

The role of the epidermal growth factor receptor in sustaining neutrophil inflammation in severe asthma

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Summary

Background The extent of epithelial injury in asthma is reflected by expression of the epidermal growth factor receptor (EGFR), which is increased in proportion to disease severity and is corticosteroid refractory. Although the EGFR is involved in epithelial growth and differentiation, it is unknown whether it also contributes to the inflammatory response in asthma.

Objectives Because severe asthma is characterized by neutrophilic inflammation, we investigated the relationship between EGFR activation and production of IL-8 and macrophage inhibitory protein-1 alpha (MIP-1 α) using *in vitro* culture models and examined the association between epithelial expression of IL-8 and EGFR in bronchial biopsies from asthmatic subjects.

Methods H292 or primary bronchial epithelial cells were exposed to EGF or H₂O₂ to achieve ligand-dependent and ligand-independent EGFR activation; IL-8 mRNA was measured by real-time PCR and IL-8 and MIP-1 α protein measured by enzyme-linked immunosorbent assay (ELISA). Epithelial IL-8 and EGFR expression in bronchial biopsies from asthmatic subjects was examined by immunohistochemistry and quantified by image analysis.

Results Using H292 cells, EGF and H₂O₂ increased IL-8 gene expression and release and this was completely suppressed by the EGFR-selective tyrosine kinase inhibitor, AG1478, but only partially by dexamethasone. MIP-1 α release was not stimulated by EGF, whereas H₂O₂ caused a 1.8-fold increase and this was insensitive to AG1478. EGF also significantly stimulated IL-8 release from asthmatic or normal primary epithelial cell cultures established from bronchial brushings. In bronchial biopsies, epithelial IL-8, MIP-1 α , EGFR and submucosal neutrophils were all significantly increased in severe compared to mild disease and there was a strong correlation between EGFR and IL-8 expression ($r = 0.70$, $P < 0.001$).

Conclusions These results suggest that in severe asthma, epithelial damage has the potential to contribute to neutrophilic inflammation through enhanced production of IL-8 via EGFR-dependent mechanisms.

Keywords asthma, chemokine, epidermal growth factor, inflammation, neutrophils, tyrosine kinase

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Introduction

The extent of epithelial injury in asthma is reflected by expression of the epidermal growth factor receptor (EGFR, HER1, ErbB1) whose levels are increased in proportion to asthma severity and are refractory to corticosteroid therapy [1]. Activation of the EGFR can elicit a range of phenotypic responses in the target cell including growth promotion, growth inhibition, protection against apoptosis, induction of differentiation, reorganization of the cytoskeleton and cell migration [2]. These

pleiotropic responses are brought about by transmission of specific intracellular signals in response to ligand-induced EGFR homo- or heterodimerization and activation of the intrinsic tyrosine kinase activity of the receptor [3].

Although the EGF ligand family are best known as mitogens, proliferating cell nuclear antigen (PCNA), a marker of proliferation, is not increased in asthmatic bronchial epithelium, even where epithelial damage is apparent [4]. This appears to be due to expression of the cyclin-dependent kinase inhibitor, p21^{waf}, which prevents cell cycle progression from G1 into S phase [5]. Animal studies have also demonstrated that the EGFR plays a role in goblet cell differentiation in the airways [6, 7]. A close correlation between EGFR activation and induction of MUC5AC mRNA expression has also been demonstrated in normal and asthmatic bronchial epithelium [8].

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Many phenotypic responses downstream of the EGFR require temporal regulation of the expression of multiple genes. Recognizing that the EGFR plays a major role in the wound-healing response, we considered the possibility that, in addition to regulating the phenotypic behaviour of the responding cell, EGFR activation may also directly co-ordinate responses of inflammatory cells within the wound environment by secretion of soluble mediators involved in cell–cell communication. Although there is little information about such a proinflammatory role for the EGFR, it has been shown that treatment of a colonic adenocarcinoma cell line with EGF caused increased IL-8 synthesis [9] and that an EGFR antibody inhibited IL-8 expression by human transitional cell carcinomas grown orthotopically in nude mice [10]. More recently, EGF has been shown to cause IL-8 release from BEAS-2B cells [11], consistent with a role for the EGFR in the pathogenesis of airway inflammation. In view of the fact that severe asthma is characterized by neutrophilic inflammation and increased production of IL-8 [12, 13], the purpose of this study was to characterize the relationship between EGFR activation and production of the neutrophil chemoattractants IL-8 and macrophage inhibitory protein 1 alpha (MIP-1 α) using bronchial epithelial cells cultured *in vitro* and to establish whether there was a relationship between the high levels of EGFR expression in asthma and epithelial IL-8 or MIP-1 α expression *in vivo*.

Materials and methods

Bronchoscopy

Bronchial biopsies and bronchial epithelial cell brushings were obtained by fiberoptic bronchoscopy, in accordance with standard guidelines [14]. Surface epithelial cells were obtained by gentle bronchial brushing and bronchial biopsies were taken from the subcarinae of the lower and middle lobes using alligator forceps. Written informed consent was obtained from all volunteers and ethical approval was obtained from the Joint Ethics Committee of Southampton University and General Hospital.

Primary cell culture

Primary bronchial epithelial cells were established from bronchial brushings obtained by fiberoptic bronchoscopy from five asthmatic volunteers (two males, three females), mean age 33.4 years (range 20–58) and mean forced expiratory volume of 1 s (FEV₁) 73.9% (range 63–83%) predicted. Subjects were receiving regular treatment with salbutamol ($n = 1$) alone or together with inhaled corticosteroids ($n = 4$). Primary cultures were also established from seven non-asthmatic control subjects (two males, five females), mean age 34.7 years (range 21–53) and mean FEV₁ 106.7% (range 95–120%) predicted. Primary cultures were grown in bronchial epithelial growth medium (BEGM; Clonetics, San Diego, CA, USA) as previously described [15].

Effects of oxidants, cytokines and growth factors on cytokine production

Serum-starved NCI-H292 cells (24 h in serum-free medium; Ultraculture, Biowhittaker, Wokingham, UK) were treated with H₂O₂ or EGF at the doses stated for 24 h in the absence

or presence of dexamethasone (1 μ M) or tyrphostin AG1478 (1 μ M). Both inhibitors were dissolved in dimethyl sulphoxide (DMSO); control incubations showed that this vehicle was without effect at the doses used. Primary bronchial epithelial cells were used at passage two and were rendered quiescent in bronchial epithelial cell basal medium (BEBM; Clonetics) supplemented with 1% bovine serum albumin, and insulin, transferrin and sodium selenite supplement (Sigma, Poole, UK) prior to use. Cells were then stimulated in the same medium with EGF (10 ng/mL) or TNF- α (10 ng/mL) in the absence or presence of AG1478 (1 μ M) for 24 h.

Following exposures, culture supernatants were harvested by centrifugation and stored at -80°C prior to assay for IL-8 and MIP-1 α by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Biosource Europe, Nivelles, Belgium). The cell monolayer was fixed in formol saline and cell number determined by the method of Oliver et al. [16]. Cytokine levels were corrected for cell number.

Analysis of EGFR phosphorylation using SDS-PAGE and western blotting

Serum-starved H292 cells were treated in serum-free medium in the absence or presence of 10 ng/mL EGF or 300 μ M H₂O₂ alone or in combination with AG1478 (1 μ M) for 0–60 min. At the end of each time point, cells were lysed and tyrosine phosphorylated proteins analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting. To confirm phosphorylation of the EGFR in the presence of EGF and H₂O₂, tyrosine phosphorylated proteins were immunoprecipitated using PY-20 agarose and EGFR detected by SDS-PAGE and western blotting with an anti-EGFR antibody, using previously described protocols [1].

Analysis of IL-8 gene expression

Serum-starved H292 cells were treated with Ultraculture $\pm$ EGF (10 ng/mL) or H₂O₂ (300 μ M) in the presence or absence of AG1478 (1 μ M) and left for 8 h. RNA was extracted using TRIzol reagent (Invitrogen, Paisley, UK), DNase-treated and reverse transcribed using random hexamers. IL-8 primer and probe sequences labelled with 5'-reporter dye FAM (6-carboxy-fluorescein) and 3'-quencher dye TAMRA (6-carboxy-N',N',N',N'-tetramethyl-rhodamine) were: forward primer, 5'-AAG-GAACCATCTCACTGTGTGTAAAC-3'; reverse primer, 5'-TTAGCACTCCTTGGCAAACTG-3'; and probe, FAM-5'-CTGCCAAGAGAGCCACGGCCAG-3'-TAMARA. Primers directed against ribosomal RNA (18S rRNA) were used as the endogenous control (Applied Biosystems, Warrington, UK). Real-time detection of the multiplex PCR reaction was followed on an ABI Prism 7700 sequence detection system (Applied Biosystems, Warrington, UK). Standard curves were generated using serial dilutions of cDNA obtained from pooled samples; the relative primer efficiencies of both systems, as indicated by the gradient of the standard curves, were within $\pm 2\%$ of each other. Data were normalized relative to the 18S rRNA.

Immunohistochemistry

For immunohistochemistry, glycol methacrylate-embedded bronchial biopsies were collected from 15 non-asthmatic subjects, 14 atopic mild asthmatics and 10 severe asthmatics. The

healthy control group (nine males, six females) had a mean age of 22.0 years (range 20–46) and a mean FEV₁ 106% (range 78–147%) predicted. The asthma groups were as defined by the GINA guidelines [17]. The mild asthmatics (12 males, two females) had a mean age of 26.4 years (range 19–54) and a mean FEV₁ 93.6% predicted (range 82–107%). The severe group (one male, nine females) with a matched mean age of 28.6 years (range 13–53) had a significantly lower ($P=0.001$) mean FEV₁ of 74.9% predicted (range 53–95%). The mild asthmatics were receiving salbutamol only and the severe group was on a mean inhaled steroid dose of 3060 µg/day (range 2000–4000) and oral corticosteroid dose of 37.5 mg/day (range 2.5–100).

Two-micron sections were cut from glycol methacrylate (GMA)-embedded biopsies and stained immunohistochemically [18]. Mouse monoclonal antibodies (mAbs) against IL-8 (Alexis, Nottingham, UK), MIP-1α (R&D Systems, Abingdon, UK) and neutrophil elastase (Dako, Cambridge, UK) were used together with a sheep polyclonal antibody against the EGFR (University of Southampton) [1]. For controls, the primary antibodies were replaced with isotype-matched immunoglobulin or buffer alone.

All slides were randomly coded and assessed by an observer blinded to the code. Positive epithelial staining was measured based on red/blue/green colour composition using computerized image analysis (Colourvision, Improvision, UK), as previously described [1]. The number of neutrophils and eosinophils were counted within the submucosa, the area measured by image analysis and number of cells/mm² submucosa calculated.

Statistical analysis

Immunohistochemistry data was analysed by the Mann–Whitney *U*-test. H292 epithelial cell culture data was analysed using Student's *t*-test, whereas primary epithelial cell data, which were not normally distributed, were analysed by Wilcoxon rank sum test. A probability value, $P < 0.05$ was considered significant.

Results

Stimulation of IL-8 expression and release from bronchial epithelial cells

To determine whether the EGFR could contribute to ongoing inflammation in asthma, we measured the ability of EGF and H₂O₂ to stimulate the expression and release of IL-8 from monolayers of H292 bronchial epithelial cells. Under the conditions tested, both EGF and H₂O₂ stimulated a rapid increase in tyrosine phosphorylation of the EGFR (Fig. 1) via ligand-dependent and ligand-independent mechanisms, respectively; this increase could be blocked by the EGFR-selective [1] tyrosine kinase inhibitor, tyrphostin AG1478. Following treatment with either EGF or H₂O₂, analysis of IL-8 gene transcription using real-time PCR showed that either treatment caused a significant increase in IL-8 mRNA and this response was also completely blocked by AG1478 (Fig. 2).

Consistent with the increased gene transcription, EGF was found to cause a dose-dependent increase in IL-8 release from the H292 cells and this was also blocked by AG1478 (Fig. 3a). As severe asthma is corticosteroid refractory, we also examined the effect of dexamethasone on EGF-induced IL-8 release. In this case, inhibition was observed but the extent of inhibition

decreased as the dose of EGF increased; at the three highest concentrations of EGF, IL-8 release in the presence of dexamethasone was significantly increased above baseline (Fig. 3a). Increased IL-8 production was also observed when cells were treated with H₂O₂, but in this case all doses were maximally stimulating (Fig. 3b). As for EGF, AG1478 completely blocked IL-8 release in response to H₂O₂ whereas dexamethasone caused 39% inhibition irrespective of H₂O₂ dose.

To investigate whether EGF might also affect release of other neutrophilic chemokines, we also examined release of MIP-1α. However, in this case no increase was observed upon treatment with either EGF or AG1478 alone, although some suppression was observed when the cells were cultured in the presence of EGF together with AG1478 (Fig. 4a); the reasons for this are unclear. In contrast, treatment with either 500 or 600 µM H₂O₂ caused a significant ($P < 0.004$) increase in MIP-1α release and this was partially suppressed by dexamethasone ($P < 0.04$, 500 µM H₂O₂; $P < 0.01$, 600 µM H₂O₂), but not by AG1478 ($P > 0.05$; Fig. 4b). The failure of AG1478 to affect MIP-1α release induced by H₂O₂ indicates that it is acting to

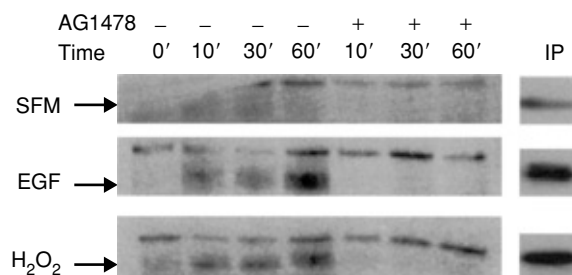


Fig. 1. Induction of EGFR phosphorylation by EGF and H₂O₂. H292 bronchial epithelial cells were treated in serum-free medium in the absence or presence of EGF (10 ng/mL) or H₂O₂ (300 µM) without or with AG1478 (1 µM) for 0–60 min. Phosphotyrosine-containing proteins in cell lysates were detected by western blotting (left panel). The 170 kDa phosphoprotein indicated by the arrow was confirmed as the EGFR by immunoprecipitation using PY-20 agarose followed by western blotting for the presence of the EGFR (right panel). The blots shown are representative of 3 individual experiments.

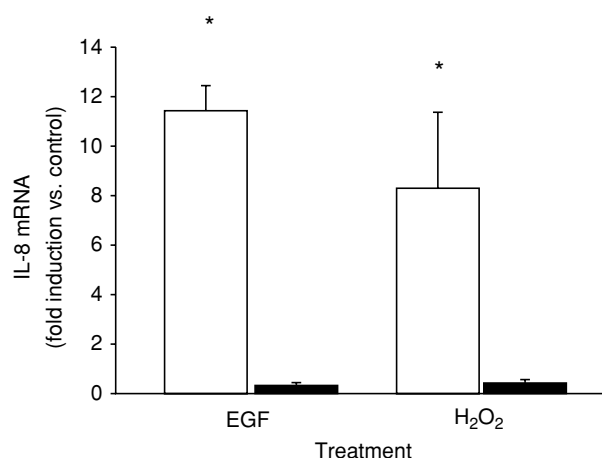


Fig. 2. Induction of IL-8 gene expression by EGF and H₂O₂. Quiescent H292 bronchial epithelial cells were treated in the presence of EGF (10 ng/mL) or H₂O₂ (300 µM) without (□) or with 1 µM AG1478 (■) for 8 h and then IL-8 mRNA analysed by real-time PCR. Data are mean ± SEM ($n=3$ for EGF-treated cells; $n=5$ for H₂O₂-treated cells) and are normalized relative to IL-8 gene expression in untreated cells. * $P < 0.05$ versus untreated control cells.

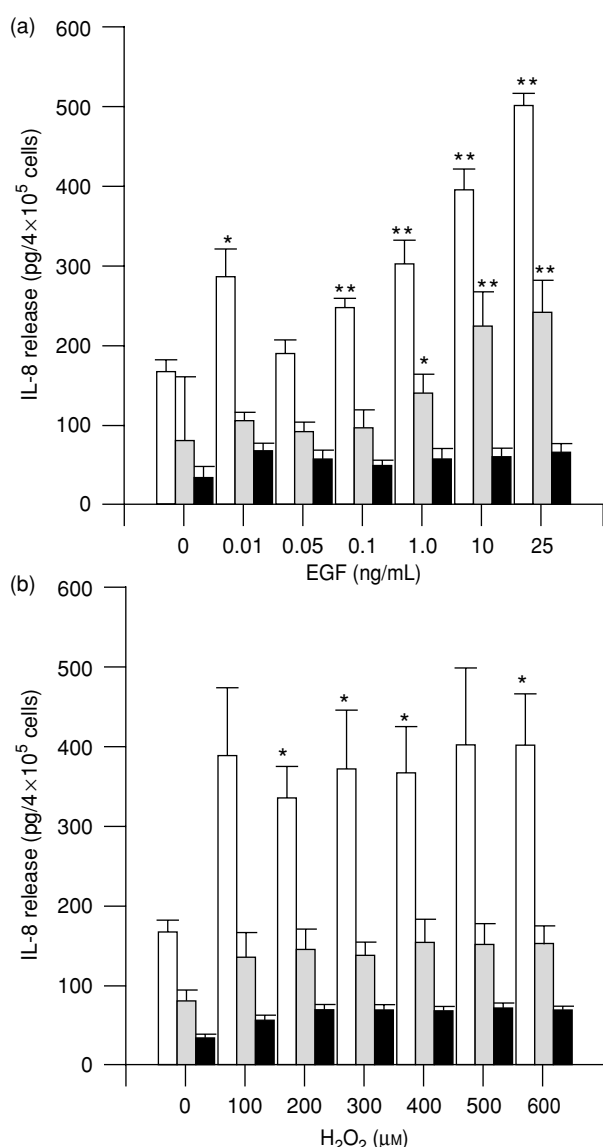


Fig. 3. IL-8 release from H292 bronchial epithelial cells. Quiescent cells were treated with increasing doses of EGF (a) or H₂O₂ (b) in the absence (open bar) or presence of 1 μM dexamethasone (grey bar) or 1 μM AG1478 (black bar) for 24 h. The graphs show IL-8 release into the culture supernatant after correcting for cell number. Data are mean ± SEM ($n = 6$); * $P < 0.05$, ** $P < 0.001$ versus appropriate baseline controls (i.e. SFM alone for comparison of EGF- or H₂O₂-treated cells; SFM + dex for comparisons in the presence of dex; or SFM + AG1478 for comparisons in the presence of AG1478).

selectively to inhibit aspects of H292 cell function mediated via the EGFR.

As the responses of cell lines are sometimes atypical, we further investigated the effect of EGF using primary asthmatic bronchial epithelial cell cultures established from bronchial brushings. As observed with the H292 cell line, EGF caused a 3 ± 1.07 -fold increase ($P < 0.05$) in IL-8 release from these asthmatic cell cultures and this was completely blocked by AG1478 (Fig. 5a); although H₂O₂ caused a 2 ± 1.0 -fold increase in IL-8 release, this failed to reach statistical significance. In the same experiments, TNF- α (10 ng/mL) caused a 3.9 ± 1.0 -fold increase ($P < 0.05$) in IL-8 release. It was also noted that AG1478 suppressed basal IL-8 release, suggesting autocrine

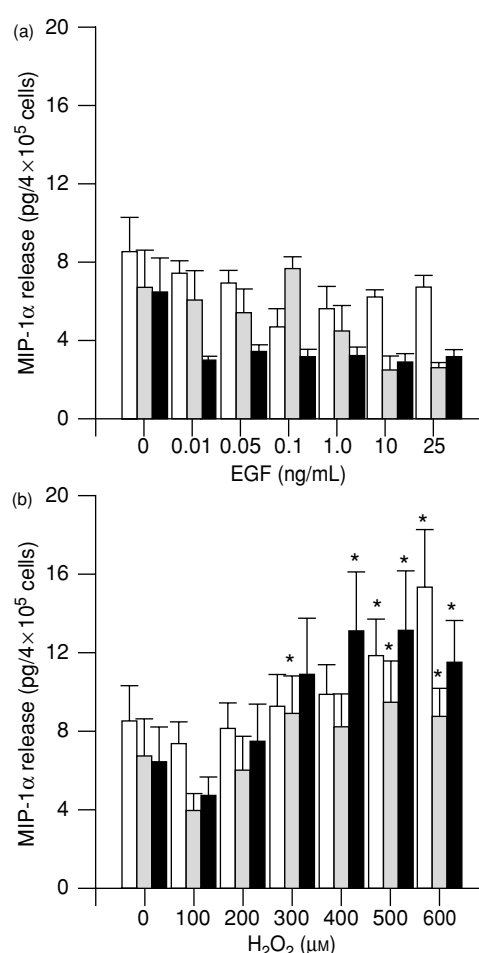


Fig. 4. MIP-1 α release from H292 bronchial epithelial cells. Quiescent cells were treated with increasing doses of EGF (a) or H₂O₂ (b) in the absence (open bar) or presence of 1 μM dexamethasone (grey bar) or 1 μM AG1478 (black bar) for 24 h. The graphs show MIP-1 α release into the culture supernatant after correcting for cell number. Data are mean ± SEM ($n = 5$ or 6); * $P < 0.05$ versus appropriate controls (i.e. SFM alone for comparison of EGF- or H₂O₂-treated cells; SFM + dex for comparisons in the presence of dex; or SFM + AG1478 for comparisons in the presence of AG1478).

stimulation of the cells. Measurement of EGF or oxidant-stimulated IL-8 release from primary cultures derived from seven normal subjects revealed responses comparable to those of the asthmatic cultures (Fig. 5b) with EGF causing a 3.3 ± 0.6 -fold increase ($P < 0.05$) but H₂O₂ failing to significantly stimulate above baseline. For comparison, there was a 7.5 ± 1.5 -fold increase ($P < 0.05$) in response to TNF- α . The slightly lower response of the asthmatic cells to EGF or TNF- α was not statistically significant ($P > 0.1$) and did not show any correlation with steroid use.

Immunohistochemical analysis of chemokine expression in bronchial biopsies from mild and severe asthmatic subjects

To assess whether there was any relationship between EGFR expression and epithelial IL-8 in asthma, we undertook immunohistochemical analysis of bronchial biopsies from asthmatic subjects with varying degrees of disease severity. As we have demonstrated previously [1], expression of the EGFR was

significantly higher in subjects with severe asthma compared to those with mild asthma or normal controls (% epithelial staining) median 18.6 (range 0.9–38.6) in severe asthma compared to median 6.4 (range 0.5–18.3; $P=0.04$) in mild asthma; or median 6.0 (range 0.0–14.3; $P=0.02$) in the non-asthmatic controls. Significantly higher levels of IL-8 and MIP-1 α were also observed in the bronchial epithelium of the severe asthmatics compared to the mild asthmatics or normal controls (Fig. 6a). Expression of IL-8 (% epithelial staining) in severe asthma was found to be median 8.5 (range 2.5–14.3) versus median 1.4 (range 0–5.5) in mild asthma ($P=0.001$) or median 0.6 (range 0.0–1.9) in normal controls ($P<0.001$), whereas that for MIP-1 α was median 17.3 (range 2.8–31.0) versus median 7.6 (range 1.1–15.7; $P=0.04$) in mild asthma and median 0.4 (range 0.0–6.2; $P=0.001$) in the non-asthmatic bronchial epithelium. There was no significant difference in either epithelial EGFR or IL-8 expression between the non-asthmatic controls and the mild asthmatic group; this may reflect the relatively mild degree of disease within this asthmatic group [mean FEV₁ 93.6% predicted (range 82–107%)]. Consistent with the higher levels of neutrophil chemoattractants detected at the severe end of the disease spectrum, significantly more neutrophils were observed in the submucosa of the severe asthmatics (24 neutrophils/mm², range 11–174) compared to the mild asthmatics (12 neutrophils/

mm², range 1–24; $P=0.007$). The expression of EGFR in the asthmatic biopsies strongly correlated with IL-8 expression ($r=0.70$, $P<0.001$; Fig. 6b), and there was a trend for a significant correlation between both EGFR and IL-8 with the number of neutrophils in the submucosa ($r=0.43$, $P=0.06$; and $r=0.41$, $P=0.08$, respectively). In those patients using corticosteroids, there was no significant correlation between any of the measured parameters and steroid dose.

Discussion

Chronic severe asthmatic patients have a greatly impaired quality of life and account for up to 50% of the health costs of this disease [19]. Although treatment with corticosteroids significantly reduces airway inflammation in mild asthma [20], this is not the case at the severe end of the spectrum where neutrophilic inflammation and high IL-8 levels persist despite high doses of both oral and inhaled corticosteroids [12, 13]. While corticosteroids may contribute to inflammation by reducing neutrophil apoptosis [21], this does not fully explain the underlying reason(s) for neutrophil influx into the airways in severe disease.

As we have previously reported [1], expression of EGFR is increased in the bronchial epithelium of severe asthmatic

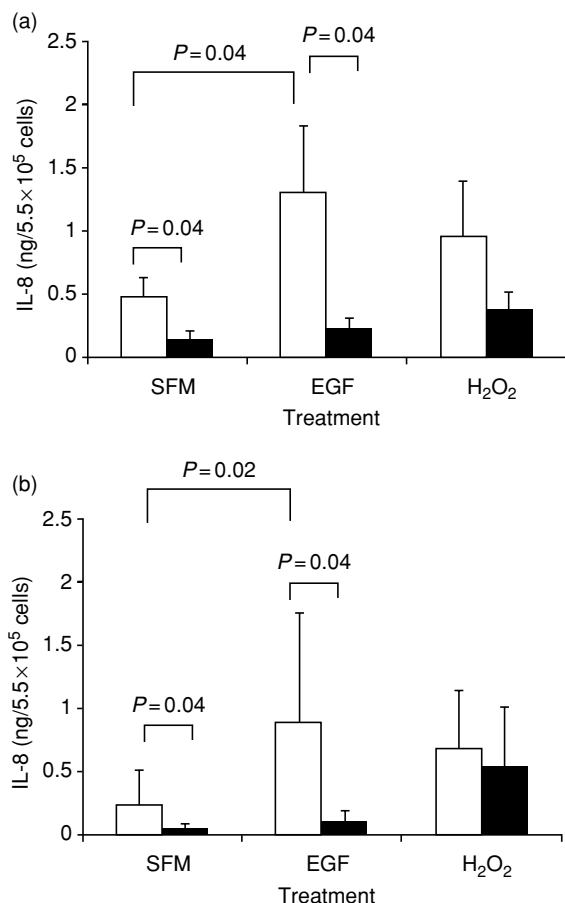


Fig. 5. IL-8 release from primary asthmatic (a) or normal (b) bronchial epithelial cells. Quiescent cells were treated with EGF (10 ng/mL) or H₂O₂ (300 μM) in the absence (□) or presence of 1 μM AG1478 (■) for 24 h. The graphs show IL-8 release into the culture supernatant after correcting for cell number. Data are mean $\pm$ SEM [$n=5$ (asthma) or 7 (normal)]; significance values were determined using Wilcoxon's rank sum test.

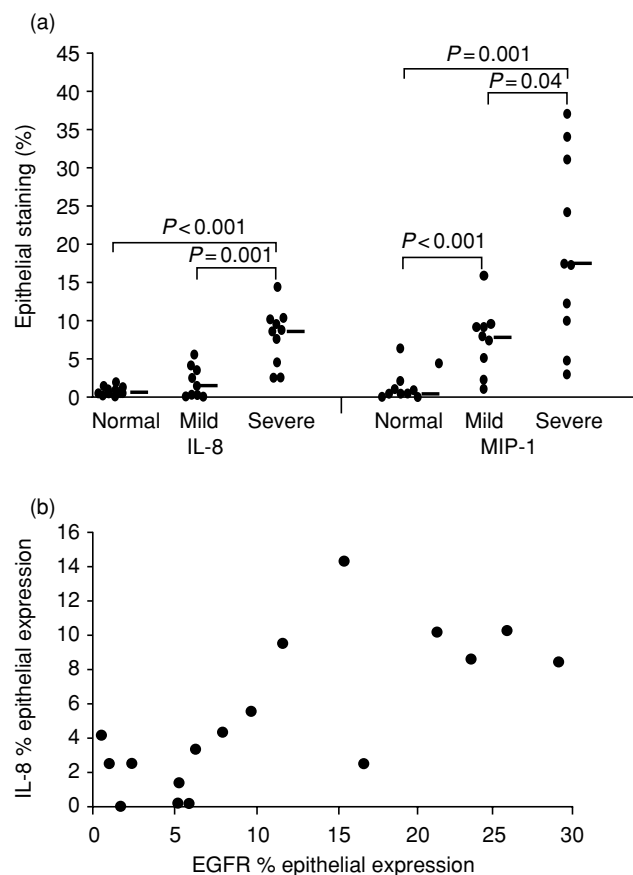


Fig. 6. (a) Comparative analysis of the epithelial expression of IL-8 and MIP-1 α in bronchial epithelium of non asthmatic control subjects or those with mild or severe asthma. Data are expressed as percentage epithelial staining which was determined by computer-aided image analysis. (b) Correlation between IL-8 and EGFR expression in asthmatic bronchial epithelium.

subjects. This increase is steroid refractory and it is not a consequence of corticosteroid treatment [1]. As EGFR levels increase in the skin [22], gastrointestinal tract [23] and lungs [24, 25] in response to tissue injury, the high levels observed throughout the epithelium of severe asthmatic subjects would suggest that there is widespread, ongoing stress and damage in the airways. In normal epithelium, EGFRs are expressed on the basolateral surface [1] and are spatially separate from their activating ligands contained in the apical lining fluid [26]. In contrast, in severe asthma EGFR immunostaining is also evident on the apical surface suggesting the potential for cognate ligand receptor interactions [1]. Thus, our finding that EGF stimulates IL-8 mRNA synthesis and release from bronchial epithelial cells provides a link between EGFR up-regulation in response to tissue injury and induction of a neutrophilic inflammatory response mediated by the resident cells. Our finding that EGFR expression in the bronchial epithelium closely correlated with the expression of IL-8, and that both EGFR and IL-8 showed a trend for correlation with neutrophil numbers further supports this cause-effect relationship. Such a response may explain the increase in epithelial IL-8 expression after exposure of volunteers to agents such as ozone, DEPs or NO₂ which also have the capacity to cause epithelial damage [27–29]. The demonstration of increased epithelial IL-8 expression in the present study, is consistent with previous studies which have shown increased levels of IL-8 in lavage fluid of severe asthmatics [12, 13]. Because EGFRs are increased in the bronchial epithelium of smokers [30], a similar mechanism may also contribute to IL-8 expression and neutrophilia in smokers and COPD patients. As the barrier to the external environment, the bronchial epithelium may have a critical role as a sensor of environmental stress, with exaggerated responses having pathological consequences.

Studies using the human epithelial cell lines, A549 and BEAS-2B, have shown that H₂O₂ induces expression of IL-8 [31, 32]; however, our studies using H292 and primary bronchial epithelial cells extend these observations by showing that IL-8 gene transcription and release stimulated by either H₂O₂ or EGF is mediated by EGFR-dependent pathways. The present study confirms the previous study with BEAS-2B cells [11] and extends it by demonstrating that EGF can overcome the inhibitory effects of corticosteroids. Corticosteroids suppress IL-8 production in mild asthma, but in the severe asthmatic epithelium increased expression of both IL-8 and MIP-1 α was evident, even though the subjects were on a mean inhaled steroid dose of 3.1 mg/day and a mean oral dose of 37.5 mg/day. As EGFR levels are elevated in severe asthma independent of therapy, these receptors offer increased signalling capacity that might contribute to the insensitivity of IL-8 release to corticosteroid therapy. The selectivity of the EGF-induced response was further demonstrated with the observation that MIP-1 α release was only provoked by H₂O₂ and this did not involve the EGFR. These differences may be due to differential activation of transcription factors. EGF has been demonstrated to activate NF- κ B [33], consistent with the known involvement of NF- κ B for IL-8 transcription and the partial suppression of IL-8 release by dexamethasone. However, the inability of dexamethasone to fully block EGF-stimulated IL-8 release suggests the activation of other transcription factors by EGF. Likely candidates are the *lef/tcf* transcription factors that are activated by EGF or Wnt signalling and are known to activate IL-8 gene transcription [34]. In contrast, MIP-1 α transcription does not

appear to involve nuclear factor kappa B (NF- κ B), but may involve other transcription factors such as activator protein (AP)-1 which is known to be activated by oxidants [31, 32].

Based on the results presented, we postulate that several self-perpetuating cycles are established in the airways in severe asthma with neutrophils occupying a central role (Fig. 7). In the first, tissue injury leads to increased surface EGFR expression with ligand-dependent EGFR activation causing IL-8 release and neutrophil influx. As neutrophil numbers and EGFR expression in severe asthma are both refractory to corticosteroid treatment [1, 21], these observations are consistent with a link between persistent neutrophil-mediated inflammation and epithelial damage. As a consequence of neutrophil influx, endogenous oxidants act directly on the epithelium to stimulate release of MIP-1 α , which attracts more neutrophils into the airways, leading to further tissue activation and injury. Superimposed on these two cycles is a third cycle in which the responses to tissue injury are amplified by TNF- α released by neutrophils and other inflammatory cells, including macrophages and mast cells. The importance of oxidants is further emphasized by the antioxidant status of the epithelium and its ability to resist oxidative stress. It has been shown that antioxidants levels are lower in the epithelium [35, 36] and bronchoalveolar lavage (BAL) [37] of asthmatic when compared to normal subjects. These decreases will be accompanied by increases in oxidant levels from the polluting stimuli and infiltrating neutrophils. Another interaction between neutrophils and EGFR activation has recently been identified where neutrophil-derived oxidants cause mucus hypersecretion [38], another feature of severe asthma.

While these self-perpetuating cycles of inflammation and tissue injury explain the chronic nature of airway inflammation in severe asthma, they do not explain how the appropriate microenvironment is created in the airway that establishes this inflammation. Nor do they account for the increased sensitivity of asthmatic subjects to innocuous components of the inhaled environment. One possible explanation can be provided by our recent studies where we have demonstrated that asthmatic epithelial cells are more susceptible to oxidant-induced apoptosis than are those from healthy controls [15]. Susceptibility to environmental oxidants may be triggering a mechanism that facilitates the initial inflammatory responses that enable the

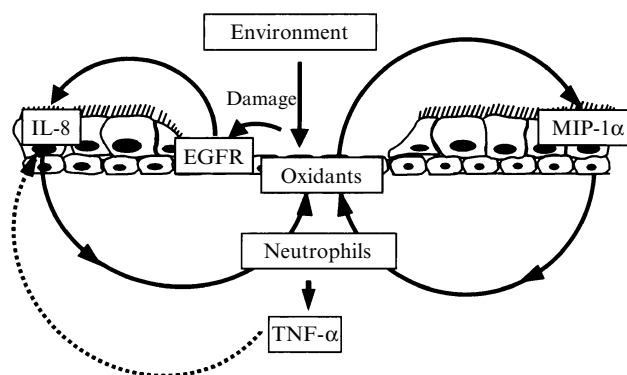


Fig. 7. Schematic representation showing how self-perpetuating cycles of chronic inflammation, tissue injury and sustained neutrophilic inflammation are established in the bronchial mucosa of severe asthmatics.

chronic cycles to become established. Together, these observations would readily account for the involvement of environmental pollutants in asthma exacerbations and the rising trends in asthma over the last 30 years.

In conclusion, our data highlight the importance of the bronchial epithelium in the regulation and modulation of inflammation, particularly in chronic severe asthma that is refractory to corticosteroid therapy. As the barrier to the external environment, we postulate that the epithelium plays a key role in translating gene by environment interactions in asthma and other inflammatory airway diseases and that it may represent a new target for therapeutic intervention.

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