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The T Cell-Specific CXC Chemokines IP-10, Mig, and I-TAC Are Expressed by Activated Human Bronchial Epithelial Cells

Alain Sauty,* Michelle Dziejman,* Rame A. Taha,‡ Albert S. Iarossi,* Kuldeep Neote,§ Eduardo A. Garcia-Zepeda,* Qutaya Hamid,‡ and Andrew D. Luster2*

Recruitment of activated T cells to mucosal surfaces, such as the airway epithelium, is important in host defense and for the development of inflammatory diseases at these sites. We therefore asked whether the CXC chemokines IFN-induced protein of 10 kDa (IP-10), monokine induced by IFN-γ (Mig), and IFN-inducible T cell α-chemoattractant (I-TAC), which specifically chemotactically activate T cells by signaling through the chemokine receptor CXCR3, were inducible in respiratory epithelial cells. The effects of proinflammatory cytokines, including IFN-γ (Th1-type cytokine), Th2-type cytokines (IL-4, IL-10, and IL-13), and dexamethasone were studied in normal human bronchial epithelial cells (NHBEC) and in two human respiratory epithelial cell lines, A549 and BEAS-2B. We found that IFN-γ, but not TNF-α or IL-1β, strongly induced IP-10, Mig, and I-TAC mRNA accumulation mainly in NHBEC and that TNF-α and IL-1β synergized with IFN-γ induction in all three cell types. High levels of IP-10 protein (>800 ng/ml) were detected in supernatants of IFN-γ/TNF-α-stimulated NHBEC. Neither dexamethasone nor Th2 cytokines modulated IP-10, Mig, or I-TAC expression. Since IFN-γ is up-regulated in tuberculosis (TB), using in situ hybridization we studied the expression of IP-10, Mig, or I-TAC in the airways of TB patients and found that IP-10 mRNA was expressed in the bronchial epithelium. In addition, IP-10-positive cells obtained by bronchoalveolar lavage were significantly increased in TB patients compared with normal controls. These results show that activated bronchial epithelium is an important source of IP-10, Mig, and I-TAC, which may, in pulmonary diseases such as TB (in which IFN-γ is highly expressed) play an important role in the recruitment of activated T cells. The Journal of Immunology, 1999, 162: 3549–3558.

T cells play a central role in host defense and mucosal immunity, particularly in the respiratory tract. Activated T cells are recruited into the lungs, where they contribute to the cell-mediated immune response following Th1-type differentiation or participate in the humoral and allergic responses following Th2-type differentiation (1). Th1 cells mainly secrete IFN-γ, while Th2 cells principally produce IL-4, IL-5, IL-10, and IL-13 (1). Th1 cells and IFN-γ expression are increased in several pulmonary diseases, including pulmonary tuberculosis (TB) (2). In TB, CD4+ T cells are required to provide a state of delayed-type hypersensitivity to Mycobacterium tuberculosis (3), and lack of CD4+ T cells, as found in AIDS patients, favors the development of TB as well as other mycobacteria diseases (4, 5).

Recruitment of T cells into sites of airway inflammation is a multistep process involving adherence to and Migration across the pulmonary endothelium, trafficking through the interstitium, and finally moving into and through the airway epithelium (6, 7). Airway epithelial cells are likely to play an important role in this process (8, 9). They can express adhesion molecules, such as ICAM-1 and VCAM-1 (10), facilitating the adherence of lymphocytes to the epithelial surface. Airway epithelial cells can also be induced to express HLA-DR (11) and therefore may present Ag to T cells. In addition, activated bronchial epithelial cells secrete a variety of mediators, including prostaglandins, platelet-activating factor, and proinflammatory cytokines, as well as chemokines important for the process of inflammatory cell recruitment into the lung interstitium and alveolar spaces (8).

Chemokines are a superfamily of 8- to 10-kDa secreted proteins that direct the recruitment of leukocytes to sites of inflammation. All but two of the chemokines belong to the CC (β-chemokine) or CXC (α-chemokine) family, defined by the primary sequence of the first two of four invariant cysteine residues (12). In general, CC chemokines chemotactact monocytes, eosinophils, basophils, and T cells and signal through the chemokine receptors CCR1 to CCR9 (reviewed in Ref. 12). The CXC chemokine family can be divided into two classes based on the presence or absence of an NH2-terminal ELR sequence (Glu-Leu-Arg). The ELR-containing CXC chemokines (e.g., IL-8) chemotact neutrophils (13), while the non-ELR CXC chemokines chemotact lymphocytes. For example, IFN-inducible protein of 10 kDa (IP-10), monokine induced by γ-IFN (Mig), and IFN-inducible T cell α-chemoattractant (I-TAC) are potent chemoattractants for activated T lymphocytes (14–18), while stromal cell-derived factor-1 is active on resting T (19) and immature B cells (20), and B cell-attracting chemokine-1 is specific for mature B cells (21). The CXC chemokines signal through the chemokine receptors CXCR1 to CXCR5.
Several members of the CC chemokine family, including monocyte chemotactic protein (MCP)-1 (22), MCP-3 (23), MCP-4 (24, 25), RANTES (23), and eotaxin (26, 27) are expressed by activated bronchial epithelial cells. Likewise, the ELR-containing CXC chemokines IL-8 (28) and epithelial cell-derived neutrophil-activating peptide-78 (29) have been shown to be inducible in bronchial epithelium. In contrast, non-ELR chemokines have not been demonstrated to be expressed in bronchial epithelium. Since bronchial epithelium is likely to regulate T cell trafficking into the lung, we asked whether human bronchial epithelial cells could express IP-10, Mig, and I-TAC.

IP-10 was initially identified as an abundantly induced mRNA in U937 cells upon IFN-γ stimulation (30), and its expression is predominantly induced by IFN-γ in endothelial cells, monocytes, fibroblasts (30), astrocytes (31), keratinocytes (32), and neutrophils (33). Human Mig, which is 37% identical to IP-10 at the amino acid level, is expressed in IFN-γ-induced THP-1 cells, PBMCs, endothelial cells, keratinocytes, and fibroblasts (17, 34). I-TAC, a newly identified non-ELR chemokine, is ~40% identical to the amino acid level to IP-10 and Mig and is expressed by activated monocytes and astrocytes (18). Both IP-10, Mig, and I-TAC chemoattract activated T cells and NK cells, but not resting T cells, B cells, or neutrophils (14–17). IP-10 expression has been found in various clinical conditions such as psoriasis (35), tuberculous leprosy (36), sarcoidosis (37), and viral meningitis (38), as well as in experimental animal models of autoimmune encephalomyelitis (31) and nephrosis (39). Mig was also found in psoriatic lesions by in situ hybridization (17). All of these diseases are associated with an increased expression of IFN-γ (Th1-type diseases) (1), which may induce IP-10 Mig and I-TAC expression in involved tissues.

IP-10, Mig, and I-TAC share a common receptor, CXCR3, which is specific for these three chemokines (15). CXCR3 is expressed on peripheral blood T cells activated in vitro and on a significant fraction of circulating CD4+ and CD8+ T cells, B cells, and NK cells (40), but not on monocytes or neutrophils. In addition, most peripheral CXCR3+ T cells expressed CD45RO+ (memory T cells) and β1 integrins (40), which are implicated in the binding of lymphocytes to endothelial cells, epithelial cells, and extracellular matrix (6). Recent studies revealed that CXCR3+ T cell clones were predominantly of the Th1 type (41–43). In addition, it was recently reported that virtually all synovial fluid T cells isolated from patients with rheumatoid arthritis expressed CXCR3 (40, 42). Furthermore, the vast majority of T lymphocytes found in colonic tissue obtained from patients with ulcerative colitis were CXCR3+ (40). These data suggest that in Th1-type diseases, in which IFN-γ is up-regulated, IP-10, Mig, and I-TAC play an important role in the recruitment of activated CXCR3+ T cells into inflamed tissue.

In this study, we demonstrate that human bronchial epithelial cells can be induced to express high levels of IP-10, Mig, and I-TAC mRNA and secrete high levels of IP-10 protein in response to IFN-γ stimulation. In addition, we show that IP-10 is expressed in bronchial epithelial cells and in cells recovered from bronchoalveolar lavage (BAL) in patients with active pulmonary TB.

Materials and Methods

Materials

BEAS-2B cells and A549 cells were obtained from the American Type Culture Collection (Manassas, VA) and cell culture reagents from Cellgro (Mediatech, Herndon, VA). Recombinant human IFN-γ, TNF-α, IL-4, IL-10, IL-13, IP-10, and Mig were obtained from PeproTech (Rocky Hill, NJ), and IL-1β from R&D Systems (Minneapolis, MN). Normal human bronchial epithelial cells (NHBEc) and their specific medium were purchased from Clonetics (Walkersville, MD). Chemicals were obtained from Sigma (St. Louis, MO).

Cell culture

BEAS-2B, an SV-40-transformed human bronchial epithelial cell line, was cultured in F12/DMEM supplemented with 2 mM L-glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, 10 mM HEPES, and 10% heat-inactivated FCS (Mediatech) (referred to as complete F12/DMEM). A549, a human lung adenocarcinoma cell line with the alveolar type II phenotype, was maintained in complete F12K in a 5% CO2 atmosphere at 37°C. NHBEc were maintained in serum-free bronchial epithelial cell basal medium (Clonetics) supplemented with 50 μg/ml bovine pituitary extract, 50 ng/ml human epidermal growth factor, 0.5 μg/ml hydrocortisone, 0.5 μg/ml epinephrine, 10 μg/ml transferrin, 5 μg/ml insulin, 0.1 ng/ml retinoic acid, 6.5 ng/ml triiodothyronine, 50 μg/ml gentamicin, and 50 ng/ml amphotericin-B. BEAS-2B and A549 cells were grown to confluence in 100-mm cell culture petri dishes, and 24 h before activation they were serum starved in their respective media without FCS. Twelve to 16 h before activation, medium from 90% confluent NHBEc was replaced by BEBM alone. Cells were then stimulated with IFN-γ, TNF-α, or IL-1β alone or in combination at indicated doses and times. In experiments involving cycloheximide (10 μg/ml) (26); dexamethasone; and IL-4, IL-13, and IL-10 (20 ng/ml), the agents were added 1 h before cell stimulation by cytokines.

RNA analysis

Total RNA was extracted from samples using Stat-60 (Tel-Test, Friendswood, TX). For Northern analysis, 20 μg of total RNA was electrophoresed on 1% agarose-formaldehyde gel and then capillary transferred to a GeneScreen membrane (NEN Life Science Products, Boston, MA). Following overnight prehybridization (50% formamide, 4× SSC, 4× Denhardt’s solution, 0.8% glycine, and 0.17 mg/ml denatured salmon sperm DNA) at 42°C, blots were hybridized at 42°C in 50% formamide, 10% dextran, 1% SDS, 5× SSC, 1× Denhardt’s solution, and 0.17 mg/ml denatured salmon sperm DNA with 1 × 10^6 cpm/ml [α-32P]dCTP-labeled cDNA probe prepared by nick translation. The following fragments were used as probes: a 1-kb Pst1 fragment from IP-10 cDNA, a 3-kb Nol1 fragment from human Mig cDNA (kindly provided by J. Farber, National Institutes of Health, Bethesda, MD), a 300-bp BamHI/Aval fragment from human I-TAC cDNA, and a 346-bp EcoRI fragment from human IL-8 cDNA (a kind gift from H. Oettgen, Children’s Hospital, Boston, MA). A glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probe was used as a control for RNA loading and to normalize signals obtained with the different probes. Signal quantitation was using a PhosphorImager (Molecular Image System, Bio-Rad, Hercules, CA). The data shown in all of the figures are representative of at least two independent experiments.

Generation of anti-human IP-10 (hIP-10) mAbs

Fifty micrograms of histidine-tagged hIP-10 (44) in CFA was injected i.p. into 6-wk-old female BALB/c mice. Mice were injected i.p. with 25 μg of His-tagged hIP-10 in IFA on days 14 and 21. On day 25, spleen cells were harvested and fused with P3X63 myeloma cells using a standard PEG 1500 protocol. Hybridoma cells were cloned by two rounds of single-cell cloning using medium supplemented with 10% IL-6-conditioned supernatant from Sp2/IL-6 cells. Direct ELISA, using recombinant non-histidine-tagged hIP-10 (PeproTech) containing only amino acids found in the mature IP-10 protein, was used to screen 750 colonies. Ten of 18 initial positive clones survived two rounds of single-cell cloning. All clones were found to be specific for rHIP-10 when tested by ELISA and dot blot analysis using the following CXC chemokines: human growth-regulated oncogene α (GROα), IL-8, Mig, neutrophil-activating peptide-2(NAP-2), platelet factor 4 (PF4), stromal cell-derived factor-1, and murine IP-10. One clone, D1D2, was chosen for use in Western blots and ELISA. D1D2 mAb was purified from serum-free supernatants using protein G chromatography (Pharmacia, Piscataway, NJ).

Western blot analysis of cell supernatants

Unconcentrated cell supernatants and different amounts of rHIP-10 were separated by SDS-PAGE using 12.5% Tris-tricine gel. Proteins were then transferred to a polyvinylidene difluoride membrane (NEN Life Science Products). The membrane was blocked with 5% dry skim milk, 0.1% Tween (PBST) at room temperature and incubated with D1D2 mAb (2 μg/ml in 1% BSA) overnight at 4°C. After the membrane was washed...
in TBST, 1/10,000 dilution of peroxidase-conjugated goat anti-mouse Ig Ab (Kirkegaard & Perry Laboratories, Gaithersburg, MD) was added for 1 h, followed by additional washes and detection solution for chemiluminescence (Amersham Life Science, Arlington Heights, IL).

Sandwich ELISA

D1D2 mAb (100 μl) at 10 μg/ml in PBS was added to wells of a high binding-efficiency 96-well ELISA plate (Costar, Cambridge, MA) and incubated overnight at 4°C. The plate was then incubated with blocking buffer (3% BSA and 3% goat serum in PBS) overnight at 4°C. After the plate was washed with PBS, 100 μl of standards or unconcentrated cell supernatants (in triplicates) were added to each well and incubated for 2 h at 37°C. The plate was washed again three times with PBST and three times with PBS and incubated with 15 ng/well of affinity-purified rabbit anti-hIP-10 (44) for 1 h at 37°C, followed by detection solution (Kirkegaard & Perry Laboratories). The plate was then read at 650 nm for 15 min. The limit of detection of this assay was ∼500 pg/ml.

BALs and bronchial biopsies

Seven control individuals (nonsmokers, nonasthmatics, and nonatopics) and seven patients with active TB (prior antituberculous chemotherapy) underwent bronchofibroscopy and BAL as previously described (2). Bronchial biopsies of the lower bronchus were performed during bronchoscopy.

In situ hybridization

BAL cells were processed as described (2). Labeled riboprobe was prepared from IP-10 cDNA using 35S-labeled UTP or digoxigenin-UTP. The prehybridization, hybridization, and posthybridization protocols were as previously described (45). Hybridization signal was visualized using autoradiography or antidigoxigenin. For negative controls, preparations were hybridized with sense probes. The specificity of the hybridization signal was confirmed by treating preparations with ribonuclease (RNase) before hybridization.

Results

Dose-response studies

Preliminary experiments using A549 and BEAS-2B cells showed that IP-10 mRNA was not found in unstimulated cells, but was

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FIGURE 1. Dose response of proinflammatory cytokine-induced IP-10, Mig, I-TAC, and IL-8 mRNA accumulation. Northern blot analysis of 20 μg of total RNA obtained from A549 and BEAS-2B cells (A) and NHBEC (B) stimulated for 8 h with increasing doses (ng) of IFN-γ, TNF-α, IL-1β, or a combination of IFN-γ and TNF-α or IL-1β. A GAPDH cDNA probe was used as a control for RNA loading.
detectable 2 h after and peaked 8 h after stimulation with IFN-γ (100 ng/ml) or TNF-α (10 ng/ml) (data not shown). We therefore used the 8-h time point for studying the effect of different doses and combinations of IFN-γ, TNF-α, and IL-1β on IP-10, Mig, and I-TAC mRNA accumulation (Fig. 1, A and B) and compared it with the expression of the ELR-containing CXC chemokine IL-8. We found that IFN-γ alone induced IP-10 in BEAS-2B but not in A549 cells and that the maximal effect was observed at 100 ng/ml. IL-1β weakly induced IP-10 in A549 but not in BEAS-2B cells. Both cell lines expressed IP-10 in response to TNF-α in a dose-dependent manner. Mig and I-TAC expression was barely detected in BEAS-2B cells treated with IFN-γ and was not seen in cells treated with TNF-α or IL-1β. TNF-α and IL-1β strongly synergized in a dose-dependent manner with IFN-γ (100 ng/ml) in inducing the accumulation of IP-10, Mig, and I-TAC mRNA. IP-10 was induced to higher levels than Mig and I-TAC in both cell lines; this can be appreciated in Fig. 1A, which shows blots for IP-10 and Mig that were exposed for similar times (25 and 26 h, respectively) and a blot for I-TAC exposed for 7 days. In contrast, IL-8 was strongly induced in A549 cells by TNF-α or IL-1β, with IFN-γ being only weakly synergistic. We also studied the effect of the proinflammatory cytokines IL-6 and granulocyte-macrophage CSF, alone or combined with IFN-γ. These two cytokines had no effect on IP-10 or Mig expression (data not shown).

The induction of IP-10, Mig, and I-TAC in NHBEC in response to cytokine stimulation was different from that observed in the two cell lines (Fig. 1B). IFN-γ induced IP-10, Mig, and I-TAC expression to higher levels than those seen in A549 and BEAS-2B cells. In addition, only 10 ng/ml of IFN-γ induced maximal IP-10, Mig, and I-TAC expression, indicating that NHBEC were more sensitive to IFN-γ than the two cell lines. TNF-α and IL-1β, alone or together, did not induce IP-10, Mig, and I-TAC expression in NHBEC, while they were potent inducers of IL-8 expression in these cells. Moreover, TNF-α and IL-1β only slightly synergized with IFN-γ in the induction of IP-10, Mig, and I-TAC, suggesting that IFN-γ alone was maximally inducing IP-10, Mig, and I-TAC in NHBEC. To determine whether TNF-α could be induced in response to IFN-γ and therefore have an autocrine effect, NHBEC were treated with IFN-γ in the presence of a neutralizing anti-hTNF-α goat polyclonal Ab (5 μg/ml) or goat IgG control (5 μg/ml). We found that these treatments had no effect on IP-10 or I-TAC mRNA expression (data not shown). However, as a control,
we found that this concentration of anti-TNF-α Ab completely inhibited the effects of 10 ng/ml TNF-α (data not shown). These data suggest that TNF-α did not contribute to the effect of IFN-γ on IP-10 or I-TAC induction in NHBEC. When the level of expression of the four chemokines was compared (Fig. 1B) according to their respective times of Northern blot exposure (IP-10, 2 h; Mig, 2 h; I-TAC, 10 h; and IL-8, 3 days), the data show that IP-10 was induced to significantly higher levels than Mig, I-TAC, or IL-8 in NHBEC.

Kinetic studies

The kinetic response of IP-10, Mig, I-TAC, and IL-8 mRNA accumulation after stimulation with combinations of IFN-γ, TNF-α, and IL-1β was also examined. Northern blot analysis of NHBEC showed that the pattern of IP-10, Mig, and I-TAC expression was very similar (Fig. 2A). IP-10, Mig, and I-TAC mRNA accumulation was detected between 1 and 2 h after cytokines were added, peaked at 8 h, and slowly decreased thereafter. In contrast, IL-8 expression was bimodal with an early peak at 1 h followed by a second peak at 16 h (Fig. 2A).

Comparable data were obtained with BEAS-2B and A549 cells, and only the PhosphorImager quantitation of Northern blots are shown in Fig. 2B. These data revealed minor differences in IP-10 and Mig expression in BEAS-2B and A549 cells compared with NHBEC. There was a suggestion of a bimodal expression of IL-8 in BEAS-2B cells; however, this was not seen in A549 cells.

Effect of protein synthesis inhibition

To examine the effect of protein synthesis inhibition on chemokine mRNA accumulation, cells were pretreated 1 h before cytokine stimulation with cycloheximide at a concentration (10 μg/ml) previously shown to inhibit >90% of protein synthesis in A549 cells (26). As shown in Fig. 3A, in NHBEC, Mig and I-TAC expression induced by IFN-γ, IFN-γ/TNF-α, and IFN-γ/IL-1β was significantly inhibited by cycloheximide. The effect of cycloheximide on IP-10 expression was weak compared with the effects of Mig and I-TAC and was even absent in IFN-γ/TNF-α-stimulated NHBEC. IP-10, Mig, and I-TAC mRNA was not detected in untreated or in TNF-α- and in IL-1β-stimulated cells and was not affected by cycloheximide treatment. The effect of cycloheximide on IP-10, Mig, and I-TAC induction in BEAS-2B cells was less pronounced than in NHBEC. However, in A549 cells we observed a superinduction of IP-10, Mig, and I-TAC mRNA accumulation in response to IFN-γ/TNF-α stimulation (Fig. 3B). IL-8 expression was strongly superinduced by cycloheximide in the three cell lines and in all situations studied (Fig. 3, A and B). As an example, in A549 cells treated with TNF-α, cycloheximide increased IL-8 expression 6.6-fold.

Effect of dexamethasone

Glucocorticoids are potent inhibitors of chemokine expression. However, treatment of NHBEC stimulated by IFN-γ, IFN-γ/
TNF-α, and IFN-γ/IL-1β with 10 μM dexamethasone (Fig. 4) was associated with no or only a weak inhibition of IP-10, Mig, and I-TAC expression. This is in contrast to the clear inhibition of IL-8 mRNA expression (Fig. 4). Data obtained with A549 and BEAS-2B cells were similar to those obtained with NHBEC (data not shown), although the effect of dexamethasone on IL-8 expression by these cells was significantly less marked.

**Effect of Th2 type cytokines**

Since IL-4 has been reported to down-regulate IP-10 in monocytes (46) and macrophages (47), we asked whether IL-4, IL-13, and IL-10 (three Th2-type cytokines) had the capacity to down-regulate IP-10, Mig, and I-TAC expression in epithelial cells. We found that IP-10, Mig, I-TAC, and IL-8 expression by NHBEC treated with IFN-γ or IFN-γ/TNF-α was not affected by pretreatment with IL-4, IL-13, or IL-10 (all at 20 ng/ml) (Fig. 5). However, NHBEC were responsive to IL-4 and IL-13, since IL-8 expression increased by 156% and 93% in TNF-α-stimulated cells pretreated with IL-4 and IL-13, respectively. Experiments performed on IFN-γ or IFN-γ/TNF-α-stimulated A549 and BEAS-2B cells pretreated with IL-4, IL-13, and IL-10 gave results comparable with those described for NHBEC (data not shown).

**Expression of IP-10 protein in bronchial epithelial cell supernatants**

To assess whether IP-10 protein was secreted by human bronchial epithelial cells, we performed Western blot analysis on unconcentrated supernatants from A549 cells, BEAS-2B cells, and NHBEC using a newly generated murine mAb (D1D2) specific for IP-10. We found a significant amount of IP-10 secreted in cell supernatants, as demonstrated by the unique band of ~10 kDa that co-migrated with rIP-10 (Fig. 6A). Using a sandwich ELISA developed in our laboratory, we detected high levels of IP-10 in unconcentrated supernatants. IP-10 levels were detected between 4 and 8 h after activation with IFN-γ/TNF-α (Fig. 6B). At 32 h, the amount of IP-10 in supernatants of A549 cells, BEAS-2B cells, and NHBEC was 253.1 ± 36.6 (SEM), 558.7 ± 62.9, and 843.8 ± 73.2 ng/ml, respectively. IP-10 levels at 8, 16, and 32 h were significantly higher than at 4 h in all three cell type supernatants. Moreover, IP-10 levels detected by ELISA were positively correlated with mRNA levels in experiments in which stimulated cells were treated with dexamethasone or with IL-4, IL-13, and IL-10 (data not shown).

**In situ hybridization**

To determine whether IP-10 was expressed in the airways of patients with pulmonary TB, a Th1-type disease in which IFN-γ has been shown to be up-regulated, we compared IP-10 mRNA expression by in situ hybridization using an IP-10 cRNA probe on BAL cells obtained from seven patients with active TB and seven normal controls. Quantitation of BAL data is shown in Table I and demonstrates a statistically significant increase in the percentage of IP-10 mRNA-positive cells in BAL from TB patients compared with controls (p < 0.001). Fig. 7, A and B, shows a representative in situ hybridization of BAL cells from one TB patient and one control. Although the type of the positive cells was not determined,
morphologically they appeared to be macrophages. In situ hybridization was also performed on bronchial biopsies obtained from patients with active TB and normal controls. The bronchial epithelium was strongly positive for IP-10 mRNA (Fig. 7D). No signal was detected using a sense probe as a control (Fig. 7C), confirming the specificity of the hybridization. Bronchial epithelium obtained from normal volunteers had little or no IP-10 signal (data not shown).

Discussion

Bronchial epithelium is an important source of mediators of lung inflammation, such as chemokines (8, 22–29, 48). Chemokines play an important role in the recruitment of leukocytes into sites of tissue inflammation. They increase leukocyte adhesion by activating specific integrins (7), and they provide a directional signal (chemical gradient) for the movement of these leukocytes into inflamed tissue (12). Among the CXC family of chemokines, IP-10, Mig, and the newly described I-TAC specifically chemoattract activated T cells through the unique chemokine receptor CXCR3 (15).

In the present study, we demonstrate that primary human bronchial epithelial cells highly express IP-10, and to a lesser extent Mig and I-TAC, in response to IFN-γ, but not in response to TNF-α or IL-1β. In addition, as has been seen in other cell types (33, 49), IL-1β and TNF-α synergized with IFN-γ in inducing IP-10 and Mig mRNA expression. Similar synergism was found with I-TAC expression. However, using the human epithelial cell lines A549 (alveolar type II phenotype) and BEAS-2B (bronchial phenotype), we found that IFN-γ, as well as TNF-α and IL-1β alone, weakly induced IP-10 but not Mig or I-TAC. This finding may reflect differences between transformed or tumor cell lines and primary cells.

We also found that significant amounts of IP-10 protein were secreted from activated bronchial epithelial cells. Greater than 800 and 500 ng/ml of IP-10 protein was detected in unconcentrated supernatants collected from NHBEC and BEAS-2B cells, respectively, activated for 32 h with IFN-γ and TNF-α. These are the highest levels reported to date for chemokine secretion from bronchial epithelial cells. In comparison, in a 24-h period, BEAS-2B treated with IFN-γ and TNF-α released 20-fold less RANTES (48), and A549 cells maximally stimulated with IL-1β secreted 10-fold less IL-8 (28) and 10,000-fold less eotaxin (26). IP-10 mRNA accumulation in bronchial epithelial cells closely reflected levels of secreted IP-10 protein, as previously shown in endothelial cells (32), suggesting that in these cells the IP-10 protein is not stored.

In contrast with IP-10, Mig, and I-TAC, IL-8 expression was weakly induced by IFN-γ but was strongly induced by TNF-α or IL-1β, confirming a previous study (28). These results show that in bronchial epithelial cells, IP-10, Mig, and I-TAC are regulated
treated for the indicated times (h) with IFN-γ from NHBEC, BEAS-2B cells, and A549 cells. Sandwich ELISA was performed to determine the levels of IP-10 in the supernatant. Three concentrations of rIP-10 were used as controls. Western blot analysis using murine anti-hIP-10 mAb (D1D2) on unconcentrated supernatants from BEAS-2B cells treated for indicated times with 100 ng/ml IFN-γ. Threshold of detection was 0.250 ng/ml. ND signifies not detected; Ctrl, control.

Differently from IL-8, this pattern of differential regulation was also seen when we explored the effects of cycloheximide, glucocorticoids, and Th2 cytokines on the expression of these four chemokines in bronchial epithelial cells.

In NHBEC, cycloheximide reduced IP-10, Mig, and I-TAC mRNA accumulation; however, in BEAS-2B and A549 cells, the effect on IP-10, Mig, and I-TAC expression was less marked. It was previously found that cycloheximide did not affect IP-10 expression on IFN-γ-treated U937 cells (30) but increased its expression in IFN-γ-induced keratinocytes (49). Therefore, the effect of cycloheximide on IP-10 expression appears to differ according to cell type. Interestingly, the effect of cycloheximide was more pronounced on I-TAC mRNA accumulation in NHBEC, suggesting that different pathways might be involved in IP-10, Mig, and I-TAC expression, even though they are all induced by IFN-γ. In contrast, cycloheximide increased IL-8 mRNA accumulation in all three cell types, suggesting again that IP-10, Mig, and I-TAC are regulated differently from IL-8.

Glucocorticoids are used in many inflammatory processes involving the lung, such as asthma and sarcoidosis. Steroids modulate cytokine gene expression and lead to an impairment of T cell function in vivo (reviewed in Ref. 50). They also down-regulate the expression of chemokines, including IL-8 (51), MCP-1 (52), RANTES (48), eotaxin (26), and MCP-4 (25). In contrast to IL-8, we found that IP-10, Mig, and I-TAC expression was not diminished by pretreatment of epithelial cells with dexamethasone.

Th2 cells secrete IL-4, IL-5, IL-10, and IL-13 and are associated with allergic and humoral-type immune responses (1). Since IL-4 down-regulates IP-10 expression in human monocytes (46) and murine macrophages (47), we asked whether IL-4, IL-13, and IL-10 would similarly modulate IP-10 and Mig expression in bronchial epithelial cells. We found that none of these cytokines affected IP-10, Mig, or I-TAC expression induced by IFN-γ or IFN-γ/TNF-α. Again in contrast, IL-8 expression was increased by IL-4 and IL-13 in TNF-α-treated NHBEC.

In addition to their differences in leukocyte selectivity and cytokine regulation, IP-10/Mig and IL-8 have opposing effects on endothelial cells. IP-10 and Mig are antiangiogenic (44, 53–56), whereas IL-8 is proangiogenic (57). This has led to speculation that the relative levels of IP-10/Mig and IL-8 may affect neovascularization in the lung following a given inflammatory process (58). For example, in idiopathic pulmonary fibrosis in which neovascularization is prevalent, pulmonary levels of IP-10 were decreased, while those of IL-8 increased compared with the levels in normal lung (58). It is interesting to note that high levels of TNF-α have been demonstrated in the lungs of patients with idiopathic pulmonary fibrosis (59–61). Our finding in bronchial epithelial cells that TNF-α was a much better inducer of IL-8 than IP-10 or Mig may explain the up-regulation of IL-8 compared with IP-10 and resultant angiogenic environment found in idiopathic pulmonary fibrosis.

Activated epithelial cells secrete high levels of IP-10 in vitro and appear to be a major source of IP-10 in vivo. IFN-γ-treated human keratinocytes secreted more IP-10 than IFN-γ-treated endothelial cells, monocytes, or fibroblasts (32). Furthermore, IP-10 was expressed in keratinocytes in psoriatic lesions, while it was not detected in normal keratinocytes (35). Similar observations were reported in fixed drug eruptions (62) and in cutaneous T-cell lymphomas (63). IFN-γ is up-regulated in these skin disorders (1, 64) and might be responsible for keratinocyte activation and IP-10 expression. Our findings extend these observations to bronchial epithelial cells, which secreted very high levels of IP-10 following IFN-γ stimulation. Furthermore, IP-10 was expressed in the bronchial epithelium in patients with active TB, in which IFN-γ and IL-12 have been shown to be up-regulated (2). IP-10 secretion from epithelial cells may play an important role in recruiting activated T cells into the epithelium, which is necessary for protective immunity at these surfaces in contact with the environment.

Table I. IP-10 mRNA expression in BAL cells

<table>
<thead>
<tr>
<th>Control (% of positive cells)</th>
<th>TB Patients (% of positive cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.5</td>
<td>25.5</td>
</tr>
<tr>
<td>7.2</td>
<td>20.7</td>
</tr>
<tr>
<td>10.5</td>
<td>16.8</td>
</tr>
<tr>
<td>12.2</td>
<td>33.7</td>
</tr>
<tr>
<td>7.0</td>
<td>29.2</td>
</tr>
<tr>
<td>5.6</td>
<td>18.4</td>
</tr>
<tr>
<td>5.0</td>
<td>15.2</td>
</tr>
<tr>
<td>7.7 ± 2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.8 ± 6.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Recovered from seven healthy control patients and seven TB patients.

<sup>b</sup> Mean ± SD.

<sup>c</sup> Significance was p < 0.001 for comparison between TB patients and control subjects using Student’s t test analysis.
IP-10 is expressed in inflamed tissues in which the Th1-type cytokine IFN-γ is up-regulated. This has been demonstrated for psoriasis (35), tuberculous leprosy (36), ulcerative colitis (65), sarcoidosis (37), and atherosclerosis (A. Sauty and F. Mach, unpublished observations), and we now present data for active TB. Interestingly, CXCR3 is found expressed predominantly on Th1 clones and on a significant fraction of memory circulating T cells (41, 42). Recent studies have implicated CXCR3+ T cells in the pathogenesis of rheumatoid arthritis (42), ulcerative colitis (40), and atherosclerosis (A. Sauty and F. Mach, unpublished observations), since the vast majority of infiltrating T cells were CXCR3+.

In addition, it has recently been shown that IP-10 selectively activated and enhanced Ag and mitogen-driven IFN-γ (but not IL-4) cytokine gene expression in T cells (66). Therefore, it is tempting to speculate that in Th1-type disease, IP-10, Mig, and I-TAC are involved in amplifying a Th1-type immune response.

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References


