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.1,4 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.8–10 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 transcription15–18 (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.25–29 This leads to activation of the HPA axis and compensatory elevations in circulating cortisol and adrenocorticotrophic 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 activity26–30 (see Figure 2).

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

Figure 1a: Nucleocytoplasmic Shutting 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 adrenocorticotropic hormone (ACTH), adrenal hyperplasia and increased secretion of adrenal steroids with mineralocorticoid and/or androgenic activity. AVP = arginine vasopressin; DOC = deoxycorticosterone.

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.

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. 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. 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 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, V571A and D641V mutations. 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.

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 hGRαR747M, which were localised in both the cytoplasm and the nucleus of cells. The addition of dexamethasone (10⁻⁶M) 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 nuclear translocation signals of the DBD that function as nuclear export signals, may account 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.

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

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

<table>
<thead>
<tr>
<th>Clinical Manifestations</th>
<th>Diagnostic Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparently normal glucocorticoid function</td>
<td>Absence of clinical features of Cushing syndrome</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>Normal or elevated plasma ACTH concentrations</td>
</tr>
<tr>
<td>Chronic fatigue (glucocorticoid deficiency?)</td>
<td>Elevated plasma cortisol concentrations</td>
</tr>
<tr>
<td>Hypokalemic alkalosis</td>
<td>Increased 24-hour urinary free cortisol excretion</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Normal circadian and stress-induced pattern of cortisol and ACTH secretion</td>
</tr>
<tr>
<td>Hypoglycaemic alcalosis</td>
<td>Resistance of the HPA axis to dexamethasone suppression</td>
</tr>
<tr>
<td>Androgen excess</td>
<td>Thymidine incorporation assays: Increased resistance to dexamethasone-induced suppression of phytomelaglutinin-stimulated thymidine incorporation compared to control subjects</td>
</tr>
<tr>
<td>Males: Acne, hirsutism, male-pattern hair loss, menstrual irregularities, oligo-anovulation, infertility</td>
<td>Dexamethasone-binding assays: Decreased affinity of the glucocorticoid receptor for the ligand compared to control subjects</td>
</tr>
<tr>
<td>Increased HPA axis activity (CRH/ACTH hypersecretion)</td>
<td>Molecular studies: Mutations/deletions of the glucocorticoid receptor</td>
</tr>
<tr>
<td>Anxiety</td>
<td>*Modified from reference 27.</td>
</tr>
<tr>
<td>Adrenal rests</td>
<td><strong>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.</strong></td>
</tr>
</tbody>
</table>

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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.
Adrenal Disorders

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

<table>
<thead>
<tr>
<th>Author (Reference)</th>
<th>Mutation Position</th>
<th>Amino acid</th>
<th>Molecular Mechanisms</th>
<th>Genotype</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chrousos et al.</td>
<td>1922 (A→T)</td>
<td>641 (D→V)</td>
<td>Transactivation ↓</td>
<td>Homozygous</td>
<td>Hypertension</td>
</tr>
<tr>
<td>Hurley et al.</td>
<td>4 bp deletion in exon-intron 6</td>
<td></td>
<td>Affinity for ligand ↓ (x 3)</td>
<td>Heterozygous</td>
<td>Hypokalemic alkalosis</td>
</tr>
<tr>
<td>Karl et al.</td>
<td>1676 (T→A)</td>
<td>559 (B→N)</td>
<td>Transactivation ↓</td>
<td>Heterozygous</td>
<td>Hypertension</td>
</tr>
<tr>
<td>Kino et al.</td>
<td>1430 (G→A)</td>
<td>477 (R→H)</td>
<td>Transactivation ↓</td>
<td>Heterozygous</td>
<td>Hirsutism</td>
</tr>
<tr>
<td>Ruiz et al.</td>
<td>2035 (G→A)</td>
<td>679 (G→S)</td>
<td>Transactivation ↓</td>
<td>Heterozygous</td>
<td>Hirsutism</td>
</tr>
<tr>
<td>Mendonca et al.</td>
<td>1712 (T→C)</td>
<td>571 (V→A)</td>
<td>Transactivation ↓</td>
<td>Homozygous</td>
<td>Ambiguous genitalia</td>
</tr>
<tr>
<td>Vottero et al.</td>
<td>2241 (T→G)</td>
<td>747 (B→M)</td>
<td>Transactivation ↓</td>
<td>Heterozygous</td>
<td>Cystic acne</td>
</tr>
<tr>
<td>Charmandari et al.</td>
<td>2318 (T→C)</td>
<td>773 (L→P)</td>
<td>Transactivation ↓</td>
<td>Heterozygous</td>
<td>Fatigue</td>
</tr>
<tr>
<td>Charmandari et al.</td>
<td>2209 (T→C)</td>
<td>737 (F→L)</td>
<td>Transactivation ↓</td>
<td>Heterozygous</td>
<td>Hypertension</td>
</tr>
</tbody>
</table>

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

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.53,64

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. 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.

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) (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 cyclooxygenase 2. 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, while IL-2 and IL-4 enhance the expression of hGR, a hGR splicing variant that acts as a dominant negative inhibitor of hGRα-mediated transactivation of glucocorticoid-responsive genes. Patients with steroid-resistant asthma may display decreased hGRβ binding affinity, abnormalities in hGR-AP-1 binding and increased expression of hGRβ. Increased hGRβ mRNA expression in peripheral lymphocytes has also been documented in patients with steroid-resistant ulcerative colitis (UC) and steroid-resistant rheumatoid arthritis.

**Inflammation and NF-κB**

A substantial part of the anti-inflammatory actions of hGRs 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

**Generalised Glucocorticoid Resistance**

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

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

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

![Figure 4a: General Mechanisms of Action of Glucocorticoids and Glucocorticoid Receptor in the Inhibition of Inflammation](image)

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

![Figure 4b: Interaction Between NF-κB and Activated Glucocorticoid Receptor](image)
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 IκBα gene,79,80 by blocking the degradation of IκBα via enhanced synthesis of IκBα 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 cytoplasm79,80 (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.77 Similar were the findings related to the activation of AP-1 and the upstream kinases p38 and JNK.78 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.81 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 therapy85 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.90 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.91 Indeed, there is a correlation between HDAC activity and the suppressive effects of glucocorticoids on cytokine release.92 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.93 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 KKKTL), 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 transcriptional activation. 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 characterized 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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