

Increased glucocorticoid receptor- β expression, but not decreased histone deacetylase 2, in severe asthma

To the Editor:

Between 5% and 10% of patients with asthma have severe disease that is unresponsive to the usual treatment regimens, including high doses of inhaled corticosteroids (ICSs).¹ Although this represents only a small percentage of patients, this group experiences significant morbidity and is responsible for a disproportionate fraction of the costs related to treating asthma. There is increasing evidence to suggest that some patients with severe asthma are biologically resistant to glucocorticoid therapy.² Glucocorticoid resistance can result from overexpression of the isoform glucocorticoid receptor (GR)- β ,³ which is a dominant negative inhibitor of the functional GR, GR α , and from decreased histone deacetylase (HDAC) activity.⁴ GR α /GR β (nonfunctional) and GR α /GR α (functional) dimers compete for GR binding elements and overexpression of the GR β isoform might result in glucocorticoid insensitivity. HDAC proteins enhance the tightening of DNA around histones, subsequently leading to trans-repression of genes. Although subjects with asthma of moderate severity achieve good control of their disease with moderate doses of ICS, subjects with severe asthma have a poor control of their disease despite high doses of ICS and often require oral steroids. Our aim was to examine the basis for glucocorticoid insensitivity. To achieve this, we measured the expression of GR α , GR β , and HDAC 1 and 2 in the airway tissues of patients with moderate and severe asthma.

From the McGill University Health Center and the Sacre-Coeur Hospital, Montreal, Canada, 13 subjects were recruited into the severe asthma group (6 male, 7 female) and 17 subjects into the moderate asthma group (11 male, 6 female). The mean age was 48 (range, 25-66) years in patients with severe asthma and 47 (range, 23-61) in patients with moderate asthma. The mean FEV₁ was 2.29 L (74% of predicted) in patients with severe asthma and 2.14 L (84% of predicted) in patients with moderate asthma. The diagnosis of asthma was based on the American Thoracic Society criteria,⁵ and selection criteria for severe and moderate asthma were as defined by an American Thoracic Society workshop on refractory asthma.¹ Exclusion criteria were noncompliance with ICS and complicating comorbidities, including other known pulmonary diseases. Airway tissues were obtained by endobronchial biopsies and preserved frozen or embedded in paraffin. Immunocytochemistry was performed according to standard protocols. GR α and GR β antibodies were obtained from ABR Affinity BioReagents (Golden, Colo), and HDAC 1 and 2 were obtained from Abcam Inc (Cambridge, Mass). Immunoreactivity was evaluated in the epithelium with a scoring technique based on the percentage of positive cells. The number of inflammatory cells in the submucosa is presented as the number of positive cells in

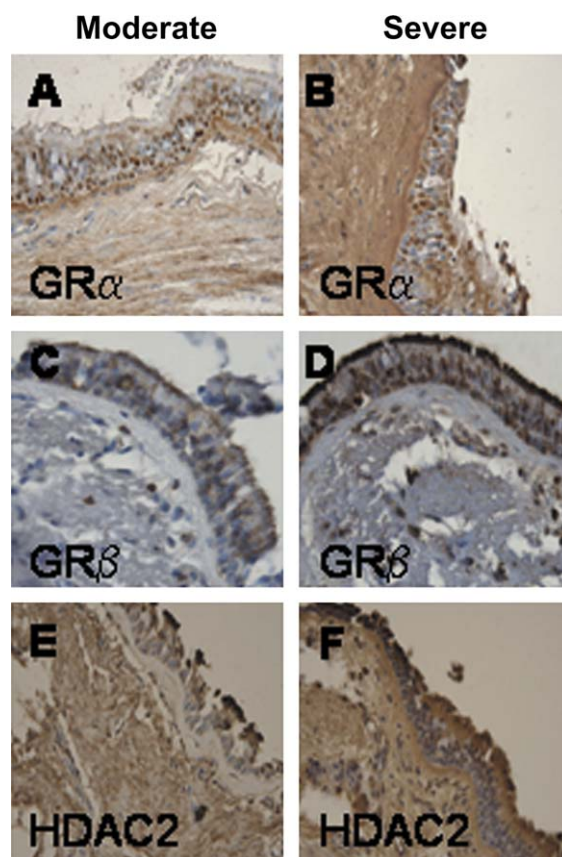


FIG 1. Representative pictures of GR α , GR β , and HDAC 2 immunostaining in moderate and severe asthmatic airways (**A and B**, GR α ; **C and D**, GR β ; **E and F**, HDAC 2). Nuclear staining is predominant for GR α , GR β , and HDAC 2. **A, B, E, F**, magnification 250 $\times$; **C and D**, magnification 400 $\times$.

the submucosa per high power field. Results are expressed as means $\pm$ SEMs. Two-tailed *t* test was performed to examine differences between groups. A *P* value of $<.05$ was considered significant.

Expression of GR α , GR β , HDAC 1, and HDAC 2 was detected in epithelial and submucosal inflammatory cells in patients with both moderate and severe asthma (Fig 1). There were no significant differences between the immunoreactivity for GR α in the epithelium and the submucosa between patients with moderate and severe asthma (Fig 2). A significantly increased expression of GR β was observed in the epithelium and the submucosal inflammatory cells of patients with severe asthma when compared to the group of patients with moderate asthma (Fig 2). In patients with moderate asthma, the ratio of the GR α /GR β in the epithelium (2.9 ± 0.5) and the submucosa (5.2 ± 0.9) was significantly higher when compared to severe group (epithelium, 1.1 ± 0.3 , $P < .05$, submucosa, 1.6 ± 0.5 , $P < .05$). No difference in the immunoreactivity for HDAC 1 and HDAC 2 was observed in the epithelium or submucosa between both groups.

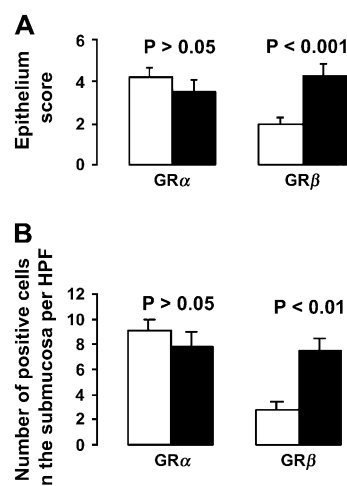


FIG 2. GR α and GR β immunoreactivities in the airway epithelium and the submucosal inflammatory cells of patients with moderate and severe asthma. **A**, Immunoreactivity score in the epithelium. **B**, Number of positive cells in the submucosa per high power field (HPF). Empty bar, patients with moderate asthma; black bar, patients with severe asthma. Data presented as means + SEMs.

We found that GR α was expressed to a similar extent in the epithelium and inflammatory cells of patients with moderate and severe asthma. From the standpoint of GR α expression, both groups therefore appear to have the same potential to respond to glucocorticoid treatment. We also observed that patients with severe asthma express more GR β in their epithelium and submucosal inflammatory cells, resulting in a reduced GR α /GR β ratio. The abundance of GR β may serve as a decoy receptor, reducing GR α availability, the functional isoform. Less GR α reduces the trans-repression of inflammatory genes and reduces the inactivation of transcription factors implicated in the expression of inflammatory genes. Airways of patients with moderate asthma expressed less GR β , consistent with the greater efficacy of glucocorticoids in these subjects and better control of asthma with moderate doses of ICS.

We detected a high expression of GR β in the airway epithelium of patients with severe asthma, and we believe this is a contribution to our understanding of glucocorticoid resistance. Epithelial cells have a major role in asthmatic inflammation, because they are a major source of chemokines recruiting leucocytes to airways. In a previous study, we demonstrated that the airway epithelium of patients with moderate to severe asthma failed to decrease its expression of CXC chemokines in response to oral glucocorticoids,⁶ again consistent with the notion of epithelial resistance to glucocorticoids as part of the pathobiology of this disease. Other studies have reported an increased GR β expression in inflammatory cells in patients with fatal and glucocorticoid-resistant asthma.^{7,8} Severe asthma is often characterized by airway neutrophilia,² and a large proportion of positive inflammatory cells could be neutrophils. Neutrophils have a high constitutive expression of GR β that may explain their resistance

to apoptosis in response to glucocorticoids⁹ and provide a mechanism for the ineffectiveness of glucocorticoid in clearing airway neutrophilia.

Modulation of histone acetylation by activated GR α has recently been described as one of the anti-inflammatory mechanisms of glucocorticoids. Previous work reported the involvement of HDAC 1 and 2 in asthmatic airways.¹⁰ In our study, patients with moderate and severe asthma appear to have the same level of HDAC 1 and HDAC 2 immunoreactivities in their epithelium and submucosa despite the high level of corticosteroid used by patients with severe asthma. Our data suggest that in patients with severe asthma, high doses of glucocorticoid are insufficient to increase further the expression of HDAC 1 or 2 and/or that a plateau in the expression of HDAC 1 and 2 is obtained with moderate steroid doses.

We can conclude that the resistance to corticosteroid therapy may be mediated by the level of GR β expression, which decreases the GR α availability not only in inflammatory cells but also in epithelial cells. Furthermore, HDAC 1 and 2 appear to have a minor role in the blunted response to glucocorticoids seen in our patients with severe asthma. Further research on the regulation of GR β expression will likely provide insight into the etiology of severe asthma and suggest a place for the investigation of mechanisms to circumvent this steroid unresponsiveness for the development of alternative anti-inflammatory drugs for these patients.

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Ghrelin and leptin: A link between obesity and allergy?

To the Editor:

Recent epidemiologic studies have demonstrated that the prevalence of asthma and obesity are both increasing concomitantly, suggesting that these factors may be causally related.¹ Asthma and obesity are both important current public health problems; however, the precise mechanisms of any association between them have not yet been established. Many hypotheses have been proposed regarding this possible link. One possibility is that obesity and allergy share common risk factors such as sedentary lifestyle and dietary factors. A second possibility is that obesity can have a significant effect on normal lung physiology by reducing chest wall compliance.² As a third possibility, accumulating evidence has implicated systemic changes in immune function in the development of obesity; several obesity-related hormones and cytokines may lead to airway hyperresponsiveness.²⁻⁴ Serum IgE levels seem to be a reliable predictor for future development of allergic disorders, including asthma,⁵ and allergic responses are markedly enhanced when IgE levels are high.⁶ In this study, we examined the relationship between obesity and serum IgE levels and then attempted to elucidate a possible link between obesity and allergy mediated by the immunomodulatory effects of 2 representative appetite-regulating peptides, leptin^{4,7} and ghrelin,⁸ based on clinical findings.

Ninety-eight children were enrolled in this study. Forty-nine children (21 females and 28 males with a mean age of 9.9 years) participated in health education classes for obese children; these subjects had a body mass index (BMI) greater than 25 or percent body fat values greater than 35% (TBF-1310 BIA Systems; Tanita Corp, Tokyo, Japan). The 49 remaining children (27 females and 22

TABLE I. Clinical characteristics of study subjects (n = 98)*

	Obese children	Nonobese children	P
Number of subjects (female/male)	49 (21/28)	49 (27/22)	.23
Age (y), mean $\pm$ SD	9.9 $\pm$ 2.6	9.7 $\pm$ 2.7	.71
BMI (kg/m ²), mean $\pm$ SD	26.3 $\pm$ 3.4	18.2 $\pm$ 2.9	<.0001
Percent body fat (%), mean $\pm$ SD	34.7 $\pm$ 7.3	19.7 $\pm$ 3.3	<.0001
Number of subjects having a history of allergic disorder (female/male)	16 (5/11)	15 (5/10)	.83

*Age, BMI, and percent body fat are summarized as the means $\pm$ SDs. For mean comparisons, we used the unpaired 2-tailed Student *t* test. χ^2 Test for independence was used to compare sex and number of subjects having a positive allergic history between 2 groups. Differences were considered statistically significant at *P* values < .05.

males with a mean age of 9.7 years) who were referred to our outpatient clinic for routine health check-ups and who had BMI values less than 22 were examined as nonobese controls. Children with more than 2 wheezing episodes in the previous 12 months or worse than moderate atopic dermatitis were considered to have a positive allergy history. These children had neither underlying illnesses, other than allergic disorders and obesity, nor a history of receiving systemic corticosteroids within the 3 months before enrollment. Clinical characteristics of participants are shown in Table I. Blood specimens were obtained after an overnight fast and immediately transferred to chilled polypropylene tubes containing EDTA-2Na (1 mg/mL) and centrifuged at 4°C. Plasma leptin levels were measured by a commercially available ELISA kit (R&D Systems, Minneapolis, Minn) according to the manufacturer's instructions. Plasma ghrelin levels were measured by double antibody radioimmunoassay system as previously described.⁹ The Pearson correlation coefficient was calculated between the log of IgE concentrations and the levels of plasma ghrelin and leptin. Informed consent was obtained from both the children and their parents. This study was approved by the Ethical Committee of Kurume University.

There were no significant differences between the 2 groups in age, sex, or prevalence of allergic disorders (Table I). Obese children had significantly higher log serum IgE levels (2.33 ± 0.77 U/mL) than nonobese children (1.86 ± 0.82 U/mL; *P* < .01). Furthermore, IgE levels were higher in obese children regardless of allergic state. No significant differences between the 2 groups were observed in the number of peripheral eosinophils, an indicator of allergic inflammation and present prevalence of allergic disorders. We confirmed the significant correlation between the levels of plasma ghrelin and leptin levels and BMI (*n* = 65; *P* < .01; *r* = -0.35; and *n* = 98; *P* < .001; *r* = 0.71, respectively). Interestingly, we also discovered a significant inverse correlation between plasma ghrelin levels and IgE levels (*n* = 65; *P* < .001; *r* = -0.51) in all subjects. In contrast, plasma leptin levels did not correlate with IgE levels. When subjects with allergy alone