

Review article

Glucocorticoid receptors in human airways

Inhaled and intranasal glucocorticoids are the most common and effective drugs for controlling symptoms and airway inflammation in respiratory diseases such as asthma, allergic rhinitis, and nasal polyposis. The last few years have seen a growing understanding of the mechanisms of glucocorticoid action and, in particular, the receptor that mediates glucocorticoid actions, the glucocorticoid receptor (GR). In this revision we present an update on the GR gene, the expression and regulation of its gene products, namely GR α and GR β , as well as their alterations in pathological states. GR α is responsible for the induction and repression of target genes, it is expressed in virtually all human cells and tissues, and its expression is known to be downregulated by glucocorticoids. GR β has been found to act as a dominant negative inhibitor of GR α -mediated transactivation in *in vitro* studies with transfected cells, but it does not appear to have a significant inhibitory effect on GR α -mediated transrepression. In addition, for most tissues the expression of GR β , at least at the mRNA level, is extremely low compared with that of GR α . Some pro-inflammatory cytokines appear to upregulate the expression of GR β , and increased GR β expression has been reported in diseases associated with glucocorticoid resistance or insensitivity, such as bronchial asthma, nasal polyposis, and ulcerative colitis. However, the possible role of GR β in modulating glucocorticoid sensitivity and/or resistance *in vivo* has been highly debated and it is not yet clear.

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Respiratory allergic diseases of the upper and lower airways share many features in common. The immunoglobulin E (IgE)-dependent allergic reaction is characterized by an early-phase reaction, involving the release of histamine and other proinflammatory mediators, and a late-phase reaction, which involves adhesion molecules, Th2 lymphocytes and numerous inflammatory cells and mediators (1–3). The major classes of drugs currently used for asthma therapy are glucocorticoids, β_2 -adrenergic agonists, theophylline and leukotriene inhibitors (2, 4–7). Along with H₁-antihistamines, glucocorticoids are also widely used in the treatment of allergic rhinitis and nasal polyposis (7–9). At present, inhaled and intranasal glucocorticoids are the most common and effective drugs for controlling symptoms and airway inflammation and have few adverse effects (7). Although most patients with allergic diseases respond to steroid treatment, there is a small subset of patients whose response to treatment is unsatisfactory even with high doses of glucocorticoids (4, 9). There is an ever-increasing interest in investigating the molecular basis that could account for glucocorticoid insensitivity of these patients, and most research has focused on the glucocorticoid receptor (GR), which mediates the anti-inflammatory effects of glucocorticoids (10–12).

Glucocorticoid receptor

The human GR gene, mRNAs and proteins

Glucocorticoid receptors belong to the superfamily of ligand-regulated nuclear receptors that also includes the receptors for mineralocorticoids, thyroid and sexual hormones, vitamin D and retinoic acid (13–15). The human GR gene is located on chromosome 5 (region 5q31-q32) and consists of nine exons (Fig. 1). Exon 1 and the first part of exon 2 contain the 5' untranslated regions (5'UTR), exons 2–9 the coding sequences, and exon 9 the 3'UTR (16, 17). The existence of two previously unidentified exons, called exon 1A and 1B, has been demonstrated (18). Both exons are located upstream from what was originally defined as exon 1, now called exon 1C. The activity of the GR gene is regulated by at least three promoters (promoter 1A, 1B and 1C), which contain binding sites for several transcription factors, including the nuclear factor- κ B (NF- κ B) and the GR itself. These promoters contain multiple GC boxes and lack characteristic TATA and CAAT boxes (18–20).

In 1985, two different human GR cDNAs, termed GR α and GR β cDNA, were cloned (21). It was demonstrated that GR α (5.5 kb) and GR β mRNAs (4.3 kb) derived from alternative splicing of exon 9 and that translation of these mRNAs gave rise to the GR α and GR β protein

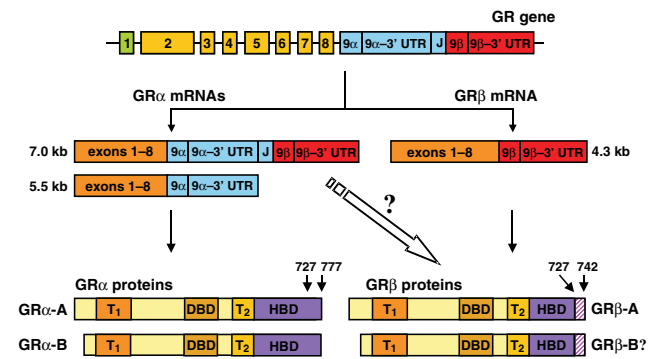


Figure 1. Structure of the GR gene, mRNAs and proteins. Alternative processing of exon 9 of the GR primary transcript generates multiple GR messages. The default splicing pathway generates the 7.0- and 5.5-kb GRα mRNAs, whereas the alternative splicing pathway generates the 4.3-kb GRβ mRNA. It has been suggested that exon skipping during translation of the 7.0-kb mRNA might generate GRβ (24). GRα and GRβ proteins are identical until amino acid 727. The A and B variants of GRα and GRβ are generated from alternative translation initiation start sites. UTR, untranslated region; T₁ and T₂, transactivating regions; DBD, DNA binding domain; HBD, hormone binding domain.

isoforms (17) (Fig. 1). It was immediately recognized that GRα was the 'classical GR', i.e. the receptor isoform that mediated glucocorticoid action. In addition to GRα and GRβ, at least five different GR mRNAs with unique 5'UTRs within exon 1 have recently been reported. These mRNAs are the result of use of the 1A, 1B and 1C promoters and also of alternative splicing of exon 1A (18). These distinct exon 1 sequences do not appear to affect the open reading frame beginning in exon 2. It has been suggested that such promoter heterogeneity gives rise to cell type-specific regulation of GR expression (18). Novel GR mRNA splicing variants, namely GR-P (or GRδ) and GRγ, have been found in certain haematological malignancies (10). These isoforms could play a role in the development of glucocorticoid resistance in cancer.

Like other members of the steroid receptor family, the GR protein contains three structural domains, i.e. a variable amino-terminal transactivation domain, a small well-conserved DNA-binding domain that comprises two repeats of a zinc finger protein motif and a relatively conserved carboxy-terminal domain responsible for hormone binding (14, 15, 22) (Fig. 1). GRα diverges from GRβ only at the carboxy terminal end. Thus, both proteins are identical until amino acid 727, with GRα having an additional 50 amino acids and GRβ having an additional 15 nonhomologous amino acids. Because of this difference in the amino acid composition at the carboxy terminal end, GRβ is unable to bind steroids (17, 21).

It has been found that alternative translation initiation results in the presence of two different GRα isoforms (A and B) (23) (Fig. 1). The use of an alternative start site for

translation at codon 27 results in a protein (the B isoform) that is 26 amino acids shorter than the originally identified 777-amino-acid-long isoform of GRα (the A isoform). These two GR isoforms show different gene transactivation capabilities. A similar mechanism of alternative translation initiation has been claimed to exist for GRβ.

Like other steroid receptors, the GR is subject to a number of post-translational modifications (24, 25). The GR, a phosphoprotein, is phosphorylated in the absence of hormone and is additionally phosphorylated after hormone binding. It appears that GR phosphorylation influences its activity. Other post-translational modifications that have been shown to alter GR function are ubiquitinylation, sumoylation and nitrosylation (24, 25).

Glucocorticoid receptor α

GRα function. In the absence of glucocorticoids, GRα is found in the cytoplasm of cells, existing as a large multiprotein complex that consists of the receptor polypeptide, two subunits of the heat shock protein hsp90, immunophilins, and several additional proteins (Fig. 2). Hsp90 associates with the ligand binding domain of the receptor preventing the nuclear localization of unoccupied receptor (26, 27). The crystal structure of the ligand binding domain of the human GRα has recently been reported (28). Hormone binding triggers a conformational change in the receptor resulting in the dissociation

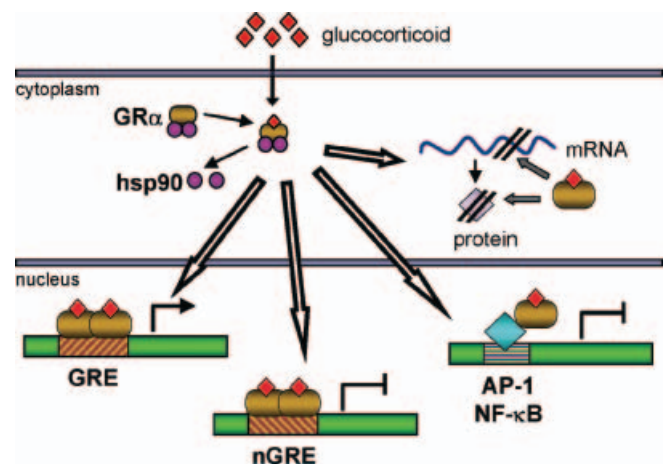


Figure 2. Mechanisms of glucocorticoid action. After passing the cell membrane by passive diffusion, glucocorticoids bind to GRα, associated heat-shock proteins are released (hsp90), and the ligand-bound receptor translocates into the nucleus. A GRα-dimer can bind glucocorticoid responsive elements (GRE) on the promoter region of target genes and activate gene transcription. Binding of GRα to a negative GRE (nGRE) leads to repression. Protein-protein interactions between GRα and transcription factors (AP-1, NF-κB) repress the transcription of pro-inflammatory genes. GRα can alter the mRNA or protein stability of inflammatory mediators.

of hsp90 and other accessory proteins. This new conformation allows translocation of the hormone-GR α complex into the cell nucleus, where it modulates the expression of target genes.

Induction of gene transcription (transactivation) is explained by the interaction of a GR α homodimer to specific DNA sequences, called glucocorticoid responsive elements (GREs), located in the promoter regions of target genes, including those coding for gluconeogenic enzymes (Fig. 2). The consensus GRE sequence consists of the palindromic 15-mer 5'-GGTACAnnnTGTTCT-3' (29, 30). As for many other transcription factors, the transcriptional activity of GR α depends on its interaction with coactivators that regulate the chromatin structure and facilitate recruitment of the basal transcription machinery (RNA polymerase II and general transcription factors) (Fig. 3). For instance, the coactivators CREB (cyclic adenosine monophosphate response element-binding protein)-binding protein (CBP), p300/CBP-associated factor (p/CAF), steroid receptor coactivator-1 (SRC-1) and glucocorticoid receptor-interacting protein-1 (GRIP-1) interact with the $\tau 1$ and $\tau 2$ domains of GR α and, through their histone acetyltransferase (HAT) activity, provoke the local unwinding of chromatin and thus facilitate gene transcription (31, 32).

Repression of gene transcription (transrepression) was initially explained by interaction of GR α with DNA sequences called negative GREs (nGREs) (29). However, the nGRE consensus binding site is variable and has only been described for relatively few genes, such as the pro-opiomelanocortin (POMC) and osteocalcin genes (33, 34). Indeed, most of the inflammatory genes that

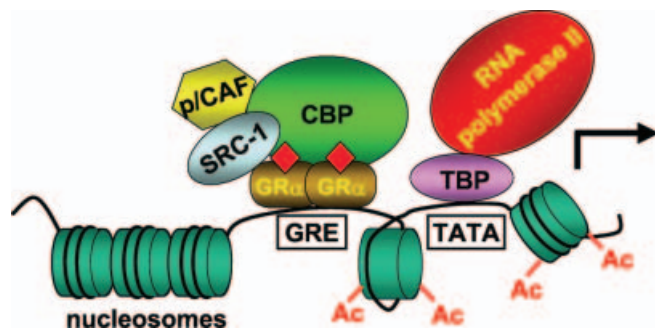


Figure 3. Mechanism of glucocorticoid-mediated activation of gene transcription. The glucocorticoid-bound GR α interacts as a dimer with glucocorticoid responsive elements (GREs), located in the promoter region of target genes, and with coactivators, such as CREB (cyclic adenosine monophosphate response element-binding protein)-binding protein (CBP), p300/CBP-associated factor (p/CAF) and steroid receptor coactivator-1 (SRC-1), which have histone acetyltransferase activity. This results in histone acetylation (Ac), local DNA unwinding, recruitment of the basal transcription machinery, i.e. RNA polymerase II and general transcription factors (TATA box-binding protein, TBP) and activation of gene transcription.

are repressed by glucocorticoids do not contain nGRE sites in their promoters. GR α -mediated inhibition of gene transcription is thought to be mainly due to direct protein-protein interactions between GR α and transcription factors that activate the expression of proinflammatory genes, such as the activator protein-1 (AP-1) and NF- κ B (35–38). Both NF- κ B and AP-1 play central roles in inflammatory airway diseases (39–41). The glucocorticoid-mediated repression of these transcription factors does not require the binding of GR α to DNA. GR α is thought to inhibit AP-1 or NF- κ B transcriptional activities once these factors are already bound to their respective specific DNA binding sites (Fig. 2). It has been reported that activated GR α inhibits CBP-associated HAT activity and favours recruitment of proteins that have histone deacetylase (HDAC) activity, thus resulting in histone deacetylation, increased tightening of DNA around histones and, ultimately, transcriptional repression of inflammatory genes (42, 43) (Fig. 4). GR α -mediated repression of gene transcription has also been explained by many other mechanisms (25, 39, 43–45). For instance, activated GR α upregulates the expression of the cytoplasmic NF- κ B inhibitor I κ B in certain cell types, which prevents nuclear translocation and DNA binding of NF- κ B by its retention in the cytoplasm (46, 47). It has also been reported that GR α can inhibit NF- κ B-induced transcription by interfering with RNA polymerase II phosphorylation (48). In addition, glucocorticoids interfere with signal transduction pathways, such as the

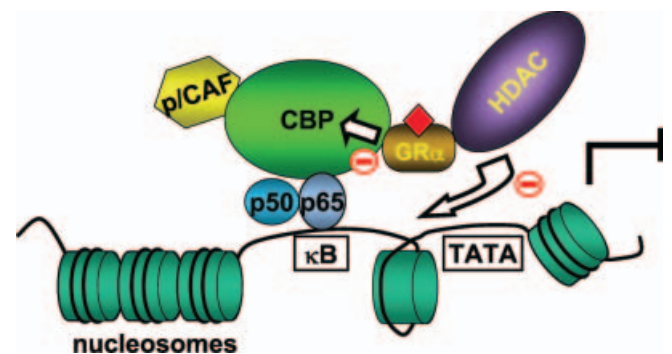


Figure 4. Mechanism of glucocorticoid-mediated repression of gene transcription. A variety of inflammatory stimuli activate the transcription factor nuclear factor- κ B (NF- κ B). Binding of the p65 subunit of NF- κ B to its specific DNA-binding site (κ B) and to coactivators with intrinsic histone acetyltransferase activity, such as CREB (cyclic adenosine monophosphate response element-binding protein)-binding protein (CBP) and p300/CBP-associated factor (p/CAF), induces histone acetylation, local DNA unwinding and transcription of proinflammatory genes. The glucocorticoid-bound GR α interacts as a monomer with the coactivators CBP and p/CAF and inhibits their histone acetyltransferase activity. The activated GR α also recruits proteins with histone deacetylase (HDAC) activity, thus resulting in histone deacetylation, increased tightening of DNA around histones and, ultimately, transcriptional repression of proinflammatory genes. Figure modified from Ito et al. (42).

mitogen activated protein kinases (MAPK) and the JAK-STAT family of receptor-associated tyrosine kinases, involved in the regulation of transcription factors (25, 39, 43). It has been shown, for example, that GR α prevents c-Jun (subunit of AP-1) phosphorylation and, consequently, AP-1 activation, by blocking the induction of the c-Jun N-terminal kinase (JNK) signalling cascade (49).

In addition to the above mentioned regulation of gene expression at the transcriptional level, the activated GR α can also regulate gene transcription at the post-transcriptional (altering mRNA stability), translational (affecting protein synthesis) and post-translational levels (altering protein processing, modification or degradation) (Fig. 2). For example, the expression of cyclooxygenase-2 (Cox-2), TNF- α and GM-CSF are regulated by one or more of these mechanisms (39, 45).

GR α expression and regulation. GR α is thought to be expressed, in differing amounts, in almost all tissues and cells. Early studies using radioligand binding techniques characterized and quantified GR α in a variety of cell types (50, 51). The expression of GR α protein has been extensively analysed using antibodies that recognize epitopes common to both GR α and GR β , located at the N-terminal region of the receptor. Within the airways, the GR has been localized in the alveolar walls, vascular endothelium, smooth muscle, airway epithelium, inflammatory cells and fibroblasts of the human lung (52, 53), and in the surface mucosa, submucosal glands, endothelial cells and inflammatory cells of nasal mucosa and nasal polyps (54). The development of antibodies specific for GR α has identified the expression and subcellular localization of GR α in different human tissues and cell lines (55, 56).

Glucocorticoid receptor expression is known to be downregulated by different glucocorticoids. Such phenomenon is thought to be a feedback protector mechanism that would avoid deleterious effects of prolonged exposure to hormone. Target tissue sensitivity to glucocorticoids has been shown to run parallel with changes in receptor levels (57, 58). For this reason, hormone-induced downregulation of GR has been proposed as one of the possible mechanisms that would explain insensitivity to glucocorticoid treatment. Both *in vitro* (59–67) and *in vivo* studies (68–73) have reported glucocorticoid-mediated downregulation of its own receptor, mostly the non-isoform-specific GR, in a variety of cells and tissues. Few studies have analysed the specific expression of GR α (Table 1).

The *in vitro* exposure of two human bronchial epithelial cell lines to glucocorticoids provoked dose- and time-dependent downregulation of GR α mRNA (67, 74). Glucocorticoids strongly downregulated the expression of GR α protein in cell lines from bronchial epithelium (BEAS-2B) and alveolar lung (A549) origin, as well as in cultured nasal mucosa epithelial cells (74). Indeed,

Table 1. Expression and regulation of GR α and GR β by glucocorticoids

Cell/tissue	Method of analysis	GC effect		References
		GR α	GR β	
Bronchial epithelial cells, alveolar macrophages	Northern	↓	↓	67
BEAS-2B, nasal mucosa epithelial cells	PCR	↓	↓	74
BEAS-2B, A549, nasal mucosa epithelial cells	WB	↓	N.D.	74
PBMCs from asthmatic subjects	WB	↓	N.D.	86
Skin biopsies from GC-sensitive asthmatics	IHC	↓	=	68
Skin biopsies from GC-insensitive asthmatics	IHC	=	=	68
IM-9 cells	PCR	↓	↓	101
CEM-C7 cells	PCR	↑	↑	101
Skeletal myoblasts	Northern	↓	↑	108
GR α / β -transfected COS-1 cells	WB	↓	=	20

GC, glucocorticoids; WB, Western blot; IHC, immunohistochemistry; N.D., not detected; BEAS-2B, human bronchial epithelial cell line; A549, human alveolar cell line; PBMC, peripheral blood mononuclear cells; IM-9, B lymphoblastoid cell line; CEM-C7, T-cell acute lymphoblastic leukemia cell line; COS-1, kidney monkey cell line.

↑, Upregulation; ↓, downregulation; =, not modified.

downregulation of GR α protein in these cells was more pronounced than downregulation of its mRNA, suggesting that, in addition to the regulation at the level of transcription, GR α is significantly regulated at the translational and/or post-translational levels. In fact, glucocorticoid treatment provokes a twofold decrease of the GR protein half-life (61, 69, 75). Studies of various systems using the protein synthesis inhibitor cycloheximide have revealed that *de novo* protein synthesis is not required for glucocorticoid-induced downregulation of GR expression (59, 63, 65, 66).

Data on the *in vivo* regulation of GR in humans is limited. Knutsson et al. (72) reported that the administration of intranasal glucocorticoids to the nasal mucosa of healthy subjects resulted in downregulation of GR mRNA expression. The authors also pointed out an inter-individual variation in GR mRNA expression. Similarly, Korn et al. (67) reported downregulation of GR α mRNA after 4 weeks of budesonide inhalation in both bronchial epithelial cells and alveolar macrophages. Both studies (67, 72) demonstrated that hormone-mediated downregulation of GR α mRNA expression was transient, with GR α expression levels returning to basal levels once glucocorticoid treatment was ceased. Indeed, Korn et al. (67) reported normalization of GR α mRNA levels within 10–12 h. Because of these findings, the authors argued that the long-term *in vivo* use of inhaled corticosteroids, which resembles a 'hit-and-run' process, is likely to represent a reversible process (67).

In contrast to the case of healthy nasal mucosa, no downregulation of GR mRNA has been found after treatment of inflamed nasal mucosa (nasal polyps) with intranasal glucocorticoids (76; L. Pujols, unpublished results). The absence of glucocorticoid-induced downregulation of GR α expression in nasal polyps might reflect a

Table 2. Expression and regulation of GR α and GR β by proinflammatory stimuli

Stimuli	Cell/tissue	Method of analysis	Effect		References
			GR α	GR β	
TNF- α , IL-1	HeLa, CEM	PCR, WB, IHC	$\uparrow$	$\uparrow$	20
IL-2 + IL-4	PBMC	IHC	N.A.	$\uparrow$	104
		PCR	$\uparrow$	=	109
		WB	=	N.D.	109
IL-8	Neutrophils	RNA dot blot	$\downarrow$	$\uparrow^*$	95
		IHC	=	$\uparrow$	95
IL-18	CEM	PCR	=	$\uparrow$	85
	PBMC	PCR	$\uparrow$	$\uparrow$	85
Bacterial superantigens	PBMC	IHC	$\uparrow$	$\uparrow$	84
	Nasal mucosa	IHC	N.A.	$\uparrow$	110
Ragweed	Nasal mucosa	IHC	N.A.	$\uparrow$	110

WB, Western blot; IHC, immunohistochemistry; N.A., not analysed; N.D., not detected; HeLa, human cervix epitheloid cell line; CEM, lymphoid cell line; PBMC, peripheral blood mononuclear cells; IL, interleukin.

$\uparrow$, Upregulation; $\downarrow$, downregulation; =, not modified

* Using a non-GR β -specific probe.

low sensitivity of this inflamed tissue to glucocorticoid treatment.

It has been known for some time that proinflammatory stimuli decrease GR binding affinity and steroid responsiveness (77–80). NF- κ B has been shown to repress the transcriptional activity of GR α (38, 81–83). It was then concluded that these effects might be due to the mutual antagonism between GR and proinflammatory transcription factors, and were in some way related to the ongoing inflammation. However, little is known about the effect of proinflammatory stimuli on GR α expression and regulation. Different proinflammatory stimuli, such as TNF- α , IL-1, or bacterial superantigens, have been shown to induce a mild increase in GR α expression in a variety of cell types (20, 84, 85) (Table 2). However, GR α does not appear to be significantly altered in various inflammatory diseases (Table 3). Thus, similar GR α levels have been reported in PBMCs (86) and skin biopsies (68) from both glucocorticoid-sensitive and -insensitive asthmatics. Similarly, no differences in GR α mRNA expression have been found between healthy PBMCs and those from patients with inflammatory bowel disease (85), and similar GR α mRNA levels have been reported in both healthy and inflamed nasal mucosa epithelial cells (87).

Glucocorticoid receptor β

GR β function. As mentioned earlier, GR β is unable to bind steroids (17, 21). Using *in vitro* transfection systems, it has been shown that GR β alone was not transcriptionally active on GRE, AP-1 and NF- κ B driven systems (17, 88–91). In different cell types, it was found that when overexpressed with respect to GR α , GR β acted as a dominant-negative inhibitor of GR α transactivational activity (17, 92–94). It was also

Table 3. Expression and regulation of GR α and GR β in diseases

Disease	Cell/tissue	Method of analysis	Effect		References
			GR α	GR β	
GC-insensitive asthma	PBMC, BAL	IHC	N.A.	$\uparrow$	104, 105
	PBMC	PCR, WB	=	=*	86, 109
	Skin	IHC	=	$\uparrow$	68
Fatal asthma	Lung	IHC	N.A.	$\uparrow$	106
Nocturnal asthma	BAL macrophages	IHC	=	$\uparrow$	112
Nasal polyposis	Nasal polyps	IHC	N.A.	$\uparrow$	107
		PCR	$\downarrow$	=	L. Pujols†
		PCR	=	$\uparrow$	87
Inflammatory bowel diseases	PBMC	PCR, WB	N.A.	$\uparrow$	100
		PCR	=	$\uparrow$	85
Chronic lymphocytic leukemia	PBMC	WB	$\downarrow$	$\uparrow$	113
Acute lymphocytic leukemia	T-lymphocytes	WB	$\downarrow$	=	114
ACTH-secreting tumours	pituitary	PCR	$\uparrow$	=	99

WB, Western blot; IHC, immunohistochemistry; N.A., not analysed; PBMC, peripheral blood mononuclear cells; BAL, bronchoalveolar lavage.

$\uparrow$, Upregulation; $\downarrow$, downregulation; =, not modified.

* No detection of GR β by Western blot; † unpublished results.

found that GR β was able to bind hsp90 and GRE, although none of these actions were affected by the addition of hormone (55, 88, 94). It was also shown that GR β forms heterodimers with GR α (94–96). It has been proposed that the mechanism of GR β dominant-negative inhibition is due to the formation of these GR α –GR β heterodimers, which would have a much lower transcriptional activity than GR α homodimers (24, 94–96). The dominant negative activity of GR β has been recently located in two residues within the 15 unique C-terminal amino acids of GR β (97).

However, the inhibitory role of GR β on GR α activity has been subject of great controversy (Table 4). For example, several authors failed to detect any dominant-negative inhibition of GR β on GR α activity (88, 89, 91, 98). Such discrepancies have been attributed to differences in the cell types and/or experimental approaches used, and also to non-specific squelching of transcriptional coactivators by GR β . It is important to take into account that most of the glucocorticoid anti-inflammatory effects are mediated through repression of genes encoding pro-inflammatory mediators by inhibiting the transcription factors AP-1 and NF- κ B. In keeping with this, GR β does not appear to inhibit (89, 91, 93), or only moderately inhibits (94), the capacity of GR α to repress AP-1- and NF- κ B-dependent activities. Interestingly, Gougat et al. (93) found that GR β , as GR α , inhibited hormone-independent AP-1- and NF- κ B-dependent activities by 25–60%. Therefore, the authors (93) suggested that overexpression of either GR α or GR β might have an anti-inflammatory effect.

GR β expression and regulation. GR β mRNA has been found to be expressed in a variety of human cells and tissues, although in much lower amounts than GR α

Table 4. *Pros* and *cons* of the GR β function and its contribution to glucocorticoid insensitivity

PROS	CONS
Transfection studies • When overexpressed with respect to GRα, GRβ is a dominant-negative inhibitor of GRα-mediated transactivation (17, 92–94) Expression and regulation • High GRβ immunoreactivity, mainly localized in inflammatory cells (68, 84, 95, 104–107) • GRβ protein half-life is twice that of GRα (20) • No downregulation of GRβ protein by GCs in GRβ-transfected cells (20) and in skin biopsies from asthmatic patients (68) • Upregulation of GRβ by proinflammatory stimuli in different cell types (20, 84, 85, 95, 104, 110) Expression in inflammatory diseases • Increased GRβ immunoreactivity in PBMCs, T cells and macrophages from GC-resistant asthmatics (68, 104, 105) • Increased GRβ immunoreactivity in inflammatory cells from fatal asthma (106) and nocturnal asthma (112) • Increased GRβ immunoreactivity in inflammatory cells from nasal polyps (107) • Increased GRβ in PBMCs from inflammatory bowel diseases (85, 100)	• GRβ is not a dominant-negative inhibitor of GRα-mediated transactivation (88, 89, 91, 98) • Little effect of GRβ on GRα-mediated transrepression (89, 91, 93, 94) • Very low levels of GRβ mRNA (GRα/GRβ > 500/1) and protein in most cells and tissues (17, 20, 53, 74, 85–88, 91, 93, 99–103) • GRβ is not differently regulated by GCs than is GRα in several cell types (67, 74, 101) • No upregulation of GRβ by IL-2 and IL-4 in PBMCs (109) • Very low levels of GRβ mRNA and no detection of GRβ protein in PBMCs from GC-insensitive asthmatics (86, 109) • Very low levels of GRβ mRNA in nasal polyps (87, Pujols*) • Very low levels of GRβ mRNA in PBMCs from inflammatory bowel diseases (85, 100)

PBMC, peripheral blood mononuclear cells; GC, glucocorticoid.

* Unpublished results.

mRNA (17, 20, 53, 85, 86, 93, 99–101). In fact, several studies report a GR α /GR β mRNA ratio greater than 500/1 (Table 4). The concentration of the GR β transcript is lower than 0.1 molecules per cell (74, 87, 101, 102). The extremely low levels of the GR β message do not always concur with the GR β protein data. De Castro et al. (55) detected GR β protein in several human tissues by Western blotting. Further studies revealed either no GR β (74, 86, 102) or low expression of GR β (88, 91, 103) in various human cell types and tissues. However, as revealed through immunohistochemistry, GR β has been detected in specific cell types, including epithelial cells in liver, thymus and lung (103), and in inflammatory cells (68, 84, 95, 104–107). Highly discrepant results have been presented with regard to the relative abundance of GR β compared with GR α (20, 55, 88, 95). There is increasing evidence that, in physiological conditions, GR α is clearly predominant over GR β in most cells and tissues (74, 88, 102, 103), although the GR α /GR β protein ratio appears to be much lower than it is at the mRNA level.

Indeed, there are great discrepancies between mRNA and protein expression data. These discrepancies could arise from the methods used. First, all currently available antibodies against GR β , and also GR α , are polyclonal and do not detect single bands through Western blotting. In most immunohistochemical analyses, the specificity of these antibodies on the target tissue has not been examined thoroughly. In addition, some studies quantify the GR α /GR β ratio of the cell based on the use of different antibodies for each GR isoform, which may not reflect the actual ratio of the cell because the antibodies may have different affinities to the epitopes. The best way

to compare the relative levels of GR α and GR β proteins would be by using a single antibody that recognized a common epitope in both isoforms of the receptor. However, the existence of the recently discovered B-isoform of GR α (23), and possibly a B-isoform for GR β , further complicates GR protein analysis, for all GR protein variants have similar weights (GR α -A, 94 kDa; GR α -B, 91 kDa; GR β , 90 kDa). It has been recently found that the GR β protein half-life is twice that of GR α (20). In our opinion, this difference would only partially explain the big discrepancy between the GR α /GR β mRNA and the GR α /GR β protein ratio. In line with this, Pedersen et al. (101) have recently confirmed the rarity of GR β transcripts in two human leukemic cell lines and, based on its low mRNA expression levels, the authors have mathematically demonstrated that the GR β /GR α protein ratio in these cells is, at most, 0.8. Finally, we cannot rule out the possibility that the mRNA vs protein discrepancies are the result of an incomplete GR gene splicing model. It has been speculated, for example, that translation of the 7.0 kb message might give rise to additional GR β through an exon skipping process (24) (Fig. 1). It could also be speculated that additional unidentified GR splicing variants might exist, whose protein products could have cross-reactivity with the anti-GR β antibodies.

The subcellular distribution of GR β is also controversial. One report (55) detected GR β in both the nucleus and cytoplasm and described the translocation of both GR β and GR α to the nucleus after exposure to glucocorticoids. In contrast, Oakley et al. (103) found GR β in the cell nucleus both in the absence and presence of

hormone. Finally, Gougat et al. (93) located GR β in both the nucleus and cytoplasm, irrespective of hormone administration.

Some reports suggest that GR β might be differently regulated by glucocorticoids than GR α (Tables 1 and 4). In a human bronchial epithelial cell line (BEAS-2B), we reported a dose-dependent downregulation of GR β mRNA after 6 h of dexamethasone administration, with GR β mRNA levels returning to basal expression levels after 24 h of hormone treatment (74). In skin biopsies from asthmatic patients, glucocorticoids did not downregulate the number of cells expressing GR β , but they did downregulate the number of GR α -immunoreactive cells (68). Dexamethasone did not downregulate GR β in COS-1 cells transfected with GR β (20). However, Pedersen et al. (101) have shown the coordinated regulation of GR β and GR α mRNAs by dexamethasone in two human leukemic cell lines. To our knowledge, only one study has reported the upregulation of GR β mRNA – as opposed to GR α downregulation – by glucocorticoids in healthy non-transformed human cells (skeletal myoblasts) (108).

Several proinflammatory cytokines have been shown to upregulate the expression of GR β (Tables 2 and 4). For instance, the combination of IL-2 and IL-4 increased the number of GR β immunoreactive PBMCs (104). However, we have recently found that this cytokine combination does not alter the expression of either the GR β mRNA or its protein product in PBMCs from both healthy and asthmatic patients, as analysed through RT-PCR and Western blot (109). It has also been reported that IL-18 increases the expression of GR β mRNA in PBMCs (85). Webster et al. (20) showed that TNF- α and IL-1 induced the expression of both GR β and GR α mRNAs in HeLa and CEM-C7 cell lines, but disproportionately increased the steady-state levels of the GR β protein over GR α . The authors also reported that the GR β protein half-life was twice as long as that of GR α (20). IL-8 induced the expression of GR β in human neutrophils (95), the stimulation of PBMC from healthy donors with phytohaemagglutinin or bacterial superantigens was found to increase the immunoreactivity for GR β (84), and the incubation of nasal tissue from both non-atopic and atopic ragweed-sensitive patients with ragweed and bacterial superantigens also increased the immunoreactivity for GR β (110).

GR β and glucocorticoid resistance. A number of reports have appeared within the last few years showing increased expression of GR β in diseases associated with glucocorticoid resistance or insensitivity (Tables 3 and 4). Corticosteroid-resistant asthma was associated with a significantly higher number of GR β -immunoreactive PBMCs and BAL cells, when compared with both healthy subjects and corticosteroid-sensitive asthmatics (104, 105). GR β expression in corticosteroid-resistant asthmatics was particularly high in airway T-cells (105). However, Gagliardo et al. (86) did not find any

association between GR β expression and glucocorticoid insensitivity in PBMCs from asthmatic patients, and we have found similar GR β expression levels in PBMCs from both healthy subjects and those from either stable or unstable asthmatics (109). An increased number of GR β -immunoreactive cells was found in both the small and large airways of patients who died of slow-onset fatal asthma (106), and an increase in the number of cells expressing GR β has also been found in skin biopsies of corticosteroid-resistant asthmatics (68).

Besides asthma, the association between increased GR β expression and corticosteroid resistance has been documented in other inflammatory diseases. For instance, an increased number of GR β -immunoreactive cells has been reported in nasal polyps, when compared with healthy nasal mucosa (107). However, we have not found a higher expression of the GR β message in nasal polyps as compared with nasal mucosa (L. Pujols, unpublished results). Increased expression of GR β has also been reported in PBMCs from patients with inflammatory bowel disease (85, 100). Honda et al. (100) detected GR β mRNA in 83% of patients with corticosteroid-resistant ulcerative colitis, but only in 10% of corticosteroid-sensitive patients or healthy subjects. GR β protein was only detected in some GR β mRNA-positive patients (100). Using a quantitative real-time PCR assay, Orii et al. (85) reported significantly higher GR β mRNA levels in the active stage of ulcerative colitis as compared with its inactive stage. However, the expression levels of the GR β message were very low compared with those of GR α (85).

Finally, a polymorphism in exon 9 β has been associated with rheumatoid arthritis (111). This polymorphism is located in the 3'UTR and is known to provide GR β mRNA with an increased mRNA stability. The authors suggested that this polymorphism might play a role in the pathogenesis of rheumatoid arthritis.

Conclusions

The last few years have seen a growing understanding of the GR gene and the expression and regulation of its gene products. GR α is expressed to varying degrees in all human cells and tissues and is responsible for the induction and repression of target genes. The *pro*- and *con*-concepts of the GR β function and expression, and its possible contribution to glucocorticoid insensitivity have been summarized in Table 4. GR β acts as a dominant negative inhibitor of GR α -mediated transactivation in certain cell types, but it does not appear to have a significant inhibitory effect on GR α -mediated transrepression. Whereas consistently low expression of GR β mRNA has been detected in a number of cells and tissues, the findings on GR β protein expression have presented great discrepancies. Glucocorticoids downregulate the expression of GR α in both *in vitro* and *in vivo* systems.

The role of glucocorticoids in the regulation of GR β expression has been less investigated but, for most cell types, GR β does not appear to be differently regulated by glucocorticoids than is GR α . Pro-inflammatory cytokines appear to upregulate the expression of GR β , and increased GR β expression has been found in diseases associated with glucocorticoid resistance or insensitivity,

such as bronchial asthma, nasal polyposis and ulcerative colitis. However, some authors have not found any association between GR β and glucocorticoid insensitivity. In addition, it remains to be elucidated to what extent an increase in GR β expression contributes to glucocorticoid resistance and/or the pathogenesis of the disease.

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