Commentary

Is there a role for glucocorticoid receptor β in asthma?

Rosalia Gagliardo*, Antonio M Vignola* and Marc Mathieu†

*Istituto di Fisiopatologia Respiratoria, Consiglio Nazionale delle Ricerche, Via Trabucco, Palermo, Italia; †Institut National de la Santé et de la Recherche Médicale U454, CHU de Montpellier, France

Correspondence: M Mathieu, INSERM U454, Hôpital A de Villeneuve, 34295 Montpellier Cedex 5, France. Tel: +33 467 41 52 13; fax: +33 467 63 28 55; email: mathieu@montp.inserm.fr

Received: 23 October 2000
Accepted: 4 December 2000
Published: 18 December 2000

Respir Res 2001, **2**:1–4

(Print ISSN 1465-9921; Online ISSN 1465-993X)

Abstract

Glucocorticoids (GCs) are routinely used as anti-inflammatory drugs in the treatment of asthma. They act through binding to glucocorticoid receptor α (GRα), which represses numerous genes encoding pro-inflammatory mediators. A hormone binding deficient GR isoform named GRβ has been isolated in humans. When overexpressed by transfection, GRβ may function as a dominant negative modulator of GRα. However, to act as such, GRβ has to be more abundant than GRα, 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, GRβ 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 GRα and GRβ in these cells were not determined. In GC-dependent asthmatics, PBMCs expressed GRα predominantly. No cells containing higher levels of GRβ than GRα have yet been reported in asthmatics. Even if the existence of such cells is demonstrated, the role of GRβ 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 α (GRα). Upon ligand-binding, GRα inhibits or stimulates gene transcription. Different mechanisms for negative transcriptional regulation by GRα have been described, but the most common is transrepression, which involves inhibitory protein–protein interactions between GRα and other transcription factors like AP-1 and NF-κB [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 GRα 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 β2-adrenergic receptor gene and may, consequently, facilitate bronchodilatory action of β2-agonists [3,4]. It should be noted that GCs induce expression of IκBα, an inhibitor of NF-κB, and may therefore counteract inflammation through transactivation. However, recent data suggest that induction of IκBα by GCs is neither required nor sufficient for the downmodulation of NF-κB 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-κB 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 GRα, a receptor isoform of 90 kDa termed GRβ is generated by alternative splicing in humans [9]. GRα and GRβ are distinguished by differences in their molecular weight and carboxy-terminal sequences [10]. Due to its different carboxy terminus, GRβ is unable to bind the hormone and to transactivate a GRE-dependent promoter [9,11].

Relative amounts of GRα and GRβ

It was reported that GRβ functions as a dominant negative inhibitor of GRα-mediated transactivation [12,13]. However, to act as such, GRβ has to be more abundant than GRα, 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, GRα 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 GRβ mRNA was 300–600 fold less represented than GRα 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 GRβ to be equal or higher than that of GRα 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 GRα, the other against GRβ, 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 GRβ 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% following 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 GRβ 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 GRα over positive cells for GRβ was reduced in GC-resistant patients compared to GC-sensitive patients [20]. Because these investigators used two different antibodies that may not recognise GRα and GRβ 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 GRα, 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 GRα over GRβ 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 GRβ in GC-dependent asthma.

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

Functional analyses of GRβ

The ability of overexpressed GRβ to alter transcription from GC-inducible, AP-1-inducible or NF-κB-inducible promoters has been examined using transient transfection and reporter gene assays. The initial observation of a dominant negative effect of GRβ on GRα-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 GRβ on transactivation is cell type specific. Indeed, GRβ 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 GRβ to prevent hormone-activated GRα from repressing NF-κB activity is also a subject of controversy. In one study, GRβ 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 GRβ

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

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 GRβ, the relative amounts of reporter gene construct, and GRα or GRβ 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-κB-inducible promoters.

Conclusion

The involvement of GRβ in physiological or pathophysiological processes will seem unlikely until this isoform is found using a recognised quantitative method at higher levels than GRα 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 GRβ**.** According to one report, GRβ 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 GRα and GRβ in cells from asthmatic patients, the establishment of cell lines containing an inducible GRβ gene and the generation of transgenic animals overexpressing GRβ will eventually help to determine if there is role for GRβ in asthma and other inflammatory diseases.

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