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Modulation of glucocorticoid receptor expression in human bronchial epithelial cell lines by IL-1β, TNF-α and LPS

M.M. Verheggen*+, P.Th.W. van Hal*+, P.W.C. Adriaansen-Soeting*, B.J.A. Goense*, H.C. Hoogsteden+, A.O. Brinkmann**, M.A. Versnel*

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ABSTRACT: Bronchial epithelium plays a major role in the regulation of inflammatory reactions in the airways. It is thought to be a possible target for glucocorticoid therapy. Glucocorticoid responsiveness requires the presence of specific glucocorticoid receptors (GR). Until now, little was known about the presence of such receptors in the human bronchial epithelium.

In this study we demonstrated the expression of GR messenger ribonucleic acid (mRNA) in two simian virus (SV)-40/adenovirus-transformed human bronchial epithelial cell lines, BEAS S6 and BEAS 2B. In a whole cell dexamethasone binding assay, BEAS S6 and BEAS 2B cells were found to possess (mean±SEM) 28.9±4.4 $\times 10^3$ and 32.1±5.7 $\times 10^3$ binding sites per cell, respectively, with dissociation constant (Kd) values of 8.2±1.5 and 8.6±2.4 nM, respectively. Using electrophoretic mobility shift assays we demonstrated the binding of nuclear translocated GR to specific sites on deoxyribonucleic acid (DNA), named glucocorticoid responsive elements (GRE).

Lipopolysaccharide (LPS) and interleukin-1β (IL-1β) significantly increased the number of GR per cell (median=312% and 171% of control, respectively; $p<0.05$), but significantly reduced the ligand affinity of these receptors, *i.e.* increased the Kd (median=410% and 145% of control, respectively; $p<0.05$) in BEAS 2B cells.

These results indicate that the bronchial epithelium may be an actual target for glucocorticoid therapy. Inflammatory mediators, such as IL-1β and LPS, modulate the number and ligand affinity of these GR. Therefore, the response of bronchial epithelium to glucocorticoid therapy may be modulated by airway diseases associated with inflammation.

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*Depts of Immunology, +Pulmonary Medicine, **Endocrinology & Reproduction, Erasmus University and Academic Hospital Dijkzigt, Rotterdam, The Netherlands.

Correspondence: M.A. Versnel
Dept of Immunology
Erasmus University Rotterdam
P.O. Box 1738
3000 DR Rotterdam
The Netherlands

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Until recently, the bronchial epithelium was considered to be a passive barrier between the environment and the internal milieu of the lung [1]. In addition to this barrier function, bronchial epithelial cells are now also known to produce various inflammatory mediators upon exposure to immunologic and nonimmunologic stimuli [1]. Cultures of human bronchial epithelial cells exposed to toluene diisocyanate or to stimuli such as acetylcholine and phorbol-12-myristate-13-acetate, release chemotactic arachidonic acid metabolites, such as 15-hydroxyeicosatetraenoic acid (15-HETE), prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) [1, 2]. Other factors produced by cultured human bronchial epithelial cells upon exposure to stimuli or during recovery from injury are interleukin-1α (IL-1α), interleukin-1β (IL-1β), interleukin-6 (IL-6), granulocyte/macrophage colony-stimulating factor (GM-CSF), interleukin-8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1) [3–6]. Therefore, the bronchial epithelium is considered to play an essential role in initiating and perpetuating inflammatory and immunological reactions as observed in pulmonary diseases such as asthma.

Glucocorticoids are widely used in the treatment of several pulmonary diseases, such as bronchial asthma [7]. They exert numerous biological effects on various cellular and metabolic processes. After entering the cell, glucocorticoids bind to an inactive cytosolic glucocorticoid receptor (GR), which subsequently translocates to the nucleus. Within the nucleus, the complex binds as a dimer to specific sites on deoxyribonucleic acid (DNA), named glucocorticoid response elements (GRE), upstream of the promoter region in steroid-responsive genes. Transcription of the target gene is enhanced or repressed by the binding of the steroid-GR complex to the GRE [8, 9]. Recent studies showed that glucocorticoids are able to inhibit the release of bronchial epithelial cell-derived cytokines [4, 10]. Bronchial epithelial cells seem to play an important role in airway inflammation and the function of these cells can be influenced by glucocorticoids. Thus, glucocorticoids may suppress airway inflammation by influencing bronchial epithelial cells. Such direct cellular effects of glucocorticoids require the presence of specific GR but the presence of these receptors has not yet been demonstrated by radioligand binding studies in human bronchial epithelial cells.

Here we report on the identification and characterization of specific GR in two human bronchial epithelial

cell lines, BEAS 2B and BEAS S6. In comparison with the data on primary cultures of human airway epithelium, BEAS cell lines have been shown to be an appropriate model [11]. These cell lines have been reported to exhibit positive immunofluorescent staining for cytokeratin, production of mucin-like glycoconjugates, and sensitivity to the differentiating effects of serum or transforming growth factor-β [11]. In addition, we examined the role of inflammatory mediators, such as IL-1β, tumour necrosis factor-α (TNF)-α and lipopolysaccharide (LPS), in modulating the number and ligand affinity of GR in BEAS cells.

Materials and methods

Cell lines and culture conditions

BEAS 2B and BEAS S6, two human bronchial epithelial cell lines, transformed by an adenovirus 12-simian virus (SV)-40 hybrid virus, were kindly provided by J. Lechner (Inhalation Toxicology Research Institute, Albuquerque, NM, USA) [12]. Cells were maintained in a serum-free, keratinocyte growth medium (KGM) containing bovine pituitary extract (Gibco, Paisley, UK) [13]. Plastic cell culture plates (Falcon, Becton Dickinson, NJ, USA) were precoated with a mixture of human fibronectin (10 $\mu g \cdot mL^{-1}$; Central Laboratory of the Blood Transfusion Service, Amsterdam, The Netherlands), collagen (Vitrogen 100, 30 $\mu g \cdot mL^{-1}$; Collagen Corp., Palo Alto, CA, USA) and bovine serum albumin (10 $\mu g \cdot mL^{-1}$; Boehringer, Mannheim, Germany) [14, 15]. For GR experiments, cells were cultured for one passage in a mixture of Dulbecco's modified Eagles medium and Ham's F12 (DMEM/F12)(1:1) (Gibco) supplemented with insulin (0.01 $mg \cdot mL^{-1}$; Sigma, St. Louis, MO, USA), hydrocortisone (1 $\mu g \cdot mL^{-1}$; Pharma Chemie, Haarlem, The Netherlands), transferrin (0.01 $mg \cdot mL^{-1}$; Behring Marburg, Germany), epidermal growth factor (EGF) (10 $ng \cdot mL^{-1}$; Collaborative Research Inc., Lexington, MA, USA), foetal calf serum (FCS) (1%), Na_2SeO_3 (50 nM), glutamine (1 mM; JT Baker bv., Deventer, The Netherlands) penicillin G sodium (100 $U \cdot mL^{-1}$; Gist-Brocades, Delft, The Netherlands) and streptomycin sulphate (0.1 $mg \cdot mL^{-1}$; Biochrom KG, Berlin, Germany).

Twenty four hours before performing GR binding experiments the medium was replaced by a basal medium of DMEM/F12 (1:1) with penicillin G sodium (100 $U \cdot mL^{-1}$) and streptomycin sulphate (0.1 $mg \cdot mL^{-1}$), but without hydrocortisone or other supplements to prevent influence of endogenous steroids on the number and affinity of GR. The effect of inflammatory mediators was studied by adding IL-1β (20 $ng \cdot mL^{-1}$; UBI, Lake Placid, NY, USA), TNF-α (20 $ng \cdot mL^{-1}$; UBI) or LPS (100 $\mu g \cdot mL^{-1}$; Difco Laboratories, Detroit MI, USA) to the basal medium.

The CV-1 cell line and the malignant mesothelioma cell line, Mero-14, were used as a negative and positive control, respectively, for GR messenger ribonucleic acid (mRNA) experiments. The CV-1 cell line, derived from the kidney of a male adult African green monkey (American Type Culture Collection, Rockville, MD, USA) was cultured on DMEM with 5% FCS. The malignant mesothelioma cell line Mero-14 was cultured as described by VERSNEL *et al*. [16].

RNA isolation and Northern blot analysis

Total ribonucleic acid (RNA) was extracted by the acid guanidium thiocyanate-phenol-chloroform extraction procedure and stored at -80°C until used [17]. Northern blotting and hybridization were performed as described previously [18]. Briefly, electrophoresis of 20 µg total RNA was performed on a 1% agarose gel with formaldehyde. After blotting onto nitrocellulose, hybridization was performed using ^{32}P labelled probes. Filters were washed twice for 20 min at 42°C with standard sodium citrate (SSC) (0.45 M NaCl and 45 mM Na-citrate, pH 7.0) and exposed to a Fuji-RX film.

The intensity of the GR and the glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) signals on the autoradiograph were determined with a handscanner (Colorscanner 2^{24}, Highscreen, Würselen, Germany) at a resolution of 100 dots per inch. Computer software described by KONING *et al*. [19] was used to analyze the intensity of the bands. Values were expressed as the ratio of GR mRNA intensity to GAPDH mRNA intensity.

Probes

The GR probe, kindly provided by J. Trapman (Department of Pathology, Erasmus University, Rotterdam, The Netherlands), was a 368 base pair (bp) HindIII-EcoRI fragment, corresponding to part of the N-terminal hypervariable region of the human GR [20]. The GAPDH probe was a 0.7 kb EcoRI-PstI fragment [21].

Electrophoretic mobility shift assay

Electrophoretic mobility shift assays were performed as described by ADCOCK *et al.* [22]. BEAS 2B cells were cultured for one passage in DMEM/F12 supplemented with insulin, hydrocortisone, transferrin, EGF, FCS and Na_2SeO_3, after which nuclear protein was isolated [23]. A double-stranded oligonucleotide encoding the consensus target sequence of GRE (5'-TCGACTGTAC-AGGATGTTCTAGCTACT-3') was endlabelled using [γ-^{32}P] adenosine triphosphate (ATP) (Amersham, Den Bosch, The Netherlands) and T4 polynucleotide kinase (Pharmacia Biotech, Roosendaal, The Netherlands). Ten micrograms of nuclear protein were incubated with 5,000 counts per minute (cpm) of labelled oligonucleotide and 3 µg unlabelled competitor poly (dI:dC)-(dI:dC) DNA (Pharmacia Biotech) in 20 µL incubation buffer (4% glycerol, 100 mM NaCl, 1 mM ethylene diamine tetra-acetic acid (EDTA), 1 mM dithiothreitol (DTT), 10 mM Tris-HCl, pH 7.5) for 20 min at 22°C. GR-GRE complexes were separated on a 6% polyacrylamide gel using a Tris base, boric acid, EDTA (TBE) running buffer (12.5 mM Tris base, 12.5 mM boric acid, 0.25 mM EDTA, pH 8.6). The retarded band was detected by autoradiography. Specificity was determined by addition of excess unlabelled double-stranded oligonucleotide.

Steroids

^3H-labelled dexamethasone (1,2,4,6,7 [^3H]-dexamethasone; specific activity 81 $Ci \cdot mmol^{-1}$) was obtained from Amersham (Buckinghamshire, UK). Nonradioactive dexamethasone was kept in a stock solution of 2×10^{-3} M in ethanol (Duchefa bv. Haarlem, The Netherlands).

Glucocorticoid receptor assay

Bronchial epithelial cells were harvested with 0.02% ethyleneglycol tetra-acetic acid (EGTA), 1% polyvinylpyrrolidine and 0.025% trypsin [13]. GR numbers and Kd values were determined according to established methods [24]. Cells were washed with phosphate-buffered saline (PBS) and resuspended at a density of 4–5×10^6 cells·mL^{-1}. Seven serial doubling dilutions (200 µL) were prepared in PBS to final ^{3}H-labelled dexamethasone concentrations of 32, 16, 8, 4, 2, 1 and 0.5 nmol·L^{-1}, respectively. For measurements of nonspecific binding, parallel serial doubling dilutions of ^{3}H-labelled dexamethasone plus a 100 fold molar excess of nonradioactive dexamethasone were prepared. To each of the 14 tubes, 200 µL of the cell suspension was added and cells were incubated for 90 min at 25°C in a waterbath under continuous shaking. Binding equilibrium was reached at all concentrations after 90 min of incubation at 25°C. Subsequently, three 100 µL aliquots of each incubation mixture (determination in triplicate) were transferred to polypropylene tubes. From the remaining incubation mixture 50 µL was used to establish the exact concentration of ^{3}H-labelled dexamethasone. To each 100 µL aliquot, 1 mL of ice-cold PBS was added and tubes were centrifuged for 5 min at 400×g at 4°C. Supernatants were removed and pellets were resuspended in 1 mL of ice-cold PBS and incubated for 30 min on ice to reduce nonspecific binding. Thereafter, samples were centrifuged and the supernatants were removed. The cell pellets were transferred to scintillation vials and radioactivity was determined by liquid scintillation counting. Specific binding was calculated as the difference between the totally bound reactivity and the nonspecifically bound reactivity at each glucocorticoid concentration. Nonspecific binding was calculated from the aliquots containing the 100 fold molar excess nonradioactive dexamethasone, assuming that nonspecific binding was nonsaturable and linearly related to the concentration of free glucocorticoid.

Analysis

Binding curves were constructed from increasing concentrations of ^{3}H-dexamethasone and dissociation constant (Kd) values and receptor binding capacity were determined by Scatchard analysis of these data. Data were analysed with the radioligand binding analysis program 'Ebda/Ligand' by GA Pherson from Elsevier-Biosoft. The Wilcoxon matched-pair signed-ranks test was used to assess the equality of GR number and Kd distributions in cells treated with IL-1β, TNF-α or LPS and in untreated cells. A p-value of less than 0.05 was considered significant.

Results

Expression of GR mRNA in BEAS cells

Total RNA was isolated from BEAS S6 and BEAS 2B cells cultured for 24 h in either KGM, or in DMEM/F12 supplemented with insulin, hydrocortisone, transferrin, EGF, FCS and Na_2SeO_3 or in DMEM/F12 without supplements. Hybridization of filters containing RNA from

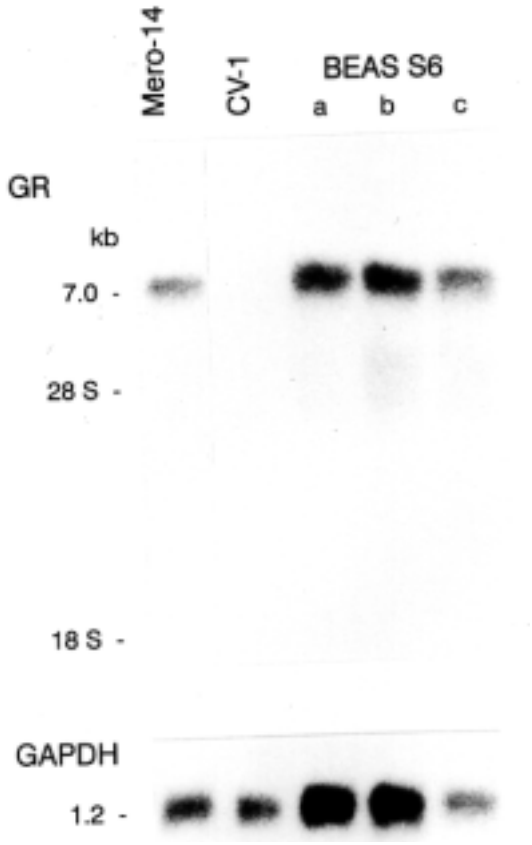

Fig. 1. – Northern blot analysis of 20 µg total ribonucleic acid (RNA) from the cell line BEAS S6. BEAS S6 cells were cultured for 24 h in a keratinocyte growth medium (lane a), in Dulbecco's modified Eagles medium/Ham's F12 (DMEM/F12) supplemented with insulin, hydrocortisone, transferrin, epidermal growth factor, foetal calf serum and Na_2SeO_3 (lane b), and in DMEM/F12 without supplements (lane c). 20 µg of total RNA from the malignant mesothelioma cell line Mero-14 was used as a positive control (lane 1) and RNA from the CV-1 cell line as a negative control (lane 2). The filter was hybridized to both ^{32}P-labelled glucocorticoid receptor (GR) and reduced glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probes.

BEAS S6 and BEAS 2B cells with the GR probe revealed the presence of a 7.0 kb GR specific transcript. Approximately similar levels of GR mRNA expression were found in the two BEAS cell lines. Figure 1 shows the GR mRNA expression in BEAS S6 cells, cultured in KGM (lane a), in DMEM/F12 with hydrocortisone and other supplements (lane b) and in DMEM/F12 without supplements (lane c). RNA from the malignant mesothelioma cell line Mero-14 was used as a positive control [17] and RNA from the CV-1 cell line as a negative control [21]. The quantity of mRNA applied was related to the constitutively expressed GAPDH mRNA. No effect of the different culture media on the GR mRNA content was observed (n=3). Typical ratios of GR mRNA intensity to GAPDH mRNA intensity, in a representative experiment in BEAS S6 cells, were 0.49 in KGM, 0.48 in DMEM/F12 with supplements and 0.42 in DMEM/F12 without supplements. A similar GR mRNA expression was found in BEAS 2B cells (data not shown). GR mRNA expression was not altered after incubation for 4, 8, 24 or 48 h with IL-1β or TNF-α (5 or 20 ng·mL^{-1}) or LPS (100 µg·mL^{-1}) (data not shown).

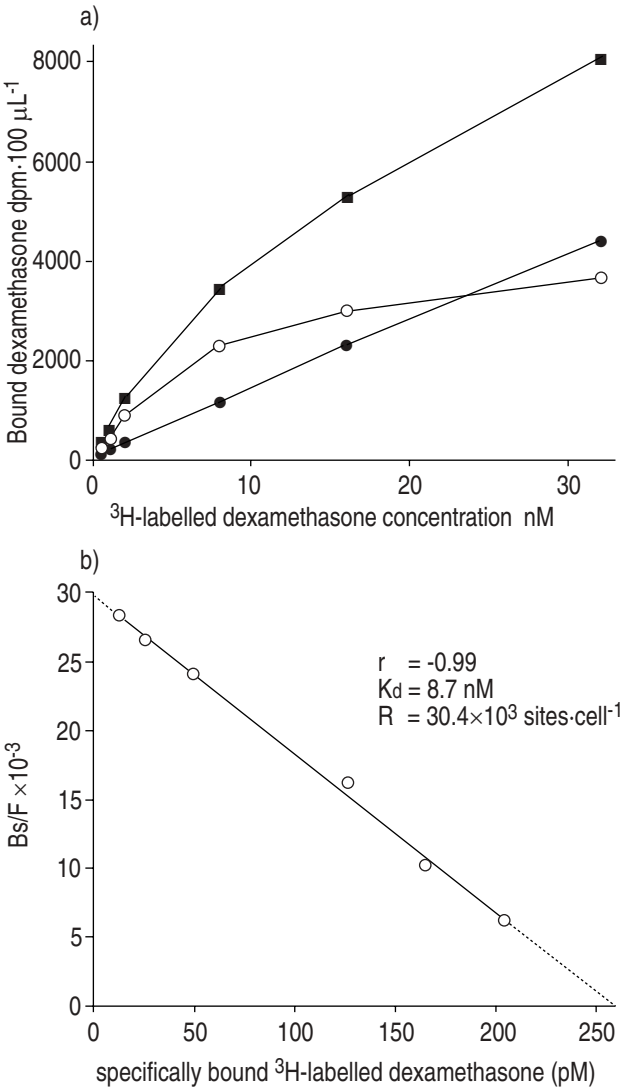

Fig. 2. – One representative experiment (out of three) showing a) Binding curve of ^{3}H-labelled dexamethasone for epithelial cell line BEAS 2B. The specific binding (○) is represented by the difference between the total (■) and nonspecific (●) binding. The data points represent mean values of quadruplicate determinations. b) Scatchard plot of the specific binding of ^{3}H-labelled dexamethasone to BEAS 2B cells. On the ordinate is given the ratio of the amount of specifically bound ^{3}H-labelled dexamethasone (B_S) to the amount of free ^{3}H-labelled dexamethasone (F). The dissociation constant (K_d) and the number of glucocorticoid receptors per cell (R) were calculated using the negative inverse of the slope and the x-intercept, respectively. dpm: disintegrations per minute.

Basal numbers and Kd of GR in BEAS cells

After demonstrating the presence of GR mRNA in BEAS cell lines, the number of GR and K_d values were determined. Using established methods to identify GR, we could demonstrate specific binding of ^{3}H-labelled dexamethasone by these cells in our final assay conditions. A typical binding curve for BEAS 2B is shown in figure 2a. After Scatchard analysis of the data, the linear regression line obtained indicated a single class of GR for both cell lines (fig. 2b). BEAS 2B cells appeared to have 32.1±5.7 ×10^3 binding sites per cell (n=3), and BEAS S6 cells were found to have (mean±SEM) 28.9± 4.4 ×10^3 binding sites per cell (n=4). K_d values, determined from Scatchard plots, were 8.6±2.4 and 8.2±1.5 nM, respectively.

GR binding to its specific DNA recognition sequence

The interaction between glucocorticoid-GR complexes isolated from the nucleus and GRE can be measured using an electrophoretic mobility shift assay. Nuclear extracts of BEAS 2B cells were incubated with a labelled double-strand (ds)DNA recognition sequence and the bound oligonucleotide was detected by its retardation in a nondenaturing gel. A clear GR/GRE binding was observed in nuclear extracts of BEAS 2B cells, (fig. 3, control lane). An excess (50 fold) of unlabelled GRE oligonucleotide produced a strong inhibition of band density (fig. 3, XS GRE oligo). No effect was observed of an excess of control unrelated oligonucleotide, corresponding to a part of the platelet derived growth factor (PDGF) B-chain promotor region (fig. 3, XS irrelevant oligo).

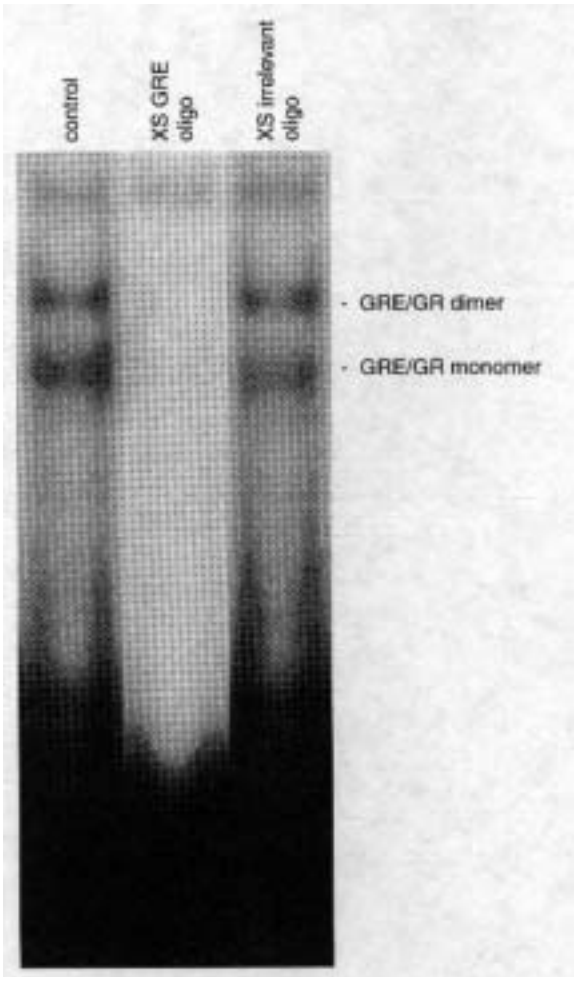

Fig. 3. – GR/GRE binding in BEAS 2B cells in electrophoretic mobility shift assay. Effect of an excess (50-fold) of unlabelled GRE oligonucleotide (XS GRE oligo) and of an excess of control unrelated oligonucleotide corresponding to part of the PDGF B-chain promotor region (XS irrelevant oligo). GR: glucocorticoid receptors; GRE: glucocorticoid responsive elements; PDGF: platelet derived growth factor.

Effects of LPS, IL-1β and TNF-α on GR binding affinity and number

To study the effect of inflammatory mediators on GR number and binding affinity, BEAS 2B cells were incubated with LPS, IL-1β, or TNF-α for 24 h. To exclude the possibility that LPS or IL-1β interfered with the ability of ^{3}H-dexamethasone to bind to the GR, LPS or IL-1β were added to the bronchial epithelial cells for 5, 15, 30 or 60 min and subsequently a ^{3}H-dexamethasone binding assay was performed. No effect on GR number or affinity was observed. Figure 4 shows a representative Scatchard plot of [^{3}H]-dexamethasone radioligand-binding in BEAS 2B cells incubated with LPS or IL-1β for 24 h, or cultured without inflammatory mediators. The radioligand-binding data of BEAS 2B treated with LPS or IL-1β (n=5) showed a significant increase in the Kd, *i.e.* a decrease in binding affinity for glucocorticoids (median=410 and 145% of control, respectively; $p<0.05$) and a significant increase in GR number (median=312 and 171% of control, respectively, $p<0.05$). In contrast, treatment with TNF-α had no significant effect on GR Kd (85.5% of control) or GR number (107.5% of control) (fig. 5).

Data were analysed with a radioligand binding analysis program. When using a two-site model for ligand binding the Scatchard results were not affected.

Discussion

In this report, we demonstrate the presence of GR mRNA and protein in two human bronchial epithelial cell lines, BEAS S6 and BEAS 2B. In nuclear extracts obtained from BEAS 2B cells cultured in hydrocortisone containing medium, electrophoretic mobility shift assays showed that nuclear translocated GR bind to their GRE. BEAS S6 and BEAS 2B cell lines were

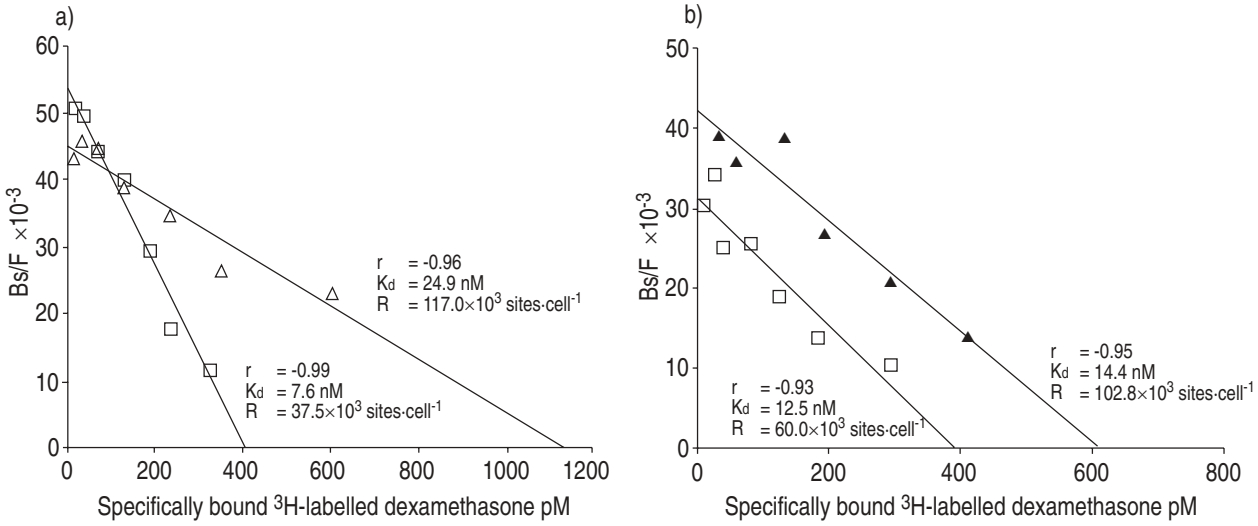

Fig. 4. – Scatchard plot (of one representative experiment out of five) of the specific binding of ^{3}H-labelled dexamethasone to epithelial cell line BEAS 2B cells treated for 24 h with a) lipopolysaccharide (LPS) (100 μg·mL^{-1}) or b) interleukin-1β (IL-1β) (20 ng·mL^{-1}). On the ordinate is given the ratio of the amount of specifically bound ^{3}H-labelled dexamethasone (Bs) to the amount of free ^{3}H-labelled dexamethasone (F). The dissociation constant (Kd) and the number of glucocorticoid receptors per cell (R) were calculated using the negative inverse of the slope and the x-intercept, respectively. ❑: control; Δ: +LPS; ▲: +IL-1β.

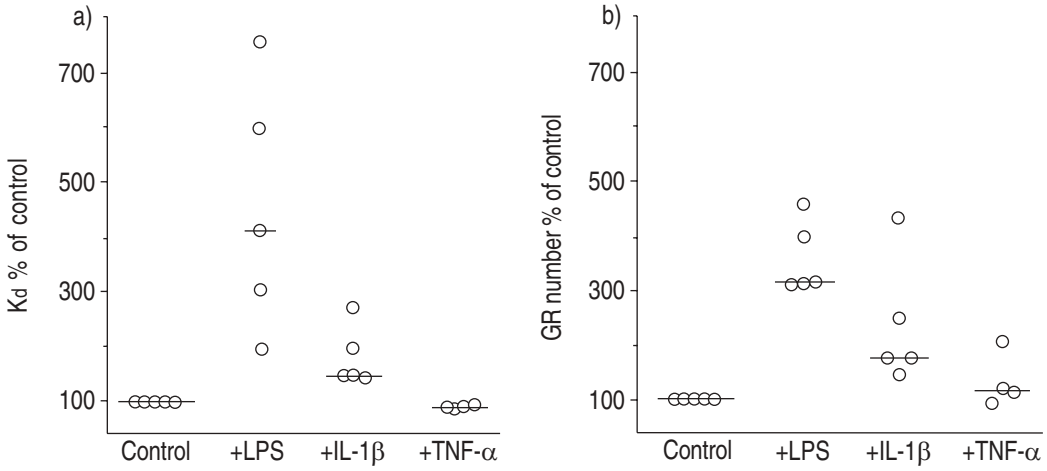

Fig. 5. – The effect of LPS, IL-1β and TNF-α on a) dissociation constant Kd and b) GR number. Incubation of epithelial cell line BEAS 2B with LPS (100 μg·mL^{-1}) or IL-1β (20 ng·mL^{-1}) resulted in a significantly increased receptor number and a decreased binding affinity of the GR for dexamethasone (n=5, p=0.05) when compared with the medium control. Incubation with TNF-α (20 ng·mL^{-1}) for 24 h had no effect on GR Kd or GR number. The solid bars indicate median values. TNF-α: tumour necrosis factor-α. For further definitions see figures 1 and 4.

found to possess 28.9±4.4 $\times 10^3$ and 32.1±5.7 $\times 10^3$ specific binding sites per cell, respectively, with Kd values of 8.2± 1.5 and 8.6±2.4 nM, respectively. Treatment of BEAS 2B cells with LPS or IL-1β resulted in a significantly higher number of GR compared to control cells, whereas GR binding affinity for glucocorticoids was reduced.

We hypothesize that the clinical response to inhaled glucocorticoids, which mainly precipitate in the larger airways, results, at least partly, from the modulation of airway epithelial cell functions. From the Kd value of the GR in bronchial epithelial cells observed in our studies, we expect that effective *in vivo* glucocorticoid concentrations should be around 5–10 nM. A recent study by VAN DEN BOSCH *et al.* [25] showed that at least 90 min after inhalation of 1.6 mg budesonide, lung tissue concentrations ranged 2.1–8.9 nM. Therefore, we can assume that inhalation of glucocorticoids may result in such concentrations of glucocorticoids in lung tissue that an interaction with GR in epithelial cells will occur.

Human bronchial epithelial cells are considered to play an important role in airway inflammation [1–6]. Inhaled glucocorticoids are used to suppress airway inflammation and, concomitantly, to improve clinical parameters. As inhaled glucocorticoids mainly precipitate in the larger airways, one of the mechanisms of action of these drugs may be the modulation of the functioning of bronchial epithelium. Cellular response to glucocorticoids demands the presence of functional GR. Only a few studies concern the presence of GR in bronchial cells, and the majority used animal studies [26–29]. Previous *in vitro* binding experiments with ^{3}H-dexamethasone performed on lung samples from adult and foetal animals and from a human foetus showed nuclear localization of ^{3}H-dexamethasone in alveolar epithelial cells, but no significant nuclear localization in bronchial epithelial cells [26–29]. In these studies autoradiographic data were confirmed with liquid scintillation counting for specific ^{3}H-dexamethasone binding in nuclear and cytosolic fractions prepared from lung tissue. Until now, only one pilot study has been performed on adult human lung, in which the localization and expression of GR mRNA was studied by *in situ* hybridization and Northern blot analysis [30]. The highest concentration of human GR mRNA was found in the alveolar walls with lesser amounts in the larger airway epithelium [30]. However, in that study no data were presented on the number of receptors and their Kd values.

The work presented here has shown, for the first time with ^{3}H-dexamethasone binding assays, that human bronchial epithelial cells possess GR and that these receptors are able to interact with GRE. Using the same ^{3}H-dexamethasone binding assay, we found in U937 cells an immature monocytic cell line, and in the NHIK 3025 cell line (derived from a carcinoma of the human uterine cervix) 18.2×10^3 and 86.0×10^3 specific binding sites per cell, respectively unpublished results. Their dexamethasone Kd values were 3.5 and 3.1 nM, respectively. The number of GR and the Kd values we found in bronchial epithelial cell lines were similar to those described for cultured dispersed adult rat lung cells [27]. Cultured L-2 cells, a cell line that originated from a type II epithelial cell of adult rat lung contained 58.2×10^3 nuclear binding sites per cell with a Kd of 8.0 nM [27]. Peripheral blood mononuclear cells (PBMC) fro normal human controls were found to possess 2.7×10^3 receptor sites per cell with a Kd of 6.7 nM [31]. Compared to PBMC, BEAS cell lines contain a relatively high number of glucocorticoid binding sites. The number and quality of GR in target cells may determine the extent of glucocorticoid responsiveness [32, 33]. Even if the fact that they are 2–3 times larger than PBMC is taken into account, bronchial epithelial cells still possess a greater number of GR.

We demonstrated that both LPS and IL-1β significantly increased the number of GR, but decreased the GR binding affinity in BEAS 2B cells. These results and the work of several other investigators indicate that cytokines produced during the course of an immune response may regulate GR number and affinity [31, 34–36]. This suggests that inflammation in itself may modulate cellular steroid responsiveness through locally produced inflammatory mediators. Inflammatory mediators can induce an increase in GR number, perhaps to control an excess of inflammatory stimuli by sensitizing the cell to feedback inhibition by glucocorticoids. IL-1, TNF-α and IL-6 have all been shown to stimulate the hypothalamic-pituitary axis to secrete corticotrophin-releasing hormone and adrenocorticotrophic hormone which, in turn, induces glucocorticoid secretion from the adrenal cortex [37, 38].

Northern blot analysis showed that GR mRNA expression was not altered after incubation with LPS or IL-1β. Therefore, the increase in GR number after treatment with LPS or IL-1β could not be explained by an increase in GR mRNA. Increased translation or increased half-life time of GR mRNA could provide an explanation for the increase in GR number. An altered phosphorylation state of the GR after treatment with LPS or IL-1β is unlikely to account for the discrepancy between GR mRNA and protein expression. Studies performed by MOYER *et al.* [39] showed no relationship between enhanced transcriptional activity and phosphorylation of the GR.

The work of several other investigators indicates that cytokines produced during the course of an immune response may regulate both GR number and affinity [31, 34–36]. KAM *et al.* [31] found that when normal T-cells and the non-T-cell fraction of PBMC were individually stimulated with IL-2 and IL-4, a significant reduction in GR binding affinity for glucocorticoids was observed only in the T-cell population. An increase in GR number was observed both in T- and non-T-cells. The reduction in PBMC GR binding affinity with IL-2 and IL-4 was associated with a reduced T-cell response to methylprednisolone. SHER *et al.* [34] demonstrated that steroid resistant patients had a significantly reduced GR binding affinity for glucocorticoids, but an increased number of nuclear GR compared with steroid-sensitive patients and normal patients. This defect was localized to T-cells and reverted to normal after 48 h in culture. Incubation with IL-2 and IL-4 sustained these abnormalities. RAKASZ *et al.* [35] have shown that glucocorticoid binding was increased by IL-1β, TNF-α and IL-6 in a B-cell line (CESS), in U937 and in a hepatoma cell line (HepG2). Interferon-γ (IFN-γ) has been shown to mediate increased GR expression in murine macrophages [36]. The decrease in GR binding affinity for

dexamethasone and the increase in GR number, which we found with IL-1β and LPS in BEAS 2B cells, is in accordance with the effects of IL-2 and IL-4 on the GR, found in T-cells by KAM *et al*. [31]. The differences in the results obtained by the different investigators mentioned above could well be due to differences in cell type, methods of ligand binding, the ligands, and the cytokines or inflammatory mediators used for different periods of time.

The actual mechanisms by which inflammatory mediators induce a decrease in GR binding affinity for glucocorticoids remain to be established. Others have shown that altered expression of glucocorticoid-regulated proteins appears to be mediated *via* interaction of the modulatory domain of the GR with transcriptional factors, such as activation protein-1 (AP-1) [40–42]. Over expression of AP-1 interferes with the function of the modulatory domain of the GR. Because cytokines can induce elevated levels of AP-1, it has been suggested that this may provide a plausible explanation for the decreased ligand binding affinity of nuclear GR for glucocorticoids, induced by IL-2 and IL-4 in T-cells [40–42]. Perhaps a similar explanation could be given for the decreased GR binding affinity induced by LPS or IL-1β in BEAS 2B cells. Another explanation for decreased GR binding affinity could be a modulating effect of LPS and IL-1β on the expression and phosphorylation of heat shock proteins, which are associated with the unliganded GR [43, 44]. Bacterial products and cytokines can regulate the expression and phosphorylation of heat shock proteins and this may modulate glucocorticoid binding to the GR [43, 44]. Further studies are necessary to clarify the exact mechanism of these LPS and IL-1β effects on GR.

In summary, we demonstrated that two human bronchial epithelial cell lines, BEAS S6 and BEAS 2B, possess a single class of specific glucocorticoid receptors, which display *in vitro* specific binding to its glucorticoid responsive element. Lipopolysaccharide and interleukin-1β significantly increased the number of glucorticoid receptors and decreased their binding affinity in BEAS 2B cells. Treatment with tumour necrosis factor-α had no effect on glucocorticoid receptor dissociation constant or receptor number. These results provide further evidence that the bronchial epithelium may be a direct target for glucocorticoid therapy in inflammatory airway diseases. Furthermore, this study suggests that inflammatory processes may influence the response of bronchial epithelium to glucocorticoid therapy *via* locally produced cytokines. As the response to glucocorticoids varies considerably among patients with asthma and chronic obstructive pulmonary disease, it is of great interest to study these patients for correlations between the clinical response to glucocorticoids and the number of glucocorticoid receptors or their dissociation constants in bronchial epithelial cells, in combination with the local expression of cytokines.

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