

## Commentary

**Is there a role for glucocorticoid receptor  $\beta$  in asthma?**

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**Abstract**

Glucocorticoids (GCs) are routinely used as anti-inflammatory drugs in the treatment of asthma. They act through binding to glucocorticoid receptor  $\alpha$  (GR $\alpha$ ), which represses numerous genes encoding pro-inflammatory mediators. A hormone binding deficient GR isoform named GR $\beta$  has been isolated in humans. When overexpressed by transfection, GR $\beta$  may function as a dominant negative modulator of GR $\alpha$ . However, to act as such, GR $\beta$  has to be more abundant than GR $\alpha$ , and conflicting data have been obtained concerning the relative levels of the two isoforms in cell lines and freshly isolated cells. Moreover, the dominant negative effect was not confirmed by independent laboratories. In GC-resistant asthmatics, GR $\beta$  was expressed by an increased number of peripheral blood mononuclear cells (PBMCs), airway T cells, and cells found in skin biopsies of tuberculin responses. However, the relative amounts of GR $\alpha$  and GR $\beta$  in these cells were not determined. In GC-dependent asthmatics, PBMCs expressed GR $\alpha$  predominantly. No cells containing higher levels of GR $\beta$  than GR $\alpha$  have yet been reported in asthmatics. Even if the existence of such cells is demonstrated, the role of GR $\beta$  in asthma will remain a matter of controversy because functional studies have given discrepant data.

**Keywords:** asthma, glucocorticoid receptor**Introduction**

Glucocorticoids (GCs) are involved in the regulation of numerous physiological processes and, as drugs, represent the cornerstone of anti-inflammatory treatment in asthma. Their effects are mediated by the glucocorticoid receptor  $\alpha$  (GR $\alpha$ ). Upon ligand-binding, GR $\alpha$  inhibits or stimulates gene transcription. Different mechanisms for negative transcriptional regulation by GR $\alpha$  have been described, but the most common is transrepression, which involves inhibitory protein–protein interactions between GR $\alpha$  and other transcription factors like AP-1 and NF- $\kappa$ B [1]. These transcription factors stimulate the expression of many genes encoding inflammatory mediators. Stimulation of gene transcription, or transactivation,

occurs after binding of the hormone-activated GR $\alpha$  to GC response elements (GREs) on DNA. These are found in another set of genes like those involved in the control of neoglucogenesis, arterial pressure and intraocular tension (genes and references are listed in [2]). GCs also transactivate the  $\beta$ 2-adrenergic receptor gene and may, consequently, facilitate bronchodilatory action of  $\beta$ 2-agonists [3,4]. It should be noted that GCs induce expression of I $\kappa$ B $\alpha$ , an inhibitor of NF- $\kappa$ B, and may therefore counteract inflammation through transactivation. However, recent data suggest that induction of I $\kappa$ B $\alpha$  by GCs is neither required nor sufficient for the downmodulation of NF- $\kappa$ B activity [5–7]. Possibly, other genes encoding anti-inflammatory proteins might be upregulated by GCs, but these

FEV1 = forced expiratory volume in one second; GC = glucocorticoid; GRE = glucocorticoid response element; PBMC = peripheral blood mononuclear cell.

remain to be identified. Thus, whereas transrepression of AP-1 and NF- $\kappa$ B activities is clearly implicated in the anti-inflammatory effect of GCs [8], transactivation probably accounts for certain beneficial (eg bronchodilatation) or adverse effects (diabetes, arterial hypertension, hydrosodic retention, hypokalemia, glaucoma) of these hormones when used as drugs.

In addition to the 94 kDa GR $\alpha$ , a receptor isoform of 90 kDa termed GR $\beta$  is generated by alternative splicing in humans [9]. GR $\alpha$  and GR $\beta$  are distinguished by differences in their molecular weight and carboxy-terminal sequences [10]. Due to its different carboxy terminus, GR $\beta$  is unable to bind the hormone and to transactivate a GRE-dependent promoter [9,11].

### Relative amounts of GR $\alpha$ and GR $\beta$

It was reported that GR $\beta$  functions as a dominant negative inhibitor of GR $\alpha$ -mediated transactivation [12,13]. However, to act as such, GR $\beta$  has to be more abundant than GR $\alpha$ , and conflicting data have been obtained concerning the relative levels of the two isoforms in cell lines and freshly isolated human cells.

Using antibodies that recognised both forms of the receptor equally well on western blots, GR $\alpha$  was found to be the predominant isoform in HeLa cells, lymphocytes [10,14], and A549 cells (Mathieu, unpublished data). In agreement with these results, northern blot analyses and quantitative RT-PCR experiments have demonstrated that GR $\beta$  mRNA was 300–600 fold less represented than GR $\alpha$  mRNA in various human tissues, a T cell line, HeLa cells, and in PBMCs of a patient with GC-resistant ulcerative colitis [13,15]. In opposition to these different reports, others have shown the amount of GR $\beta$  to be equal or higher than that of GR $\alpha$  in HeLa cells, various human tissues, and in transformed lymphocytes from a patient with systemic GC resistance [16,17]. In these latter studies, the investigators used two different antisera, one directed against GR $\alpha$ , the other against GR $\beta$ , together with standards created by coupling the immunogenic peptides to albumin. As mentioned by Hecht and collaborators [14], it is unclear to what extent coupling efficiency for the different peptides is controlled in this experiment and whether a quantitative comparison between the two isoforms in this fashion is really valid. The use of a single antibody that recognises a common epitope in both forms of the receptor seems more appropriate for quantitative comparison of the isoforms, as no differences in affinity to this epitope between isoforms will be expected in western blotting.

Expression of GR $\beta$  was investigated both in GC-resistant and in GC-dependent asthma. GC-resistant asthmatics are defined as patients who have a baseline prebronchodilation forced expiratory volume in 1 s (FEV1) of less than 70–80% predicted which improves by less than 15% fol-

lowing 1–2 weeks of 40 mg prednisolone daily. It should be noted that this definition does not consider the other clinical benefits of GCs and, in most studies, GC-resistant patients often use inhaled and/or systemic GCs. In GC-resistant asthma, immunohistochemical analyses have shown that GR $\beta$  was expressed by an increased number of PBMCs, airway T cells, and cells found in skin biopsies of tuberculin responses [18–20]. The ratio of positive cells for GR $\alpha$  over positive cells for GR $\beta$  was reduced in GC-resistant patients compared to GC-sensitive patients [20]. Because these investigators used two different antibodies that may not recognise GR $\alpha$  and GR $\beta$  with the same efficiency, it was not possible to determine by this approach which isoform was predominantly expressed. It should be noted that resistance to GCs may result from the reduced expression of GR $\alpha$ , or its lower affinity towards GCs as observed in certain patients [20,21].

GC-dependent asthmatics have a severe form of the disease and require long-term treatment with oral, as well as inhaled, GCs. Systemic GCs are not entirely satisfactory because they produce deleterious side effects. Moreover, despite this treatment, some inflammation may persist in these patients ([22] and references therein). Using comparative RT-PCR and antibodies that recognise both forms of the receptor equally well on western blots, a predominance of GR $\alpha$  over GR $\beta$  was found in PBMCs isolated from normal subjects and asthmatic patients, including GC-dependent asthmatics [22]. Thus, there is no convincing evidence as yet for a role of GR $\beta$  in GC-dependent asthma.

Unless cells containing higher amounts of GR $\beta$  than GR $\alpha$  are isolated from patients with GC-dependent or GC-resistant asthma, it will be difficult to assign a role to GR $\beta$  in these forms of the disease.

### Functional analyses of GR $\beta$

The ability of overexpressed GR $\beta$  to alter transcription from GC-inducible, AP-1-inducible or NF- $\kappa$ B-inducible promoters has been examined using transient transfection and reporter gene assays. The initial observation of a dominant negative effect of GR $\beta$  on GR $\alpha$ -mediated transactivation in COS-7 [12] and HeLa cells [13] was not reproduced in Jurkat T cells [23], COS-7 cells [14, 24] and COS-1 cells [25]. Our results suggest that the dominant negative effect of GR $\beta$  on transactivation is cell type specific. Indeed, GR $\beta$  inhibited GC-induced gene expression in COS-1 cells and to a lesser extent in A549 cells, but not in HeLa cells (Mathieu, unpublished data). The capacity of GR $\beta$  to prevent hormone-activated GR $\alpha$  from repressing NF- $\kappa$ B activity is also a subject of controversy. In one study, GR $\beta$  had this capacity, suggesting that it may reduce the anti-inflammatory effects of GCs [26], whereas in another study, it did not have such a capacity [24]. Data obtained in our laboratory indicate that GR $\beta$

does not behave as a dominant negative receptor for transrepression of either AP-1 or NF- $\kappa$ B activities. On the contrary, GR $\beta$  significantly repressed these activities (Mathieu, unpublished data). This result suggests that overexpression of GR $\beta$  could form part of a cellular response destined to counteract inflammation. In line with this, GC-resistant asthmatics expressed higher amounts of GR $\beta$  and developed a weaker reaction to tuberculin as compared to GC-sensitive asthmatics [20]. GR $\alpha$  also had a hormone-independent inhibitory effect on AP-1 and NF- $\kappa$ B activities ([27] and Mathieu, unpublished data), indicating that the contribution of GR $\beta$  would be significant only if found at similar or higher levels within cells than GR $\alpha$ .

Overall, the functional data are conflicting and further studies will be necessary to clarify this controversy. The discrepant results may be explained by differences in experimental procedures. For example, the promoter used to overexpress GR $\beta$ , the relative amounts of reporter gene construct, and GR $\alpha$  or GR $\beta$  expression vectors were not the same in every study. Moreover, in many studies [13,14,23,25,26], the reporter gene assay did not include a constitutive reporter gene to correct values for variations in transfection efficiency. This control is of crucial importance because efficiency of transient transfection may vary. Without such a control, a variation in transfection efficiency may be misinterpreted as a regulation of the GC-inducible, AP-1-inducible or NF- $\kappa$ B-inducible promoters.

## Conclusion

The involvement of GR $\beta$  in physiological or pathophysiological processes will seem unlikely until this isoform is found using a recognised quantitative method at higher levels than GR $\alpha$  in the same cells. Even if the existence of such cells is demonstrated in future studies, there will be no clear indication as to the role of GR $\beta$ . According to one report, GR $\beta$  would be expected to decrease sensitivity to the anti-inflammatory action of glucocorticoids [26]. In contrast, other studies have predicted that it would have no effect at all [23,24], or that it would have an anti-inflammatory effect (Mathieu, unpublished data). Development of quantitative immunoassays like ELISA or RIA to determine the relative amounts of GR $\alpha$  and GR $\beta$  in cells from asthmatic patients, the establishment of cell lines containing an inducible GR $\beta$  gene and the generation of transgenic animals overexpressing GR $\beta$  will eventually help to determine if there is role for GR $\beta$  in asthma and other inflammatory diseases.

## References

- Cato A, Wade E: **Molecular mechanisms of anti-inflammatory action of glucocorticoids.** *Bioessays* 1996, **18**:371–378.
- Jaffuel D, Demoly P, Gougat C, Balaguer P, Mautino G, Godard P, Bousquet J, Mathieu M: **Transcriptional potencies of inhaled glucocorticoids.** *Am J Respir Crit Care Med* 2000, **162**:57–63.
- Emorine L, Marullo S, Delavier-Klutchko C, Kaveri S, Durieu-Trautmann O, Strosberg A: **Structure of the gene for human  $\beta$ 2-adrenergic receptor: expression and promoter characterization.** *Proc Natl Acad Sci USA* 1987, **84**:6995–6999.
- Mak J, Nishikawa M, Shirasaki H, Miyayasu K, Barnes P: **Protective effects of a glucocorticoid on downregulation of pulmonary  $\beta$ 2-adrenergic receptors in vivo.** *J Clin Invest* 1995, **96**:99–106.
- Brostjan C, Anrather J, Sizmadia V, Stroka D, Soares M, Bach F, Winkler H: **Glucocorticoid-mediated repression of NF $\kappa$ B activity in endothelial cells does not involve induction of IkappaBalpha synthesis.** *J Biol Chem* 1996, **271**:19612–19616.
- Heck S, Bender K, Kullmann M, Gottlicher M, Herrlich P, Cato A: **I kappaB alpha-independent downregulation of NF-kappaB activity by glucocorticoid receptor.** *EMBO J* 1997, **16**:4698–4707.
- Bosscher KD, Schmitz M, Berghe WV, Plaisance S, Fiers W, Haegeman G: **Glucocorticoid-mediated repression of nuclear factor-kappaB-dependent transcription involves direct interference with transactivation.** *Proc Natl Acad Sci USA* 1997, **94**:13504–13509.
- Di Croce L, Okret S, Kersten S, Gustafsson JA, Parker M, Wahli W, Beato M: **Steroid and nuclear receptors. Villefranche-sur-Mer, France, May 25–27, 1999.** *EMBO J* 1999, **18**:6201–6210.
- Hollenberg S, Weinberger C, Ong E, Cerelli G, Oro A, Lebo R, Thompson E, Rosenfeld M, Evans R: **Primary structure and expression of a functional human glucocorticoid receptor cDNA.** *Nature* 1985, **318**:635–641.
- Oakley R, Webster J, Sar M, Parker CJ, Cidlowski J: **Expression and subcellular distribution of the  $\beta$ -isoform of the human glucocorticoid receptor.** *Endocrinology* 1997, **138**:5028–5038.
- Giguere V, Hollenberg SM, Rosenfeld MG, Evans RM: **Functional domains of the human glucocorticoid receptor.** *Cell* 1986, **46**:645–652.
- Bamberger CM, Bamberger AM, de Castro M, Chrousos GP: **Glucocorticoid receptor  $\beta$ , a potential endogenous inhibitor of glucocorticoid action in humans.** *J Clin Invest* 1995, **95**:2435–2441.
- Oakley R, Sar M, Cidlowski J: **The human glucocorticoid receptor  $\beta$  isoform. Expression, biochemical properties, and putative function.** *J Biol Chem* 1996, **271**:9550–9559.
- Hecht K, Carlstedt-Duke J, Stierna P, Gustafsson J-Å, Brönnegård M, Wikström A-C: **Evidence that the  $\beta$ -isoform of the human glucocorticoid receptor does not act as a physiologically significant repressor.** *J Biol Chem* 1997, **272**:26659–26664.
- Honda M, Orii F, Ayabe T, Imai S, Ashida T, Obara T, Kohgo Y: **Expression of glucocorticoid receptor  $\beta$  in lymphocytes of patients with glucocorticoid-resistant ulcerative colitis.** *Gastroenterology* 2000, **118**:859–866.
- de Castro M, Elliot S, Kino T, Bamberger C, Karl M, Webster E, Chrousos G: **The non-ligand binding  $\beta$ -isoform of the human glucocorticoid receptor (hGR $\beta$ ): tissue levels, mechanism of action, and potential physiologic role.** *Mol Med* 1996, **2**:597–607.
- Shahidi H, Vottero A, Stratakis CA, Taymans SE, Karl M, Longui CA, Chrousos GP, Daughaday WH, Gregory SA, Plate JM: **Imbalanced expression of the glucocorticoid receptor isoforms in cultured lymphocytes from a patient with systemic glucocorticoid resistance and chronic lymphocytic leukemia.** *Biochem Biophys Res Commun* 1999, **254**:559–565.
- Leung D, Hamid Q, Vottero A, Szeffler S, Surs W, Minshall E, Chrousos G, Klemm D: **Association of glucocorticoid insensitivity with increased expression of glucocorticoid receptor  $\beta$ .** *J Exp Med* 1997, **186**:1567–1574.
- Hamid Q, Wenzel S, Hauk P, Tsiocopoulos A, Wallaert B, Lafitte J, Chrousos G, Szeffler S, Leung D: **Increased glucocorticoid receptor  $\beta$  in airway cells of glucocorticoid-insensitive asthma.** *Am J Respir Crit Care Med* 1999, **159**:1600–1604.
- Sousa AR, Lane SJ, Cidlowski JA, Staynov DZ, Lee TH: **Glucocorticoid resistance in asthma is associated with elevated in vivo expression of the glucocorticoid receptor beta-isoform.** *J Allergy Clin Immunol* 2000, **105**:943–950.
- Sher ER, Leung DY, Surs W, Kam JC, Zieg G, Kamada AK, Szeffler SJ: **Steroid-resistant asthma. Cellular mechanisms contributing to inadequate response to glucocorticoid therapy.** *J Clin Invest* 1994, **93**:33–39.
- Gagliardo R, Chanez P, Vignola AM, Bousquet J, Vachier I, Godard P, Bonsignore G, Demoly P, Mathieu M: **Glucocorticoid receptor  $\alpha$  and  $\beta$  in glucocorticoid dependent asthma.** *Am J Respir Crit Care Med* 2000, **162**:7–13.

23. Bamberger C, Else T, Bamberger A, Beil F, Schulte H: **Regulation of the human interleukin-2 gene by the  $\alpha$  and  $\beta$  isoforms of the glucocorticoid receptor.** *Mol Cell Endocrinol* 1997, **136**: 23–28.
24. Brogan JJ, Murray IA, Cerillo G, Needham M, White A, Davis JR: **Interaction of glucocorticoid receptor isoforms with transcription factors AP-1 and NF-kappaB: lack of effect of glucocorticoid receptor  $\beta$ .** *Mol Cell Endocrinol* 1999, **157**:95–104.
25. de Lange P, Koper JW, Brinkmann AO, de Jong FH, Lamberts SW: **Natural variants of the  $\beta$  isoform of the human glucocorticoid receptor do not alter sensitivity to glucocorticoids.** *Mol Cell Endocrinol* 1999, **153**:163–168.
26. Oakley RH, Jewell CM, Yudit MR, Bofetiado DM, Cidlowski JA: **The dominant negative activity of the human glucocorticoid receptor  $\beta$  isoform. Specificity and mechanisms of action.** *J Biol Chem* 1999, **274**:27857–27866.
27. Mathieu M, Gougat C, Jaffuel D, Danielsen M, Godard P, Bousquet J, Demoly P: **The glucocorticoid receptor gene as a candidate for gene therapy in asthma.** *Gene Ther* 1999, **6**:245–252.