):2050–55.
95. Irusen E, Matthews JG, Takahashi A, et al., *J Allergy Clin Immunol*, 2002;109(4):649–57.