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Importance of glucocorticoid receptors in upper and lower airways

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1. ABSTRACT

Glucocorticoids (GCs) are the most common and effective drugs for treating inflammatory airway diseases, but some patients respond poorly to them. GC effects are mediated through the glucocorticoid receptor (GR). We present an update on the GR gene, the GR alpha and GR beta splicing variants, their translational and post-translational modifications, as well as their alterations in disease. GR alpha is ubiquitously expressed and is responsible for the induction and repression of target genes. GR beta acts as a dominant negative inhibitor of GR alpha-mediated transactivation and transrepression in certain cell types. The GR beta message is expressed at low levels in numerous tissues and its protein is only expressed in specific cell types. Increased GR beta expression has been reported in bronchial asthma, nasal polyposis and inflammatory bowel diseases (IBD), and after incubation of cells with certain proinflammatory stimuli. In addition to GR beta, other mechanisms explaining GC resistance include alterations in GR binding to ligand, nuclear translocation, and binding to GRE, and/or a defective cross-talk with transcription factors and cofactors.

2. INTRODUCTION

Glucocorticoids (GCs) are the most common and effective drugs for treating inflammatory and immune diseases, such as rheumatoid arthritis, IBD, and respiratory diseases from the lower and upper airway, including asthma (1), allergic rhinitis (2), and chronic rhinosinusitis and nasal polyposis (3).

GCs were first synthesized between the 1930s and 1940s. In 1950, the same year that Dr Philip Hench was awarded the Nobel Prize for Medicine for using cortisol in the treatment of rheumatoid arthritis, cortisol was also used in the treatment of bronchial asthma. The development of topical GCs in the mid 1970s drastically reduced the adverse effects of systemic GCs. The anti-inflammatory effect of GCs is exerted through a reduction in both the cell number and the function of immune cells (4). Despite their widespread use, a subset of patients suffering from inflammatory diseases shows a limited clinical response to even high doses of GCs. Understanding the molecular mechanisms involved in GC insensitivity in these patients may help us to develop new strategies for

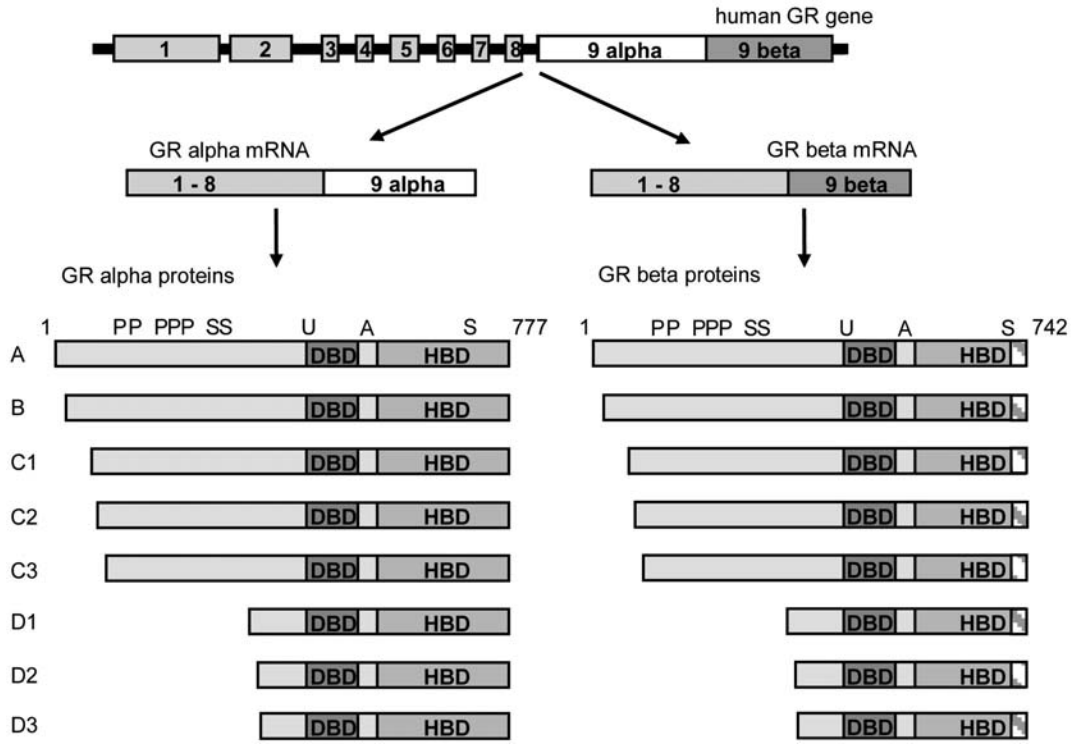

Figure 1. Structure of the human GR gene, mRNAs and proteins. Alternative splicing of exon 9 generates GR alpha and GR beta mRNAs. Each GR alpha mRNA, and presumably also GR beta mRNA, produces multiple GR alpha or GR beta proteins by alternative translation initiation. Numbers denote the first and last amino acid for each GR isoform. Each GR protein is subject to posttranslational modifications, including phosphorylation (P), ubiquitination (U), SUMOylation (S), and acetylation (A). DBD, DNA binding domain; HBD, hormone binding domain.

reating airway inflammatory diseases. In the last few decades, one of the main focuses of research has been the receptor that mediates GC actions, i.e. the glucocorticoid receptor (GR). Numerous outstanding reviews have been recently published along these lines (4-12). The widespread distribution of the GR within the body explains the efficacy of GC treatment in most patients and in numerous inflammatory diseases. The discovery of another variant of the receptor, namely GR beta, with negative effects on the functioning of the classical GR alpha receptor (13), raised enormous interest in analyzing whether an imbalance between GR alpha and GR beta could explain GC insensitivity in inflammatory diseases. In addition, the recent discovery of multiple GR alpha variants as well as their numerous post-translational modifications has contributed to our increasing understanding of the diversity in the GR signaling pathway (7, 8, 14).

3. THE GLUCOCORTICOID RECEPTOR

3.1. The human GR gene and mRNA products

GRs belong to the super-family of ligand-regulated nuclear receptors that also includes the receptors for mineralocorticoids, thyroid and sexual hormones, vitamin D and retinoic acid (7-9). The human GR gene is located on chromosome 5q31-32 and is composed of 9 exons. The protein-coding region is contained in exons 2 to 9. Upstream of exon 2, there is a 3.1 kb CpG rich region that comprises multiple first exons, apparently each with its own promoter region. Alternative mRNA transcript variants are obtained by splicing of these alternative first exons to a common acceptor site in exon 2. This 5'-heterogeneity is untranslated and therefore does not affect the sequence of the GR protein (15). The promoter regions of the GR gene contain binding sites for several transcription factors, including the nuclear factor (NF)-kappa B and the GR itself, but lack characteristic TATA and CCAAT boxes. The last exon of the human GR gene is, together with exon 1, subject to alternative splicing, resulting in mature GR alpha and GR beta mRNAs (7-9, 13) (Figure 1). Other GR mRNA splicing variants, namely GR-P (or GR delta) and GR gamma, have been found in certain hematological malignancies and could play a role in the development of GC resistance in cancer (8).

3.2. Multiple GR proteins

Translation of GR alpha and GR beta mRNAs generates GR alpha and GR beta proteins. GR alpha diverges from GR beta only at the carboxy terminal end. Thus, both proteins are identical until amino acid 727, with GR alpha having an additional 50 amino acids and GR beta having an additional 15 non-homologous amino acids. Structurally, both proteins, like other members of the steroid receptor family, contain a variable amino-terminal trans-activation 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 (7-9, 11) (Figure 1). The difference at the carboxy terminal end of GR beta impairs its binding to steroids (13). However, it has recently been found that GR beta can bind the GC antagonist RU-486 (*see* chapter 5.1) (16).

In the last few years, multiple new GR isoforms, designated in capital letters from A to D, have been found for GR alpha and proposed for GR beta. These GR alpha variants are derived from alternative translation initiation from the GR alpha mRNA, through mechanisms involving ribosomal leaky scanning or ribosomal shunting from alternative translation initiation sites located in exon 2 (7, 8, 14) (Figure 1). All these GR alpha protein variants are functional but exhibit differences in their sub-cellular localization and trans-activation capabilities. For instance, the GR alpha D isoform displays half the activity of the wild-type GR alpha. Interestingly, it has been found through microarray analysis that while all GR alpha proteins regulate a set of about 200 common genes, each of these GR alpha isoforms can additionally regulate unique sets of genes. In addition, different tissue expression patterns in these GR alpha isoforms have been found in both rats and mice. The existence and unique properties of these GR alpha isoforms in humans would provide a novel mechanism for tissue-specific GC responses (7, 8, 14). It has recently been observed that all these GR alpha isoforms can elicit apoptosis of human bone cells (17). The distribution of these GR alpha variants in human cells and tissues, and their relevance in determining the clinical response of patients to GC, are as yet unknown.

Like other steroid receptors, the GR contains specific sites for phosphorylation, ubiquitination, SUMOylation, and acetylation (Figure 1). These posttranslational modifications are known to affect the functional activity of the receptor (*see* next chapter) (7, 8).

4. GLUCOCORTICOID RECEPTOR ALPHA

GR alpha is expressed, in differing amounts, in all human cells and tissues, including those from the lower and upper airways (9, 18-24), where it functions as a hormone-dependent transcription factor. In the absence of GCs, GR alpha is retained in the cytoplasm of cells as part of a chaperone-containing multiprotein complex that prevents the nuclear localization of unoccupied receptor. Hormone binding triggers a conformational change in the receptor that provokes its dissociation from chaperone proteins and its translocation into the cell nucleus. Once there, GR alpha regulates the transcription of target genes through several mechanisms discussed below (6, 9, 11, 18) (Figure 2). Once the GC action is culminated, GR alpha is polyubiquitinated and degraded through the proteasome (8, 25).

GR alpha can activate gene transcription by interacting as a homodimer with glucocorticoid response elements (GREs) located in the promoter regions of target genes. In common with many other transcription factors, the transcriptional activity of GR alpha depends on its interaction with co-activators, such as CREB (cyclic adenosine monophosphate response element-binding protein)-binding protein (CBP), p300, p300/CBP-associated factor (p/CAF), and steroid receptor coactivator-1 (SRC-1), which through their histone acetyltransferase (HAT) activity provoke the local unwinding of chromatin, thus facilitating the recruitment of the basal transcription machinery (RNA polymerase II and general transcription factors) and inducing gene transcription (6, 26). Until recently, it was believed that GC-mediated activation of gene transcription (transactivation) scarcely contributed to the anti-inflammatory effects of GC. However, the increasing number of GC-activated genes with anti-inflammatory effects found through microarray technology reinforces the role of transactivation in mediating the GC anti-inflammatory function (27-29). Expression of the anti-inflammatory molecules lipocortin-1, IL-10, IL-1 receptor antagonist, mitogen-activated protein kinase phosphatase-1 (MKP-1), the NF-kappa B inhibitor I-kappa B alpha, GC-induced leucine zipper, and the RNA-binding protein tristetraprolin (TTP) is induced by GC via GRE-dependent gene transcription (6, 8, 10, 28).

GR alpha can inhibit gene transcription by interacting with negative GREs (nGREs), as is the case for the proopiomelanocortin (POMC) and osteocalcin genes. However, most of the inflammatory genes that are repressed by GCs do not contain nGRE sites in their promoters. GR alpha-mediated inhibition of gene transcription appears to be mainly due to direct protein-protein interactions between GR alpha and transcription factors, particularly activator protein-1 (AP-1) and NF-kappa B, which activate the expression of pro-inflammatory genes. The repressive effect of GR alpha on these transcription factors is mutual, since NF-kappa B and AP-1 also repress GR alpha-mediated transcription. The exact mechanisms behind this mutual antagonism are still subject to debate (10). GR alpha does not appear to decrease the binding of NF-kappa B or AP-1 to its cognate response elements. In addition, in most cases the crosstalk between GR alpha and NF-kappa B or AP-1 does not result from a competition for the binding to common co-activators but involves, instead, the remodeling of chromatin. It has been proposed that activated GR alpha mediates the recruitment of transcriptional co-repressors, such as histone deacetylases (HDACs), thus resulting in histone deacetylation, increased tightening of DNA around histones and, ultimately, transcriptional repression of inflammatory genes. However, the involvement of HDACs in the negative crosstalk between GR alpha and other transcription factors is still a matter of debate (6, 10). It has also been reported that GR alpha can inhibit NF-kappa B-induced transcription by interfering with RNA polymerase II phosphorylation. Finally, it has been suggested that GR alpha might repress NF-kappa B- or AP-1-dependent transcription by interfering with other histone tail modifications, such as phosphorylation and methylation (10).

GCs have been shown to exert numerous rapid (i.e. lasting a few minutes) anti-inflammatory and immunosuppressive effects on different cells, tissues, and

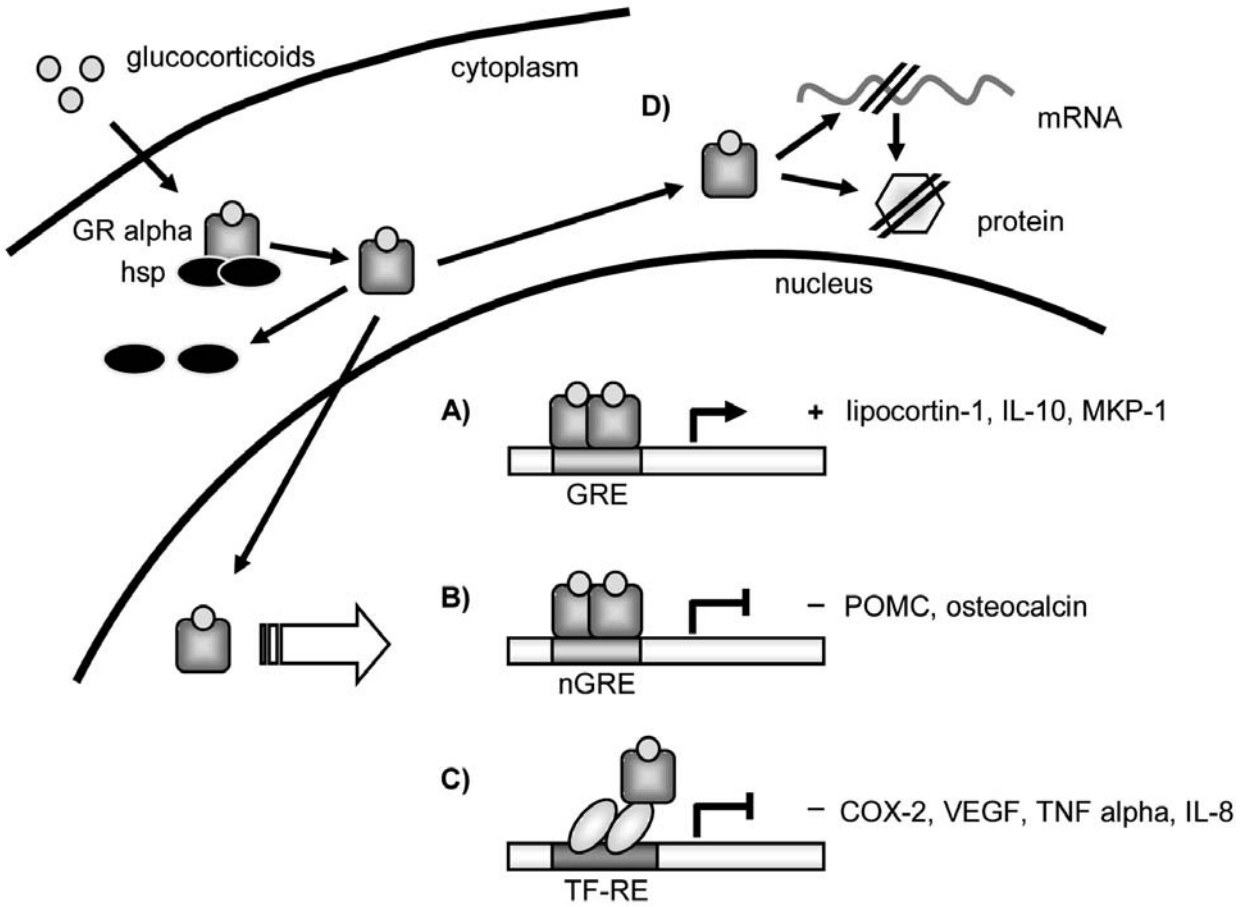

Figure 2. Mechanisms of glucocorticoid action. After passing the cell membrane by passive diffusion, GCs bind to GR alpha, associated heat-shock proteins (hsp) are released, and the ligand-bound receptor translocates into the nucleus. A) A GR alpha-dimer can bind GC responsive elements (GRE) on the promoter region of target genes and activate gene transcription. B) Binding of GR alpha to a negative GRE (nGRE) leads to repression. C) Protein-protein interactions between GR alpha and transcription factors, such as NF-kappa B and AP-1, repress the transcription of pro-inflammatory genes. D) GR alpha can alter the mRNA or protein stability of inflammatory mediators. IL: interleukin, MKP-1: mitogen-activated protein kinase phosphatase-1, POMC: proopiomelanocortin, COX-2: cyclooxygenase-2, VEGF: vascular endothelial growth factor, TNF alpha: tumor necrosis factor alpha, TF-RE: transcription factor-response element.

organs (30). These rapid GC effects are incompatible with the genomic regulation discussed above and they are indeed mediated by non-genomic mechanisms, involving both the classical cytosolic GR alpha and a distinct membrane-associated GR signaling via G protein-dependent mechanisms (4, 6, 10, 30).

GR alpha also mediates anti-inflammatory actions by decreasing the mRNA stability of proinflammatory genes, such as cyclooxygenase-2, tumor necrosis factor (TNF) alpha, and vascular endothelial growth factor (6, 28) (Figure 2). Posttranscriptional control via regulation of mRNA turnover is conferred by adenylate-uridylate-rich elements (ARE) located in the 3' untranslated regions of transcripts encoding various inflammatory molecules and the action of trans-acting factors, such as TTP, which bind to the ARE and promote mRNA deadenylation and subsequent degradation. It has been reported that GC induce the synthesis of TTP mRNA and protein, enabling the posttranscriptional decrease of TNF alpha mRNA expression (31). This mechanism represents a novel inductive signaling pathway for GC to exert their anti-inflammatory actions.

The functional activity of GR alpha is significantly affected by the posttranslational modifications to the receptor mentioned above (Figure 1) (7, 8). The human GR is phosphorylated on specific serine residues after hormone binding and is also phosphorylated by cyclins/cyclin-dependent kinases, p38 mitogen-activated protein kinase (MAPK), and c-Jun N-terminal kinase (JNK) (32, 33). It has been recently reported that GR phosphorylation at specific serines determines the transcriptional response of the GR. Thus, GR phosphorylation at serine 211 activates the transcriptional activity of the receptor, while GR phosphorylation on serine 226 inhibits GR transcriptional activation (34). GR phosphorylation is also known to affect the stability of the

receptor, its sub-cellular localization, and its interaction with coregulators (33, 34). A more biological example that demonstrates that GR phosphorylation affects its function was provided by Irusen and coworkers (35), who reported that GR phosphorylation induced by IL-2 and IL-4, inductors of the p38 MAPK pathway, resulted in a reduced capacity of dexamethasone to repress LPS-stimulated GM-CSF release and increase IL-10 release in PBMCs. It was also reported that nitrosylation of the GR at an hsp90 interaction site induced by nitric oxide decreased GR ligand binding affinity (36). Ito and coworkers (37) have recently reported that the GR is acetylated after ligand binding and deacetylated by HDAC2. GR deacetylation by HDAC2 appears to be a prerequisite for suppression of NF-kappa B and, subsequently, suppression of inflammatory gene expression. The activity of the GR may also be indirectly modulated by chemical reactions, such as acetylation and methylation, that occur in proteins that interact with the GR (7, 8, 19).

5. GLUCOCORTICID RECEPTOR BETA

5.1. GR beta function

Owing to its altered ligand-binding domain, GR beta cannot bind GCs, and although it can bind GRE, it cannot activate the transcription of GRE, AP-1 and NF-kappa B promoters (13, 38-41). However, it has recently been shown that GR beta binds the antiglucocorticoid RU-486, but not dexamethasone, and translocates to the cell nucleus following RU-486 administration (16). In addition, the authors demonstrate that GR beta alone regulates gene expression, and that binding of RU-486 to GR beta has an antagonistic effect on gene regulation. In certain cell types, when over-expressed with respect to GR alpha, GR beta inhibits both the transactivation activity of GR alpha (13, 41-43) and the capacity of GR alpha to repress AP-1- and NF-kappa B-dependent promoters (41). However, other investigators failed to reproduce one or more of these findings (38, 40, 43, 44). The molecular basis for the dominant-negative activity of GR beta appears to lay on the formation of GR alpha-GR beta heterodimers, which would hinder the formation of transcriptionally active GR alpha homodimers (41, 45, 46). The dominant-negative activity of GR beta has been located in two residues within the 15 unique C terminal aminoacids of GR beta (47). In addition, it has been reported that GR beta competes with GR alpha for binding to the co-activator glucocorticoid receptor-interacting protein 1 (GRIP1) at the N terminus, which is required for full GR alpha activity (48). It has recently been found that GR alpha nuclear translocation and transactivation are reduced in murine cells virally transduced with the GR beta gene (49). Surprisingly, using transient transfections, it has recently been reported that, as GR alpha, GR beta acts as a transcriptional repressor of the Th2 cytokines IL-5 and IL-13, effect mediated through the recruitment of HDAC complexes. This implies that in this circumstance GR beta does not act as a dominant-negative inhibitor of GR alpha (50).

5.2. GR beta expression

The GR beta message is expressed in numerous human cells and tissues, though in much lower levels than the GR alpha message (13, 20-22, 49, 51-55). With regard to the GR beta protein, low expression of GR beta is detected by Western blot in untreated cells or physiological conditions (38, 40, 55-57), although the GR alpha/GR beta protein ratio appears to be much lower (8:1) (57) than that of their mRNAs. Other researchers failed to detect GR beta by Western blot, in either healthy cells or tissues (20, 21, 51, 58) or in PBMCs from asthmatic subjects (51, 58). The expression of GR beta appears to be restricted to specific cell types. Thus, positive immunostaining for GR beta has been detected in epithelial cells in the liver, thymus and lung (56), in inflammatory cells, including PBMCs, T lymphocytes, neutrophils, monocytes, macrophages, and eosinophils (45, 49, 55, 59-64), and in bronchial (65) and nasal epithelium (66). Contradictory results have been reported with regard to the sub-cellular distribution of GR beta. It was initially reported that GR beta was only localized in the cell nucleus, irrespective of hormone treatment (56). However, the same group (16) has recently detected GR beta in both the nucleus and cytoplasm, depending on the cell type and the method used for transfecting GR beta. Other researchers have also localized GR beta in both cell compartments (43, 49, 55, 66). The sub-cellular distribution of GR beta appears to be cell-type specific, for GR beta has been detected in both the nucleus and cytoplasm of blood monocytes (55) and BAL macrophages (49), but only in the nucleus of T-cells (55).

6. GR AND GLUCOCORTICOID INSENSITIVITY

Different hypotheses have been postulated to explain the resistance that some patients show to the therapeutic effects of GCs. This resistance or insensitivity is not only found in respiratory diseases such as asthma and nasal polyposis, but also in other inflammatory diseases, including IBD and rheumatoid arthritis. The molecular mechanisms that explain GC resistance have been ascribed to alterations in different points of the GR signaling pathway, including changes in GR alpha and GR beta expression levels, defects in GR binding to ligand, GR translocation to the cell nucleus, or GR binding to GREs, and/or a defective cross-talk with transcription factors. As opposed to familial GC resistance, in which there are mutations in the GR gene and a subsequent resetting of basal cortisol levels, the GC resistance found in patients with asthma and other airway inflammatory diseases does not appear to involve defects in the GR gene structure, and these patients have normal cortisol levels and are not addisonian (6).

6.1. GR alpha and GR beta expression levels

Since target tissue sensitivity to GCs directly correlates with receptor levels, insufficient expression of GR alpha might lead to GC resistance. However, findings reporting similar GR alpha levels in both healthy and diseased cells/tissues do not support this hypothesis (Table 1) (9, 18). Down-regulation of GR alpha by its own ligand might also contribute to GC insensitivity in chronically GC-treated patients. Such hormone-induced down-regulation of GR alpha has been amply demonstrated *in vitro* in different cell types and involves both transcriptional and post-transcriptional mechanisms (18,

Table 1. Expression and regulation of GR alpha and GR beta in airway diseases

Disease	Cell/tissue	Method of analysis	Effect on GR alpha	Effect on GR beta	References
GC-insensitive asthma	PBMC, BAL	IHC	Not analyzed	Increase	49, 61, 62
	PBMC	PCR, WB	No change	No change[1]	51, 58
	BAL	PCR	No change	Increase	49
	Peripheral blood monocytes	PCR	No change	No change	49
	Skin	IHC	No change	Increase	59
	Endobronchial biopsies	IHC	No change	Increase	65
Fatal asthma	Lung	IHC	Not analyzed	Increase	63
Nocturnal asthma	BAL macrophages	IHC	No change	Increase	72
Nasal polyposis	nasal polyps	IHC	Not analyzed	Increase	64
		IHC	Decrease[2]	Increase	66
		PCR	Increase	No change	24
		PCR	Decrease	No change	66
	epithelial cells	PCR	No change	Increase	52

WB: Western blot, IHC: immunohistochemistry, PBMC: peripheral blood mononuclear cells, BAL: bronchoalveolar lavage. [1]No detection of GR beta by Western blot, [2]using and antibody recognizing both GR alpha and GR beta

20, 67). However, it is not clear whether hormone-induced down-regulation of GR alpha occurs *in vivo* in patients chronically treated with GCs. *In vivo* treatment of healthy nasal mucosa (68) and bronchial epithelial cells and alveolar macrophages (67) with intranasal or inhaled GCs resulted in a transient down-regulation of GR alpha mRNA, with GR alpha levels returning to basal values once GC treatment was ceased. In contrast, no down-regulation of GR alpha was reported in inflamed nasal mucosa (nasal polyps) after systemic or intranasal GCs (66, 69), whereas one study reported down-regulation of GR alpha in nasal polyps after a 2-week treatment with oral GCs (24).

Given the above mentioned inhibitory function of GR beta on GR alpha activity, over-expression of GR beta in the inflamed airways might lead to GC resistance. In this regard, numerous reports show increased expression of GR beta in airway diseases associated with GC insensitivity (Table 1) (9, 18, 70). Significantly higher number of GR beta-immunoreactive PBMCs and BAL cells were first reported in corticosteroid-resistant asthmatics, compared to both healthy subjects and corticosteroid-sensitive asthmatics (61, 62). GR beta expression in corticosteroid-resistant asthmatics was particularly high in airway T-cells (62). However, other groups (51, 58, 71) could not find any association between GR beta expression and GC insensitivity in PBMCs from asthmatic patients. An increased number of GR beta-immunoreactive cells was found in both the small and large airways of patients who died of slow-onset fatal asthma (63), and an increase in the number of cells expressing GR beta was also reported in skin biopsies of corticosteroid-resistant asthmatics (59). An increased number of GR beta-positive cells has been found in both the epithelium and sub-mucosal inflammatory cells of patients with severe asthma, compared with moderate asthmatics (65). Also, increased GR beta immuno-reactivity was reported at night in BAL macrophages from patients with nocturnal asthma (72). More convincing evidence of the association between GR beta and GC insensitivity has been reported by Goleva and coworkers (49). The authors showed that BAL macrophages, but not PBMCs, from GC-insensitive asthmatics had higher GR beta mRNA and protein levels than those from GC-sensitive patients, though GR beta mRNA levels were still 400 times lower than those of GR alpha. Interestingly, RNA silencing of GR beta expression in BAL macrophages from GC-insensitive asthmatics enhanced dexamethasone-induced GR alpha transactivation (49). An increased number of GR beta-immuno-reactive inflammatory cells has also been found in nasal polyps, compared to healthy nasal mucosa (64, 66).

The association between increased GR beta and GC resistance has also been reported in IBD patients, including ulcerative colitis and Chron's disease (73-76). Thus, higher GR beta mRNA expression was detected in PBMCs from patients with corticosteroid-resistant ulcerative colitis compared to corticosteroid-sensitive patients (74), and higher GR beta mRNA levels were detected in the active stage of disease (73, 75). In contrast to these results, in a prospective and retrospective study, Hausmann and coworkers (77) have recently reported no correlation between GR beta mRNA expression and the efficacy of GC treatment in IBD, thus excluding GR beta as a predictive marker of steroid treatment response in this disease.

Numerous *in vitro* studies have reported increased GR beta expression after incubation with pro-inflammatory stimuli (9, 18). For instance, increased immuno-reactivity for GR beta has been found in PBMCs after co-incubation with IL-2 and IL-4 (61), although we were unable to reproduce these findings (51). IL-18 increased GR beta mRNA expression in PBMCs (73), and TNF-alpha and IL-1 disproportionately increased the steady-state levels of GR beta protein over GR alpha in HeLa and CEM-C7 cell lines (78). IL-8 induced GR beta expression in human neutrophils (45), phytohaemagglutinin and bacterial superantigens increased the number of GR beta immunoreactive PBMCs (60), and the incubation of nasal tissue from both non-atopic and atopic ragweed-sensitive patients with ragweed and bacterial superantigens also increased GR beta immuno-reactivity (79). Finally, co-incubation with TNF-alpha and IFN-gamma enhanced GR beta mRNA and protein expression in airway smooth muscle cells, with a GR alpha/GR beta protein ratio of 8:1 in untreated cells and 1:3 in TNF-alpha/IFN-gamma-treated cells (57).

6.2. Ligand binding, nuclear translocation, and binding to GRE

A decreased GC binding affinity (dissociation constant, Kd) for GR alpha was found in the nucleus of PBMCs from patients with GC-resistant asthma, compared

to GC-sensitive asthmatics or control subjects (6, 35, 80). This abnormality was reversed with serum deprivation and was mimicked by incubation of cells with IL-2 and IL-4, or IL-13 alone (35, 80), which suggested that the abnormality in GR alpha was the result of the ongoing inflammation seen in these GC-resistant asthmatic patients. The alterations in GR binding characteristics induced by IL-2 and IL-4, or IL-13 have been attributed to post-translational modifications of GR alpha induced by these cytokines. Thus, Irusen and coworkers (35) showed that activation of p38 MAPK by IL-2 and IL-4 resulted in serine phosphorylation of the GR, as well as a reduced capacity of dexamethasone to repress LPS-stimulated GM-CSF release and to increase IL-10 release. Similarly, nitrosylation of the GR at an hsp90 interaction site decreased GR ligand binding affinity (36).

An impaired nuclear translocation of GR alpha has been reported in PBMCs from GC dependent and GC-resistant asthmatic patients (81) and in BAL cells from patients with GC-insensitive asthma (49). Also, a decreased GR binding affinity to DNA (GRE) was found in GC-insensitive asthmatics (61). Other authors reported no change in GR binding affinity to GRE but showed instead a reduced number of GRs available for binding to GREs in GC-resistant asthmatics, probably resulting from the decreased GR nuclear translocation (6). Leung's group has attributed both impaired GR alpha nuclear translocation and reduced GR binding to DNA to the increased expression of GR beta (49, 61). Alterations in GR-GRE binding have also been attributed to excessive activation of AP-1, increased c-Fos expression, and JNK activity in response to inflammatory stimuli (6).

In some asthmatic patients, GR translocation is normal, but there is an altered histone acetylation pattern in response to dexamethasone, i.e., a reduction in histone H4 K5 acetylation, which is a marker of GC transactivation (81). The enzyme MKP-1, which dephosphorylates and inactivates p38 MAPK, is one of the genes transactivated by GC, and its induction by GC is partly responsible for the anti-inflammatory effects of GC (82, 83). Interestingly, it has been recently reported that alveolar macrophages from patients with asthma have an impaired inducibility of MKP-1 (84). Thus, changes in p38/MKP-1 homeostasis appear to be important in contributing to GC insensitivity.

6.3. Interaction with transcription factors and cofactors

The pro-inflammatory transcription factor AP-1 significantly contributes to the expression of numerous Th2 cytokines. AP-1 is composed of heterodimers of different Jun and Fos subunits. It is induced by a variety of cytokines, growth factors and by oxidative stress, and is activated through the phosphorylation of c-Jun and the transcriptional regulation of c-Fos. c-Jun phosphorylation is mediated by JNK, a member of the MAPK family (85). Adcock and coworkers (86) initially reported increased AP-1 binding to DNA and a reduced capacity of GR to interact and repress AP-1 in PBMC from GC-resistant asthmatics. In addition, an increased expression of c-Fos and an increased phosphorylation of c-Jun and JNK have been reported in cells and tissues from patients with GC-resistant asthma (6, 87, 88). GC treatment failed to decrease c-Jun and JNK activation in GC-resistant asthmatic patients, as opposed to GC-sensitive asthmatics (87, 88). It has also been reported that c-fos, but not c-jun or GR beta mRNA expression, inversely correlates with GC sensitivity in PBMC from asthmatic patients (71). The reason for the failure of GC to inhibit the activation of JNK and AP-1 in GC-resistant asthmatic patients is unknown, but might relate to the excessive production of a unique pattern of Th2 cytokines.

It has been hypothesized that GC insensitivity might be the result of a reduced capacity of GR alpha to recruit key transcriptional cofactors, such as the co-repressor HDAC2 or the chromatin remodeling ATPase Brahma-related gene (Brg) 1 (89, 90). Along these lines, decreased HDAC activity and decreased expression of HDAC1 and HDAC2 proteins have been reported in bronchial biopsies from asthmatic patients (91). The same group later reported reduced HDAC activity in PBMC from patients with severe asthma, compared with patients with non-severe asthma, and further demonstrated that this reduced HDAC activity directly correlated with GC insensitivity (92). However, other studies do not show decreased HDAC2 expression in the airways of patients with severe asthma (65). Bilodeau and coworkers (90) demonstrated that Brg1 was essential for GR alpha-mediated transrepression of the POMC gene, and also found that around 50% of GC-resistant human and dog corticotroph adenomas were deficient in nuclear expression of either Brg1 or HDAC2.

Finally, other factors contributing to GC resistance that have been proposed and reviewed recently (6) include the local immune milieu, cigarette smoking, genetic predisposition, viral infection, allergen exposure, microbial superantigens, and neutrophilia. With regard to the influence of the local immune milieu, Tliba and coworkers (57) demonstrated that the expression of CD38 was insensitive to GC action in airway smooth muscle cells co-incubated with TNF-alpha and IFN-gamma for 24 h – an effect that involved the up-regulation of GR beta. The same group has recently reported that short-term exposure (6 h) of these cells to this same cytokine mixture also induces GC resistance – an effect that is independent of GR beta but instead involves the transcription factor interferon regulatory factor 1 (93).

7. CONCLUSIONS

GR alpha and GR beta are the main products derived from alternative splicing of a unique GR gene. GR alpha has widespread distribution and, acting as a transcription factor and with the participation of numerous cofactors, it is responsible for the induction and repression of target genes. GR beta acts as a dominant negative inhibitor of GR alpha-mediated trans-activation and trans-repression in certain cell types transfected with GR beta. While the GR beta message is expressed in numerous tissues, although at much lower levels than the GR alpha mRNA, the expression of the GR beta protein appears to be limited to specific cell types. Increased expression of GR

beta has been reported in different inflammatory diseases, including bronchial asthma, nasal polyposis, and IBD, and after incubation of cells with certain pro-inflammatory stimuli. Because of this, over-expression of GR beta in the inflamed airways has been proposed as one of the mechanisms explaining GC resistance. However, some crucial findings concerning GR beta expression and function have not been reproduced by other researchers, which question its active role in modulating the sensitivity to GCs. Other hypotheses that could account for GC resistance include alterations in GR binding to ligand, nuclear translocation, and binding to GRE, and/or a defective cross-talk with transcription factors and cofactors. Finally, the recent discovery of numerous translational and post-translational forms of GR alpha adds further complexity to the GR signaling pathway, and may also account for the differential fine tuning of GC action in human cells and tissues. With the purpose of bringing new insights into the development of novel therapeutic treatments of patients with airway inflammatory diseases, it is of major importance to uncover which is the true involvement of these GR translational and post-translational variants in healthy and diseased airways, as well as to investigate the molecules that interact with the GR and alter its function.

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Abbreviations: AP-1: activator protein-1, ARE: adenylate-uridylate-rich elements, BAL: bronchoalveolar lavage, Brg: Brahma-related gene, CBP: CREB-binding protein, COX-2: cyclooxygenase-2, CREB: cyclic adenosine monophosphate response element-binding protein, GC: glucocorticoid, GR: glucocorticoid receptor, GRE: glucocorticoid response element, GRIP1: glucocorticoid receptor-interacting protein 1, HAT: histone acetyltransferase, HDAC: histone deacetylases, IBD: inflammatory bowel disease, IL: interleukin, JNK: c-Jun N-terminal kinase, MAPK: mitogen-activated protein kinase, MKP-1: mitogen-activated protein kinase phosphatase-1, NF: nuclear factor, p/CAF: p300/CBP-associated factor, PBMC: peripheral blood mononuclear cells, POMC: proopiomelanocortin, SRC-1: steroid receptor coactivator-1, TNF alpha: tumor necrosis factor alpha, TF-RE: transcription factor-response element, TTP: tristetraprolin, VEGF: vascular endothelial growth factor

Key Words: Glucocorticoid, Glucocorticoid Receptor Isoforms, Airway Respiratory Diseases, Airway Inflammation, Review

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