

Original article

Differential expression of IL-10 receptor by epithelial cells and alveolar macrophages

Background: Interleukin (IL)-10 is a pleiotropic cytokine with a broad spectrum of immunosuppressive and anti-inflammatory effects. IL-10 secretion from alveolar macrophages is defective in patients with asthma and lower concentrations of IL-10 are found in bronchoalveolar lavage (BAL) from asthmatic patients than in normal control subjects. Reduced IL-10 may result in exaggerated and more prolonged inflammatory responses in asthmatic airways. IL-10 acting through the IL-10 receptor (IL-10R) stimulates the transcription factors STAT1 and STAT3.

Methods: We investigated IL-10 and IL-10R expression in normal and asthmatic bronchial epithelium and BAL macrophages using reverse transcription-polymerase chain reaction, immunohistochemistry and Western blotting. The functional effect of IL-10 was examined using granulocyte-macrophage-colony stimulating factor, enzyme-linked immunosorbent assay and Western blotting for phosphorylated STAT1 and STAT3.

Results: IL-10 was not expressed in epithelial cells; furthermore these cells did not express the IL-10R and had no functional response to exogenous IL-10. Bronchial epithelial cells expressed variable levels of phosphorylated STAT1 and STAT3 with no change in expression between normal subjects and asthmatics. IL-10 protein and IL-10R expression was detected in alveolar macrophages from all subjects.

Conclusion: Our study suggests that the bronchial epithelium is not a source of IL-10 and cannot respond to exogenous IL-10 because of a lack of IL-10R expression.

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Interleukin (IL)-10 is an anti-inflammatory cytokine, which is predominantly produced by macrophages and T cells (1). Constitutive IL-10 secretion occurs in the healthy lung and is a potent inhibitor of monocyte/macrophage function, suppressing the production of a number of proinflammatory cytokines, including tumour necrosis factor- α (TNF- α), IL-1 β , IL-6, macrophage inflammatory protein- α (MIP-1 α) and IL-8 (1, 2). IL-10 inhibits monocyte major histocompatibility complex class II, B7.1/B7.2 and CD23 expression and accessory cell function, which is essential for T-cell activation (3). IL-10 also inhibits eosinophil survival and IL-4-induced IgE synthesis, and there is evidence for reduced expression of both IL-10 protein and mRNA in alveolar macrophages of asthmatic subjects compared with those from non-asthmatic individuals (4, 5). Treatment with inhaled glucocorticoids leads to an increase in IL-10 production, together with a decrease in granulocyte-macrophage-colony stimulating factor (GM-CSF) production by alveolar macrophages (4).

IL-10 exerts its anti-inflammatory actions through binding to the IL-10 receptor (IL-10R). Binding to the IL-10R stimulates phosphorylation and activation of intracellular signalling pathways, specifically STAT1 and STAT3 (6). Indeed, targeted disruption of STAT3 in macrophages and neutrophils results in defective IL-10 function, exaggerated inflammatory responses and pro-inflammatory cytokine production (7).

Airway epithelial cells are barrier cells, which protect the airway mucosa against inhaled macromolecules (8). The bronchial epithelium, because of its direct contact with the external environment, is exposed to a wide variety of airborne particles, such as allergens, toxic gases and pollutants. Some of these particles are rapidly cleared from the respiratory tract whilst others may result in recruitment and activation of immunocompetent and inflammatory cells.

The epithelium is immunologically and metabolically active, with evidence to suggest that epithelial cells have the capacity to act as effector cells in response to

endogenous and exogenous stimuli. For example, epithelial cells are capable of producing a variety of inflammatory mediators, including lipid mediators, oxygen radicals and cytokines and chemokines in response to inflammatory stimuli (9, 10). Epithelial cells also express receptors for inflammatory mediators and adhesion receptors, and this combination of factors makes the epithelium a focus for inflammatory cell recruitment (11). The production of these inflammatory mediators implicates the bronchial epithelium in the initiation and/or maintenance of airway inflammatory diseases such as asthma.

Although the epithelium possesses proinflammatory activities, it can also exhibit anti-inflammatory effects. For example, expression of IL-1 receptor antagonists (IL-1ra) has been described (12). It has previously been reported that IL-10 mRNA and protein is expressed by epithelium obtained from normal subjects but not by epithelium derived from patients with cystic fibrosis (13). Therefore, we investigated the ability of alveolar macrophages, primary bronchial epithelial cells and human epithelial cells to express the anti-inflammatory cytokine IL-10, and its receptor IL-10R. We also investigated the expression of STAT1 and STAT3 as markers of IL-10 induced cell activation.

Materials and methods

Materials

A549 and BEAS-2B cell lines were obtained from the American Type Culture Collection (Rockville, MD, USA). 16HBE cells were a kind gift from D. Gruenert (Cardiovascular Research Institute, University of California-San Francisco, CA, USA). Keratinocyte serum-free medium, epidermal growth factor (EGF), bovine pituitary extract, Dulbecco's modified Eagle's Media, Ham's F12 nutrient media, Hank's balanced salt solution were purchased (Gibco Ltd, Paisley, UK). IL-1 β and TNF- α were also purchased (R&D Systems Europe Ltd, Abingdon, UK). Enzyme-linked immunosorbent assay (ELISA) kits for GM-CSF and IL-10 (Quantikine) were purchased from R&D Systems). Qiagen RNeasy mini kit was purchased from Qiagen Ltd (Crawley, UK). All materials pertaining to reverse transcription-polymerase chain reaction (RT-PCR) were purchased from Promega (Southampton, UK). All other reagents were supplied by Sigma (Poole, UK).

Subjects

Ten mild stable asthmatic patients (Table 1) receiving only intermittent treatment with the inhaled β_2 -adrenergic agonist aerosol, salbutamol, for relief of wheeze were recruited. All patients demonstrated a >15% improvement in forced expiratory volume (FEV₁) following inhalation of 200 μ g of salbutamol and airway hyper-responsiveness to methacholine with a provocative concentration of methacholine producing a 20% fall in FEV₁ (PC₂₀) of <4 mg/ml. All patients were atopic as defined by two or more positive skin prick tests to common allergens. None of the subjects studied had received oral or inhaled glucocorticoids for the preceding 12 months, or any other treatment apart from inhaled β_2 -agonists. Current smokers or ex-smokers of more than five pack years and patients with FEV₁ <80% predicted were excluded. Six

Table 1. Patient characteristics

Patient	Sex	Age	FEV ₁	PC ₂₀	Atopy	NO
Normal subjects						
1	M	27	94	>64 mg/ml	No	5
2	M	24	91	>64 mg/ml	No	6.2
3	M	22	102	>64 mg/ml	No	1
4	M	23	90.6	>64 mg/ml	No	7.3
5	F	23	105	>64 mg/ml	No	6.2
6	M	24	105	>64 mg/ml	No	6.6
Asthmatic patients						
1	M	22	110	0.114	Yes	40.5
2	M	24	92	0.25	Yes	27
3	M	27	101	1.035	Yes	14.9
4	F	23	70.9	0.093	Yes	43
5	F	27	93.7	0.5	Yes	25.3
6	F	23	100	1.123	Yes	5.5
7	F	27	96	1.16	Yes	17.5
8	M	29	93	0.5	Yes	25
9	M	24	103	0.0232	Yes	33.5
10	F	23	84.7	1.2	Yes	20.1

FEV₁, forced expiratory volume in first second expressed as a percentage of predicted; PC₂₀, concentration of methacholine required to produce a 20% drop in FEV₁; NO, exhaled nitric oxide; ppb, parts per billion.

normal nonatopic, nonasthmatic controls were also recruited for control data in this study. All asthmatic subjects had elevated levels of exhaled nitric oxide (NO). The study was approved by the Royal Brompton Hospital Ethics Committee and all subjects gave their written informed consent.

Bronchoscopy

Subjects attended the bronchoscopy suite after overnight fasting and bronchial biopsies, brushings and alveolar macrophages obtained exactly as previously described (4, 14). All biopsies were frozen within 20 min of collection. Sections (10 μ m) were placed on poly-L-lysine coated microscope slides (BDH, Poole, UK), air dried for 30 min then wrapped in aluminium foil and stored at -70°C prior to immunostaining.

Identification of cells

Cytospins (10 000 cells/slide) were made using a centrifuge (Shandon Cytospin 3, Southend, UK). The cells were airdried onto the slides and fixed for 10 min in cold acetone and stored at -20°C.

Immunofluorescent study of IL-10 and IL-10R expression in bronchial epithelial cells from brushings, bronchial biopsies and alveolar macrophages

Slides were brought back to room temperature and left to air dry for 20 min, fixed in cold acetone and left again to air dry for 5 min. Staining was performed as previously described using saponin (0.1%) to permeabilize cells (4, 14). Rabbit anti-human IL-10 and rabbit anti-human IL-10R (both at 1 : 100; Santa Cruz, Wembley, UK) were used. In addition, some sections were stained using an antibody directed against COS cell-expressed IL-10 (Pharmingen, JES3-12G8). Sections were counterstained with haematoxylin and the coverslip mounted on slides with citifluor and sealed with mounting medium (distyrene, plasticizer, xylene [DPX]). Stained cells were observed by confocal microscopy.

Isolation and culture of primary human bronchial epithelial cells

Primary human bronchial brushing cells were isolated and cultured as previously described (15). The cells were cultured until confluence (approximately 5 days) and then for a further 24 h in serum-free media prior to stimulation with cytokines IL-1 β 10 ng/ml (alone) and IL-1 β plus TNF- α 10 ng/ml. After 24 h the supernatants were removed and the cells lysed for RNA isolation. We also cultured and stimulated primary epithelial cells in the presence of IL-10 (0.1–10 ng/ml) and dexamethasone (10^{-9} and 10^{-6} M), which was added to the media 1 h prior to stimulation. The supernatants were removed after 24 h for cytokine immunoassays.

Western blot analysis

Western blotting was performed exactly as previously described (16). At least 40 μ g/lane of whole-cell proteins were subjected to 18 (IL-10) or 8% (IL-10R, STAT1, pSTAT1, STAT3 and pSTAT3) sodiumdodecyl sulphate-polyacrylamide gelelectrophoresis (SDS-PAGE). All antibodies were used at 1 : 1000 dilution (anti-IL-10 and anti-IL-10R 1 : 500 dilution) and detected by enhanced chemiluminescence (ECL) (Amersham Pharmacia Biotech, Little Chalfont, UK). To control for protein loading each filter was subsequently probed with an anti-human actin antibody (Santa Cruz). The bands were visualized at approximately 43 kDa (actin), 14.5 kDa (mouse IL-10), 18 kDa (human IL-10), 110 kDa (IL-10R), 91 and 84 kDa (STAT1 and pSTAT1 p91 and p84 isoforms) and 92 kDa (STAT3 and pSTAT3).

Culture of cell lines

A549, BEAS-2B and 16HBE cells were cultured as previously described (17, 18). Once confluence was achieved, the cells were then cultured for 24 h in serum-free media prior to stimulation with cytokines IL-1 β (1 ng/ml) alone, TNF- α (10 ng/ml) alone and IL-1 β plus TNF- α . Following these experimental treatments, the supernatants were removed and the cells lysed for RNA isolation. All experiments were carried out on six duplicates for each cell line. Each cell line was also treated with IL-10 (0.1–10 ng/ml) or dexamethasone (10^{-9} and 10^{-6} M), for 1 h prior to stimulation. The supernatants were removed after 24 h for cytokine immunoassays.

GM-CSF, IL- and IL-10 measurement by ELISA

GM-CSF and IL-8 were measured by sandwich ELISA according to the manufacturer's instructions (R&D Systems Europe). The detection limit of both assays is 16 pg/ml. IL-10 was assayed using a commercially available kit exactly as described by the manufacturer (R & D systems).

RNA isolation

RNA was isolated from epithelial cells using the Qiagen RNeasy mini kit according to the manufacturer's instructions (Qiagen Ltd).

Reverse transcription-polymerase chain reaction

Reverse transcription of 0.5 μ g of total RNA was performed using avian myeloblastosis virus (AMV)-reverse transcriptase 15 U, 10 mM of dATP, dCTP, dGTP and dTTP, oligo dT15 primer (0.2 μ g), RNase inhibitor 30 U and 5x AMV buffer in a total volume of 8.5 μ l RNA was denatured at 65°C for 10 min. The

remaining reagents were then added as a master mix and samples were incubated at 42°C for 60 min followed by 4 min at 90°C. The cDNA was subsequently diluted to a final volume of 100 μ l in nuclease-free water.

Polymerase chain reaction

For PCR, 5 μ l of the cDNA solutions were used. PCR was performed using 0.5 μ g/ μ l of forward and reverse primers, dATP, dGTP, dTTP, dCTP at a concentration of 10 mM each, *Taq* polymerase 0.5 U and buffer A in a final volume of 25 μ l set for 30 cycles for IL-10, 35 cycles for GM-CSF and 30 cycles for glyceraldehyde-3-phosphate dehydrogenase (GADPH) at a denaturing temperature of 94°C for 30 s, specific annealing temperature of 65°C for IL-10 and GM-CSF and 58°C for GADPH and extension temperature of 72°C for 30 s. Primers for IL-10 were 5'-ATG/CCC/CAA/GCT/GAG/AAC/CAA/GAC/CCA and 3'-TCT/CAA/GGG/GCT/GGG/TCA/GCT/ATC/CCA giving a product of 351 bp. Primers for GAPDH were: 5'-TCT/AGA/CGG/CAG/GCT/AGG/TCC/ACC and 3'-CCA/CCC/ATG/GCA/AAT/TCC/ATG/GCA giving a product of 598 bp. For GM-CSF, the primer sequence was 5'-ATG/TTT/GAC/CTC/CAG/CCG and the 3'-GTC/CGT/ACC/GTC/TTT/TGT/A giving a product of 369 bp.

Polymerase chain reaction was carried out in a Techne multiwell thermocycler (Techne, Cambridge, UK). Products were distinguished by electrophoresis on a 1.5% agarose ethidium bromide stained gel and then visualized using ultraviolet luminescence.

Statistical analysis

Data are reported as mean $\pm$ SEM. Data obtained from the epithelial cell lines were analysed using one-way ANOVA. In order to compare the data between asthmatics and normal, an unpaired *t*-test was used. A *P* < 0.05 was considered to be significant.

Results

IL-10 and IL-10R expression in primary bronchial epithelial cells and bronchial biopsies

Using fluorescein isothiocyanate (FITC)-labelled secondary antibodies we found that both normal and asthmatic epithelium had significant autofluorescence (Fig. 1). We therefore used rhodamine red-X linked secondary antibodies, which had no detectable autofluorescence from the tissue. Using both Santa Cruz and Pharminogen antibodies we were unable to detect staining for IL-10 within the bronchial epithelium of normal and asthmatic subjects above that seen with isotype control antibodies (Fig. 1). In addition, IL-10R expression was not detected in any samples from normal or asthmatic subjects.

In order to confirm a lack of IL-10 production by epithelial cells, primary epithelial cells obtained by bronchial brushing were cultured from both normal and asthmatic subjects. These cells did not express IL-10 protein as measured by ELISA either under basal conditions or following IL-1 β - or TNF α -stimulation (data not shown). In addition, none of the cell lines studied (A549, BEAS-2B or 16-HBE) produced any IL-10 protein either constitutively or following stimulation (data not shown).

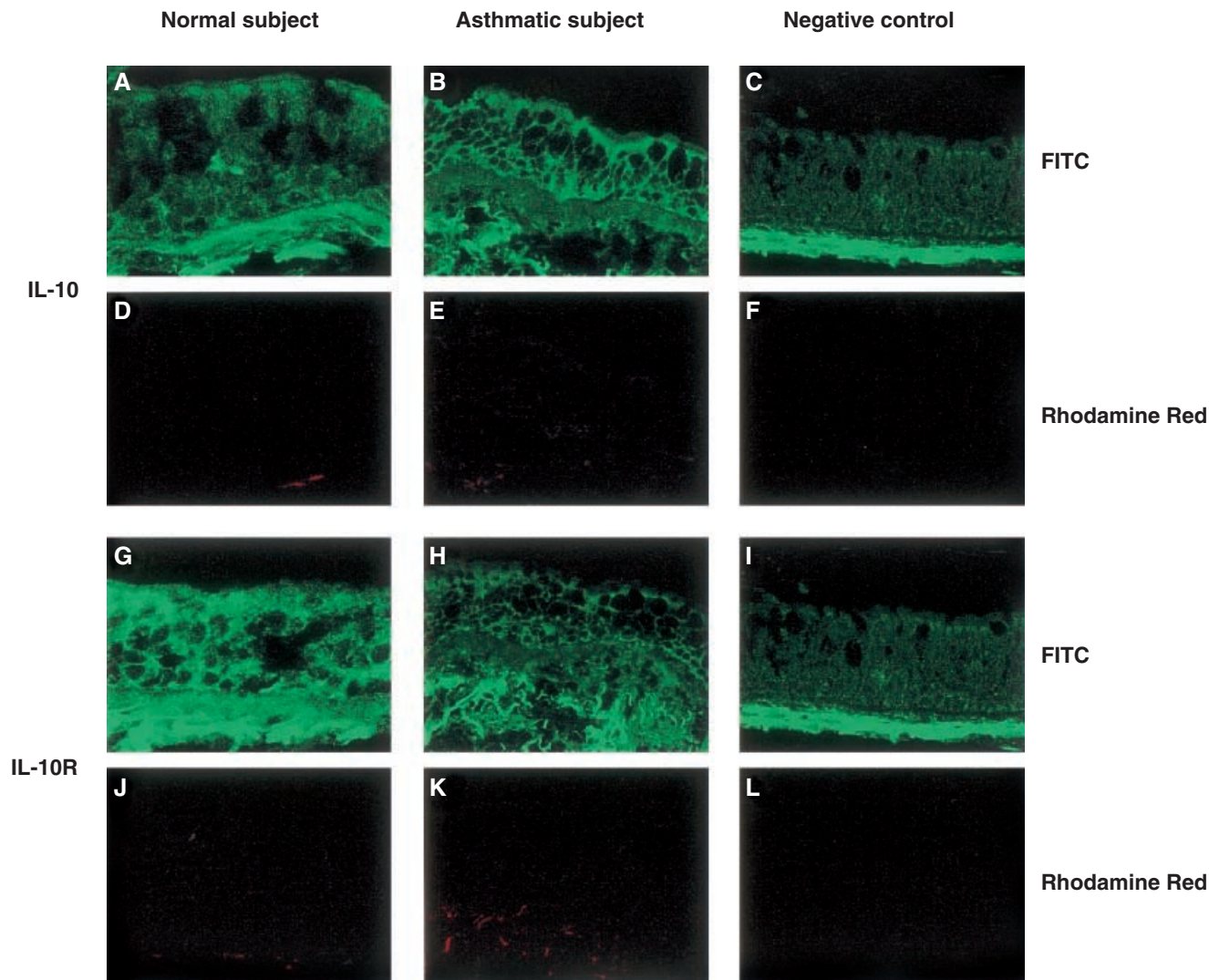


Figure 1. Expression of IL-10 and IL-10R in bronchial biopsies from normal and asthmatic subjects. Sections were cut from bronchial biopsies from normal (panels A, C, D, F, G, I, J and L) and asthmatic subjects (panels B, E, H and K). Sections were stained with anti-IL-10 antibodies (panels A, B, D, E) and anti-IL-10R antibodies (panels G, H, J, K). Fluorescence in the flourescein isothiocyanate wavelength (panels C and I) or with rhodamine Red (panels F and L) using an isotype control antibody is also shown. Results are representative of those for six normal subjects and 10 asthmatic patients.

Western blotting also failed to detect IL-10 expression in primary epithelial cells or cells lines (data not shown). Furthermore, we were unable to detect IL-10 mRNA either constitutively or following stimulation with proinflammatory cytokines (Fig. 2A). There was also no evidence of IL-10 or IL-10R mRNA expression from A549, BEAS-2B or 16-HBE epithelial cell lines (Fig. 2A).

Expression of GM-CSF and IL-8 in primary bronchial epithelial cells

The failure to induce IL-10 expression was not because of a lack of IL-1 β or TNF- α receptors as these cells significantly increased GM-CSF mRNA and protein and IL-8 protein expression following stimulation with these cytokines. GM-CSF release from primary epithelial

cells was significantly greater than in A549 cells, BEAS-2B cells or 16HBE cells (Fig. 2B,C). There was no difference in the levels of GM-CSF released from normal subjects or asthmatic patients either at baseline or following stimulation with cytokines (Fig. 2C). All three cell lines studied were able to express GM-CSF mRNA and protein following stimulation with inflammatory cytokines (Fig. 2B,C).

Effect of IL-10 on release of GM-CSF from primary epithelial cells

Despite being unable to detect IL-10R in these cells by immunocytochemistry and RT-PCR it is possible that the detection methods used were not sensitive enough to detect low, but functionally relevant, levels of IL-10R. We

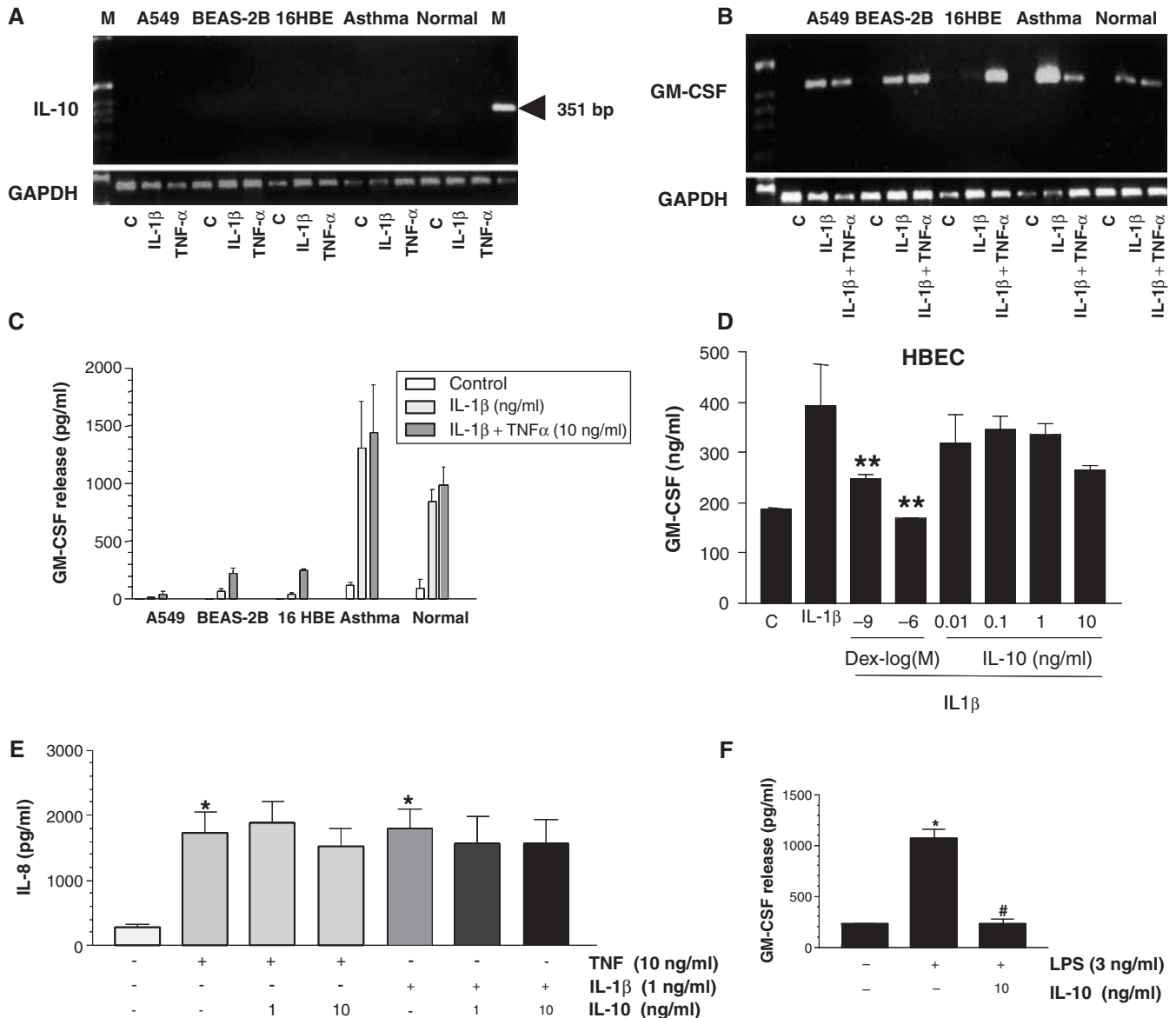


Figure 2. (A) Expression of IL-10 mRNA from bronchial epithelial cells. RNA was extracted from A549, BEAS-2B, 16HBE and primary bronchial epithelial cells from normal and asthmatic subjects before and after stimulation with IL-1 β (1 ng/ml) or TNF- α (10 ng/ml). IL-10 transcripts were detected by RT-PCR and GAPDH expression was used as control for RT. RNA isolated from alveolar macrophages (*M ϕ*) was used as a positive control for IL-10 expression (351 bp). Results are representative of at least six independent observations (M, molecular weight ladder). (B) Expression of GM-CSF mRNA and protein in bronchial epithelial cells. RNA was extracted and GM-CSF mRNA detected by RT-PCR. Results are representative of three independent observations. (C) GM-CSF released into the media was detected by sandwich ELISA. Results are given as mean $\pm$ SEM from six normal and 10 asthmatic patients and five independent observations for cell lines. (D) Effect of dexamethasone (10^{-9} and 10^{-6} M) and IL-10 (0.01–10 ng/ml) on IL-1 β -stimulated GM-CSF release in primary human epithelial cells. Results are given as the mean $\pm$ SEM of three independent observations (** P < 0.01). (E) Effect of IL-10 (1 and 10 ng/ml) on IL-1 β - and TNF- α -stimulated IL-8 release in primary human epithelial cells. Results are given as the mean $\pm$ SEM of three independent observations. * P < 0.05 compared with unstimulated cells. (F) Effect of IL-10 (10 ng/ml) on LPS (3 ng/ml)-stimulated GM-CSF release in alveolar macrophages. Results are given as the mean $\pm$ SEM of three independent observations. * P < 0.05 compared with unstimulated cells and P < 0.05 compared with LPS-stimulated cells.

therefore determined whether exogenous IL-10 could suppress cytokine-induced GM-CSF and IL-8 release in primary human bronchial epithelial cells obtained by bronchial brushing. Primary human bronchial epithelial cells were

cultured in the presence or absence of IL-10 (0.1–10 ng/ml), which was added to the supernatant 1 h prior to stimulation with IL-1 β and TNF- α . Supernatants were harvested after 24 h. GM-CSF and IL-8 release from cell

lines or primary epithelial cells were not inhibited by IL-10 *in vitro* (Fig. 2D,E). In contrast, dexamethasone caused a marked inhibition of GM-CSF release in primary epithelial cells (Fig. 2D). We confirm previous data (4, 19–22) showing that cytokine release can be suppressed by IL-10 in alveolar macrophages (Fig. 2F).

Expression and activation of STAT1 and STAT3 in bronchial epithelial cells

To examine the possibility that IL-10 was acting on other pathways, distinct from those regulating GM-CSF release, through the IL-10R to stimulate intracellular signalling we further examined the presence and phosphorylation status of STAT1 and STAT3 in bronchial biopsies from normal and asthmatic subjects. Variable expression of STAT1 and STAT3 was observed in bronchial biopsies as measured by Western blotting. However, no differences in total or phosphorylated STAT1 and STAT3 expression were observed in bronchial biopsy samples from normal or asthmatic subjects (Fig. 3A,B). STAT1 and STAT3

expression and phosphorylation levels in primary epithelial cells were extremely low, not different between normal and asthmatic subjects and were not modified by IL-10 treatment (data not shown).

IL-10 and IL-10R expression in alveolar macrophages

To confirm that the antibodies were capable of detecting IL-10 and IL-10R, we measured their expression in alveolar macrophages from normal and asthmatic subjects by immunocytochemistry (Fig. 4A) and by Western blot analysis (Fig. 4B,C). IL-10 expression, as determined by Western blotting, was reduced in alveolar macrophages from asthmatic patients compared with normal controls ($P < 0.05$). There was no difference in IL-10R expression between normal and asthmatic subjects as measured by Western blotting. Immunocytochemistry showed a similar site and level of IL-10R expression. Both normal and asthmatic alveolar macrophages showed significant autofluorescence using FITC-labelled secondary antibodies (data not shown).

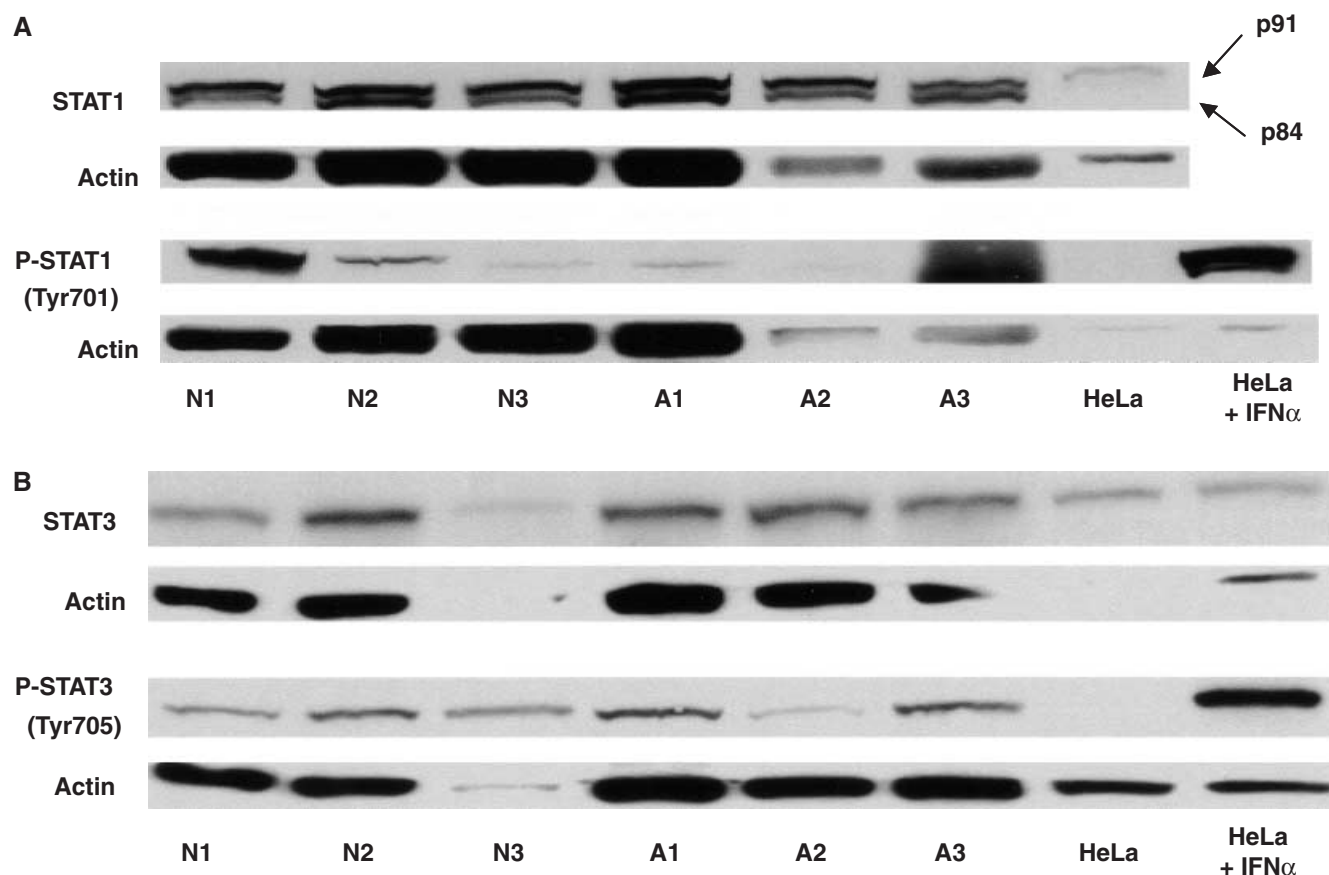


Figure 3. Western blot analysis of STAT1 and three and phospho(P)-STAT1 and three in bronchial biopsies from normal (N) and asthmatic (A) subjects. Total cellular proteins were size-fractionated by SDS-PAGE and STAT1, STAT3 and P-STAT1 and P-STAT3 were detected using specific antibodies. As positive controls for STAT and P-STAT1 (Tyr 701) and P-STAT3 (Tyr705) we used total cell extracts from HeLa cells prepared in the absence or presence of interferon alpha (IFN α). Results are representative of samples from six normal and 10 asthmatic patients.

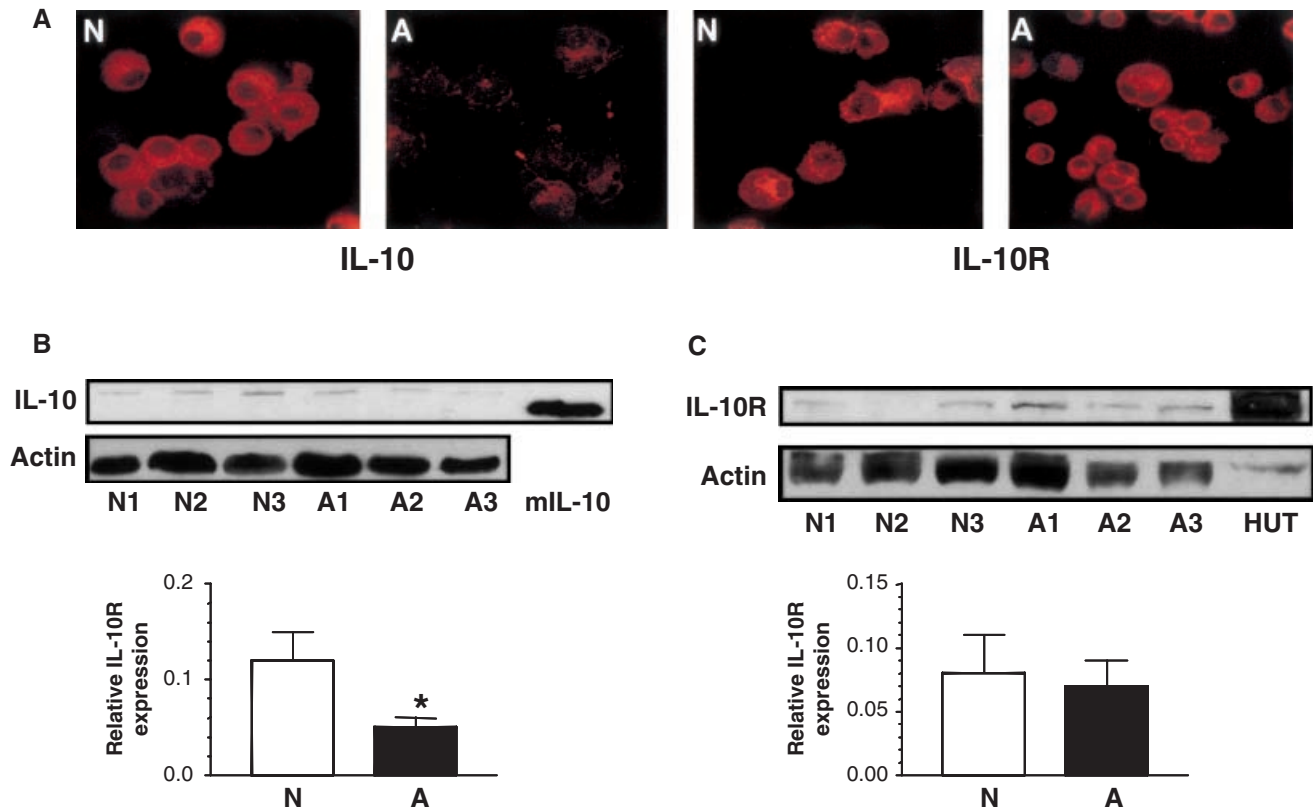


Figure 4. (A) Immunocytochemical localization of IL-10 and IL-10R in macrophages. Cytospin samples of alveolar macrophages isolated from normal (N) and asthmatic (A) subjects were stained for IL-10 (panels A and B) and IL-10R (panels C and D) using rhodamine red-X-linked secondary antibodies. Results are representative of those from six normal and ten asthmatic patients. (B) Western blot analysis of IL-10 and IL-10R in alveolar macrophages from normal (N) and asthmatic (A) subjects. Total cellular proteins were size-fractionated by SDS-PAGE and IL-10 (left panel) or IL-10R (right panel) were detected using specific antibodies. Recombinant murine IL-10 and HUT cell extract were used as positive controls for IL-10 and IL-10R, respectively. Results are represented graphically below and shown as mean $\pm$ SEM of IL-10 or IL-10R expression relative to actin expression.

Discussion

This study demonstrates that human bronchial epithelial cells express no detectable levels of the anti-inflammatory cytokine IL-10 under either basal or stimulated conditions. In addition, cells from neither patient group under any condition expresses the IL-10R *in vivo* and *in vitro*. Functionally, IL-10 was not able to suppress basal or stimulated GM-CSF release from primary bronchial epithelial cells and human bronchial epithelial cell lines. This was in contrast to the marked effect seen with dexamethasone. We have also confirmed that alveolar macrophages from asthmatic subjects have reduced expression of IL-10 compared with those isolated from normal subjects.

This data suggests that although the bronchial epithelium has a central position in allergic disorders of the respiratory tract, both as a target for allergen and as a route for therapeutic intervention, it is probable that alveolar macrophages rather than epithelial cells determine the levels of IL-10 within the airways. In addition,

our data suggests that although IL-10 may be important in maintaining a state of immune nonresponsiveness within the airway, this effect is not mediated via a direct targeting of epithelial cell function.

The lack of IL-10 effect on GM-CSF and IL-8 release could have been due to TNF α - and IL-1 β -induced GM-CSF and IL-8 not being a target for IL-10 in epithelial cells in contrast to monocytes and macrophages. We, therefore, examined whether the activation status of components of the IL-10R signal-transduction pathway. We were able to detect low but variable amounts of STAT1 and STAT3 phosphorylation in cells and biopsies from both subject groups. This data is in contrast to that seen in the study of Holtzman et al. (19) which showed an increase in nuclear STAT1 expression in asthmatic bronchial epithelial cells. This may be due to the presence of nonphosphorylated STAT1 within the nuclei of these cells or that STAT1 activation can occur following phosphorylation at sites other than tyrosine 701. Alternatively, the mild nature of the asthmatic subjects studied may have influenced the results. In

addition, STAT1 and STAT3 are also capable of being phosphorylated by other cytokines acting through the gp130 cytokine receptor family (20, 21) and by growth factors such as EGF (22). Variable expression of these mediators in our subject groups may also account for the levels of phosphorylated STAT1 and STAT3 seen in this study.

The results presented here show differences from those reported by Bonfield et al. (13). In this study 40 cycles of nested PCR was used to detect IL-10 transcripts in primary epithelial cells suggesting an extremely low level of IL-10 mRNA expression. Furthermore, immunohistochemical staining by Bonfield et al. detected low IL-10 expression in epithelial cells using FITC-labelled antibodies. In our studies these antibodies produced a high level of nonspecific fluorescence. Additionally, immunofluorescent studies are not readily quantifiable except when used in conjunction with other methods such as Western blotting and we were unable to detect IL-10 by Western blotting in this study. Finally, Bonfield et al. did not report IL-10 release from epithelial cells *per se* but showed IL-10 release into the epithelial lining fluid *in vivo* and hypothesized that this be due to epithelial release of IL-10. In contrast, our data would suggest that macrophages and not epithelial cells are the major source of IL-10 in the airways.

Other authors have also reported expression of IL-10 within airway epithelial cells. Thus, Bianco et al. (23) report that IL-10 induces a 2.7-fold increase in I-CAM1 expression in human mucoepidermoid carcinoma NCI-H292 cells. Similarly, Tazi et al. (24) have also reported markedly enhanced IL-10 expression in epithelial carcinoma cells and some normal epithelial cells. In the later study it is possible that the epithelial cells staining for IL-10 are from different sized airways as the tissue was obtained from lung resection compared with bronchoscopy samples used here. Striz et al. (25) report a positive effect of IL-10 on IL-8 expression in primary human bronchial epithelial cells, however, this effect is due primarily to the media used. Furthermore, the data from Tabary et al. (26) examines IL-10 expression in cultured primary epithelial submucosal gland cells rather than columnar surface bronchial epithelial cells.

The production of IL-10 is governed by regulatory elements in the promoter of the IL-10 gene. Polymorphisms determine the production of IL-10 and there is evidence to suggest that patients with more severe steroid-dependent asthma have an impaired ability to express IL-10 (27, 28). Reduced IL-10 expression within the airways may contribute towards the development of an allergic inflammatory response, through successful recognition of inhaled allergens, which could initiate the production of proinflammatory cytokines within the airways. Reduced levels of IL-10 have been reported in asthma compared with normal subjects (5). Treatment *in vivo* with IL-10 is associated with the down-regulation

of cytokine expression and may attenuate inflammation by influencing the synthesis of mediators (29). Studies of asthmatic patients treated with inhaled glucocorticoids have demonstrated that inflammatory cell populations are significantly reduced and the epithelium is restored to a state comparable with that seen in normal control subjects (30).

Early reports (31) suggested that IL-10 expression was elevated in T-cells from asthmatic patients. In contrast, we, and others, have reported reduced expression of IL-10 in asthmatic subjects. Recent data from Paul O'Byrne et al. (32) has suggested an explanation for this apparent conflict that may also explain, at least in part, the data presented here. Allergen inhalation leads to two distinct bronchoconstrictor phenotypes: early responders and dual responders. Persistence of allergen-induced airway inflammation is a feature of dual responders. Matsumoto (32) showed that dual responders had significantly higher IL-10 positive CD4+ T-cells before allergen challenge but following allergen challenge this was reduced. In contrast, in early responders allergen challenge increased the number of IL-10+ CD4+ T-cells. These results suggest that the contrasting profiles of IL-10 production may be associated with the different time course of allergen-induced airway inflammation between allergen-induced early and dual responders. The subjects studied here were not tested as to whether they were early or late responders.

It is likely, therefore, that the ability of IL-10 to modulate the expression of inflammatory mediators is not orchestrated directly through the bronchial epithelium. In monocytes and macrophages, IL-10 inhibits the production of proinflammatory cytokines, such as IL-1 β , TNF- α and GM-CSF (33) and we have used concentrations of IL-10 that have been previously shown to be inhibitory on monocytes and macrophages. Our results suggest that IL-10 production and IL-10R expression are cell-specific and IL-10 may have differential effects on different cell types within the respiratory tract (34).

Our data also confirms previous results from our group (4) and others showing release of IL-10 from alveolar macrophages of asthmatic subjects. For example, Magan et al. (35) reported that alveolar macrophages from asthmatic subjects, particularly those taking inhaled corticosteroids, had a higher level of basal IL-10 release than alveolar macrophages from normal subjects. Furthermore, lipopolysaccharide (LPS)-stimulated IL-10 release was also greater in alveolar macrophages from asthmatic patients although the increase was less in patients on corticosteroid therapy (35). Although alveolar macrophages are clearly a source of IL-10, other cells in the sputum of allergic asthmatics have been reported to be a potential source of IL-10. Thus, Bettiol et al. reported that following allergen challenge there is an increase in sputum IL-10 production that correlates with enhance sputum eosinophilia (36).

In conclusion, the inability of the bronchial epithelium to express the IL-10R and its expression on alveolar macrophages and in submucosal cells suggests that these later cells may be the targets for IL-10 therapy delivered by aerosol. Altered pro- or anti-inflammatory mediator synthesis may result in secondary targeting of the epithelium.

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