Airway inflammation in severe COPD. Relationship with lung function and radiologic emphysema.

Airway Inflammation in Severe Chronic Obstructive Pulmonary Disease

Relationship with Lung Function and Radiologic Emphysema

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The lung pathology of severe chronic obstructive pulmonary disease (COPD) has been poorly investigated. We examined surgical specimens obtained from patients with severe (forced expiratory volume in 1 second [FEV_1] = 29 ± 3% predicted, n = 9) or mild/no airflow limitation (FEV_1 = 86 ± 5% predicted, n = 9) and similar smoking history. With histochemical and immunohistochemical methods we quantified the structural changes and the inflammatory cells in small airways and in muscular pulmonary arteries. As compared with smokers with mild/no COPD, smokers with severe COPD had an increased number of leukocytes in the small airways, which showed a positive correlation with the radiologic score of emphysema and with the value of residual volume, and a negative correlation with the values of FEV_1 and carbon monoxide diffusing capacity. The inflammatory process was characterized by an increase in $CD8^+$ and $CD4^+$ T-lymphocytes in the airway wall and by an increase in macrophages in the airway epithelium. When all smokers were considered together, the smoking history was correlated with both the airway wall and smooth muscle thickness, suggesting that smoking itself may play a role in the development of structural changes. No structural and cellular differences were observed in pulmonary arteries between smokers with severe COPD and smokers with mild/no COPD. In conclusion, in the small airways of smokers with severe COPD, there is an increased number of leukocytes, which is correlated with reduced expiratory flow, lung hyperinflation, carbon monoxide diffusion impairment, and radiologic emphysema, suggesting a role for this inflammatory response in the clinical progression of the disease.

Keywords: airway inflammation; airflow obstruction; smoking

The majority of studies examining the lung pathology in chronic obstructive pulmonary disease (COPD) has been focused on patients with a mild or moderate disease (1–6), whereas only a few pathologic reports have examined living patients with severe COPD (7–9). Investigating these patients may be of interest because, even if they represent only a small percentage of smokers, they require an enormous amount of health care resources (10). Furthermore, a better characterization of lung pathology in these patients may help to clarify why, among patients with a similar smoking history, only a minority develops a severe disease.

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Lung specimens from patients undergoing lung volume reduction surgery (LVRS) or lung transplantation for severe COPD represent a unique opportunity to examine lung pathology in living patients who develop a severe stage of disease. Moreover, the fact that these patients perform pulmonary function tests before surgery allows investigation of the relationships between measurements of lung pathology and pulmonary function.

The pioneering study by Retamales and coworkers (7) was the first to examine and quantify the lung pathology in living patients undergoing LVRS for severe COPD. The authors demonstrated that there is an increase in the intensity of the inflammatory response in the alveolar walls and alveolar spaces of these patients and concluded that the lung inflammation induced by cigarette smoking is amplified in severe emphysema. That report was focused on the lung parenchyma but did not investigate the small airways, which, together with the lung parenchyma, are the major sites responsible for the development of airflow limitation in smokers (11). The only study that examined the small airways in patients with severe COPD undergoing lung transplantation is that by Pilette and coworkers (9), who demonstrated a reduced epithelial expression of secretory component associated with neutrophil infiltration in these patients. However, in that report, the investigators limited their analysis to neutrophils and did not attempt a complete characterization of the inflammatory cells infiltrating the small airways.

The aim of our study was to characterize and quantify the inflammatory process present in the small airways of smokers with severe COPD and to compare it with that present in patients who, despite a similar smoking history, did not develop a severe disease. We also examined the inflammatory process present in pulmonary arteries because vascular remodeling could play a role in the progression of the disease.

METHODS

Patients

We examined lung specimens obtained from nine smokers with severe COPD (forced expiratory volume in 1 second [FEV_1] < 50% predicted) undergoing LVRS for emphysema and compared them with those obtained from nine smokers with no or mild airflow limitation ($FEV_1 \geq 70\%$ predicted) undergoing thoracotomy for localized pulmonary lesions (12).

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

Pulmonary Function Tests

Pulmonary function tests were performed as previously described (13). They included measurements of forced vital capacity (FVC),

FEV_1, functional residual capacity (FRC), residual volume (RV), total lung capacity (TLC), and carbon monoxide diffusing capacity ($D_{L_{CO}}$).

Chest Radiography

Chest radiographs (posteroanterior and lateral) were obtained within 48 hours of lung function studies. Scores for emphysema were calculated on the basis of criteria for evaluation of roentgenographic signs of emphysema as previously described (13). The scores ranged from 0 to 16.

Histology

Four to six randomly selected tissue blocks were taken from the subpleural parenchyma obtained at surgery (avoiding areas involved by tumor in control groups). Samples were fixed in 4% formaldehyde using vacuum inflation, embedded in paraffin wax, and processed for histochemical and immunohistochemical analysis of small airways and pulmonary arteries as previously described (4–6). Briefly, 4 to 10 intact airways, transversally cut, with an internal perimeter of less than 6 mm (corresponding to a diameter of about 2 mm) were identified for each patient (4). The internal perimeter (defined by the basement membrane), the total wall area (everything between the basement membrane and the outer border of the airway wall), and the smooth muscle area were measured on sections stained with hematoxylin–eosin. Fifteen muscular pulmonary arteries with a perimeter of less than 1.5 mm (corresponding to a diameter of about 0.5 mm) and a double elastic lamina visible for at least half the circumference were selected for each patient (5). Measurements of arterial perimeter and intimal, medial, and adventitial thickness were performed on sections stained with elastic van Gieson. In both small airways and muscular pulmonary arteries, the morphometric measurements were normalized by perimeter (4, 5).

The infiltration of total leukocytes ($CD45^+$ cells), $CD4^+$ T-lymphocytes, $CD8^+$ T-lymphocytes, neutrophils ($elastase^+$ cells), eosinophils ($Eg2^+$ cells), mast cells (tryptase+ cells), and macrophages ($CD68^+$ cells) in the small airway wall and epithelium was quantified using immunohistochemical methods (4, 6). Furthermore, $CD45^+$ and $CD8^+$ cells were counted in the adventitia of pulmonary arteries (5). The results were expressed as number of cells per millimeter of basement membrane in the epithelium and as number of cells per square millimeter of tissue examined in the airway wall and in the pulmonary artery adventitia. The final result (per patient) is the average of the number of inflammatory cells present in each airway or artery of that patient. The cases were coded, and the measurements made without knowledge of clinical data.

Statistical Analysis

Group data were expressed as means and SEM or as medians and range when appropriate. Differences between groups were analyzed using the nonparametric Mann–Whitney *U* test for morphologic data and the unpaired Student's *t* test for clinical data. Correlation coefficients were calculated using Spearman's rank method. Probability values of $p < 0.05$ were accepted as significant.

RESULTS

Clinical Findings

The characteristics of smokers with severe COPD and with mild/no COPD are reported in Table 1. The latter group included five smokers with normal lung function ($FEV_1 \geq 80\%$ predicted) and four smokers with mild airflow limitation (70% predicted $< FEV_1 <$ 80% predicted). In two patients with mild COPD, the emphysema score was not obtained because the quality of posteroanterior and lateral chest radiographies was not good. In one patient with severe COPD, the measurements of TLC, FRC, RV, $D_{L_{CO}}$, and transfer coefficient of carbon monoxide (K_{CO}) were not performed. As expected from the selection criteria, smokers with severe COPD had a significantly lower value of FEV_1 (% predicted) and FEV_1/FVC ratio (%) than did smokers with mild/no COPD. The values of TLC (% predicted), FRC (% predicted), RV (% predicted), and radiologic score for emphysema were greater in severe COPD than in smokers with mild/no COPD, whereas the values of $D_{L_{CO}}$ (% predicted), K_{CO} (% predicted), and arterial oxygen pressure (mm Hg) were lower in smokers with severe COPD than in smokers with mild/no COPD. The two groups of patients were similar with regard to age, sex, smoking history (pack-years), and arterial carbon dioxide pressure values (mm Hg).

TABLE 1. PATIENT CHARACTERISTICS*

	Severe COPD	Mild/No COPD	p
Patients examined†, n	9	9	
Age, yr	63 ± 3	60 ± 2	NS
Sex†, M/F	6/3	8/1	
Smoking history, pack-yr	49 ± 10	43 ± 6	NS
FEV_1, % predicted	29 ± 3	86 ± 5	< 0.0001
FEV_1/FVC, %	32 ± 2	68 ± 2	< 0.0001
TLC, % predicted	114 ± 6	95 ± 6	< 0.05
FRC, % predicted	144 ± 9	98 ± 7	< 0.005
RV, % predicted	163 ± 13	94 ± 10	< 0.001
$D_{L_{CO}}$, % predicted	29 ± 4	77 ± 10	< 0.001
K_{CO}, % predicted	30 ± 5	82 ± 11	< 0.005
Pa_{O_2}, mm Hg	65 ± 6	83 ± 2	< 0.05
Pa_{CO_2}, mm Hg	41 ± 2	40 ± 1	NS
Emphysema score‡	9 (7–15)	4 (0–5)	< 0.001

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; $D_{L_{CO}}$ = carbon monoxide diffusing capacity; FEV_1 = forced expiratory volume in 1 s; FRC = functional residual capacity; K_{CO} = transfer coefficient of carbon monoxide; NS = not significant; RV = residual volume; TLC = total lung capacity.

* Values are expressed as mean ± SEM.

† Values are expressed as absolute number.

‡ Values are expressed as median (range).

Histologic Findings

The number of airways examined was 61 in smokers with severe COPD and 68 in smokers with mild/no COPD, with an average number of 7.1 airways per patient.

The results of the morphometric measurements of small airways are illustrated in Table 2. The airway perimeter was similar in smokers with severe COPD and in those with mild/no COPD, indicating that we were comparing airways of similar size. The total wall area normalized by perimeter and the smooth muscle area normalized by perimeter were not significantly different in the two groups of patients examined.

The results of the cell counts in the small airways are illustrated in Figure 1 and Table 3. In smokers with severe COPD, the number of $CD45^+$ cells was increased in both airway epithelium and airway wall as compared with smokers with mild/no COPD (Figures 1, 2 and Table 3). When we examined the different cell types, we found that in smokers with severe COPD there was an increased number of $CD4^+$ and $CD8^+$ T-lymphocytes in the airway wall and an increased number of macrophages ($CD68^+$ cells) in the airway epithelium as compared with smokers with mild/no COPD (Table 3). No significant differences were observed between the two groups of patients

TABLE 2. MORPHOMETRIC RESULTS IN SMALL AIRWAYS*

	Severe COPD	Mild/No COPD	p
Airways examined†, n	61	68	
Perimeter, μm	1,885 (1,180–2,730)	1,875 (1,500–2,350)	NS
Wall area/perimeter, μm	70 (48–151)	83 (46–27)	NS
Muscle area/perimeter, μm	13 (6–21)	12 (5–16)	NS

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; NS = not significant.

* Values are expressed as median (range).

† Values are expressed as absolute number.

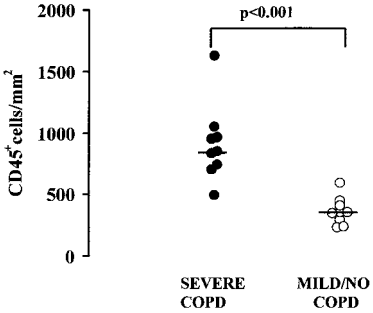

Figure 1. Individual counts for total leukocytes ($CD45^+$ cells) in the small airway wall of smokers with severe COPD and smokers with mild/no COPD. The results are expressed as the number of cells per square millimeter of tissue examined. *Horizontal bars* represent median values.

in the numbers of eosinophils, neutrophils, and mast cells infiltrating the airway wall or the epithelium (Table 3).

The mean "between-airways" coefficient of variation for inflammatory cells ranged between 0.39 and 1.20 in the airway wall and from 0.57 to 2.10 in the airway epithelium. With respect to the individual inflammatory cells that showed a significant difference between the two groups, the mean between-airways coefficient of variation was 0.46 for $CD45^+$ cells, 0.60 for $CD4^+$ cells, and 0.50 for $CD8^+$ cells in the airway wall. In the epithelium it was 0.59 for $CD45^+$ cells and 0.93 for macrophages. Except for epithelial macrophages, the between-airways variability was smaller than the betweeen-groups differences. A possible explanation for the high between-airways variability of epithelial macrophages is that their absolute number is low (on average 2 cells/mm of epithelium), and therefore a small difference in absolute terms (i.e., 2 cells/mm of epithelium) corresponds to a 100% difference.

The results of the morphometric and cellular measurements in pulmonary arteries are illustrated in Table 4. The arterial perimeter was similar in smokers with severe COPD and in those with mild/no COPD, indicating that we were comparing arteries of similar size. The intimal, medial, and adventitial thicknesses normalized by perimeter, as well as the number of $CD45^+$ and $CD8^+$ cells infiltrating the adventitia, were not significantly different in the two groups of patients examined.

Correlations

When all the smokers were considered together, the value of airway wall area normalized by perimeter showed a positive correlation with smoking history ($p < 0.005$, $r = 0.76$) (Figure 3) as did the value of smooth muscle area normalized by perimeter ($p < 0.005$, $r = 0.72$) (Figure 4).

The number of $CD45^+$ cells infiltrating the airway wall showed a positive correlation with the values of TLC ($p < 0.01$, $r = 0.68$), FRC ($p < 0.005$, $r = 0.84$), RV ($p < 0.005$, $r = 0.71$; Figure 5), and the radiologic score for emphysema ($p < 0.005$, $r = 0.80$; Figure 6), and a negative correlation with the values of FEV_1 ($p = 0.005$, $r = -0.70$), FEV_1/FVC ($p < 0.005$, $r = -0.73$), D_{LCO} ($p = 0.05$, $r = -0.62$), and K_{CO} ($p = 0.05$, $r = -0.65$).

TABLE 4. MORPHOMETRIC AND CELLULAR RESULTS IN PULMONARY ARTERIES*

	Severe COPD	Mild/No COPD	p
Perimeter, μm	740 (530–1,130)	680 (530–1,250)	NS
Intimal thickness, %	8 (2–11)	6 (2–10)	NS
Medial thickness, %	5 (2–7)	4 (3–8)	NS
Adventitial thickness, %	10 (3–16)	10 (4–17)	NS
$CD45^+$ cells, cells/mm^2, (total leukocytes)	466 (44–710)	387 (35–580)	NS NS
$CD8^+$ cells, cells/mm^2, (CD8 T-lymphocytes)	34 (6–150)	43 (17–381)	NS NS

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; NS = not significant.
* Values are expressed as median (range).

DISCUSSION

This study shows that, in small airways of smokers with severe COPD, there is a nearly threefold increase in the number of leukocytes, even though these patients had not smoked more than patients with mild COPD. The enhanced inflammatory response in severe COPD is characterized by an increase in T-lymphocytes in the airway wall as well as by an increase in macrophages in the airway epithelium and is correlated with the degrees of airflow limitation, lung hyperinflation, carbon monoxide diffusion impairment, and radiologic emphysema.

Previous studies have shown that smokers with mild airflow limitation already have an inflammatory process in their lungs and that this inflammatory process is mainly characterized by an increased number of T-lymphocytes and macro-

TABLE 3. CELLULAR RESULTS IN SMALL AIRWAYS*

	Wall (*cells/mm²*)		Epithelium (*cells/mm*)	
	Severe COPD	Mild/No COPD	Severe COPD	Mild/No COPD
$CD45^+$ cells, Total leukocytes	847 (494–1,629)†	354 (235–591)	8.1 (1.8–41.1)‡	2.8 (0.8–10.1)
$CD4^+$ cells, CD4 T-lymphocytes	326 (127–873)‡	155 (60–346)	1.8 (0.0–6.0)	0.6 (0.3–0.9)
$CD8^+$ cells, CD8 T-lymphocytes	336 (194–581)§	190 (93–332)	4.1 (1.8–21.3)	5.4 (2.0–17.1)
$CD68^+$ cells, Macrophages	156 (55–584)	123 (63–207)	3.7 (1.4–29.4)§	0.8 (0.4–3.5)
$Eg2^+$ cells, Eosinophils	28 (10–85)	16 (4–91)	0.1 (0.0–1.2)	0.2 (0.0–0.7)
$Elastase^+$ cells, Neutrophils	68 (16–248)	59 (36–245)	0.1 (0.0–2.3)	0.4 (0.0–5.6)
$Tryptase^+$ cells, Mast cells	218 (157–458)	182 (60–363)	1.0 (0.3–4.2)	1.1 (0.1–2.0)

Definition of abbreviation: COPD = chronic obstructive pulmonary disease.
* Values are expressed as median (range).
† $p < 0.001$.
‡ $p < 0.05$.
§ $p < 0.005$.

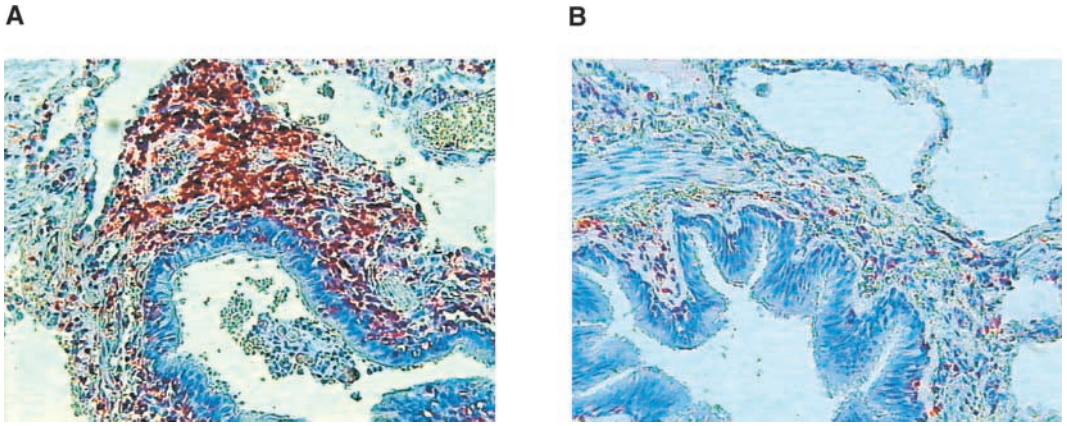

Figure 2. Microphotograph showing leukocyte infiltration in a small airway of a smoker with severe COPD (*A*) and in that of a smoker with mild COPD (*B*). Immunostaining with monoclonal antibody anti-CD45. Positive cells are stained in *red*. Original magnification: ×400.

phages (3, 4, 14–19). The present study, by demonstrating that when the disease progresses, there is an amplification of this inflammatory response, extends to peripheral airways the recent findings of Retamales and coworkers, who reported an enhanced inflammation in the alveolar walls and alveolar spaces of smokers with severe emphysema (7). The results of the present study may appear to be in contrast with our previous finding of a similar number of $CD45^+$ cells in smokers with COPD and smokers with normal lung function (6). However, in that report, we examined only smokers with a mild degree of airflow limitation, and we did not include patients with severe disease. Our present findings extend our earlier observation by showing that when the disease becomes severe, there is an amplification of the inflammatory process.

Among the different inflammatory cell types contributing to the inflammatory response in small airways of smokers with severe COPD, $CD8^+$ and $CD4^+$ T-lymphocytes in the airway wall and macrophages in the airway epithelium appeared to be the most relevant cells (Table 3). Traditionally, the rapid resolution of acute viral infections has been considered the major activity of $CD8^+$ cytotoxic T-lymphocytes, and viral infections are a frequent occurrence in patients with COPD. As pointed out in the pioneering study of O'Shaughnessy and coworkers (3), it is conceivable that, in response to repeated viral infections, an excessive recruitment of $CD8^+$ T-lymphocytes may occur and damage the lung in susceptible smokers, possibly through the release of perforins and tumor necrosis factor-α (20). The recent observation of a latent adenoviral infection in the lungs of smokers with severe emphysema supports this hypothesis (7). As recently suggested (21), it is possible that individuals with a genetically determined low CD4/CD8 T-cell ratio would be more likely to have an exaggerated CD8 T cytotoxic response to viral infections. On the other hand, it is also possible that $CD8^+$ T-lymphocytes are able to directly damage the lung even in the absence of a viral infection, as shown by Enelow and coworkers (22), who clearly demonstrated that recognition of a lung "autoantigen" by cytotoxic lymphocytes may directly produce a marked lung injury. On the basis of these findings, it can be hypothesized that the $CD8^+$ T-cell accumulation observed in severe COPD could be a response to an "autoantigenic" stimulus originating in the lung and induced by cigarette smoking (19, 23).

Our observation of a similar number of neutrophils and eosinophils between severely and mildly affected smokers may appear to be in contrast with the report by Retamales and coworkers (7), who demonstrated that not only T-lymphocytes and macrophages, but also neutrophils and eosinophils, contribute to the amplification of the inflammatory response observed in smokers with severe COPD. However, there are significant differences between the two studies that may have influenced the results. At variance with our study, in that report, patients with severe COPD had significantly greater cigarette consumption than patients with mild COPD, and it is known that smoking *per se* may induce an inflammatory response (24). Furthermore, Retamales and coworkers measured the inflammatory process in the alveolar walls and in the alveolar spaces, where neutrophils accumulate (24), whereas we measured it in the walls of small airways, where lymphocytes are the predominant cells (4, 6).

A recent study that examined the small airways in patients with severe COPD undergoing lung transplantation (9) demonstrated a reduced epithelial expression of secretory component that was associated with an increased number of neutrophils. Even if this finding may appear to be in contrast with our results, comparisons between our study and that report are difficult because of the different control populations. In particular, in our study, smokers with severe COPD were

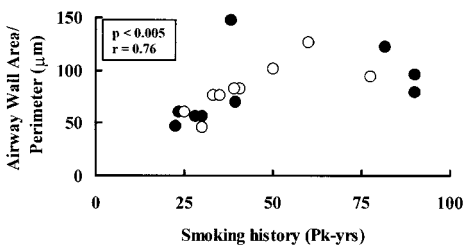

Figure 3. Relationship between smoking history and value of airway wall area normalized by perimeter (Spearman's rank correlation, $p < 0.005$, $r = 0.76$). *Closed circles* indicate smokers with severe COPD; *open circles* indicate smokers with mild/no COPD.

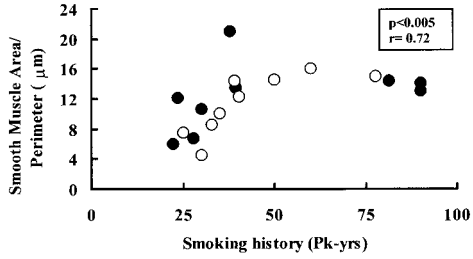

Figure 4. Relationship between smoking history and value of smooth muscle area normalized by perimeter (Spearman's rank correlation, $p < 0.005$, $r = 0.72$). *Closed circles* indicate smokers with severe COPD; *open circles* indicate smokers with mild/no COPD.

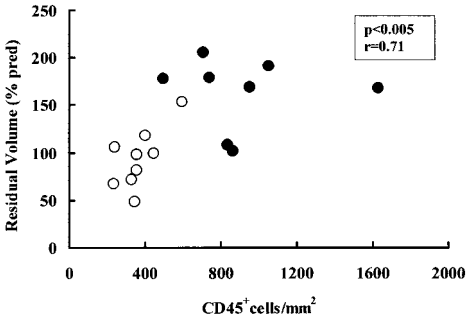

Figure 5. Relationship between the number of total leukocytes ($CD45^+$ cells) infiltrating the small airway wall and RV (% predicted) (Spearman's rank correlation, $p < 0.005$, $r = 0.71$). *Closed circles* indicate smokers with severe COPD; *open circles* indicate smokers with mild/no COPD.

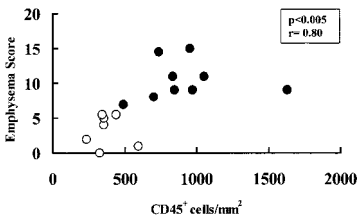

Figure 6. Relationship between the number of total leukocytes ($CD45^+$ cells) infiltrating the small airway wall and radiologic score for emphysema (Spearman's rank correlation, $p < 0.005$, $r = 0.80$). *Closed circles* indicate smokers with severe COPD; *open circles* indicate smokers with mild/no COPD.

compared with smokers with mild COPD, whereas in that report, they were compared with nonsmoking control subjects.

The observation that not only $CD8^+$ T-lymphocytes but also $CD4^+$ T-lymphocytes and macrophages contributed to the nearly threefold increase in the number of total leukocytes in the small airways of subjects with severe COPD suggests that, as recently pointed out by Shapiro (25), our challenge now is more to find out how these inflammatory cells interact and contribute to the disease than to detect the specific role of a single cell type.

A possible way to evaluate the contribution of inflammatory cells to the development of the disease is to investigate the correlation between inflammatory cells and clinical parameters, such as pulmonary function tests and chest radiography. A potential bias of these correlations is that they combine two polar groups of smokers, who were essentially selected for very different lung function, and this may have influenced the results. Despite this limitation and even if we are well aware that correlations do not imply cause–effect relationships, we believe that the significant correlations observed in our study between increased number of leukocytes and reduced lung function suggest a possible role for this enhanced inflammatory response in the clinical progression of the disease.

The increase in the number of inflammatory cells was not paralleled by a structural remodeling of small airways, as shown by the lack of differences in airway structure between severely and mildly affected smokers. However, when all the smokers were considered together, the airway wall thickness and the smooth muscle thickness showed a positive correlation with the smoking history, suggesting that smoking itself may play a role in the development of airway remodeling, regardless of the severity of airflow limitation.

The lack of a significant cellular and structural difference in pulmonary arteries between smokers with severe COPD and smokers with mild COPD may appear to be in contrast with previous observations (5, 26, 27), showing the presence of vascular remodeling and $CD8^+$ T-lymphocyte infiltration in COPD. However, because cigarette smoking itself is able to induce vascular changes even in the absence of airflow limitation (5, 26, 27), the lack of a significant difference in our study may be because of the fact that both patients with severe COPD and those with mild COPD were heavy smokers.

A potential bias in this study performed on surgically resected specimens is that smokers with mild COPD had lung cancer whereas smokers with severe COPD had not, and the presence of cancer itself may influence the results by enhancing inflammation (28). However, if this is the case, the difference in inflammatory cells would be even greater between two better matched groups of patients, thus confirming that the finding of an enhanced inflammatory response in severe COPD is valid. Another potential bias of this study is that, in LVRS, the surgeon selects the worst part of the lung, and this may have influenced the results by exaggerating the differences in inflammatory cells between the two groups. However, surgical specimens from patients undergoing LVRS are precious specimens because they allow the examination of lung pathology in living patients who have developed a severe stage of the disease.

Because the internal perimeter has been shown to remain constant despite changes in smooth muscle tone and lung volume (29), we used the internal perimeter as a marker of airway size, and we normalized the wall area and the smooth muscle area by this parameter. In our study, the internal perimeters of the small airways were similar in severely and mildly affected smokers, indicating that despite the possible different lung volumes caused by tissue preparation and the possible different smooth muscle tone in the two groups of patients, we were comparing bronchioles of similar size.

In conclusion, patients with severe COPD have an enhanced inflammatory response in small airways as compared with patients who, despite a similar smoking history, did not develop a severe disease. The number of leukocytes is correlated with reduced expiratory flow, lung hyperinflation, carbon monoxide diffusion impairment, and radiologic emphysema, suggesting a role for this inflammatory response in the clinical progression of the disease.

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