Increased Expression of the Chemokine Receptor CXCR3 and Its Ligand CXCL10 in Peripheral Airways of Smokers with Chronic Obstructive Pulmonary Disease

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CXCR3 is a chemokine receptor preferentially expressed on lymphocytes, particularly on type-1 T-lymphocytes. Smokers who develop chronic obstructive pulmonary disease (COPD) have a chronic bronchopulmonary inflammation that is characterized by an increased infiltration of T-lymphocytes, particularly CD8$^+$, in the airways and lung parenchyma. To investigate the expression of CXCR3 and its ligand interferon-induced protein 10/CXCL10 in COPD, we counted the number of CXCR3$^+$ cells and analyzed the expression of CXCL10 in the peripheral airways of 19 patients undergoing lung resection for localized pulmonary lesions. We examined lung specimens from seven smokers with fixed airflow limitation (COPD), five smokers with normal lung function, and seven nonsmoking subjects with normal lung function. The number of CXCR3$^+$ cells was immunohistochemically quantified in the epithelium, in the submucosa, and in the adventitia of peripheral airways. The number of CXCR3$^+$ cells in the epithelium and submucosa was increased in smokers with COPD as compared with nonsmoking subjects, but not as compared with smokers with normal lung function. Immunoreactivity for the CXCR3-ligand CXCL10 was present in the bronchiolar epithelium of smokers with COPD but not in the bronchiolar epithelium of smoking and nonsmoking control subjects. Most CXCR3$^+$ cells coexpressed CD8 and produced interferon γ. These findings suggest that the CXCR3/CXCL10 axis may be involved in the T cell recruitment that occurs in peripheral airways of smokers with COPD and that these T cells may have a type-1 profile.

Keywords: inflammation; type-1 lymphocytes; airflow limitation; smoking

Smokers with chronic obstructive pulmonary disease (COPD) have a chronic bronchopulmonary inflammation characterized by an increased infiltration of T-lymphocytes, particularly CD8$^+$, both in the airways and in the lung parenchyma (1, 2). The peripheral airways are the major site responsible for the development of airflow limitation in smokers (3). Interestingly, in smokers, the number of CD8$^+$ T-lymphocytes in peripheral airways is significantly correlated with the degree of airflow limitation, suggesting an important role for these cells in the pathophysiology of COPD (4). However, the cytokine profile of these T-lymphocytes and their chemokine receptor expression has not been fully investigated.

A current paradigm in immunology is that the nature of an immune response to an antigenic stimulus is determined largely by the pattern of cytokines produced by activated T cells (5). Type-1 T cells express cytokines, such as interferon gamma (IFNγ), crucial in the activation of macrophages and in the response to viral and bacterial infections, whereas type-2 T cells express cytokines, such as interleukin (IL)-4 and IL-5, involved in IgE-mediated responses and eosinophilia characteristic of allergic diseases. A type-1 profile has been hypothesized in COPD, particularly because viral and/or bacterial infections have been associated with the development of the disease (2, 6). Indeed, a type-1 cytokine phenotype has been demonstrated in peripheral blood (7) and qualitatively described in bronchial biopsies of patients with COPD (8).

Several *in vitro* studies have demonstrated that type-1 and type-2 T cells express distinct sets of chemokine receptors, which regulate the recruitment of these T-lymphocytes subsets to inflammatory sites (9–11). In particular, it has been shown that the chemokine receptor CXCR3 is preferentially expressed on type-1 cells, which then selectively migrate to its ligand CXCL10 (12, 13).

As chemokines play an important role in the recruitment of leukocytes to sites of tissue inflammation, in this study we investigated the expression of CXCR3 and its ligand CXCL10 in the peripheral airways of smokers with chronic airflow limitation. The results were compared with those obtained in smokers without chronic airflow limitation and in normal nonsmoking control subjects.

METHODS

Subjects

We recruited three groups of subjects to the study who were undergoing lung resection for a solitary peripheral carcinoma: seven smokers with both symptoms of chronic bronchitis and chronic airflow limitation (smokers with COPD), five asymptomatic smokers with normal lung function, and seven asymptomatic nonsmoking subjects with normal lung function. Chronic bronchitis was defined as cough and sputum production occurring on most days of the month for at least 3 months a year, during the 2 years before the study (1). Chronic airflow limitation was defined as forced expiratory volume in 1 second (FEV_1)/forced vital capacity (FVC) less than 70% associated with a FEV_1 less than 80% predicted (1), with a FEV_1 reversibility of less than 15% after inhalation of 200 μg of salbutamol. No subject had an exacerbation of his disease within 1 month of surgery.

All the subjects had been free of acute upper respiratory tract infections and none had received glucocorticoids or antibiotics within the month preceding surgery, or bronchodilators within the previous 48 hours. They were nonatopic (i.e., they had negative skin tests for common allergen extracts) and had no past history of asthma or allergic rhinitis.

The study conformed to the Declaration of Helsinki, and informed written consent was obtained for each subject undergoing surgery. Each patient underwent an interview, a chest radiography, an electrocardiography, routine blood tests, skin tests with common allergen extracts, and pulmonary function tests in the week before surgery.

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Pulmonary function tests. Pulmonary function tests were performed as described previously (14). Briefly, they included measurements of blood gas analysis, FEV_1, and FVC. The predicted normal values used were those from Communité Europeenne du Carbon e de l'Acier (15). To assess the reversibility of the airway obstruction in subjects with a baseline FEV_1 less than 80% predicted, the FEV_1 measurement was repeated 15 minutes after the inhalation of 200 μg of salbutamol.

Immunohistochemistry

Four to six randomly selected tissue blocks (template size 1 × 2 × 2 cm) were taken from the subpleural parenchyma of the lobe obtained at surgery, avoiding areas involved by tumor. Samples were fixed without inflation in 4% formaldehyde in phosphate-buffered saline (PBS) at pH 7.2 and, after dehydration, embedded in paraffin wax. Tissue specimens were oriented, and 5-μm–thick sections were cut for immunohistochemical analysis.

Immunohistochemical analysis of $CXCR3^+$ cells. Sections were deparaffinated in xylene, hydrated through an alcohol series, washed twice in PBS, incubated in 0.3% H_2O_2/PBS to quench endogenous peroxidase activity, and then blocked with 10% normal goat serum/PBS (blocking solution). The slides were then incubated overnight at 4° C with the primary mouse monoclonal antibody anti-CXCR3 (R&D Systems Inc., Minneapolis, MN) diluted in blocking solution in a humidified chamber. After five washes with PBS, sections were incubated with a goat anti-mouse biotinylated secondary antibody (Vector Laboratories Inc., Burlingame, CA) followed by a preformed peroxidase conjugated avidin–biotin complex (Vectastain ABC Elite kit; Vector). The staining was revealed with the Vector SG (Vector) as a substrate for peroxidase. Nuclei were counterstained with Vector Nuclear Fast Red (Vector), and then sections were dehydrated through an alcohol series and permanently mounted with Vectamount mounting medium (Vector). Negative controls were performed omitting the primary antibody or using an isotype control antibody from the same species.

Analysis of peripheral airways was performed using a light microscope (Leica DMLB; Leica, Cambridge, UK) connected to a video recorder linked to a computerized image system (Software: Casti Imaging SC processing; Casti Imaging, Venice, Italy). The cases were coded, and the measurements made without knowledge of clinical data.

From 3 to 12 noncartilaginous peripheral airways with an internal perimeter less than 6 mm were examined for each patient. To avoid measurements in longitudinally cut airways, bronchioles with a short/long diameter ratio less than one-third were excluded from the study. In each airway we measured the internal perimeter along the subepithelial basement membrane, and we quantified $CXCR3^+$ cells in the epithelium, in the submucosa, and in the adventitia. The submucosa was defined as the area that extends from the distal edge of the basement membrane to the internal edge of the smooth muscle, whereas the adventitia was defined as the area that extends from the outer edge of the smooth muscle to the alveolar attachments (16). For each airway, all the $CXCR3^+$ cells present in the epithelium, submucosa, and adventitia were counted and expressed as number of cells per unit volume (mm^3) in the three compartments. The volume of epithelium, submucosa, and adventitia was determined first by measuring the area of each compartment and then by converting the area to volume by multiplying by the thickness of the section (5 μm), according to the method described by O'Byrne and coworkers (17). Then, to overcome the problem that the diameter of the $CXCR3^+$ cells (5.63 ± 1.09 μm) was greater than the thickness of the section (5 μm), we corrected the values of the cell counts by a factor of 0.458, calculated using a method described previously (18).

Sequential sections and double immunofluorescent staining. Examination of sequential sections and double staining were performed in a subgroup of three smokers with COPD chosen because of their high number of $CXCR3^+$ cells. The purpose of this was to determine whether $CXCR3^+$ cells colocalized to CD3, CD4, and/or $CD8^+$ cells and whether they coexpressed IFNγ.

Groups of three serial sections for each patient were stained with the following mouse anti-human antibodies: anti-CD4 (Dako SpA, Milan, Italy), anti-CXCR3, and anti-CD8 (Dako). A 30-minute pretreatment of sections with an antigen retrieval solution (Dako) was used for CD8 staining. The immunoreactivity was revealed as described in the previous paragraph, and colocalization of the staining was confirmed by analyzing the same cell in CD4 and CXCR3-stained sequential sections or in CD8 and CXCR3–stained sequential sections.

To determine whether $CXCR3^+$ cells colocalized to CD3 and whether they coexpressed IFNγ, double immunofluorescent staining was performed (8). After hydration, slides were post-fixed with 4% paraformaldehyde in PBS, washed twice in PBS, and blocked first with 0.1% glycine/PBS and then with 15% fetal bovine serum /PBS (blocking solution). After five washes with wash buffer (0.45 M NaCl, 0.24 M Na_2HPO_4, 0.3% Triton X-100), the slides were incubated overnight at 4° C with the primary antibodies mouse anti-CXCR3 and rabbit anti-CD3 (Dako) or mouse anti-CXCR3 and goat anti-IFNγ (R&D Systems) diluted in blocking buffer in a humidified chamber. The slides were washed again five times with wash buffer and incubated with the fluorochrome (Rhodamine Red-X or Cy2)-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) diluted in blocking buffer for 1 hour at room temperature. After 5 washes in wash buffer and 2 in PBS, slides were mounted with 90% glycerol/PBS. Control stainings using only one primary antibody and both secondary antibodies in every possible combination, and negative controls, omitting both primary antibodies, were performed. Once mounted, slides were analyzed with a confocal microscope (MRC-1024; BioRad Laboratories) equipped with a 15-mW krypton/argon laser emitting at 488 and 647 nm, mounted on a Nikon Eclipse E600 microscope. Images were acquired and analyzed with Laser Sharp 3.2 software.

Immunohistochemical analysis of $CXCL10^+$ cells. Immunohistochemical analysis of $CXCL10^+$ cells was performed with a rabbit anti-human CXCL10 antibody (Peprotech EC, London, UK) in sections obtained from all the subjects of the study: seven smokers with both symptoms of chronic bronchitis and chronic airflow limitation, five asymptomatic smokers with normal lung function, and seven asymptomatic nonsmoking control subjects with normal lung function. After overnight incubation with the primary antibody, immunoreactivity was determined as described previously with a biotinylated goat anti-rabbit antibody followed by Vectastain ABC Elite kit (Vector Laboratories) and Nuclear Fast Red (Vector Laboratories) counterstaining. Controls were performed as described previously.

The presence or absence of CXCL10 expression was evaluated in peripheral airways and in pulmonary arteries.

Statistical Analysis

Group data were expressed as means and standard errors of the mean or as medians and ranges when appropriate. Analysis of variance for clinical data and the Kruskall–Wallis test for histologic data were used to determine differences between groups. The Mann–Whitney *U* test was performed after the Kruskall–Wallis test when appropriate. Spearman's rank correlation test was used to examine the association between immunohistochemical measurements and clinical data. Probability values of 0.05 or less were accepted as significant. At least 3 replicate measurements of $CXCR3^+$ cells were performed by the same observer in 10 randomly selected slides, and the intraobserver reproducibility was assessed with the coefficient of variation and with the intraclass correlation coefficient. The intraobserver coefficient of variation was 7%, and the intraobserver correlation coefficient was 0.87.

TABLE 1. CHARACTERISTICS OF THE SUBJECTS

	Nonsmokers	Smokers With Normal Lung Function	Smokers With COPD
Subjects examined, n, sex	1M:6F	5M	7M
Age, yr	63 ± 5	66 ± 2	70 ± 3
Smoking starting age, yr	—	20 ± 2	16 ± 1
Smoking history, packs-yr	—	42 ± 8	56 ± 8
FEV_1, % of predicted	109 ± 7	100 ± 4	65 ± 3*
FEV_1/FVC, %	81 ± 1	76 ± 3	63 ± 2*
Pa_{O_2}, mm Hg	87 ± 8	88 ± 4	79 ± 2
Pa_{CO_2}, mm Hg	38 ± 1	39 ± 1	42 ± 2

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity.

Values are expressed as means ± SEM.

* Significantly different from nonsmokers and from smokers with normal lung function ($p < 0.01$).

TABLE 2. $CXCR3^+$ CELLS IN SMOKERS WITH COPD, SMOKERS WITH NORMAL LUNG FUNCTION, AND NONSMOKERS

	Nonsmokers (*n* = 7)	Smokers With Normal Lung Function (*n* = 5)	Smokers With COPD (*n* = 7)
$CXCR3^+$ cells in the epithelium, 10^3 cells/mm^3*	0 (0–1.5)	0 (0–28.4)	1.7 (0–21)†
$CXCR3^+$ cells in the submucosa, 10^3 cells/mm^3*	9.7 (0.9–31.9)	34.8 (5.5–50.1)	62.8 (4.8–104.2)†
$CXCR3^+$ cells in the adventitia, 10^3 cells/mm^3*	21.1 (9.5–56.9)	24.2 (1.6–57.7)	35.2 (6.1–81.0)
Airways examined per patient‡	7.00 ± 0.06	9.80 ± 0.03	6.14 ± 0.04

Definition of abbreviation: COPD = chronic obstructive pulmonary disease.
* Results are expressed as medians with ranges shown in parentheses.
† Significantly different from nonsmokers ($p < 0.05$).
‡ Results are expressed as mean ± SEM.

RESULTS

Clinical Findings

Table 1 shows the characteristics of the subjects examined. The three groups of subjects were similar with regard to age, Pa_{O_2}, and Pa_{CO_2} values. There was no significant difference in smoking history between smokers with COPD and smokers with normal lung function. In smokers with COPD, whose FEV_1 ranged from 54 to 78% predicted, the average response to bronchodilator was 5%.

Immunohistochemical Findings

The average number of airways examined per patient was 6.14 ± 0.04 in smokers with COPD, 9.80 ± 0.03 in smokers with normal lung function, and 7.00 ± 0.06 in nonsmokers, with no statistical difference between groups (Table 2). Because the internal perimeter has been shown to remain constant despite changes in lung volume due to different degrees of inflation and in smooth muscle tone (19), we used the internal perimeter as a marker of airway size. The airway internal perimeter was similar in smokers with COPD (median, range: 1,747, 1,471–1,997 μm), smokers with normal lung function (2,052, 1,215–2,225 μm), and nonsmokers (1,570, 1,044–2,189 μm), indicating that, despite the possible different lung volumes caused by tissue preparation and the possible different smooth muscle tone in the three groups of subjects, we compared airways of similar size.

As compared with nonsmoking subjects, in the epithelium there was a significant increase in the number of $CXCR3^+$ cells in smokers with COPD ($p = 0.02$) but not in smokers with normal lung function. In the submucosa $CXCR3^+$ cells showed a trend to increase in smokers with normal lung function ($p = 0.06$), and this trend became statistically significant in smokers with COPD ($p = 0.02$) (Figures 1 and 2). In the adventitia there were no significant differences between smokers with COPD, smokers with normal lung function, and nonsmoking subjects (Table 2).

Examination of sequential sections and double staining of selected specimens showed that, in smokers with COPD, CXCR3 immunoreactivity colocalized to $CD3^+$ and $CD8^+$ but not to $CD4^+$ cells (Figures 3 and 4). Furthermore, $CXCR3^+$ cells coexpressed IFNγ (Figure 3). In the three cases in which we performed sequential sections, the proportion of $CD8^+$ cells expressing CXCR3 was 29.4%, 41.4%, and 23.7%, with an average value of 31.5 ± 5.2%.

When all the smokers (those with COPD and those with normal lung function) were considered together, they showed an increased number of $CXCR3^+$ cells in both epithelium (median and range: 0.37 and 0–28.4 versus 0 and 0–1.5 × 10^3 cells/mm^3; $p = 0.05$) and submucosa (35.4 and 4.8–104.2 versus 9.7 and 0.9–31.9 × 10^3 cells/mm^3; $p = 0.01$) as compared with nonsmokers. No significant differences were observed in the adventitia between smokers and nonsmokers (33.6 and 1.6–80.9 versus 21.1 and 9.5–56.9 × 10^3 cells/mm^3).

In this study we did not measure the number of $CXCR3^+$ cells in the lung parenchyma, but we qualitatively observed the presence of a clear CXCR3 immunoreactivity in the lung parenchyma of three out of seven smokers with COPD, but not in the lung parenchyma of smoking and nonsmoking control subjects.

When all the subjects (smokers with COPD, smokers with normal lung function, and nonsmokers) were grouped together,

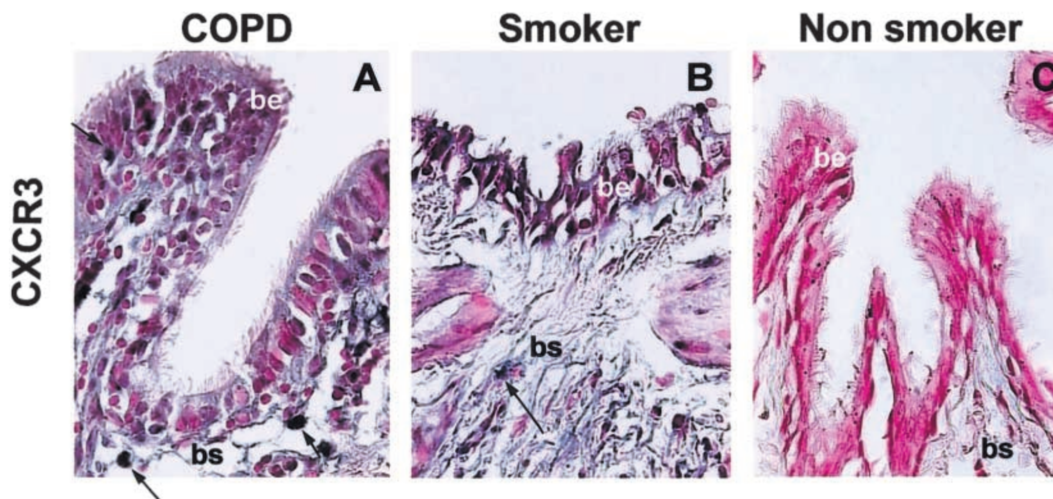

Figure 1. Expression of CXCR3 in the bronchiolar epithelium (*be*) and in the bronchiolar submucosa (*bs*) of a smoker with COPD (*A*), a smoker with normal lung function (*B*), and a nonsmoking subject with normal lung function (*C*). *Arrows* indicate $CXCR3^+$ cells. Nuclei were counterstained with Nuclear Fast Red. Original magnification: ×40.

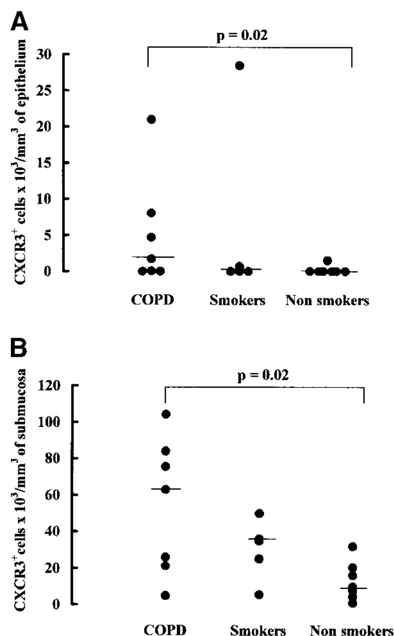

Figure 2. Individual counts for CXCR3$^+$ cells in the bronchiolar epithelium (*A*) and submucosa (*B*) of peripheral airways in smokers with COPD (COPD), smokers with normal lung function (*smokers*), and nonsmoking subjects with normal lung function (*non smokers*). The results are expressed as number of cells $\times$ 10^3 per cubic millimeter of tissue examined. *Horizontal bars* represent median values. Differences between groups were analyzed using the Kruskall–Wallis test and the Mann–Whitney *U* test.

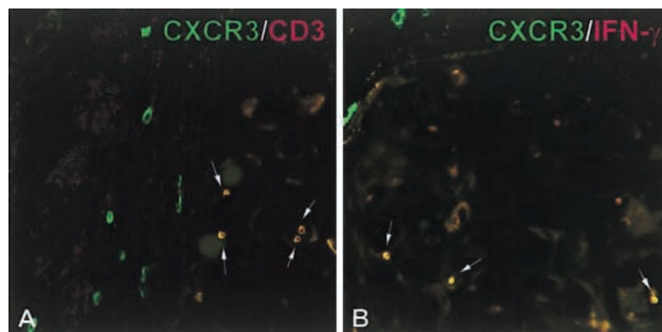

Figure 3. Double immunofluorescence staining with anti-CXCR3 monoclonal antibody (*green*) and anti-CD3 polyclonal antibody (*red*) (*A*), and with anti-CXCR3 monoclonal antibody (*green*) and anti-IFNγ polyclonal antibody (*red*) (*B*), in the bronchiolar submucosa of a smoker with COPD. *Arrows* indicate double positive T-lymphocytes (*yellow*). Original magnification: ×40.

the value of FEV_1/FVC (%) showed a negative correlation with the number of CXCR3$^+$ cells in the epithelium ($r = -0.67$; p = 0.001) and in the submucosa ($r = -0.63$; p = 0.003) (Figure 5). This latter correlation remained significant when only smokers with COPD were considered ($r = -0.81$; p = 0.02). When all the subjects were grouped together, the value of FEV_1 (% predicted) also showed a negative correlation with the number of CXCR3$^+$ cells in the submucosa ($r = -0.58$; p = 0.01), but not in the epithelium.

Immunohistochemical staining with an anti-CXCL10 antibody revealed a strong expression of this chemokine in peripheral airway epithelium of smokers with COPD. In these subjects, CXCL10 was also expressed in the wall, and particularly in the medial layer, of pulmonary arteries. In contrast to COPD, CXCL10 was not expressed on peripheral airways or on pulmonary arteries of smoking and nonsmoking control subjects (Figure 6).

DISCUSSION

This study shows that the T cells infiltrating the peripheral airways of smokers with COPD have an increased expression of the chemokine receptor CXCR3, which is paralleled by a strong epithelial expression of its ligand CXCL10.

We have shown previously that smokers with COPD have a chronic inflammatory process in peripheral airways and that this inflammatory process is characterized by an increased infiltration of T-lymphocytes, particularly CD8$^+$ (4). However, the cytokine profile of these T-lymphocytes and their chemokine receptor expression were not fully investigated.

The fact that smokers with COPD have an increased number of CXCR3$^+$ cells in peripheral airways and that these cells are CD8-positive and produce IFNγ confirms and extends the previous observation of a predominant CD8$^+$ (20) type-1 (8) response in the central airways of smokers with chronic airflow limitation.

The tissue localization of the CXCR3$^+$ T-cells in peripheral airways revealed that smokers with COPD had an increased expression of the chemokine receptor in the epithelium and in the submucosa, but not in the adventitia, suggesting that different microenvironments within the airway wall may influence the inflammatory response. Epithelium and submucosa are closer to the airway lumen, where inhaled agents may trigger an inflammatory response in the adjacent tissue.

In COPD, CXCR3 expression was paralleled by a strong epithelial expression of its ligand CXCL10, suggesting that the CXCR3/CXCL10 axis may be involved in the recruitment of type-1 T cells in peripheral airways of smokers with COPD. Interestingly, a strong expression of CXCL10 was also present in pulmonary arteries, which have been shown previously to be infiltrated by T cells in this disease (21). In contrast to COPD, CXCL10 was not expressed on peripheral airways or on pulmonary arteries of smoking and nonsmoking control subjects.

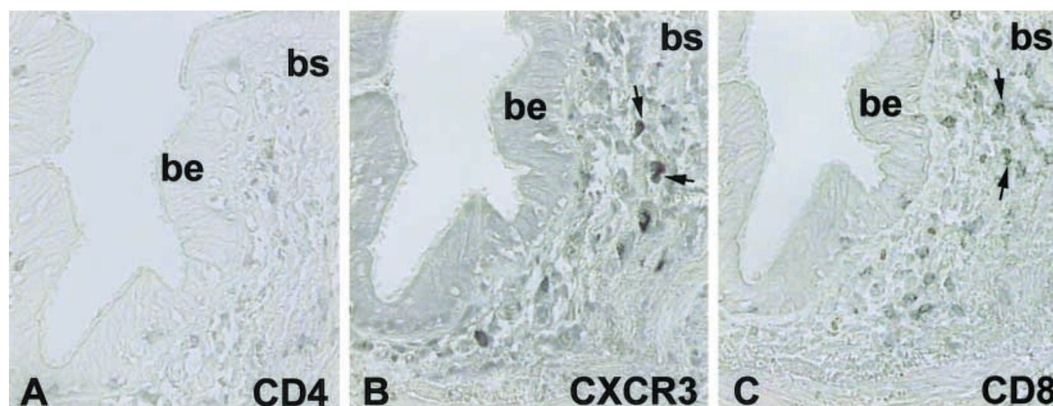

Figure 4. Immunohistochemical staining with anti-CD4 (*A*), anti-CXCR3 (*B*), and anti-CD8 (*C*) monoclonal antibodies on serial sections of a peripheral airway from a smoker with COPD. *Arrows* indicate CXCR3$^+$ and CD8$^+$ cells. *be*, bronchiolar epithelium; *bs*, bronchiolar submucosa. Original magnification: ×40.

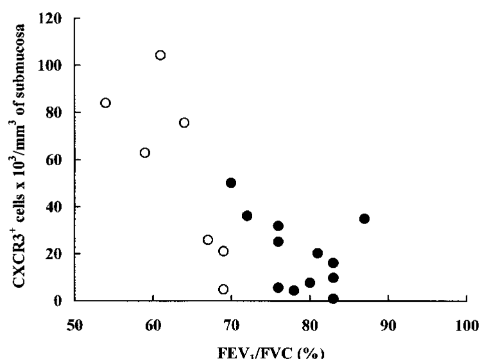

Figure 5. Relationship between the number of $CXCR3^+$ cells infiltrating the submucosa of peripheral airways and the value of FEV_1/FVC in all the subjects of the study ($r = -0.63$; $p = 0.003$). This relationship remained significant when only smokers with COPD (*open circles*) were considered ($r = -0.81$; $p = 0.02$).

Because CXCL10 expression can be induced and upregulated by IFNγ (22), our results may suggest a possible mechanism by which the type-1 inflammatory response in the lung of patients with COPD is maintained and amplified. A currently unidentified initiating inflammatory event could cause airway resident cells to release CXCL10, which recruits CXCR3-bearing type-1 T-lymphocytes. These cells, through the production of IFNγ, could further upregulate CXCL10 production, thus establishing a mechanism for the amplification and maintenance of a type-1 dominated inflammatory response in the lung.

Type-1 T-lymphocytes are crucial in the response to viral infections (23), and viral infections are a frequent occurrence in patients with COPD. It is possible that an excessive recruitment of type-1 T-lymphocytes may occur in response to repeated viral infections in some smokers, leading to lung damage in susceptible individuals (20). This hypothesis is supported by the observation that a latent adenoviral infection can be found in smokers who develop COPD (6). A viral infection, through the activation of IFNγ-producing T cells, could indeed sustain and amplify the inflammatory process present in lungs of these subjects. On the other hand, it is also possible that type-1 T-lymphocytes are able to damage the lung even in the absence of a stimulus such as viral infection, as shown by Enelow and coworkers (24), who clearly demonstrated that recognition of a lung "autoantigen" by T-lymphocytes may directly produce a marked lung injury. These findings suggest that the type-1 cell accumulation observed in COPD could be a response to an "autoantigenic" stimulus originating in the lung and induced by cigarette smoking.

Our results of a predominant type-1 response in COPD may appear to be in contrast with the eosinophilia and the "allergic profile" recently reported in some smokers with chronic bronchitis (25), particularly during an exacerbation of the disease (14, 26). A possible explanation for this discrepancy is that COPD is a heterogeneous disease that includes a subgroup of patients characterized by "asthmatic features," such as airway eosinophilia. Whether this subset of patients with COPD has a different disease or a different stage of the same disease still remains to be investigated.

At variance with the airway wall, where the T-lymphocytes predominate, in the airway lumen of smokers with COPD the neutrophil is the predominant cell (27). Notably, neutrophils have been shown to produce, among other chemokines, all the three ligands for CXCR3: CXCL10, monokine induced by IFNγ/CXCL9, and IFNγ-inducible T cell chemoattractant/CXCL11 (28, 29). These observations suggest that neutrophils might also contribute to the initial chemotactic signals that participate in the tissue-specific homing of type-1 T-lymphocytes. This scenario is supported by a recent *in vivo* study showing that depletion of neutrophils in a mouse model of *Chlamydia* infection results in a severe reduction in the number of type-1 $CD8^+$ T cells and in a delay in the resolution of the infection (30).

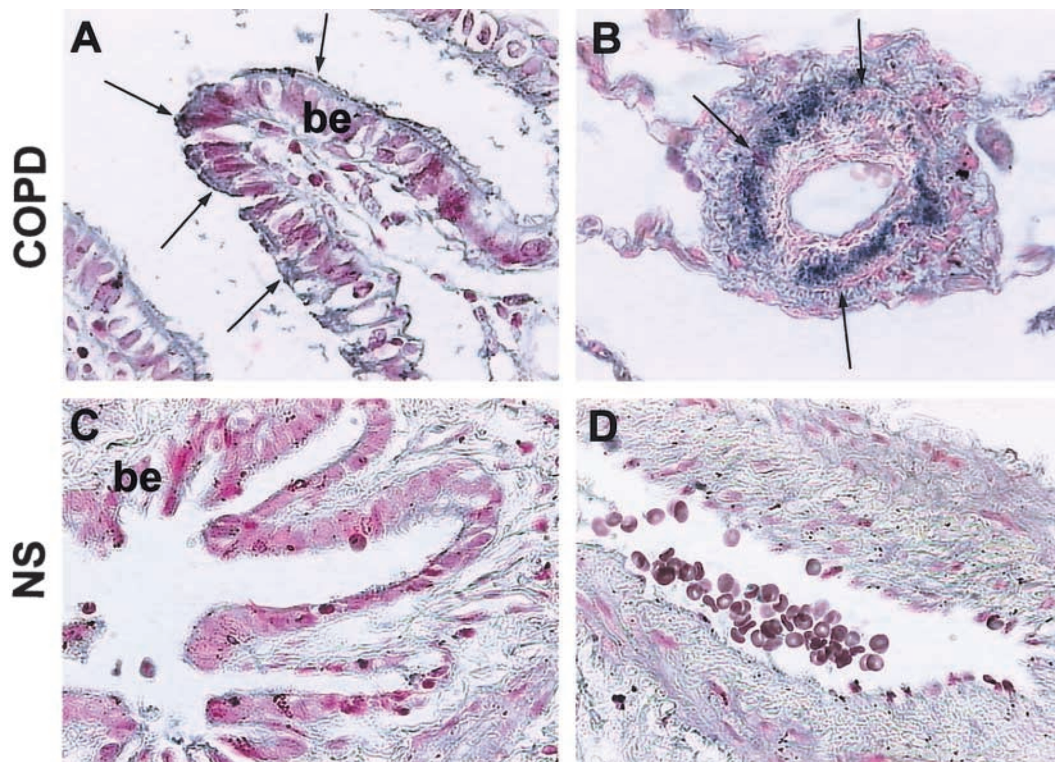

Figure 6. Immunohistochemical staining with anti-CXCL10 monoclonal antibody of a peripheral airway and of a pulmonary artery from a smoker with COPD (*A* and *B*, respectively) and from a nonsmoking subject (*C* and *D*, respectively). *Arrows* indicate CXCL10 immunoreactivity. *be*, bronchiolar epithelium; *COPD*, smoking subject with COPD; *NS*, nonsmoking subject. Original magnification: ×40.

An interesting finding of our study is the significant correlation between increased CXCR3 expression and reduced expiratory flow (Figure 5). An *r* value of 0.63 (which implies an r^2 value of 0.40) indicates that the number of $CXCR3^+$cells only explains a relatively small proportion of the variance of airflow limitation. However, when we restricted the analysis only to smokers with COPD, the *r* value increased to 0.81, suggesting a stronger relationship between $CXCR3^+$ cells and degree of airflow limitation when the disease is established. Further studies are needed to clarify this point because we are well aware that a correlation does not imply a cause–effect relationship, but just suggests a possible role of $CXCR3^+$ cells in the pathogenesis of smoking-induced airflow limitation.

A confusing element in any study performed on surgically resected specimens of patients with lung cancer is that the presence of cancer itself may influence the results. However, surgical specimens are the only specimens that allow for the examination of peripheral airways in patients who are well characterized in terms of pulmonary function, and peripheral airways are the major site responsible for the development of chronic airflow limitation in smokers. Moreover, as a result of our having examined only tissue away from the tumor site, and having included subjects with lung cancer in the control groups, we feel rather confident that our finding of an increased CXCR3 expression in the peripheral airways of smokers with COPD is valid.

In conclusion, this study shows that the T cells infiltrating the peripheral airways of smokers with COPD have an increased expression of the chemokine receptor CXCR3, which is paralleled by a strong epithelial expression of its ligand CXCL10. These findings suggest that the CXCR3–CXCL10 axis may be involved in the T cell recruitment that occurs in the peripheral airways of smokers with COPD and that these T cells may have a type-1 profile. Taken in their entity, these data imply specific roles for CXCR3 and CXCL10 in the inflammatory process underlying COPD and suggest that these molecules may serve as targets for novel therapeutic strategies.

References

1. Pauwels RA, Buist AS, Calverley PM, Jenkins CR, Hurd SS. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) Workshop summary. *Am J Respir Crit Care Med* 2001;163:1256–1276.
2. Saetta M, Turato G, Maestrelli P, Mapp CE, Fabbri LM. Cellular and structural bases of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2001;163:1304–1309.
3. Hogg JC, Macklem PT, Thurlbeck WM. Site and nature of airway obstruction in chronic obstructive lung disease. *N Engl J Med* 1968;278: 1355–1360.
4. Saetta M, Di Stefano A, Turato G, Facchini FM, Corbino L, Mapp CE, Maestrelli P, Ciaccia A, Fabbri LM. CD8+ T-lymphocytes in peripheral airways of smokers with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1998;157(3 Pt 1):822–826.
5. Romagnani S. The Th1/Th2 paradigm. *Immunol Today* 1997;18:263–266.
6. Retamales I, Elliott WM, Meshi B, Coxson HO, Pare PD, Sciurba FC, Rogers RM, Hayashi S, Hogg JC. Amplification of inflammation in emphysema and its association with latent adenoviral infection. *Am J Respir Crit Care Med* 2001;164:469–473.
7. Majori, M, Corradi M, Caminati A, Cacciani G, Bertacco S, Pesci A. Predominant TH1 cytokine pattern in peripheral blood from subjects with chronic obstructive pulmonary disease. *J Allergy Clin Immunol* 1999;103(3 Pt 1):458–462.
8. Panina-Bordignon P, Papi A, Mariani M, Di Lucia P, Casoni G, Bellettato C, Buonsanti C, Miotto D, Mapp C, Villa A, *et al.* The C–C chemokine receptors CCR4 and CCR8 identify airway T cells of allergen-challenged atopic asthmatics. *J Clin Invest* 2001;107:1357–1364.
9. D'Ambrosio D, Mariani M, Panina-Bordignon P, Sinigaglia F. Chemokines and their receptors guiding T lymphocyte recruitment in lung inflammation. *Am J Respir Crit Care Med* 2001;164:1266–1275.
10. Baggiolini M. Chemokines and leukocyte traffic. *Nature* 1998;392:565–568.
11. Luster AD. Chemokines–chemotactic cytokines that mediate inflammation. *N Engl J Med* 1998;338:436–445.
12. Bonecchi R, Bianchi G, Bordignon PP, D'Ambrosio D, Lang R, Borsatti A, Sozzani S, Allavena P, Gray PA, Mantovani A, *et al.* Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. *J Exp Med* 1998;187:129–134.
13. Sallusto F, Lenig D, Mackay CR, Lanzavecchia A. Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes. *J Exp Med* 1998;187:875–883.
14. Saetta M, Di Stefano A, Maestrelli P, Turato G, Ruggieri MP, Roggeri A, Calcagni P, Mapp CE, Ciaccia A, Fabbri LM. Airway eosinophilia in chronic bronchitis during exacerbations. *Am J Respir Crit Care Med* 1994;150(6 Pt 1):1646–1652.
15. CECA. Aide-memoire of spirographic practice for examining ventilatory function, 2nd ed. Industrial Health and Medicine, Luxembourg; 1971.
16. Saetta, M, Turato G, Baraldo S, Zanin A, Braccioni F, Mapp CE, Maestrelli P, Cavallesco G, Papi A, Fabbri LM. Goblet cell hyperplasia and epithelial inflammation in peripheral airways of smokers with both symptoms of chronic bronchitis and chronic airflow limitation. *Am J Respir Crit Care Med* 2000;161(3 Pt 1):1016–1021.
17. O'Byrne PM, Walters EH, Gold BD, Aizawa HA, Fabbri LM, Alpert SE, Nadel JA, Holtzman MJ. Neutrophil depletion inhibits airway hyperresponsiveness induced by ozone exposure. *Am Rev Respir Dis* 1984;130:214–219.
18. Smolen AJ, Wright LL, Cunningham TJ. Neuron numbers in the superior cervical sympathetic ganglion of the rat: a critical comparison of methods for cell counting. *J Neurocytol* 1983;12:739–750.
19. James AL, Hogg JC, Dunn LA, Pare PD. The use of the internal perimeter to compare airway size and to calculate smooth muscle shortening. *Am Rev Respir Dis* 1988;138:136–139.
20. O'Shaughnessy TC, Ansari TW, Barnes NC, Jeffery PK. Inflammation in bronchial biopsies of subjects with chronic bronchitis: inverse relationship of CD8+ T lymphocytes with FEV1. *Am J Respir Crit Care Med* 1997;155:852–857.
21. Saetta M, Baraldo S, Corbino L, Turato G, Braccioni F, Rea F, Cavallesco G, Tropeano G, Mapp CE, Maestrelli P, *et al.* CD8+ve cells in the lungs of smokers with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1999;160:711–717.
22. Taub DD, Lloyd AR, Conlon K, Wang JM, Ortaldo JR, Harada A, Matsushima K, Kelvin DJ, Oppenheim JJ. Recombinant human interferon-inducible protein 10 is a chemoattractant for human monocytes and T lymphocytes and promotes T cell adhesion to endothelial cells. *J Exp Med* 1993;177:1809–1814.
23. Whitmire JK, Ahmed R. Costimulation in antiviral immunity: differential requirements for CD4(+) and CD8(+) T cell responses. *Curr Opin Immunol* 2000;12:448–455.
24. Enelow RI, Mohammed AZ, Stoler MH, Liu AN, Young JS, Lou YH, Braciale TJ. Structural and functional consequences of alveolar cell recognition by CD8(+) T lymphocytes in experimental lung disease. *J Clin Invest* 1998;102:1653–1661.
25. Brightling CE, Monteiro W, Ward R, Parker D, Morgan MD, Wardlaw AJ, Pavord ID. Sputum eosinophilia and short-term response to prednisolone in chronic obstructive pulmonary disease: a randomised controlled trial. *Lancet* 2000;356:1480–1485.
26. Zhu J, Qiu YS, Majumdar S, Gamble E, Matin D, Turato G, Fabbri LM, Barnes N, Saetta M, Jeffery PK. Exacerbations of bronchitis: bronchial eosinophilia and gene expression for interleukin-4, interleukin-5, and eosinophil chemoattractants. *Am J Respir Crit Care Med* 2001;164:109–116.
27. Keatings VM, Collins PD, Scott DM, Barnes PJ. Differences in interleukin-8 and tumor necrosis factor-alpha in induced sputum from patients with chronic obstructive pulmonary disease or asthma. *Am J Respir Crit Care Med* 1996;153:530–534.
28. Cassatella MA, Gasperini S, Calzetti F, Bertagnin A, Luster AD, McDonald PP. Regulated production of the interferon-gamma-inducible protein-10 (IP-10) chemokine by human neutrophils. *Eur J Immunol* 1997;27:111–115.
29. Gasperini S, Marchi M, Calzetti F, Laudanna C, Vicentini L, Olsen H, Murphy M, Liao F, Farber J, Cassatella MA. Gene expression and production of the monokine induced by IFN-gamma (MIG), IFN-inducible T cell alpha chemoattractant (I-TAC), and IFN-gamma-inducible protein-10 (IP-10) chemokines by human neutrophils. *J Immunol* 1999;162:4928–4937.
30. de Oca RM, Buendia AJ, Del Rio L, Sanchez J, Salinas J, Navarro JA. Polymorphonuclear neutrophils are necessary for the recruitment of CD8(+) T cells in the liver in a pregnant mouse model of Chlamydophila abortus (Chlamydia psittaci serotype 1) infection. *Infect Immun* 2000;68:1746–1751.