Expression Of Glucocorticoid Receptor Beta (GCR B) In Asthmatic Patients And Its Correlation With Clinical Severity And Pulmonary Functions

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ABSTRACT
Background: Glucocorticoids are the gold standard treatment of bronchial asthma. Although the majority of patients with asthma respond favorably to inhaled and systemic steroid therapy, a subset of asthmatics failed to demonstrate a satisfactory response even to systemic glucocorticoid therapy. GCR β (glucocorticoid receptor beta) is a hormone binding deficit isoform of GCR (glucocorticoid receptor) which has been isolated in humans and when over expressed, it may function as a dominant negative modulator of GCR. **Aim of the work:** This study was designed to determine the percentage of expression of GCRβ on PBMCs: (peripheral blood mononuclear cells)of asthmatic patients and to correlate it with the clinical severity and pulmonary functions**. Subjects and Methods**: 60 asthmatic patients (41 males, 19 females) and 20 healthy controls were enrolled in this study. Asthmatics were classified according to GINA guidelines (2002) into mild, moderate and severe asthma. They were subdivided into asthmatic on inhaled corticosteroid (ICS) (n=35) and those not on ICS (n=25). For all studied groups, spirometeric pulmonary functions and immunohisto-chemistry staining of $PBMC_S$ were performed to analyze percentage of expression of GCRβ on PBMCs. **Results:** It showed that the percentage of expression of GCRβ on $PBMC_S$ were statistically higher in all asthmatic patient groups compared to control, with higher % of expression in those not on ICS. Also a statistical significant higher % of expression of GCR β in severe asthmatics compared to both mild and moderate groups was detected. **In conclusion:** This study highlights the importance of glucocorticoid receptor beta isoform in pathogenesis of bronchial asthma and this may be directly linked to asthma severity and can affect the response to medications especially ICS. [New York Science Journal 2010;3(5):54-62]. (ISSN: 1554-0200).

Key words: Bronchial asthma, Glucocorticoid Receptor beta, Glucocorticoid therapy.

Introduction

Glucocorticoids are the gold standard treatment of asthma affecting most of the components involved in bronchial inflammation as inflammatory cells and bronchial structure cells.[21] Glucocorticoids modulate a large number of metabolic, cardiovascular, immune, and behavioral functions. The biological action of glucocorticoids is mediated through the activation of intracellular glucocorticoid receptors (GR). The GR belongs to the superfamily of steroid/thyroid/retinoid acid receptor proteins that function as ligand-dependent transcription factors. [12]

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.[24] 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). The human glucocorticoid receptor gene encodes two protein isoforms; GCRα which binds glucocorticoids, translocates to the nucleus and regulates gene transcription, GCRβ which does not bind GC and alters GCRα action and may therefore interfere with action of glucocorticoid. Increased expression of GCR β in inflammatory cells might be a critical mechanism for conferring glucocorticoid resistance.[8]

Understanding the mechanisms that control GC insensitivity will be important in developing alternative therapies for these patients whose disease tends to be severe and in minimizing side effects from long-term systemic GC therapy, by allowing early identification of biomarkers that predict GC insensitivity.[20] In this revision we present an update on the GR gene, the expression and regulation of its gene products, namely GRalpha and GRbeta, as well as their alterations in pathological states. GRalpha has a widespread distribution and is responsible for the induction and repression of target genes, and the one that shows steroid binding activity, it is expressed in virtually all human cells and tissues, and its expression is known to be downregulated by glucocorticoids, whereas GRβ can act as a dominant negative inhibitor of GRα-mediated transactivation and transrepression. [5] GRbeta has been found to act as a dominant negative inhibitor of GRalpha-mediated transactivation in vitro studies with transfected cells, but it does not appear to have a significant inhibitory effect on GRalpha-mediated transrepression. In addition, for most tissues the expression of GRbeta, at least at the mRNA level, is

extremely low compared with that of GRalpha. [12] Some pro-inflammatory cytokines appear to upregulate the expression of GRbeta, and increased GRbeta Glucocorticoid (GC) insensitivity is a challenging clinical problem associated with life-threatening disease progression. Glucocorticoid-resistant asthma is associated with enhanced mononuclear cell–mediated airway inflammation, which is unresponsive to the anti-inflammatory effects of glucocorticoids. [21]

Pro-inflammatory cytokines appear to upregulate the expression of GRbeta, and increased GR beta 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 GRbeta in modulating glucocorticoid sensitivity and/or resistance in vivo has been highly debated and it is not yet clear. [6]

Although the majority of patients with asthma respond favorably to inhaled and systemic steroid therapy, a subset of asthmatics failed to demonstrate a satisfactory response even to systemic glucocorticoid therapy. [20)] It is important to recognize those patients early because failure to respond to steroids leads to prolonged courses of high dose of glucocorticoid therapy and serious adverse effects despite persistent airway compromise.[23]

Glucocorticoid-resistant bronchial asthma is characterized by failure of corticosteroids to supresss key asthma-relevant, cell-mediate inflammatory responses in the airways. Although this phenomenon is relatively uncommon, it poses a difficult therapeutic problem because few alternative therapies are available.[12]

Glucocorticoid-sensitive asthma was defined as an increase in FEV1 of at least 30% after a 2-week course of oral prednisolone 40 mg daily, corrected for body surface area. Glucocorticoid-resistant asthma was defined as a less than 15% improvement in FEV1 after a similar course of corticosteroids. [6]

Aim of the work: This study was carried to determine the role of glucocorticoid receptor beta (GCRβ) in asthmatic patients through assessment of its percentage of expression on peripheral blood mononuclear cells (PBMCs) and its relation to the clinical severity of the disease, their pulmonary functions and their response to steroid therapy.

Subjects and methods: 60 asthmatic patients were enrolled in this study . They were selected from patients attending the chest and internal medicine clinics, Ain Shams University for follow up and treatment.

All participants were subjected to:

A- Full history taking and thorough clinical examination, especially stressing on episodic wheezing (has the patient had an attack or recurrent attacks of wheezing?), does the patient have a troublesome cough at night? does the patient wheeze or cough after exercise?, does the patient experience wheez, chest tightness, or cough after exposure to airborne allergens or pollutants?, do the patient's colds go to the chest" or take more than 10 days to clear up?, are symptoms improved by appropriate asthma treatment?

B- Chest x-ray: To exclude other pulmonary diseases.

C- Spirometric pulmonary function tests: Spirometric pulmonary function tests (FVC, FEV1, and FEV1/FVC) were measured using spirometer. Simple spirometry is the recommended method of measuring airflow limitation and reversibility to establish a diagnosis of asthma. Measurements of FEV1 and FVC are undertaken during a forced expiratory maneuver using a spirometer. Recommendations for the standardization of spirometry have been published. [26]

Spirometry is effort-dependent. Therefore, proper instructions on how to perform the forced expiratory maneuver must be given to patients, and the highest value of three recordings taken. As ethnic differences in spirometric values have been demonstrated, appropriate predictive equations for FEV1 and FVC should be established for each patient. Because many lung diseases may result in reduced FEV1, a useful assessment of airflow limitation is the ratio of FEV1 to FVC. The FEV1/FVC ratio is normally greater than 0.75 to 0.8. Any values less than these suggest airflow limitation.

The data obtained from this maneuver were:

* FVC : Forced vital capacity (L).
* FEV1 : Forced expiratory volume in first second (L).
* FEV1/FVC: percentage of expired volume in first second (FEV1%) divided by forced vital capacity.
* PEFR: Peak expiratory flow rate (L/S).

These parameters were expressed as percentage of predicted values. The predictive values for each subject based on sex, age and height were obtained from standard tables. Abnormal values were considered to be < 80% for each of them of the predicted values. [26]

Patients with a diagnosis of asthma, were based on The American Thoracic Society criteria were selected for evaluation. They were included if they had ≥ 12% increase (reversibility) in forced expiratory volume in one second (FEV_1) (or ≥ 200 ml) after two inhalations of 400 μg salbutamol (i.e, from the pre-bronchodilator value).[13]

They were classified according to asthma severity based upon GINA guidelines 2006 into:

1-Mild persistent asthma: 20 patients they were 15 males and 5 females.

2- Moderate persistent asthma: 20 patients, they were 13 males and 7 females.

3-Severe asthma: 20 patients, they were 12 males and 8 females.

Patients were subdivided according to the intake of inhaled steroids into:

35 asthmatics on inhaled steroids (25 males and 10 females) with a mean age 31±3.05 years.
25 asthmatics not on inhaled steroids (16 males, 9 females) with a mean age of 30.35±2.26 years.
Age and sex matched 20 healthy individuals were chosen as control group to be included in the study.
Patients were excluded if they had evidence for other types of lung diseases, and asthmatic patients who were given systemic steroids within the last 8 weeks.
D- Complete blood picture laying stress on total leucocytes count and absolute eosinophilic count.
E- Estimation of GCRβ in PBMCs by immuno-histochemistry: Venous blood samples were drawn from each patient, mono-nuclear cells (mnc) were carefully collected from phosphate buffer solution (PBS) / Ficoll-Hypaque interface with a Pasteur pipette and transferred to a new tube. Immuno-cyto-chemical staining by Polyclonal antibody (SC-1003) and Histo-stain SP universal kit used for staining of cell smears. [13]
Statistical analysis:
After collection of data, each was coded and entered into a personal computer statistical analysis was done using SPSS program version 8 (Statistical Package for Social Science).
The following Statistical tests were used: Mean (X) and standard deviation (SD) to describe quantitative data. e.g age .Simple frequency tables include number (N) and percent (%) to describe qualitative data. e.g. sex. Chi-Square test (X2) to compare two independent groups in qualitative variables. Student "t" test was used to compare mean of two groups .ANOVA (Analysis Of Variance) (F value) was used to compare mean of more than two groups. Pearson correlation (r) to correlate two variables in the same group. P>0.05 was considered insignificant, $p < 0.05$ was considered significant. $p < 0.01$ was considered highly significant .

RESULTS
This study included 60 asthmatic patients and 20 healthy individuals as a control group. The asthmatic patients were 41males and 19 females, their age ranged from 21 to 45 years with a mean age of 31.34±5.57 years. The control group was age and sex matched. They were 11 males, 9 females, their age ranged from 20 to 44 years, with a mean age of 31.62±2.60 years.
The asthmatic patients were classified according to asthma severity based upon GINA guidelines 2006 into:
1-Mild persistent asthma: 20 patients (15 males and 5 females) with a mean age of 31.54±5.54 years.
2- Moderate persistent asthma: 20 patients (13 males and 7 females) with a mean age of 32.41±6.97 years.
3-Severe asthma: 20 patients (12 males and 8 females) with a mean age of 30.91±1.57 years.
Patients were subdivided according to the intake of inhaled steroids into:
• 35 asthmatics on inhaled steroids (25 males, 10 females) with a mean age 31±3.05 years.
• 25 asthmatics not on inhaled steroids (16 males, 9 females) with a mean age of 30.35±2.26 years.
Comparing the clinical groups of asthmatic patients (mild, moderate, and severe asthma), this work showed that there was a statistical significant difference ($P<0.05$) between the three groups as regards the absolute eosinophilic count but there is no statistical significant difference ($P>0.05$) between them regarding TLC/m3 (table 2). Also it showed that there was a statistical highly significant difference ($P<0.001$) between the three groups as regards spirometric pulmonary functions tests with lower values in moderate and severe groups (table 2).
Our study showed a statistically significant increase in percentage of expression of GCR β on PBMC's in asthmatic group (8.64±I4.35%) as compared to control group (2.05±0.99) ($p<0.01$) (table 1).
Also it showed that there was a statistical significant difference ($P<0.001$) in % of expression of GCR β in severe group as compared to mild and moderate groups (table 3).
This work showed that there was no statistical significant difference ($P>0.05$) between asthmatics on ICS and asthmatics not on ICS as regards total leucocytes count and absolute eosinophilic count (table 4). However the level of pulmonary function tests were significantly higher in asthmatics on ICS compared to asthmatics not on ICS ($p<0.001$) (table 4).
The percentage of expression of GCR β on PBMCs was statistically higher in asthmatic pateints whether they are on ICS or not on ICS compared to control ($p<0.001$) with significantly higher percentage of expression in asthmatics not on ICS compared to asthmatics on ICS. ($p<0.001$) (table 5 & fig.1).
There was statistical significant negative correlation between GCR β expression and PEFR (table 6, fig.2). On the other hand, there were insignificant correlation between GCR β expression and FEV1 (table 6).

Table (1):Comparison between asthmatic group and controls as regards % of expression of GCR β on PBMCs.

	Control n=20	Asthmatic group n=60	P
GCR β(% of expression)	2.05 ±0.99	8.64 ±14.35	<0.01

Table (2): Comparison between asthmatic subgroups as regards clinical parameter, some, laboratory parameters and spirometric pulmonary functions tests

Variable	Mild persistent n=20	Moderate persistent n=20	Severe persistent n= 20	F	P
Absolute eosinophils /m3	271± 138	412 ±210	540 ±230	2.87	<0.05
FEV1 (% of predicted)	81.29 ± 7.69	64.38 ±4.20	41.20±12.28	29.99	<0.001
FVC(% of predicted)	86.57±5.32	73.62±6.23	53.40 ±12.24	26.24	<0.001
PEFR (% of predicted)	72.29± 27.22	62.50±25.93	36.80±14.72	3.21	<0.05

Table (3): Statistical comparison between asthmatic subgroups as regards % of expression of GCR β on PBMCs.

	Mild persistent n=20	Moderate persistent n=20	Severe persistent n= 20	F	P
GCR β (% of expression)	8.28 ± 1.11	12.11±1.81	15.80 ±4.44	17.10	<0.001

Table (4): comparison between asthmatics on ICS and asthmatics not on ICS as regards some laboratory parameters and spirometric pulmonary functions tests.

Variable	Asthmatics on ICS n=25	Asthmatics not on ICS n=20	t	P
Absolute eosinophilis/mm^3	480 ±225	395 ±211	1.29	>0.05
FEV1 **(% of predicted)**	80.68 ±8.62	64.50 ±17.79	4.01	<0.001
FVC (% of predicted)	87.68 ±7.98	73.10 ±14.96	4.19	<0.001
PEFR **(% of predicted)**	78.10 ±14.8	59.50 ±26.96	2.92	<0.001

Table (5): Statistical comparison between asthmatics on ICS and asthmatics not on ICS) and control as regards % of expression of GCR β on PBMCs.

	Control n=20	Asthmatics on ICS n=35	Asthmatics not on ICS N= 25	r	P
GCR β(% of expression)	2.050.99	6.20 ±3.20	11.70 ±3.62	5.32	<0.001

Table (6): Statistical correlation between % of expression of GCR β and pulmonary function tests in asthmatic patients

GCR β		
Variable	R	P
FEV1 (% of predicted)	-0.00	>0.05
PEFR (% of predicted)	- 0.42	<0.05

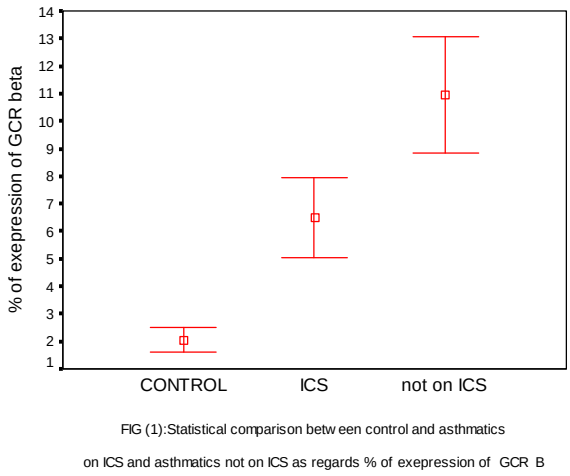

FIG (1):Statistical comparison betw een control and asthmatics on ICS and asthmatics not on ICS as regards % of exepression of GCR B

Fig. (2): Pearson correlation between GCRβ and PEFR in severe asthmatic cases

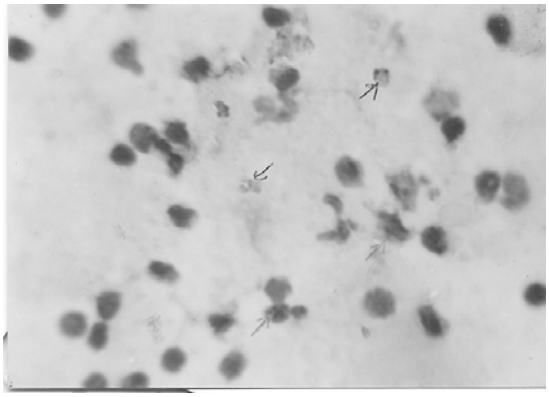

Fig. (3): Immuno-histochemical assay of % of expression of GCR β on PBMCs of male patient. He was diagnosed as severe persistent asthma not on inhaled steroids, his % of expression of GCR β on PBMCs was 17 % .

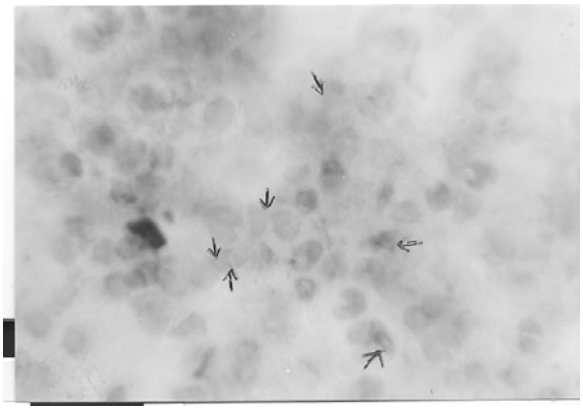

Fig. (4): Immuno-histochemical assay of % of expression of GCR β on PBMCs of male patient. He was diagnosed as mild persistent asthma and he was on inhaled steroids, his % of expression of GCR β on PBMCs was 2 %.

Discussion: GCR β is a hormone binding deficit isoform of GCR which has been isolated in humans and when over expressed, it may function as a dominant negative modulator of GCR. [10] This study showed a statistically significant increase in percentage of expression of GCR β on PBMC's in asthmatic group (8.64±I4.35%) as compared to control group (2.05±0.99) ($p<0.01$).This was in agreement with Leung et al.,(1997) [16] and Hamid et al.,(1999) [13] who studied PBMC's from 15 asthmatics and 8 controls by immuno-histochemistry for expression of GCR β,they found significantly higher expression of GCR β in asthmatics compared to control, with higher level of expression in those less responsive to steroids. More recently GCRα and GCR β were studied in BAL cells of asthmatics and control .Lower level of GCRα and up regulation of GCRβ in asthmatics with different grades of severity were detected.[5,12] The exact mechanism for the increase in GCR β expression in peripheral blood cells of asthmatics is still under extensive studies but many investigators suggested that inflammatory milieu in asthmatic airways is contributing to expression of GCR β and that increased expression of GCR β is cytokine inducible.[3,7,19,25]

Bantel et al., (2000) [3] suggested that different cytokines may affect the rate of alternative splicing of glucocorticoid receptor or prolong half life of β isoform. Webster et al., (2001) [25] suggested that inflammatory cytokines may regulate GCR mRNA stability leading to a state where GCRα receptors are destabilized and GCRβ become more stable. Loke et al.2002[19] attributed the selective expression of GCRβ isoform in asthmatics to be both dose and time dependent on level of TNF-α with resulted increase in GCRβ specific mRNA. De Bosscher et al, 2003 [7] studied the antagonistic effect between glucocorticoids and NF-κB. They proved that this factor antagonize the transactivation function of corticosteroids at the nuclear level by mediating changes in the level of GCRα and/or GCRβ with net result of increasing GCRβ. This provide new insights into the mechanism by which inflammation induces GC resistance and how defects in GCR may contribute to inflammation, creating the visious cycle leading to chronic inflammatory diseases .[20]

On the contrary, Gagliardo et al, 2000 [9] reported that, persistent release of other cytokines as IL-8 and GM-CSF in glucocorticoid -dependent asthma is not associated with low expression of GCRα or overexpression of GCRβ.

This study showed a statistical significant higher percentage of expression of GCRβ isoform in severe asthmatics compared to both mild and moderate asthmatics and a statistical significant negative correlation between GCR β expression and PEFR.

This is in agreement with Barnes (2006) [4] who stated that severe asthmatics are at risk of continuous exposure to allergens with release of different patterns of cytokines which reduce GCR binding affinity in mononuclear cells via increasing GCRβ .This was explained by increasing transcription factor activity in severe asthmatics which inhibit GCR/DNA interactions and may contribute to severity of the disease due to glucocorticoid insensitivity.[5,17] Loke et al., (2002) [19] categorized a subset of asthmatics who do not respond to clinically relevant doses of GCs, and proved to have increased bronchial hyperreactivity, lower morning PEFR and a longer duration of symptoms. Those patients had alternative splicing isoform of GCR which is β isoform. More recently, PBMCs of patients with severe asthma were shown to express relative corticosteroid insensitivity due to over expression of GCR β. [14] Also it was observed increased expression of GCRβ on BAL macrophages at night in asthmatics with nocturnal worsening which was not observed in asthmatics without nocturnal exacerbations.[6]Their observations may contribute to nocturnal airway inflammation by inhibiting anti-inflammatory effects of glucocorticoids at night. [15]

In this study there was a higher statistical significant increase in percentage of expression of GCR β in asthmatic group not on inhaled steroid (11.70 ±3.62) comapred to asthmatics on inhaled steroids (6.20 ±3.20) ($p<0.001$).

This is in agreement with Andersson et al., (1999)[2] who studied in vivo modulation of GCR mRNA expression in 10 mild atopic asthmatics treated with fluticasone propionate (FP) 250 µg twice/day for 4 weeks. A significant reduction of GCR mRNA in endobronchial biopsy and more striking down regulation in peripheral blood were noticed after treatment due to control of inflammation and suppression of cytokines.

On the other hand, Leung and Chrousos, (2000) [17] and Gagliardo et al.,(2001) [10] noticed that neutrophils in asthmatics on large doses of steroids, constitutively have higher level of expression of GCRβ. Thus failure of the patients to resolve their airway inflammation may be due to persistent neutrophilic inflammation with relatively higher level of GCR β.

Sousa et al.,(2000)[22] and Miller and Chin(2006)[20] found that asthmatics who need higher doses and poorly responding to ICS showed a resistance of peripheral blood T-cells and monocytes to glucocorticoids in vitro and failure of ICS therapy to reduce expression of asthma relevant cytokines on airways in vivo. Those patients showed no abnormalities in glucocorticoid pharmacokinetics and are susceptible to development of cushingoid side effects of glucocorticoid therapy. This indicates that the defect in GC action in asthmatics is not generalized but it is attributed to T-cell and other inflammatory cells defects or abnormalities.

The variation in steroid response in asthmatics can not be explained by pharmacokinetic mechanisms, by a defect in binding of steroids to its receptors, nor by defective nuclear translocation of this receptor. Chronic exposure to GC leads to down regulation of GCRα at both mRNA and protein level.[1] Uncontrolled asthmatics on frequent interrupted courses of systemic steroids had reduced expression of GCRα relative to GCRβ which will render inflammatory cells subsequently insensitive to steroids. [22] On the contrary other studies do not support the hypothesis that increased GR-beta expression can contribute to cytokine-induced glucocorticoid insensitivity. [24]

Various data had been published about the percentage of expression of GCR β which could result in glucocorticoid insensitivity state. PBMCs with approximately 20% reactivity with GCRβ are associated with functional insensitivity to glucocorticoid.[16] However other investigators found that much lower level of GCRβ protein can achieve dominant negative regulative function. [25]

The clinical implication of higher expression of GCRβ has been related to the progressive increase in morbidity and mortality due to asthma in the past 3 decades. Increase number of GCRβ in airway cells of fatal asthma suggest that GCRβ may be a contributing factor to steroid insensitivity, leading to more deterioration in pulmonary functions and asthma mortality. [12] This has been attributed to increasing of airway inflammation which induces GCRβ expression with subsequent reduction in functional response to glucocorticoids. This proves the concept that inflammation dampens response to endogenous and exogenous glucocorticoid . [21]

The binding affinity of glucocorticoids to its receptor has clinical implication because ICS is the mainstay of treatment of severe asthma which is characterized by extensive inflammatory cytokines network. Some asthmatics are using higher doses of steroids for prolonged time, despite an initial response, any additional improvement may be impeded because the cells are now expressing predominant GCRβ which would not be anticipated to be steroid responsive. So it is important to recognize those patients as early as possible to avoid high dose therapy and serious adverse effects despite persistent airway compromise. [22]

In conclusion GCR beta may be important in the

pathogenesis of bronchial asthma which may be linked to asthma severity and can affect the response to ICS. So it is important to recognize those patients with high GCR beta to avoid high dose glucocorticoids serious adverse effects.

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