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Expression and generation of interleukin-8, IL-6 and granulocyte–macrophage colony-stimulating factor by bronchial epithelial cells and enhancement by IL-1β and tumour necrosis factor-α

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SUMMARY

We have tested the hypothesis that the bronchial epithelium has the capacity to generate and release cytokines that could contribute to inflammatory events associated with inflammatory lung diseases. Messenger RNA (mRNA) for interleukin-6 (IL-6), IL-8 and granulocyte–macrophage colony-stimulating factor (GM-CSF) was identified in human bronchial epithelial cell primary cultures, characterized on the basis of staining for cytokeratin, using both *in situ* hybridization and Northern blotting. Using *in situ* hybridization we have shown that the majority of the cells expressed mRNA for IL-6 and IL-8, whereas fewer than 20% of cells expressed message for GM-CSF. The numbers of cells expressing message were increased by culture with tumour necrosis factor-α (TNF-α) (20 ng/ml, 24 hr). These observations were substantiated by Northern blotting, which showed that both TNF-α and IL-1β were able to induce a dose-dependent increase in IL-8-specific mRNA. Immunoreactive IL-6 and GM-CSF were detected and quantified in the culture supernatants by ELISA, and IL-8 by radioimmunoassay. The levels of immunoreactivity were increased by incubation of epithelial cells with either IL-1β or TNF-α for 24 hr. A transformed tracheal epithelial cell line (9HTEo$^-$) expressed mRNA for IL-6, IL-8 and GM-CSF but, whereas levels of immunoreactive IL-6 in culture supernatants were comparable with those in primary cell cultures, levels of IL-8 were low and GM-CSF trivial. These observations indicate that the bronchial epithelium has the potential to be a major source of IL-8 and a number of other cytokines, and that production can be amplified substantially by IL-1β and TNF-α. The bronchial epithelium is ideally situated to modulate inflammatory and immunological events in and around the airways, and these observations suggest that it could contribute to promote and sustain inflammatory and immunological processes in inflammatory lung diseases such as asthma.

INTRODUCTION

The bronchial epithelium represents the primary interface between the lung and inspired air. Its roles as a physical barrier for the exclusion and removal of particulate matter and the protection of underlying tissue, together with the control of airway humidity and lining fluid composition, are well recognized.[1] The epithelial cells are vulnerable to the actions of the various mediators and cytokines generated by cells involved in the inflammatory component of the asthmatic response, in particular eosinophils, mast cells and T lymphocytes.[2,3] Epithelial damage and loss of integrity is a recognized feature of asthma,[4] and is thought to be associated with the increased bronchial hyper-responsiveness characteristic of the disease.[5] The hyper-responsiveness may be attributable in part to factors such as exposure of afferent nerve endings,[6] facilitated access to mediator-producing cells by allergens and loss of capacity to produce epithelial cell-derived relaxing factor.[7] In addition epithelial cell-derived arachidonic acid metabolites[8] and endothelin[9] may also contribute. Furthermore the epithelium can be viewed as being in a key position to induce and sustain inflammatory and immunological events in lung diseases such as asthma through the production of effector substances such as cytokines. In this regard granulocyte–macrophage colony-stimulating factor (GM-CSF), interleukin-6 (IL-6) and IL-8 are of particular interest.

GM-CSF significantly augments DNA synthesis in human alveolar macrophages, and human lung-conditioned medium has a similar effect that is inhibitable by anti-GM-CSF.[10] GM-CSF is recognized as an important factor for stimulating haematopoiesis and prolonging eosinophil survival, and it also

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activates neutrophils, eosinophils, monocytes and basophils enhancing their responsiveness to other physiological stimuli for secretion, lipid mediator generation, cytotoxicity, etc.[11] Numerous activities have been ascribed to IL-6 including its ability to contribute to the stimulation of humoral and cellular defence mechanisms; to facilitate the terminal differentiation of B cells into immunoglobulin-secreting cells; to act as a helper factor in primary antigen-receptor-dependent T-cell activation and subsequent proliferation; and to enhance proliferation of multipotential haematopoietic progenitors by stimulating proliferation and shortening the G_0 phase.[12,13] IL-8 is recognized principally as a neutrophil chemoattractant and activator[14] and has been shown to induce neutrophil adherence and transendothelial migration.[15] IL-8 is also chemotactic for T lymphocytes,[16] and although eosinophils from normal donors appear not to respond to this interleukin, preincubation of the cells with either GM-CSF or IL-3 induces a significant chemotactic response.[17]

For these reasons we have investigated the ability of epithelial cells recovered from the bronchi from surgically resected human lung to express mRNA and immunoreactivity for IL-6, IL-8 and GM-CSF, using *in situ* hybridization, Northern blotting and immunoassays. The possibility of modulating cytokine generation has also been examined by studying the influence of IL-1β and TNF-α on the response of the cells.

MATERIALS AND METHODS

Isolation and culture of dispersed bronchial epithelial cells

Samples of human lung were obtained from patients undergoing surgery for peripheral lung cancer. Tissue was collected into Ca^{2+}/Mg^{2+}-free HBSS (Gibco-BRL Ltd, Uxbridge, U.K.) immediately after excision, and processing for tissue culture was initiated within 1 hr. Sections of grossly normal bronchus were dissected out from surrounding tissue, opened longitudinally, rinsed with Ca^{2+}/Mg^{2+}-free HBSS and pinned-out on sterile gauze on an expanded polystyrene sheet. Longitudinal parallel incisions were made in the epithelial surface approx. 2 mm apart and strips of epithelium were pulled off with forceps. Dispersed cell preparations were prepared after the method of Widdicombe *et al.*[18] by digestion with type II collagenase (Sigma, Poole, U.K.), in the presence of 0·04%/200 U/ml type IV deoxyribonuclease (Sigma) and 5 mM dithiothreitol (Sigma), and cells were collected after 90 and 120 min and pooled. Cells were washed once with F12/DMEM and then resuspended in F12/DMEM supplemented with glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 μg/ml), hydrocortisone (1 μM) and LPSR-1 (1%) (Sigma) at a concentration of 2×10^5/ml. Cells were transferred to four-chamber Lab-Tek plastic chamber slides (Gibco-BRL Ltd) and cultures were maintained at 37°, 5% CO_2 for up to 10 days with medium changes at 3-day intervals using supplemented F12/DMEM omitting hydrocortisone, but including foetal calf serum (FCS) (1%). Hydrocortisone was included in the initial culture to inhibit fibroblast growth and stimulate fibronectin production.[19] Fibronectin could facilitate epithelial cell adherence to the culture plastic and explain our empirical observation that cultures were established more consistently in the presence of hydrocortisone. Cultures were terminated before reaching confluence, and processed for *in situ* hybridization. All culture media and supplements were obtained from Gibco-BRL Ltd unless otherwise indicated.

Epithelial cell outgrowths from bronchial tissue

Tissue pieces stripped from the bronchial wall were cut into 2-mm^2 pieces which were then orientated epithelial surface uppermost on 35-mm plastic tissue culture dishes that had been previously rinsed with serum-free supplemented F12/DMEM, including hydrocortisone (1 μM). These tissue explants were allowed to stick down onto the plastic at 37° 5% CO_2 for 16 hr, after which medium was added gently so as not to dislodge the tissue. Medium was changed at 3-day intervals with hydrocortisone-free supplemented F12/DMEM until the outgrowths of cells reached the edge of the dish after 18–28 days. The explant tissue was removed, and the cells were cultured for a further 24 hr in fresh medium after which samples of supernatant were taken for immunoassay and the cells were processed to recover RNA. Numerous ciliated cells were observed in all the cultures and the epithelial nature of the cells was confirmed by positive cytokeratin staining.

Characterization of epithelial cells

Cells were fixed in methanol for 10 min, rinsed with Tris-buffered saline (TBS) and stained using the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique. Briefly, cells were over-layered with an anti-cytokeratin monoclonal antibody (Clone LP34; M717, Dako, High Wycombe, U.K.) at a dilution of 1 in 50 in TBS for 30 min at room temperature. Slides were then rinsed briefly in TBS and rabbit anti-mouse IgG (Dako) (1 in 40 dilution) applied. After 30 min the slides were again rinsed in TBS and APAAP (1 in 40 dilution) was applied. The reaction was visualized using Fast Red substrate (Sigma). Slides were counterstained for 2 min with Harris's haematoxylin (BDH Ltd, Poole, U.K.) and mounted in the water-soluble mountant Glycergel (Dako). Monoclonal antibodies against fibroblasts (Clone 505; M877, Dako) and macrophages (Clone EMB11; M718, Dako) were used with the same protocol to exclude the possibilities of contamination by these cell types. In all cultures investigated conclusive cytokeratin staining was seen in at least 98% of the cell population.

Transformed human tracheal epithelial cell line—9HTEo$^-$

A human tracheal epithelial cell line (9HTEo$^-$) transformed with an origin-defective simian virus 40 {841} was a gift from Dr D. Gruenert (Cardiovascular Research Institute, University of California, San Francisco, CA). Cells in passages 5–8 were cultured in MEM containing Earle's salts, with 5% FCS, L-glutamine, penicillin and streptomycin.

Cytokine stimulation of cells in culture

Recombinant human IL-1β or TNF-α (British Biotechnology Ltd, Oxford, U.K.) in the dose range 0·2 to 20·0 ng/ml were added to confluent cell cultures of comparable surface area and approximately 5×10^6 cells/plate in fresh medium after removal of the explant tissue. After 24 hr in culture the supernatants and cells were processed for immunoassay and RNA extraction.

Probe construction

A GM-CSF plasmid was a gift from the Glaxo Institute for Molecular Biology (Geneva, Switzerland). IL-6 cDNA was a gift from Professor T. Hirano (Osaka University Medical School, Osaka, Japan). IL-8 cDNA was a gift from Dr K. Matsushima (NIH, Bethesda, MD). The cDNA were subcloned in various PGEM vectors, linearized with appropriate

restriction enzymes, and then transcribed *in vitro* in the presence of [^{35}S]UTP (Amersham International, Amersham, U.K.) and SP6 or T7 polymerase (Promega, Southampton, U.K.) to produce radiolabelled antisense (cRNA) and sense (identical sequence to the mRNA) probes respectively, as previously described.[20]

Tissue preparation and in situ *hybridization*

Epithelial cells cultured on plastic slides were fixed in a freshly prepared solution of 4% paraformaldehyde in PBS (pH 7·4) for 30 min at 4° and then washed with PBS containing 15% sucrose before air drying at 37° for 12 hr. Slides were stored at −80° and processed within 1 month. Non-confluent cell preparations were used in order to facilitate subsequent evaluation of the autoradiographs. The *in situ* hybridization protocol was as previously published in detail.[21,22] Cytospin preparations of phytohaemagglutinin (PHA)-stimulated mononuclear cells were used as a positive control for GM-CSF, and 9HTEo$^-$ cultures were used as controls for IL-6 and IL-8.

Northern blot analysis

Total RNA was extracted from epithelial cells using an established technique.[23] Cells were lysed in 4 M guanidine isothiocyanate in the presence of Sarkosyl and 2-mercaptoethanol. The lysate was acidified with sodium acetate pH 4·0, and extracted with phenol and chloroform. The RNA was precipitated with ethanol, washed, redissolved in guanidine isothiocyanate solution and reprecipitated. The yield and purity of the RNA was assessed by absorption spectrometry, and the material stored at −80°. Samples of the RNA (20 μg) were denatured with formaldehyde/formamide and subjected to electrophoresis on 1% agarose formaldehyde gel prior to blotting onto a nylon membrane (Hybond-N; Amersham International) and cross-linking by exposure to ultraviolet (UV) radiation. Membranes were subjected to prehybridization treatment for 6 hr at 42° in 50% formamide buffer containing 5 × Denhardt's reagent and 100 μg/ml sonicated salmon sperm DNA. Specific mRNA sequences in the membrane-bound RNA were detected by hybridization with specific cDNA probes excised from pGEM vectors and labelled by random hexanucleotide priming using [^{32}P]deoxycytidine triphosphate and a Multiprime DNA labelling System (Amersham International) adopting the method of Feinberg and Vogelstein.[24,25] Hybridization was performed using fresh prehybridization buffer with the addition of 10% w/v dextran sulphate and labelled probe for 20 hr at 42°. Membranes were rinsed with 2 × SSC and subjected to four 15-min washes at 50°, twice with 0·2 × SSC containing 0·1% SDS and twice with 0·1 × SSC containing 0·1% SDS, prior to autoradiography. Autoradiographs were assessed by scanning densitometry and the intensity of bands was measured on a scale of 256 grey-levels. Expression of β-actin was used to correct for minor variations in the RNA loading between lanes on the blot. Results were expressed relative to β-actin and as a percentage of the maximum response by determining the ratio of cytokine mRNA : β-actin mRNA and defining the maximum response as 100% in order to assess possible enhancement. The method was based in part on that described by Akashi *et al.*[26] RNA from a human fibroblast cell line served as a control in these experiments. All chemicals for nucleic acid processing were obtained from Gibco-BRL Ltd and Sigma.

Cytokine immunoassays

IL-8 was measured by radioimmunoassay. IL-8 was iodinated using Iodogen reagent (Life Sciences Laboratories Ltd, Luton, U.K.) as described previously.[27] Briefly, recombinant human IL-8 (5 μg) (a gift from Dr K. Matsushima) was incubated for 10 min at room temperature with 500 μCi Na^{125}I (Amersham International) in 20 μl 0·2 M sodium phosphate pH 7·4 in a tube coated with 10 μg Iodogen reagent. Following the addition of 500 μl PBS, containing 2 mg/ml gelatin, 0·25 mM NaI and 28 mM L-tyrosine, the [^{125}I]IL-8 was purified by gel filtration on Sephadex G-25M equilibrated in PBS. The specific activity of the [^{125}I]IL-8 was 65·5 μCi/μg. The radioimmunoassay (RIA) buffer used was 10 mM PBS containing 0·1% sodium azide, polyethylene glycol 6000 (22% w/v), protamine sulphate (1% w/v), EDTA (10 mM) and 0·2% gelatin. Samples were mixed with an equal volume of buffer, incubated for 1 hr at 4° and centrifuged (5400 ***g***, 10 min, 4°). Duplicate microcentrifuge tubes containing 100-μl sample, standard or assay buffer were incubated for 30–32 hr at room temperature together with 100 μl goat anti-human IL-8 antibody (1:3600), raised against 72 amino acid synthetic IL-8 (a generous gift from Dr H. J. Showell, Pfizer Central Research, Groton, CT) and 50 μl [^{125}I]IL-8 (0·5 ng). This was followed by the addition of 50 μl donkey anti-goat IgG (Nordic Immunological Laboratories, Maidenhead, U.K.) and a further incubation of 14–16 hr at room temperature. After addition of 1 ml PBS containing 0·1% sodium azide and immediate centrifugation (5400 ***g***, 10 min, 24°) supernatants were removed by suction and antibody-bound radioactivity in the pellets was counted in a multiwell γ-counter. Control binding in the absence of IL-8 was 30·3 ± 1·4% ($n=3$) and non-specific binding was 4·2 ± 0·09% ($n=3$). A standard curve was constructed using a spline-fit program and the lower detection limit of the assay was 0·33 pg/ml. IL-6 and GM-CSF were assayed using commercially available ELISA kits, with quoted sensitivities of 3·5 and 1·5 μg/ml respectively, from R & D Systems (Minneapolis, MN) supplied by British Biotechnology Ltd.

RESULTS

In situ hybridization

Specific mRNA encoding IL-6, IL-8 and GM-CSF was detected by hybridization with the respective ^{35}S-labelled cRNA probes and visualized as deposits of silver grains in the photographic emulsion overlying the cells. Preparations were examined under both phase-contrast and dark-field illumination, and Fig. 1(a,b) shows positive signals for IL-6 and IL-8 respectively obtained with the anti-sense probes. The corresponding sense probes and RNase pretreatment were used as negative controls and in all cases these gave negative results. The phase-contast picture obtained with the IL-6 sense probe is shown in Fig. 1(c). Hybridization signals were scored on the basis of the percentages of cells exhibiting a positive signal as indicated in Table 1. mRNA for IL-6 and IL-8 was detected in more than 20% of the epithelial cells derived from three subjects, and mRNA for GM-CSF was found in fewer than 20% of the cells cultured from two of the same three subjects. The identity of the cells was confirmed on the basis of positive immunocytochemical staining for cytokeratin, which was expressed by > 98% of the cells in the cultures. mRNA encoding IL-6 and IL-8 was also identified in a

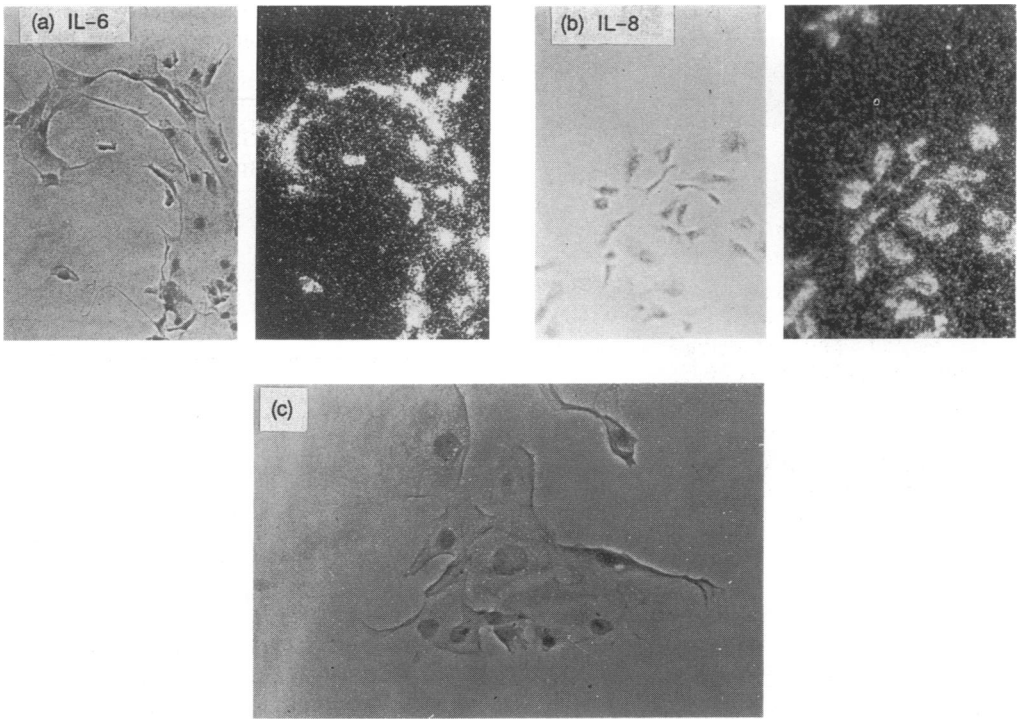

Figure 1. Autoradiographs of cultured human bronchial epithelial cells after *in situ* hybridization with ^{35}S-labelled riboprobes. Hybridization signals achieved with antisense probes for (a) IL-6 and (b) IL-8 in cells viewed under both phase-contrast and dark-field illumination. (c) Example of a negative control with cells hybridized with an IL-6 sense probe and viewed under phase contrast.

Table 1. Cytokine mRNA expression in cultured epithelial cells assessed using *in situ* hybridization

mRNA	GM-CSF		IL-6		IL-8	
Culture conditions	Medium	TNF-α	Medium	TNF-α	Medium	TNF-α
Patient 1	−	+	++	+++	++	+++
Patient 2	+	++	++	+++	+++	+++
Patient 3	+	++	++	+++	++	++

\+ <20% of cells expressing mRNA.
++ 20–80% of cells expressing mRNA.
+++ >80% of cells expressing mRNA.

transformed human tracheal epithelial cell line (9HTEo$^-$), and these cells were included in all experiments as positive controls.

Culture in the presence of TNF-α (20 ng/ml, 24 hr) enhanced expression of mRNA for IL-6 (3/3 subjects) and IL-8 (1/3 subjects) (Table 1), and induced expression of mRNA for GM-CSF in one subject whilst enhancing the expression in the other two cases.

Northern blotting

The presence of mRNA encoding IL-6, IL-8 and GM-CSF was identified using Northern blotting in total RNA extracts prepared from primary bronchial epithelial cell cultures in each of four individual patients. In addition, culture in the presence of TNF-α or IL-1β for 24 hr, in the concentration ranges 0·2–20·0 ng/ml, induced dose-dependent increases in the amounts of IL-8-specific mRNA detected (Figs 2 and 3), confirming and extending the observations made with *in situ* hybridization. The extent of enhancement was assessed relative to β-actin (see Materials and Methods) to correct for any variation in loading of RNA between lanes. In addition there was some evidence for enhancement of IL-6 and GM-CSF mRNA, but the extent of the enhancement was not as great as for IL-8, and 9HTEo$^-$ cells were found to express mRNA encoding the three cytokines (data not shown).

Immunoassays

Low levels of GM-CSF immunoreactivity were detected by ELISA in the culture supernatants from cells derived from three

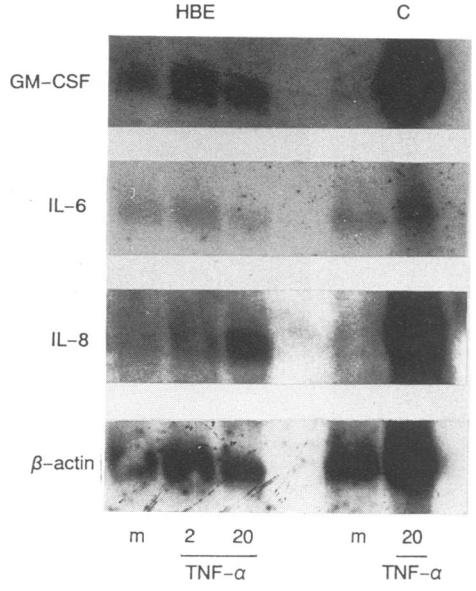

Figure 2. An example of expression of mRNA for GM-CSF, IL-6, IL-8 and β-actin in human bronchial epithelial cells (HBE) detected by Northern blotting. RNA recovered from cells from one individual cultured for 24 hr in the presence of either medium alone (m) or TNF-α at 2·0 and 20·0 ng/ml. Controls (C) were a human fibroblast line cultured either in the presence of medium alone (m) or TNF-α at 20·0 ng/ml.

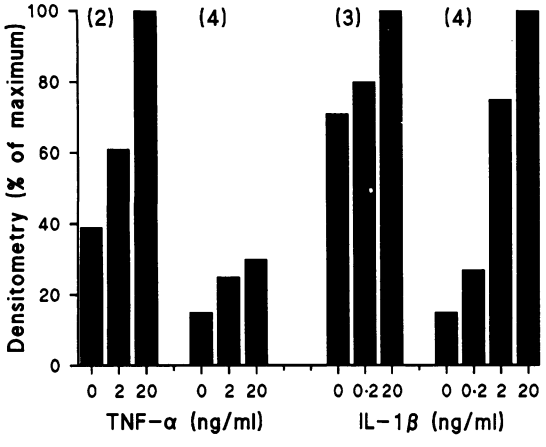

Figure 3. Densitometric analysis of Northern blots for GM-CSF, IL-6 and IL-8. The data are expressed relative to the maximum response seen from cells cultured with TNF-α or IL-1β, and relative to mRNA for β-actin to correct for minor variations in RNA loading on the blot. Cells recovered from subjects 2, 3 and 4 were cultured in the presence of fresh medium alone (m) and various concentrations of either TNF-α or IL-1β for 24 hr prior to harvesting of RNA and subsequent Northern blot analysis.

subjects (Table 2), and culture with increasing doses of TNF-α or IL-1β resulted in an increase in GM-CSF concentrations (Fig. 4). The degree of enhancement varied considerably for cells derived from different subjects. IL-6 immunoreactivity was also detected by ELISA (Table 2) and expression of the cytokine was enhanced by both TNF-α and IL-1β in a dose-dependent

Table 2. GM-CSF, IL-6 and IL-8 immunoreactivity in unstimulated epithelial cell culture supernatants

	GM-CSF (pg/ml)*	IL-6 (pg/ml)	IL-8 (ng/ml)
Patient 1	ND	ND	12·75
Patient 2	7·6	45·3	2·55
Patient 3	44·5	49·1	10·62
Patient 4	373·0	105·7	4·42
9HTEo⁻	4·1	50·3	0·77

* Concentration/ml of culture supernatant being the product of approximately 2×10^6 cells.

ND, not determined.

fashion (Fig. 4), although in one instance with TNF-α there was a negative correlation with IL-6 (patient 4), as opposed to a positive correlation seen with GM-CSF and IL-8. Samples were reassayed and the observation confirmed. IL-8 was detected in cell cultures derived from four subjects (Table 2) and again TNF-α and IL-1β induced a dose-dependent enhancement of generation of the cytokine (Fig. 4). The variations in the patterns of cytokine production seen between cultures from different subjects appeared not to be associated with clinical parameters relating to their lung cancer, and there was no suggestion that they were asthmatic.

IL-6 levels generated by the tracheal epithelial cell line (9HTEo⁻) were comparable with those from the primary cultures and appeared to be influenced by treatment of the cells with TNF-α and IL-1β (Fig. 4). Secretion of IL-8 was similarly augmented, but to a lesser extent than in the primary cell cultures, and whilst low concentrations of GM-CSF were detectable (Table 2) there was no evidence that TNF-α and IL-1β could influence the amount secreted by the cells (Fig. 4).

DISCUSSION

It is well recognized that the bronchial epithelium plays an important role as a physical barrier protecting the underlying tissue and in maintaining the local environment in the airways. Another possibility that has received little attention is that the epithelium may actually contribute to inflammatory and immunological reactions in the lung, such as those associated with ongoing asthma. This notion is supported by the present observations that bronchial epithelial cells can express mRNA for IL-8, IL-6 and GM-CSF and secrete the corresponding proteins, and that both phenomena can be enhanced by cytokines such as IL-1β and TNF-α.

In situ hybridization provides an elegant way of demonstrating mRNA expression in single cells, and in conjunction with immunocytochemical staining the identity of the cells can be established with some certainty. The technique enabled us to identify specific mRNA for GM-CSF, IL-6 and IL-8 in cultured bronchial epithelial cells and indicated that it is possible to enhance mRNA expression by prior culture in the presence of TNF-α (Fig. 1 and Table 1). Quantification of *in situ* hybridization is problematic and accurate enumeration of silver grains in association with cells growing in close proximity one with

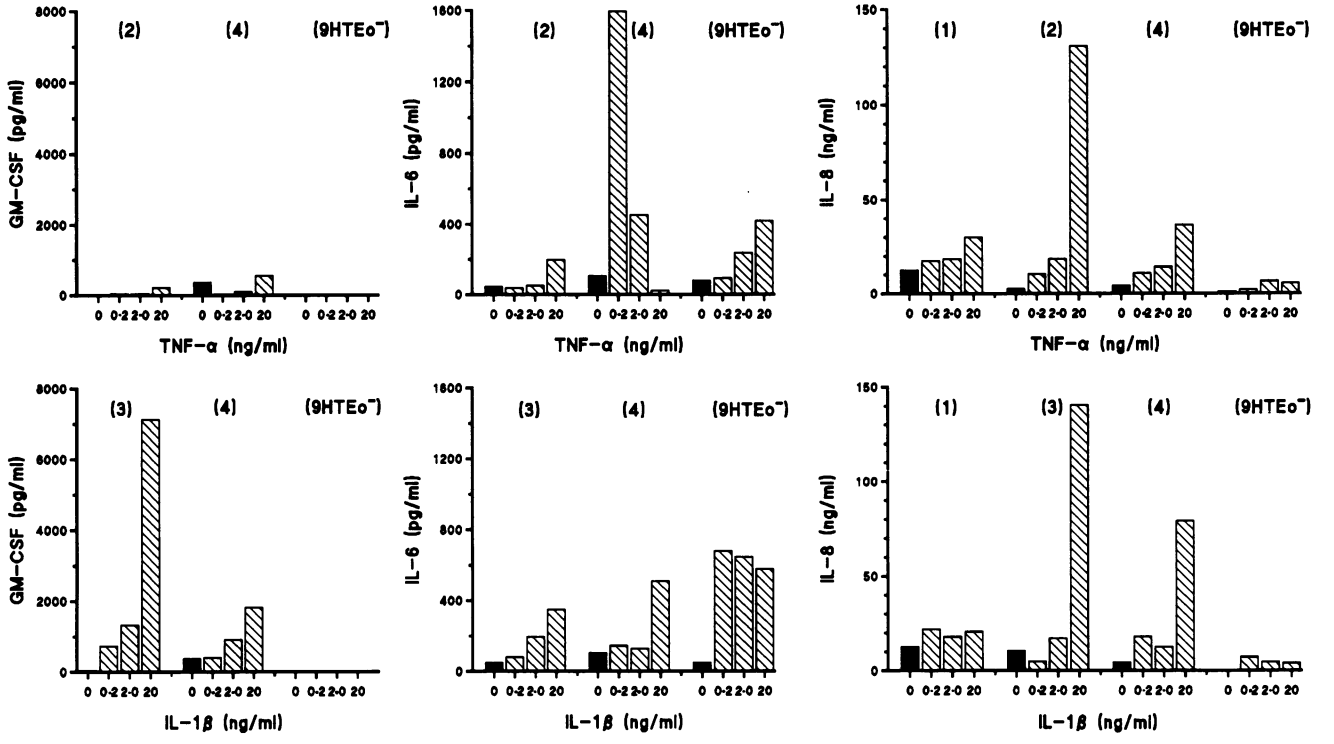

Figure 4. Immunoreactive GM-CSF, IL-6 and IL-8 in culture supernatants from bronchial epithelial cells and a transformed tracheal epithelial cell line (9HTEo⁻) stimulated with TNF-α and IL-1β. Bars represent cytokine concentrations/ml of culture supernatant/2×10^6 cells, measured in unstimulated cultures (■) (see Table 2) and after 24 hr culture in the presence of doses of TNF-α and IL-1β from 0·2 to 20·0 ng/ml (▨).

another was not always feasible. For this reason we restricted ourselves to counting the percentage number of cells giving a positive hybridization signal, and on this basis the proportion of cells expressing mRNA for GM-CSF in unactivated cultures was consistently less than the numbers expressing message for IL-6 and IL-8. This may reflect functional heterogeneity of the epithelial cells or variations in cytokine expression during the course of the cell cycle, although the present study cannot distinguish these possibilities.

In order to confirm the observations with *in situ* hybridization and to achieve a greater appreciation of the extent of cytokine-induced enhancement, RNA extracts were subjected to Northern blot analysis. This enabled us to demonstrate that both TNF-α and IL-1β are able to augment expression of mRNA encoding IL-8 (Fig. 3), suggesting that the cytokine generation is regulated, at least in part, at the transcriptional level. The actions of IL-1β and TNF-α also manifest themselves in dose-dependent increases in levels of immunoreactive material recovered from the culture supernatants (Fig. 4). Whilst there was some variation in the pattern of cytokine production between subjects there is no immediate explanation for this. One possibility is that different epithelial cell types, such as ciliated cells, secretory cells, basal cells, etc., may have various capacities to generate cytokines and that variations reflect the cellular composition of the cultures. Overall the observations suggest that the bronchial epithelial cells have the capacity to play an interactive role with other cell types through the cytokine network.

The transformed tracheal epithelial cell line (9HTEo⁻) appears to behave similarly to the freshly cultured cells in so far as it expresses mRNA encoding GM-CSF, IL-6 and IL-8, but its abilities to secrete immunoreactive GM-CSF and IL-8 appear to be impaired.

Gene expression for IL-8 has been demonstrated in a pulmonary type II cell line,[28] and our present observations show that bronchial epithelial cells also produce the cytokine. The fact that IL-8 is chemotactic for T lymphocytes[16] and primed eosinophils[17] suggests that the cytokine may be a contributing factor to the pathology of asthma in which both cell types feature prominently.[3] Similarly a role for bronchial epithelial cell-derived GM-CSF can be envisaged particularly in terms of promotion of eosinophil longevity and priming neutrophils, eosinophils, basophils and monocytes for enhanced generation of inflammatory mediators.[11] Endothelial cells, fibroblasts and macrophages/monocytes have been regarded as the principal sources of IL-6 because of their high capacities to synthesize the cytokine and their relative abundace.[13] The ability of supernatants from toluene diisocyanate-stimulated bronchial epithelial cells to induce activation and proliferation of T cells has previously been attributed to IL-6 and IL-1 on the basis of inhibition of the effects by specific antibodies.[29] Toluene diisocyanate provokes asthmatic responses with accompanying mucosal inflammation in sensitized subjects, and promotion of IL-6 production by airway epithelial cells is a possible contributory factor.

The response of the epithelial cells is likely to be modulated by cytokines derived from other cell types in and around the airways. Alveolar macrophages, which account for 85% or more of the cells recovered from the airway lumen by bronchoalveolar lavage,[30] produce IL-6, TNF-α and IL-1β consecutive to allergen induced late asthmatic reactions.[31] Generation of these cytokines by macrophages and other cell types[32] may be a key

factor in the modulation of cytokine gene expression and production. IL-1 receptors have previously been demonstrated on human bronchial epithelial cells and their functional activity demonstrated through modulation of GM-CSF synthesis and release.[33] An interesting observation in the present study is the extent of the enhancement of cytokine generation that can be achieved with both TNF-α and IL-1β, suggesting a sensitive mechanism whereby epithelial cell function may be controlled and influence inflammatory events. A report, which adds weight to this suggestion is one that has shown up-regulation of synthesis and release of GM-CSF by bronchial epithelial cells in asthma.[34] Human bronchial[34] and tracheal[35] epithelial cells have previously been shown to produce GM-CSF, which is susceptible to inhibition by steroids,[34] and to promote eosinophil survival. These factors may help explain in part the mechanisms whereby the eosinophilic inflammatory response is sustained in asthma and the basis of the success of corticosteroids in the treatment of the disease.

The pleiotropic nature of the cytokines makes it difficult to decide what their precise roles might be in any one particular situation. However, bronchial epithelial cells appear to have an established position in the cytokine network that provides a basis for intercellular communication and control. The cells are potentially a major source of IL-8, and together with the production of IL-6 and GM-CSF may contribute to the inflammation that is a characteristic feature of on-going asthmatic reactions by virtue of the various abilities of the cytokines to promote lymphocyte responses, inflammatory cell recruitment and eosinophil survival and activation.

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