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The Nature of Small-Airway Obstruction in Chronic Obstructive Pulmonary Disease

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ABSTRACT

BACKGROUND

Chronic obstructive pulmonary disease (COPD) is a major public health problem associated with long-term exposure to toxic gases and particles. We examined the evolution of the pathological effects of airway obstruction in patients with COPD.

METHODS

The small airways were assessed in surgically resected lung tissue from 159 patients — 39 with stage 0 (at risk), 39 with stage 1, 22 with stage 2, 16 with stage 3, and 43 with stage 4 (very severe) COPD, according to the classification of the Global Initiative for Chronic Obstructive Lung Disease (GOLD).

RESULTS

The progression of COPD was strongly associated with an increase in the volume of tissue in the wall ($P<0.001$) and the accumulation of inflammatory mucous exudates in the lumen ($P<0.001$) of the small airways. The percentage of the airways that contained polymorphonuclear neutrophils ($P<0.001$), macrophages ($P<0.001$), CD4 cells ($P=0.02$), CD8 cells ($P=0.038$), B cells ($P<0.001$), and lymphoid aggregates containing follicles ($P=0.003$) and the absolute volume of B cells ($P=0.03$) and CD8 cells ($P=0.02$) also increased as COPD progressed.

CONCLUSIONS

Progression of COPD is associated with the accumulation of inflammatory mucous exudates in the lumen and infiltration of the wall by innate and adaptive inflammatory immune cells that form lymphoid follicles. These changes are coupled to a repair or remodeling process that thickens the walls of these airways.

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THE GLOBAL INITIATIVE FOR CHRONIC Obstructive Lung Disease (GOLD) has introduced a five-stage classification for the severity of chronic obstructive pulmonary disease (COPD) based on measurements of airflow limitation during forced expiration.[1,2] Each stage is determined by the volume of air that can be forcibly exhaled in one second (FEV_1) and by the ratio of FEV_1 to the forced vital capacity (FVC); lower stages indicate less severe disease. Abnormalities in these tests reflect both the reduction in the force available to drive air out of the lung as a result of emphysematous lung destruction[3] and obstruction to airflow in the smaller conducting airways.[4-6]

COPD is attributed to long-term exposure to toxic gases and particles,[1,2] most often related to cigarette smoking. The primary host defenses against this stimulus are the innate and adaptive inflammatory immune responses.[7,8] The innate defense system of the lung includes the mucociliary clearance system[9] and the epithelial barrier, supported by the acute inflammatory response that follows tissue injury.[7,10,11] This response system reacts quickly but lacks specificity, has very limited diversity, and has no memory.[7] The adaptive response provided by the humoral and cellular components of the immune system evolves much more slowly but is highly specific and very diverse and has an exquisite memory for previous insults.[8] The repair process associated with both types of response remodels damaged tissue by restoring the epithelium and microvasculature and adding connective-tissue matrix in an attempt to return the tissue to its previous state. We evaluated the relationship between the progression of COPD, as reflected by the GOLD stage, and the pathological findings in airways less than 2 mm in internal diameter, which are located from the 4th to the 12th generation of airway branching in the lung.[4-6,12]

METHODS

SPECIMENS AND PATIENT POPULATION

Specimens were obtained from two groups of patients: patients enrolled in Vancouver, Canada, who required surgical treatment of small, peripheral lung tumors,[13-15] and patients enrolled in Pittsburgh, Denver, and Houston,[16-18] who participated in the National Emphysema Treatment Trial (NETT). Table 1 shows the number of patients in each GOLD stage, the clinical and demographic characteristics, and the type of tissue examined, including the source, the number of airways examined per patient, and the mean length of the basement membrane of airways in each group.

PULMONARY FUNCTION

The measurements of FEV_1 and FEV_1:FVC met the American Thoracic Society standard and have been described previously.[13-18]

HISTOLOGIC ANALYSIS

The lung tissue obtained from the patients in the NETT was fixed by immersion in formalin, whereas the lungs and lobes resected for tumor in patients in Vancouver were first inflated and then fixed by immersion in formalin. Samples of fixed tissue were processed into paraffin blocks, cut into sections that were 4 to 5 μm thick, placed on glass slides, and stained with Movat's pentachrome technique.[19] Six complete sets of slides from a subgroup of 40 patients were stained separately to identify polymorphonuclear neutrophils (NP57, Dako-Cytomation), macrophages (CD68, Dako-Cytomation), eosinophils (Hansel's stain), T-cell subtypes (CD4 and CD8, NovoCastra Laboratories), and B cells (CD20, Dako-Cytomation). Staining was carried out on an automatic immunostainer (Dako Autostainer) according to a standard alkaline phosphatase–antialkaline phosphatase method with the use of naphthol AS-BI phosphate (Sigma) and New Fuchsin (Sigma) as substrate. Positive and negative controls were included with each run.

Digital images of the small conducting airways were obtained with the use of a light microscope (Nikon Microphot) equipped with a digital camera (JVC3-CCD KY F-70, Diagnostic Instruments) linked to a computer and then analyzed with the use of Image Pro Plus digital-image-analysis software (Media Cybernetics). Airways less than 2 mm in diameter were cross-sectioned and examined.[20,21] The maximal luminal area was calculated by determining the area enclosed by a circle formed by the full length of the basement membrane minus the area taken up by the epithelium (this process is termed expansion).[20,21] The luminal content was expressed as a fraction of the maximal luminal area to correct for the uncontrolled collapse of the lumen that occurs when lung tissue is fixed in different ways. Wall thickness included the area bound by the epithelial luminal sur-

face and the connective tissue at the outer limit of the adventitia. The fractional areas (Vv) taken up by epithelium (from the basement membrane to the luminal surface), lamina propria (from the basement membrane to the outer edge of the smooth muscle), and adventitia (from the outer edge of the smooth muscle to the outer edge of the adventitia) were also measured. Because histologic analysis reduces three-dimensional structures to two dimensions, in which volumes become areas and surfaces become lines, the ratio of tissue area to the length of the basement membrane was used to express the ratio of the volume to the surface area (V:SA) or the thickness of the airway wall and its compartments.

We determined the extent of the infiltration of the small airways by each type of inflammatory cell by counting the airways as positive if they contained the inflammatory cells and negative if they did not. We estimated the total number of each type of inflammatory cell by measuring their accumulated volume using a multilevel cascade sampling design.[22-24] The reference volume (level 1) for this cascade is the total volume of lung tissue estimated from the electronic record of the preoperative computed tomographic (CT) scan. Lung weight was determined by multiplying the volume of each CT voxel by CT density and then summing the values for the entire lung. We determined the total tissue volume by dividing this lung weight by the gas-free tissue density (1.065).[22] We determined the Vv of the total lung tissue taken up by small airways by counting the number of points occupied by small airways in whole-mount images of the histologic sections (level 2), and we determined the Vv of each compartment in the airway wall by counting the number of points at the next level of magnification (level 3). We then determined the Vv of specifically stained cells present in each airway compartment by counting the number of points at the next level of magnification (level 4). We calculated the absolute volume of a cell of interest in the entire airway wall and compartments by multiplying the Vv values from the highest level of magnification (level 4) through the other levels to the reference volume.[22-24]

Table 1. Clinical Characteristics of the Patients According to the GOLD Stage of COPD.*

Characteristic	GOLD Stage 0 (At Risk)	GOLD Stage 1 (Mild)	GOLD Stage 2 (Moderate)	GOLD Stage 3 (Severe)	GOLD Stage 4 (Very Severe)
Center (no. of patients)					
Vancouver	39	39	22	0	0
NETT	0	0	0	16	43
FEV_1 (% of predicted)	98.1±1.7	93.0±1.3	67.5±1.5	35.0±1.0	21.9±0.6
FEV_1:FVC (% of FVC)	0.78±0.009	0.65±0.006	0.58±0.016	0.35±0.014	0.30±0.008
Age (yr)	63±1	67±1	66±2	67±1	66±1
Sex (no. of patients)					
Male	26	22	17	8	29
Female	13	17	5	8	14
Smoking history					
Pack-years	40±13	50±5	51±6	60±5	67±5
Current (no. of patients)	22	22	14	0	0
Former	17	15	8	12	43
Years since quitting	6±2	4±1	5±2	9±2	9±1
Unknown	0	2	0	4	0
Corticosteroid treatment (no. of patients)†	1	2	4	3	33
No. of airways examined/patient	10±1	9±1	7±1	7±1	13±2
Basement-membrane length (mm)	2.69±0.14	2.56±0.09	2.57±0.18	2.69±0.22	2.57±0.11

* Plus–minus values are means ±SE. NETT denotes National Emphysema Treatment Trial, FEV_1 forced expiratory volume in one second, and FVC forced vital capacity.

† A total of 43 of the 159 patients were confirmed to have taken corticosteroids at some point during their preoperative course. Five patients received oral corticosteroids only, 23 received inhaled corticosteroids only, and 13 received both inhaled and oral corticosteroids. In two additional patients, the route of administration was uncertain. The times and durations of the treatments received varied and were difficult to summarize.

STATISTICAL ANALYSIS

The correlation between the FEV_1 and the numbers of airways positive for each type of inflammatory immune cell was determined with the use of Poisson regression analysis, after adjustment for the total number of airways examined for each type of cell.[25] The correlation between FEV_1 and the volume of each type of inflammatory cell, wall, or lumen variable was determined with the use of a univariate analysis based on Spearman's rank correlation.[26] The strongest correlates with FEV_1 from the lumen, wall compartments, and lymphocyte subtypes were then included simultaneously in a multiple linear-regression model.[26] All statistical tests performed were two-sided and used a type I error of 0.05.

RESULTS

The mean (±SE) number of airways examined per patient and the mean basement-membrane length were similar in all five GOLD groups (Table 1). Figure 1A shows an airway from a patient with GOLD stage 4 in which an inflammatory exudate containing mucus nearly fills the airway lumen. Figure 1B shows the same airway after the lumen

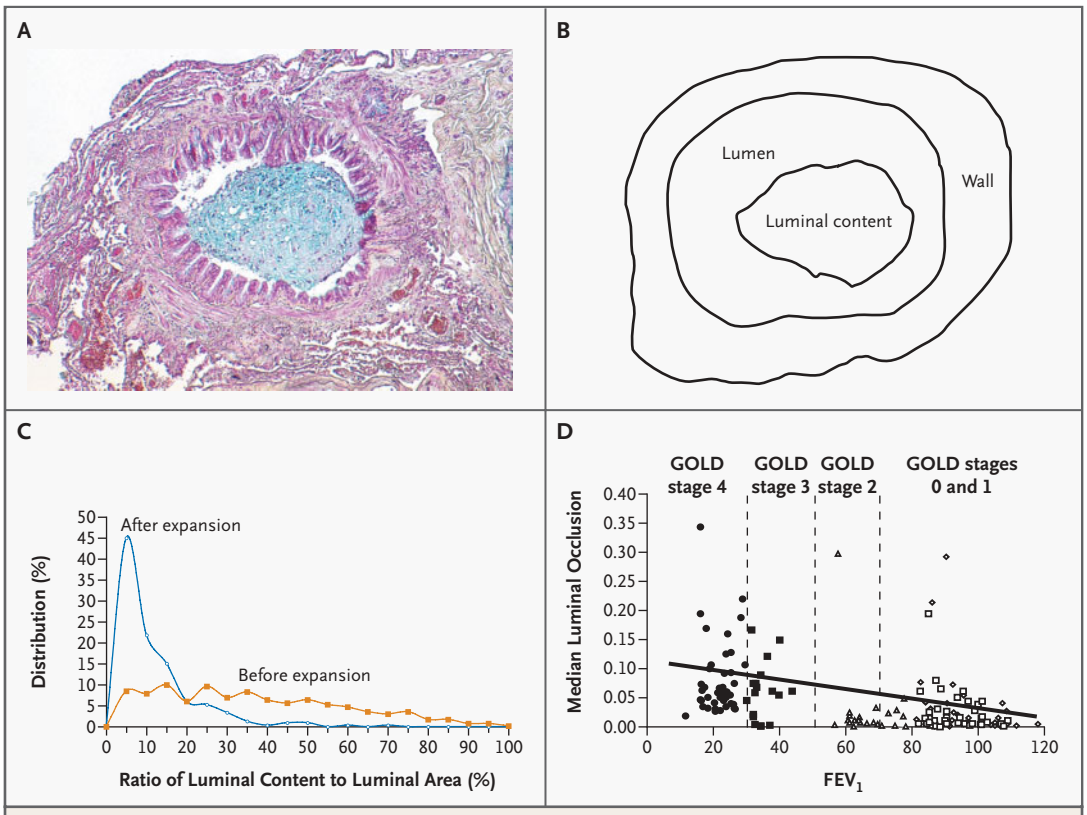

Figure 1. Airway from a Patient with GOLD Stage 4 COPD before (Panel A) and after (Panel B) Expansion, the Frequency Distribution of the Ratio of the Luminal Content to the Total Luminal Area in 42 Patients with GOLD Stage 4 COPD (Panel C), and the Relationship between the Forced Expiratory Volume in One Second (FEV_1) and the Median Luminal Occlusion in All 159 Patients (Panel D).

Panel A shows a single airway from a patient with the most severe stage of COPD (GOLD stage 4) in which the mucosa is folded because the lung was fixed in a collapsed state (Movat's stain, ×4). Panel B shows a reconstructed diagram of the same airway shown in Panel A after the lumen was fully expanded by manipulation of the digital image (Movat's stain, ×4).[20] Panel C shows the frequency distribution of the ratio of the luminal content to the total luminal area for 562 airways from 42 patients with GOLD stage 4 COPD before and after the luminal area was fully expanded. Although expansion of the lumen shifts the distribution curve to the left, many airways remain partially occluded. Panel D shows the relationship between FEV_1 and the median value of luminal occlusion for each of the 159 patients after the luminal area was fully expanded (R=0.505, P=0.001).

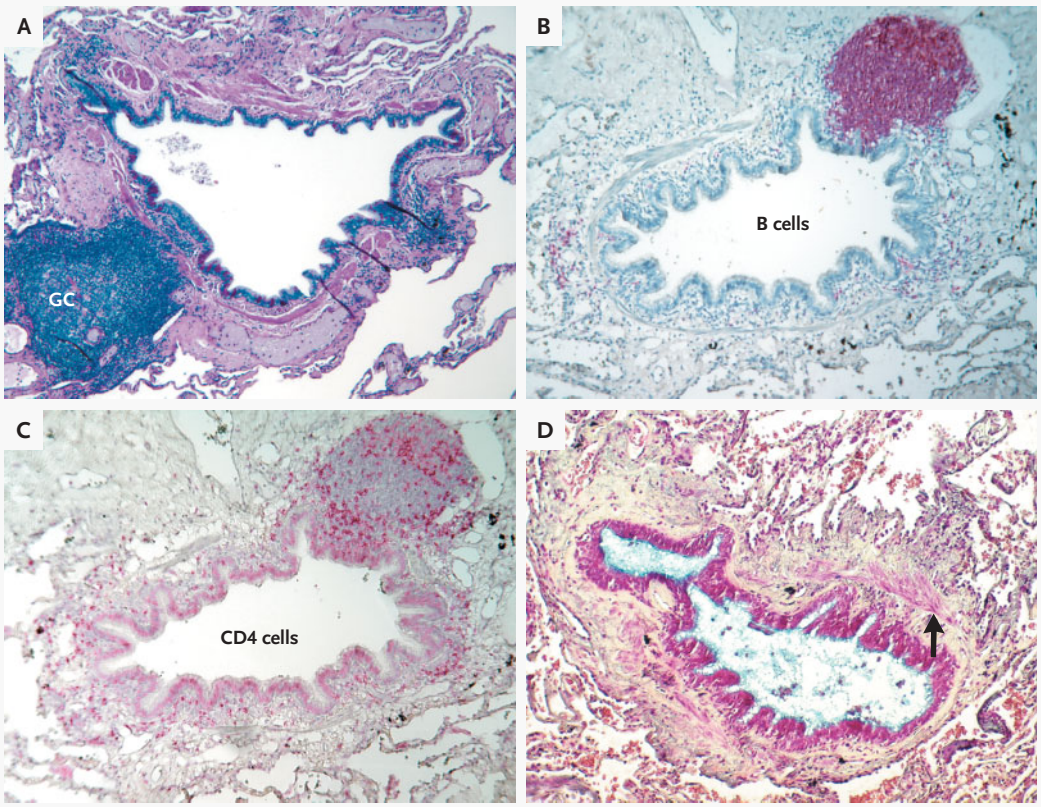

Figure 2. Pathological Findings in Patients with COPD.

Panel A shows a collection of bronchial lymphoid tissue with a lymphoid follicle containing a germinal center (GC) surrounded by a rim of darker-staining lymphocytes that extend to the epithelium of both the small airway and alveolar surface (Movat's stain, ×6). Panel B shows another follicle, in which the germinal center stains strongly for B cells (×6), and Panel C shows a serial section of the same airway stained for CD4 cells, which are scattered around the edge of the follicle and in the airway wall (×6.5). Panel D shows an airway that has been extensively remodeled by connective-tissue deposition in the subepithelial and adventitial compartments of the airway wall. The arrow points to the smooth muscle that separates the subepithelial from the adventitial compartments (Movat's stain, ×6).

has been fully expanded by smoothing out the mucosal folds.[20] Figure 1C shows the frequency distribution of the ratio of the area of the luminal content to the expanded area of the lumen before and after correction to full expansion of the lumen in all the patients with GOLD stage 4. Figure 1D shows the relationship between the severity of the luminal occlusion, calculated after the airway lumen had been fully expanded, and FEV_1 for all 159 patients in the study.

Figure 2A shows an airway with a lymphoid follicle containing a germinal center. Figure 2B shows that these structures stained strongly for B cells, and Figure 2C shows that the area surrounding the follicles stained strongly for CD4 cells. Figure 2D shows a remodeled airway in which connective tissue has been deposited in the subepithelium and adventitia of the airway wall.

Figures 3A and 3B show the number of airways that were positive for polymorphonuclear neutrophils, macrophages, eosinophils, CD4 cells, CD8 cells, and B cells, expressed as a percentage of the total number of airways examined for each type of cell. Figure 3C shows the relationship between FEV_1 and total wall thickness over the entire range of FEV_1, and Figure 3D shows the V:SA ratio or thickness of each airway compartment and the percentage of the airways with lymphoid follicles in each GOLD stage.

Table 2 summarizes the analysis of the sub-

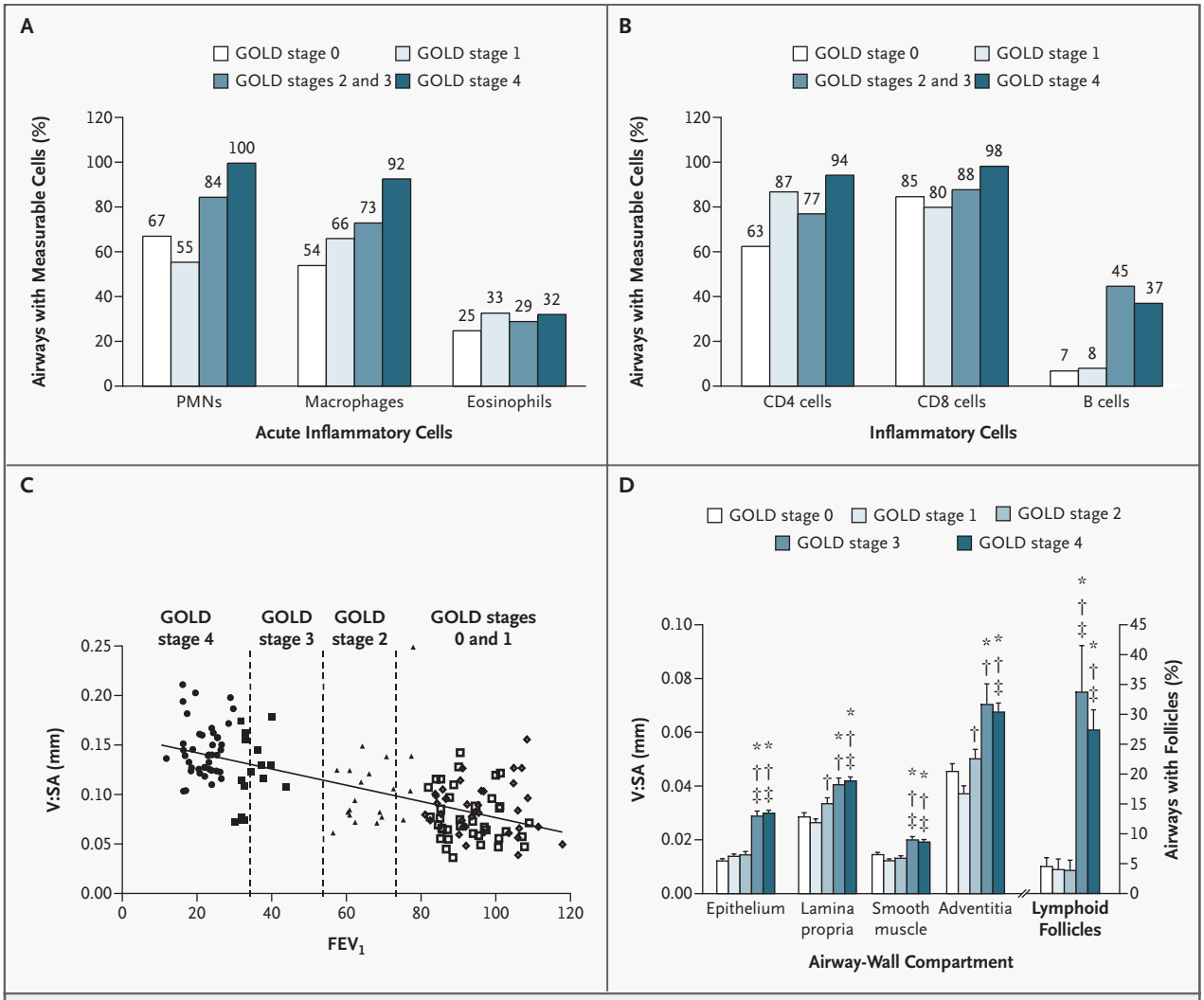

Figure 3. Clinical Findings in Patients with COPD According to the GOLD Stage.

Panel A shows the extent of the airway inflammatory response, as measured by the percentage of the airways containing polymorphonuclear neutrophils (PMNs), macrophages, and eosinophils, among patients in each GOLD stage of COPD. Panel B shows similar data for CD4 cells, CD8 cells, and B cells. Panel C shows the association between total wall thickness, measured as the ratio of the volume to the surface area (V:SA), and forced expiratory volume in one second (FEV_1) for all 159 patients. Panel D shows the mean (+SE) volume of epithelium, lamina propria, smooth muscle, and adventitial tissue expressed per unit of basement-membrane surface area (V:SA) and the percentage of airways that contained lymphoid follicles in all 159 patients. Patients with GOLD stages 2 and 3 have been combined in Panels A and B to make the number of patients similar in each group. Asterisks indicate P<0.001 for the comparison with patients with GOLD stage 0. Daggers indicate P<0.001 for the comparison with patients with GOLD stage 1. Double daggers indicate P<0.001 for the comparison with patients with GOLD stage 2.

group of 40 patients in whom inflammatory immune cells were measured. The extent of the response, as reflected by the number of airways containing polymorphonuclear neutrophils, macrophages, CD4 cells, CD8 cells, B cells, and lymphoid follicles, increased with disease progression, whereas the total accumulated volume of cells only increased for B cells and CD8 cells. The univariate analysis involving all 159 patients (Table 2) shows strong associations between the progression of COPD and the percentage of airways containing lymphoid follicles, the occlusion of the fully expanded lumen by inflammatory mucous exudates, total wall thickness, and the thickness of

Table 2. Relationship of FEV_1 to Small-Airway Abnormalities.*

Variable	All 159 Patients		Subgroup of 40 Patients	
	R Value	P Value	Coefficient of Variation	P Value
Univariate analysis				
Extent of inflammation†				
% of airways with PMN			−0.0065	<0.001
% of airways with macrophages			−0.0068	<0.001
% of airways with eosinophils			−0.0060	0.19
% of airways with CD4 cells			−0.0035	0.02
% of airways with CD8 cells			−0.0029	0.038
% of airways with B cells			−0.0245	<0.001
% of airways with lymphoid follicles	−0.575	<0.001	−0.467	0.003
	R Value	P Value	R Value	P Value
Degree of infiltrate‡				
Accumulated volume of PMN	—	—	−0.0118	0.91
Accumulated volume of macrophages	—	—	−0.26	0.11
Accumulated volume of eosinophils	—	—	−0.049	0.76
Accumulated volume of CD4 cells	—	—	−0.25	0.12
Accumulated volume of CD8 cells	—	—	−0.36	0.02
Accumulated volume of B cells	—	—	−0.35	0.03
Expanded lumen	−0.505	<0.001	−0.359	0.02
Wall thickness				
Epithelium	−0.723	<0.001	−0.689	<0.001
Lamina propria	−0.583	<0.001	−0.542	<0.001
Adventitia	−0.544	<0.001	−0.428	0.006
Total	−0.687	<0.001	−0.607	<0.001
	R^2 Value	P Value	R^2 Value	P Value
Multivariate analysis	0.515		0.476	
Luminal content	—	<0.001	—	0.76
Wall thickness	—	<0.001	—	0.004

* PMN denotes polymorphonuclear neutrophils.
† Poisson regression analysis was used.
‡ Spearman's rank correlation was used.

each of the wall compartments. The multivariate analysis for both the entire group of patients and the subgroup of 40 patients indicates that thickening of the airway walls had the strongest association with the progression of COPD.

DISCUSSION

Our results extend those of previous reports[4-6] by providing quantitative information about the nature of the pathological findings at the site of airway obstruction in relation to the GOLD stage of COPD.[1,2] The multivariate analysis indicates that progression of COPD from GOLD stage 0 to GOLD stage 4 was most strongly associated with thickening of the airway wall and each of its compartments by a repair or remodeling process. The degree to which the lumen was filled with mucous exudates; the extent of the inflammatory response, as reflected by the number of the airways containing acute inflammatory cells (polymorphonuclear leukocytes and macrophages) and lymphocytes (CD4 cells, CD8 cells, and B cells) organized into follicles; and the severity of this response, as reflected by the absolute volumes of CD8 cells and B cells, were more weakly associated with disease progression.

Our results expand on previous reports that the epithelial barrier of the innate defense system is breached in cigarette smokers[10,11] by showing that small airways become occluded by inflammatory exudates containing mucus as COPD progresses. Hypersecretion of mucus is the defining feature of chronic bronchitis and is associated with an inflammatory process involving the epithelium, gland ducts, and glands of the larger central airways.[27,28] Although the accumulation of inflammatory exudates in the small-airway lumen might be attributed to the extension of chronic bronchitis into the small airways, several studies suggest that this is not the case. At least two large clinical trials have shown that the presence of chronic bronchitis does not predict the development of airflow limitation,[29,30] and pathological studies indicate that central and peripheral airway inflammation can occur quite independently of each other.[27] Collectively, these data suggest that the cough and sputum production that defines chronic bronchitis is independent of the disease process in the small airways that is responsible for airway obstruction in patients with COPD.

The Poisson regression analysis of the number of airways containing inflammatory cells shows that progression of COPD is associated with increasing infiltration of the airways by polymorphonuclear neutrophils, macrophages, CD4 cells, and lymphocyte subtypes. However, the cascade analysis showed that the accumulated volume of inflammatory cells was increased only in the case of CD8 cells and B cells. The absence of the accumulation of polymorphonuclear neutrophils, macrophages, and CD4 cells in the airway tissue may be related to the fact that the patients with severe (GOLD stage 3) and very severe (GOLD stage 4) COPD had all stopped smoking an average of nine years earlier and a high percentage had received some form of corticosteroid therapy.

The observed increase in the absolute volume of CD8 cells and B cells as COPD progressed is consistent with previous results[31-33] and extends such findings by showing an even stronger association with the percentage of airways containing lymphoid follicles. The increase in lymphocytes and their organization into follicles are consistent with increased immune surveillance of the mucosal surface in patients with COPD, in whom close collaboration among the epithelium, antigen-presenting cells, and lymphocytes organized into follicles facilitates antigen presentation.[34,35] Although the innate immune response can mobilize T cells and B cells, with respect to their organization into follicles, we believe that an adaptive immune response develops in relation to the microbial colonization and infection known to occur in the later stages of COPD.[36]

The strongest association with disease progression was an increase in the volume of the airway wall tissue owing to an increase in epithelium, lamina propria, muscle, and adventitial compartments. The increase in tissue between the epithelial surface and the muscle layer is thought to contribute to nonspecific airway responsiveness,[37] which is one of the best predictors of the rapid decline in FEV_1 in patients with COPD.[38] The observed increase in connective tissue in the adventitial compartment is similar to that reported by Matsuba and Thurlbeck[39] and could contribute to fixed airway obstruction by preventing the airways from opening properly during lung inflation. Experiments in transgenic mice have shown that overexpression of cytokines such as interleukin-13 results in the activation of transforming growth factor β, leading to subepithelial and peribronchiolar fibrosis very similar to that reported here.[40] A more complete understanding of the cytokine pathways that control the deposition of connective tissue in human disease might lead to effective treatments.

We conclude that obstruction of the small airways in COPD is associated with a thickening of the airway wall by means of a remodeling process related to tissue repair and a malfunction of the mucociliary clearance apparatus of the innate host defense system, which results in the accumulation of inflammatory exudates in the lumen. We also postulate that colonization and infection of the lower airways are associated with an adaptive immune response that accounts for the increase in lymphocytes and their organization into lymphoid follicles in patients with severe (GOLD stage 3) and very severe (GOLD stage 4) COPD.

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