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Increased galectin-3 expression and intraepithelial neutrophils in small airways in severe chronic obstructive pulmonary disease.

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ABSTRACT

Galectins-1 and -3 regulate epithelial proliferation/apoptosis and neutrophil activation, and are implicated in lung cancer and asthma. The role of galectins in chronic obstructive pulmonary disease (COPD), characterized by epithelial changes and neutrophil infiltration, remains unknown.

Galectin-1 and -3 expression was assessed by immunohistology in the bronchial epithelium of lung specimens from eight severe COPD patients, as compared to 9 nonsmokers and 6 smokers without COPD. Findings were related to epithelial proliferation (Ki-67), tissue inflammation and lung function.

Epithelial galectin-3 immunostaining was increased only in small airways of COPD as compared to nonsmokers ($p<0.001$) and smokers ($p=0.002$). In contrast, galectin-1 was significantly increased only in smokers' small airways ($p<0.001$). Ki-67+ epithelial cells were increased in COPD small airways as compared to smokers ($p=0.05$), as well as neutrophils. Moreover intraepithelial neutrophils correlated in small airways with Ki-67+ epithelial cells ($p=0.03$, $r=0.76$) and with the FEV_1/FVC ratio ($p=0.001$, $K=-0.93$), whereas no correlation was observed with galectin expression.

This study supports the hypothesis that distal airways represent an important site for detecting changes in COPD: in patients with severe disease we demonstrate increased galectin-3 expression and neutrophil accumulation in the small airway epithelium, correlating with epithelial proliferation and airway obstruction.

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a frequent disorder characterized by mostly irreversible airway obstruction that develops in susceptible smokers [1]. During the course of the disease COPD airways are infiltrated by neutrophils, macrophages and T-cells (especially $CD8^+$), through the up-regulation of adhesion molecules such as intercellular adhesion molecule (ICAM)-1 or chemokine receptor CXCR3 [2,3]. However, pathways involved in the persistence of recruited leukocytes, especially neutrophils, in the bronchi of COPD patients remain to be identified. Moreover, there are differences in the leukocyte phenotypes present in COPD airways with regard to disease severity and to microlocalisation within bronchial tissues [4].

In addition to inflammatory changes, COPD airways also undergo structural remodelling [5] which includes increased number and size of mucous acini in submucosal glands, hyperplasia and metaplasia of surface mucus-secreting goblet cells in large and small airways as well as squamous metaplasia [5]. The mechanisms leading to these changes are not known. In addition, bronchial epithelium function is affected in COPD, with upregulated expression of cytokines/chemokines (reviewed in [6]) such as interleukin (IL)-8 or macrophage inflammatory protein-1α and -1β and downregulation of proteins such as the polymeric immunoglobulin receptor [7] probably following degradation by neutrophil enzymes [8].

Galectins are galactose-binding proteins that are involved in lung physiology [9-12]. Galectin-3 can enhance the proliferation of respiratory epithelial cells, by limiting their adhesion to the basement membrane through competition with integrin receptors for the binding of laminin in the extracellular matrix [13]. Galectins-1 and -3 are also involved in apoptosis regulation *via* binding to cell surface or cytoplasmic ligands such as glycans of the fibronectin receptor, CD7 or intracellular proteins such as bcl-2 [10,14]. The nuclear and/or cytoplasmic pattern of galectin-3 expression could play a role during malignant transformation, as suggested in breast cancer [15]. In addition, galectin-3 triggers the release of oxygen metabolites and elastase by neutrophils and macrophages, enhances macrophage phagocytosis, stimulates natural killer cell activity and IL-1 production [14,16], and represents an adhesion molecule independent from selectins and β2-integrins that underlie neutrophil recruitment to the lung during streptococcal pneumonia [17,18]. In contrast, galectin-1 inhibits tumour necrosis

factor-α production by monocytes/macrophages [14], suggesting that galectin-1 and -3 have balanced, opposite effects on cell apoptosis/proliferation and phagocyte activation. Of note, these two lectins are abundantly expressed in the lung [11,18].

The role of galectin-3 has been evaluated in lung cancer and asthma, whereas its role in COPD remains unknown. Therefore we evaluated the expression of galectins-1 and -3 in the bronchial epithelium by immunohistochemistry from eight patients with severe COPD undergoing lung transplantation, in both the large and small airways. Lung specimens from 9 nonsmokers and 6 smokers without COPD were used as controls. Findings on galectin expression were related to clinical parameters of airflow limitation, to epithelial proliferation (Ki-67 expression) and to tissue accumulation of neutrophils, macrophages and T-cells in the epithelium and lamina propria, in both large and small airways.

MATERIALS AND METHODS

Patients

COPD patients were recruited from a population undergoing lung transplantation, as previously described [7]. Eight very severe COPD patients (stage IV, according to GOLD classification [1]) ranging from 42 to 60 years old (mean: 52) were included. All were ex-smokers and had symptoms of chronic bronchitis, and all except one were treated by inhaled corticosteroids. Controls consisted of 6 patients (ranging from 42 to 74 years old, mean: 60) recruited from a population of smokers without COPD and 9 nonsmokers (mean age: 62), undergoing lung surgery for a solitary peripheral lung tumor. All patients were clinically stable with no evidence of lung infection at the time of surgery, and pulmonary function tests were performed 37±26 days (mean±SD) before surgery. For each patient, random tissue blocks (3 to 11 per patient) were sampled in central and peripheral areas from each lobe of one or both lungs (single or bipulmonary transplantation), fixed under constant inflation pressure (30cm water) in 4% formaldehyde, and processed for immunohistochemistry. Patient characteristics are presented in table 1.

Immunohistochemistry

Tissue sections were processed for galectin immunostaining. Endogenous peroxidase activity was inhibited by incubation in 0.03% v/v H_2O_2 and sections were blocked by normal horse serum and avidin/biotin blocking kit (Dako). Sections were then treated for 30 min with 1:100 rabbit polyclonal anti-galectin-1 or anti-galectin-3 antibodies, which had been rigorously tested for specificity by ELISA and Western blotting [12,19,20]. Control sections were incubated with normal rabbit serum (1:100). Biotinylated horse anti-rabbit IgG (1:100) was used as secondary antibody, and sections were incubated with avidin-biotin-horseradish peroxidase (ABC standard kit of Vectorstain, Dako, Glostrup, Denmark). After revelation with 3,3'diaminobenzidine tetrahydrochloride and 0.03% H_2O_2, sections were counterstained with Toluidine blue.

Leukocytes were detected using specific antibodies to neutrophil elastase (neutrophils), CD68 (monocytes/macrophages) and CD3 (T-lymphocytes). For macrophages and T-cells, *antigen*

retrieval was performed by using microwave treatment (5 cycles of 3 min, 350 Watts) in 0.01M citrate buffer, pH 5.7. Endogenous peroxidase activity was inhibited by incubation in 0.03% H_2O_2 and sections were blocked with 1% BSA. Sections were then incubated with mouse monoclonal antibodies to neutrophil elastase (mIgG1κ, clone NP57; Dako) or CD68 (mIgG3κ, clone PG-M1; Dako) or with polyclonal rabbit antibody to CD3 (Dako). Control sections were treated with appropriate normal rabbit serum or mouse IgG isotype. Secondary antibodies were biotinylated goat anti-rabbit IgG or goat anti-mouse IgG (Dako). The reaction was amplified by incubation with streptavidin-peroxidase conjugate and revealed by using diaminobenzidine and H_2O_2. Sections were counterstained with Mayer's hemalum and mounted with coverslips in Eukitt medium (Vel, Leuven, Belgium).
Ki-67 antigen staining was carried out by using MIB-1 mAb (Dako) and anti-mouse detection method as described above.

Protocol for computer-assisted quantification

Quantification of immunostaining was performed following the same methodology as previously published [7]. Galectin and Ki-67 stainings were studied in 10 randomly selected areas of well-preserved bronchial epithelium (defined by the presence of basal and columnar cells without detachment from the basement membrane) on each section (3 to 11 per subject), in both large and small airways. Leukocyte infiltration was evaluated in 10 randomly selected areas of epithelium, 10 areas of lamina propria and 10 areas of submucosal glands on each section. Large airways were defined as cartilaginous bronchi with submucosal glands and small airways as membranous bronchioles, without cartilage or glands and with an internal diameter less than 2 mm, as previously described [21]. Two to 9 different airways per slide and a total of 10 to 21 large airways and 9 to 28 small airways per subject were evaluated; 30, 34 and 45 tissue sections were analyzed in the nonsmokers, smokers and COPD groups, respectively.

Computer-assisted quantification of the staining in the selected areas was carried out by a SAMBA 2005 system (Alcatel TITN, Grenoble, France) equipped with a color camera and an optical microscope (Olympus BX 50), using a final magnification of 400x. Results were expressed as the mean optical density (MOD, arbitrary units, a.u.), representing the mean intensity of staining in the considered area, and as the labelling index (LI, %) referring to as

the percentage of surface staining to the total selected area and therefore reflecting relative numbers of positive cells.

Statistical methods

The staining indices in the different sections from each patient were subjected to statistical analysis. Two types of comparisons were performed. First, a mean value was calculated for each patient from the staining indices obtained in the different sections, and comparisons between groups (COPD *versus* controls) were performed using the Mann-Whitney *U*-test. Second, within-group comparisons were performed on paired data (large *vs* small airways) obtained in the same section (where thus both large and small airways were present) using the Wilcoxon matched pairs test. Correlations were tested using the non-parametric Kendall test or Spearman's method as appropriate, and p values less than 0.05 were considered significant. Results were expressed as medians and ranges. The statistical analyses were carried out using Prism (GraphPad software, version 4.0 2003).

RESULTS

Airway expression of galectin-1 and -3 in COPD and control patients

Galectin-1 expression was detected in the bronchial epithelium from control and COPD patients, as well as in the lamina propria notably in endothelial cells (not shown). Galectin-3 immunostaining was observed in bronchial epithelial cells, especially in the cytoplasm of ciliated and sero-glandular cells (fig. 1B), whereas staining was also observed in macrophages (fig. 1B, arrows).

While no change in galectin expression was observed in large airways, the bronchial epithelium of smokers exhibited increased galectin-1 expression in small airways, as compared to nonsmokers (~3-fold increase; $p<0.001$) (fig. 2). In COPD galectin-1 expression also tended to increase ($p=0.06$, as compared to nonsmokers), but to a much lower extent than in smokers (fig. 2).
In contrast, expression of galectin-3 in the small airway epithelium - which tended to increase in smokers ($p=0.06$) - was strongly increased in COPD (~2-fold increase; $p<0.001$) (fig. 2).

Ki-67 expressing epithelial cells in COPD and control airways

Numbers of Ki-67 expressing epithelial cells were significantly increased in small airways of patients with severe COPD, as compared to smokers ($p=0.05$) (fig. 3). No significant changes in Ki-67 staining were observed in COPD large airways (not shown).

Neutrophil, macrophage and T-cell infiltration of COPD airways

Highly significant increases in neutrophil numbers were observed in severe COPD as compared to controls ($p<0.001$) (Table 2). When compared to smokers, neutrophils were elevated in submucosal glands and in small, but not large, airways (Table 2). A trend for increased neutrophil numbers was thus observed within the epithelium of large airways from smokers, as compared to nonsmokers (Table 2).

$CD68^+$ monocytes/macrophages were also observed in COPD airways (occasionally clustered in foci; not shown), whereas staining values largely overlapped with those observed in

controls (Table 2). Relative numbers of macrophages were however significantly increased in smokers and COPD, as compared to nonsmokers (Table 2).

Lymphoid foci were observed around COPD airways (not shown), with increased T cell numbers in the epithelium and lamina propria of small airways, as compared to smokers ($p=0.003$ and $p<0.001$, respectively) (Table 2).

Relationships between galectins, epithelial proliferation, airway inflammation and lung function

Epithelial galectin-3 expression in small airways did not correlate in COPD with epithelial proliferation or neutrophil infiltration. In small airways Ki-67^+ cell numbers correlated with neutrophils ($p=0.03$, $r=0.76$) (fig. 4). Moreover intraepithelial neutrophils correlated with airflow obstruction assessed by the FEV_1/FVC ratio ($p=0.001$, $K=-0.93$), and subepithelial monocyte/macrophages correlated with FEV_1 (fig. 5).

DISCUSSION

Galectins are secreted lectins that participate in the regulation of neutrophil recruitment and cell apoptosis/proliferation, notably during lung development and inflammatory responses [10-14]. This study shows for the first time that in severe COPD epithelial expression of galectin-3 is increased in small airways. In contrast, in smokers without COPD increased galectin-1 in small airways was observed. By assessing large numbers of tissue specimens containing various airway generations (whole explanted lungs and surgical specimens), which probably improved the power to detect between-group differences [22], we also show that in severe COPD neutrophils are preferentially microlocalized to the epithelium of small airways, where they correlate with increased epithelial proliferation and airflow obstruction.

It has been shown that galectin-1 expression is induced in nasal polyps upon corticosteroid therapy and that this may limit tissue eosinophilia [23]. Decreased galectin-1 and increased galectin-3 expression has been associated with defective apoptosis of synovial mononuclear cells in juvenile idiopathic polyarthritis [24]. A recent study showed that galectin-3 is mitogenic for cardiac fibroblasts, induces collagen deposition and may lead to ventricular dysfunction [25]. In the lung, epithelial expression of galectin-3 is increased in non-small lung cancer [26,27], while in murine asthma models gene therapy targeted to galectin-3 inhibits allergen-induced airway inflammation (including IL-5 expression) and epithelial mucous metaplasia [28-30]. Secretion of galectin-3 is also upregulated in a rat model of radiation-induced lung fibrosis [31,32].

Changes in galectin expression observed in our study could be relevant to COPD pathogenesis, as galectins control apoptosis of epithelial and inflammatory cells as well as neutrophil and macrophage activation. Thus galectin-3 participates to the recruitment of neutrophils, and triggers their release of oxygen radicals and elastase [14] which are thought to mediate the lung destruction induced by cigarette smoking [33]. This study focused however on the airways, and not on the lung parenchyma where an increased apoptotic rate of alveolar epithelial and endothelial cells could underly the development of emphysema [34], including in end-stage COPD [35,36]. The altered galectin balance between galectin-1 and galectin-3 in the small airways could favor hyperplasia of the bronchial epithelium observed in COPD. Increased numbers of proliferating (expressing Ki-67) epithelial cells were

observed in COPD small airways, as previously reported in squamous metaplastic areas of large bronchi [37]. Elevated Ki-67 expression was also previously observed in the lung alveolar wall from severe COPD patients [38], whereas it remained unchanged from controls in another study [36]. Interestingly, in the present study Ki-67+ epithelial cell numbers in small airways correlated with neutrophils, suggesting a link between inflammation and tissue remodelling. Accordingly, increased Ki-67+ epithelial cells following allergen challenge were related in asthma to airway eosinophilia [39]. Alternatively, these correlations could be due to the presence of intraepithelial inflammatory cells expressing Ki-67 antigen. Although we can not exclude this possibility, by examining serial sections of COPD stained for Ki-67 and neutrophils, we could not relate Ki-67 staining to epithelial infiltration by neutrophils (data not shown).

Smokers without COPD display increased galectin-1 expression in the bronchial epithelium in small airways, in parallel to a trend for increase in galectin-3, suggesting that the impaired balance between galectin-1 and galectin-3 favoring airway inflammation and remodelling is only observed in susceptible smokers who develop COPD. It is tempting to speculate that in nonsusceptible smokers galectin-1 could help to suppress signals of inflammatory and proliferative responses. Our study was however not designed to address the mechanisms of these changes, which could relate to intrinsic changes of the COPD bronchial epithelium or to non-specific factors associated with severe disease, such as hypoxia [40]. Also a putative effect of the treatment of COPD can not be ruled out, although it seems unlikely that inhaled corticosteroid treatment could account for the observed changes in galectin expression [23].

Our study did not document a correlation between epithelial galectin-3 expression and neutrophil accumulation in severe COPD patients. As previously demonstrated [41-45], we observed that neutrophils infiltrate the airways of severe COPD patients. We show that neutrophils are more particularly localized in severe COPD in submucosal glands and small airways, a latter feature in contrast to mild stage [46-48]. Smokers were characterized by neutrophil infiltration of proximal bronchi, particularly within the epithelium. As previously reported [43,45], macrophage numbers largely overlap those observed in smokers, although a trend for increase was observed in our study in small airways.

Regarding T cells, we did not observe in large airways a significant difference between groups. This is in contrast to previous studies of mild-to-moderate COPD showing increased subepithelial CD3 [42, 44, 45] and CD8 cells (44,45] in large airways. Conversely, in a study in severe COPD by Di Stefano et al [51] CD3 and CD8 cell numbers were decreased and inversely correlated with the degree of airflow limitation, suggesting that cigarette smoke-induced bronchial inflammation is downregulated as the disease progresses. Alternatively, severe COPD could represent a particular subgroup of COPD patients with a distinct immunological phenotype. Our findings confirm however that T cells infiltrate small airways of these severe COPD patients, in agreement with a previous study in severe COPD [52] and as reported previously in mild COPD [48]. Moreover, we show that a decrease in T cell numbers characterizes smokers without COPD as compared to nonsmokers. As previously suggested [45], this observation could relate to the selection of a subpopulation of smoking subjects with low CD3 cell counts who are more resistant to the effect of cigarette smoke.

Microlocalisation of neutrophils to smooth muscle has recently been reported in COPD [53], and related to local expression of CXC chemokines [54]. Similarly, localisation of mast cells within peribronchial smooth muscle in asthma [55] has been associated with the release of CXCL10 by smooth muscle cells [56]. We show that intraepithelial (in contrast to subepithelial) microlocalisation of neutrophils in severe COPD correlates with airway obstruction. In addition to the production of galectin-3 reported here, bronchial epithelial cells can express CXC chemokines (and adhesion molecules) that may facilitate the trafficking of neutrophils to the airway epithelium. Conversely, neutrophil-derived products, such as elastase, can contribute in COPD to locally activate epithelial cells and to trigger transcriptional programmes leading to epithelial expression of proinflammatory mediators. We observed that subepithelial macrophages also correlate with airflow limitation, suggesting that in COPD inflammatory cells have different functional implications within the various airway microcompartments [57].

Although the small numbers of patients limit the conclusions, this study provides further evidence of important changes occurring in the bronchial epithelium of patients with severe COPD. Up-regulated galectin-3 expression – unbalanced by galectin-1 - is observed in small airways of smokers who have developed severe COPD, in contrast to smokers without COPD who tend to display the opposite expression profile. Although other factors appear involved,

changes in galectin expression could contribute in COPD to epithelial hyperplasia and neutrophil accumulation in the airways. Of note, the emerging involvement of galectin-2 in T-cell regulation and the pathogenesis of myocardial infarction highlights the importance of further analysis of the galectin network [58-60].

We show also for the first time that in severe COPD the small airway epithelium is a particular site of neutrophil infiltration, related to epithelial proliferation and airflow limitation. Our findings support previous studies [61] which proposed an important role for small conducting airways in the pathophysiology of COPD. We suggest that chronic damage to the epithelium is associated in susceptible smokers with increased galectin-3 expression, and that microlocalisation of neutrophils to the small airway epithelium is a particular and functionally relevant feature in severe COPD.

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FIGURE LEGENDS

Figure 1. Galectin-1 (*A*) and galectin-3 (*B*) staining in small airways from a COPD patient; original magnification 400x, and control staining with normal rabbit serum (*C*). Galectin-3 expression is also observed in alveolar macrophages (*D*, arrows).

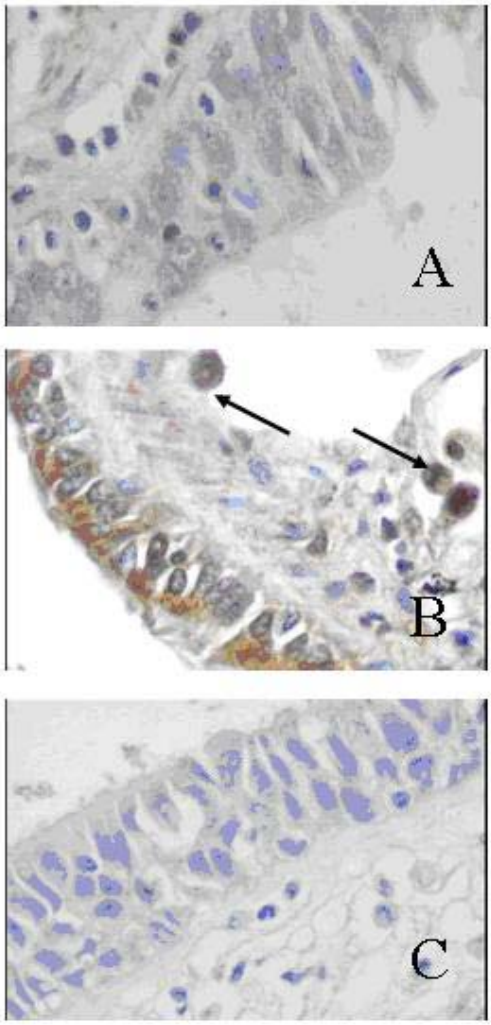


Figure 2. Staining indices of galectin-1 and galectin-3 expression in the bronchial epithelium. Epithelial expression of galectin-1 (upper panel) and galectin-3 (lower panel) in large and small airways from COPD patients versus nonsmokers (Nonsm) and smokers (Sm) is shown in terms of Mean Optical Density (MOD). Bars represent medians. * $p<0.01$, °$p=0.06$ (Mann-Whitney *U*-test).

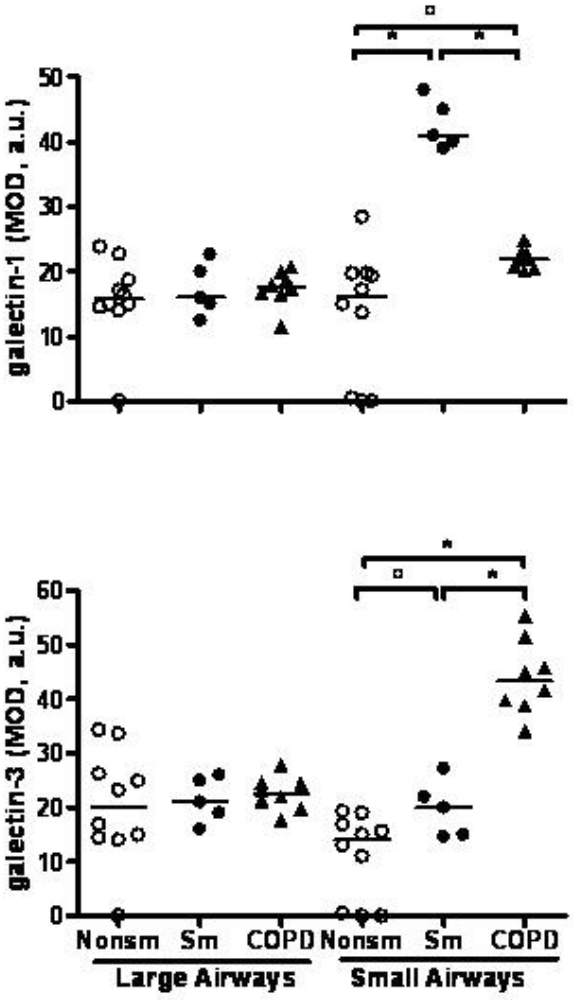


Figure 2

Figure 3. Epithelial proliferation (Ki-67 staining) in the bronchial mucosa of COPD and control patients. Relative numbers (labelling index, LI) of Ki-67 expressing epithelial cells are shown in the small airways of patients with severe COPD, nonsmokers (Nonsm) and smokers (Sm). Bars represent medians. *p=0.05 (Mann-Whitney *U*-test).

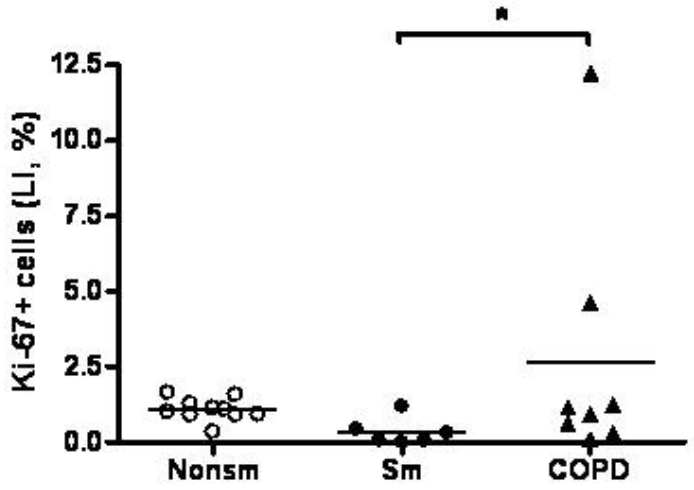


Figure 3

Figure 4. Relationship between neutrophils and Ki-67^{+} epithelial cells in small airways of severe COPD patients. Neutrophils (brown-stained cells, *A*) were plotted against Ki-67^{+} proliferating epithelial cells (brown-stained cells, *B*) in small airways of severe COPD patients according to Spearman's method. Panel C shows a representative isotypic (mouse IgG1κ) control.

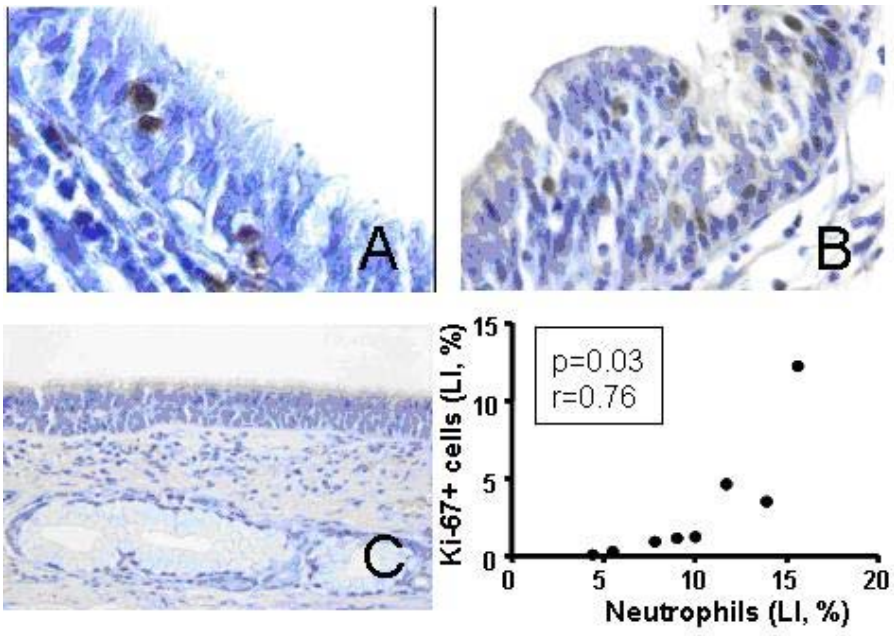


Figure 4

Figure 5. Relationship between neutrophils, macrophages and lung function. Functional parameters of airway obstruction (FEV_1, FEV_1/FVC ratio) plotted against intraepithelial neutrophils (upper panel) and subepithelial macrophages (lower panel) (expressed as MOD values) in small airways of severe COPD patients; K (Kendall's tau) and p values according to Kendall test.

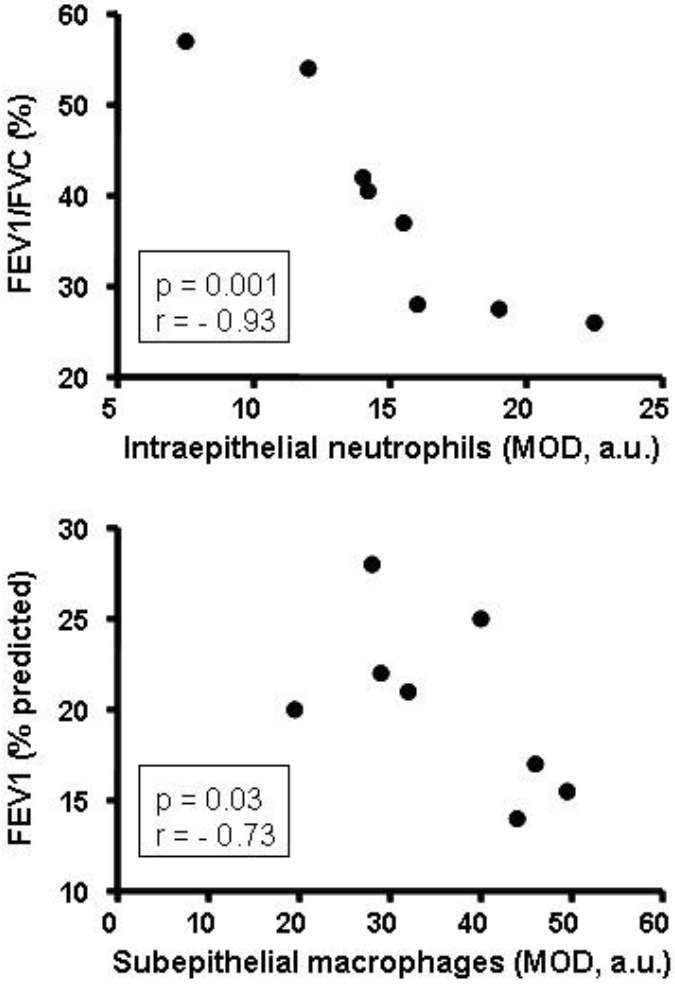

FEV1/FVC (%)
60
50
40
30
20
5
10
15
20
25
Intraepithelial neutrophils (MOD, a.u.)
p = 0.001
r = - 0.93
FEV1 (% predicted)
30
25
20
15
10
0
10
20
30
40
50
60
Subepithelial macrophages (MOD, a.u.)
p = 0.03
r = - 0.73

Figure 5

TABLES

TABLE 1. Patient characteristics.

	Nonsmokers	Smokers	COPD
Subjects examined (n)	9	6	8
Age (yr)	62 ± 14	60 ± 7	53 ± 2
Sex (M :F)	4M:5F	4M:2F	5M:3F
Smoking history (pack-yr)	0	39±15*	35 ± 4*
FEV_1 (% of predicted)	104 ± 10	103± 3	23 ± 2*
FEV_1/FVC (%)	77 ± 6	73 ± 5	39 ± 4*
TLC (% pred)	113 ± 5	100 ± 4	122 ± 7*
FRC (% pred)	113 ± 16	96 ± 1	196 ± 11*
RV (% pred)	136 ± 31	94 ± 2	236 ± 25*
DL_{CO} (% pred)	86 ± 10	80 ± 7	24 ± 3*
PaO_2 (mm Hg)	na	na	61 ± 3
$PaCO_2$ (mm Hg)	na	na	44 ± 2

Values are means ± SD. Lung function tests including DL_{CO}, and blood gases were evaluated before lung surgery ; only blood gas values under oxygen supplementation were available in the months preceding lung transplantation. *significantly different from nonsmokers, $p<0.05$ (Mann-Whitney *U* test) ; na : not available.

TABLE 2. Leukocytes immunostaining data.

	Neutrophils			Macrophages			T-lymphocytes		
	Nonsm	Sm	COPD	Nonsm	Sm	COPD	Nonsm	Sm	COPD
Large Airways									
Epithelium	0.03 (0-5.7)	0.9 (0.2-4.7)	2.9 (2.6-4.5)	0.2 (0-0.3)	0.8 (0.5-1)	0.5 (0.4-0.8)	16.7 (14.2-23.7)	19.1 (15.7-24.5)	18.3 (16.1-25.9)
Lamina	0.1 (0-5.5)	0.1 (0-6.8)	2.6 (1.9-2.8)	0.4 (0.2-0.8)	0.9 (0.4-1.5)	0.8 (0.7-0.8)	21.5 (17.4-23.1)	21.8 (18.1-22.7)	22.1 (19-23.8)
Glands	0.1 (0-0.8)	0.4 (0.1-1.2)	4.3** (3.8-5.1)	0.3 (0.1-0.5)	0.4 (0.2-0.6)	0.6 (0.5-0.9)	17.9 (14.8-25.3)	20 (17.4-27.4)	21.9 (15-24.8)
Small Airways									
Epithelium	0.1 (0-1.5)	0.1 (0-0.8)	5.9** (3.2-6.4)	0.1 (0-0.3)	0.8* (0.5-1.1)	0.9 (0.6-1)	32.8 (30.1-37.9)	20.8* (16.9-24.1)	35.2** (22-40.7)
Lamina	0.2 (0-0.6)	0.2 (0.1-0.5)	9.5** (7.2-12.3)	0.2 (0.1-0.5)	0.9° (0.7-1.2)	1.8 (1.6-1.9)	30.1 (30-35.2)	20.1* (18.2-21.3)	46.1** (35.2-50.8)

Values represent medians (range) of labelling indices (LI, %) for neutrophils, macrophages and T-cells in the microcompartments of large and small airways from the 3 groups of patients. °p=0.06, *p<0.05, smokers versus nonsmokers; **p<0.05, COPD versus smokers (Mann-Whitney *U*-test).

ABBREVIATIONS

COPD : chronic obstructive pulmonary disease

DL_{CO} : diffusing capacity of carbon monoxide

FEV_1 : forced expiratory volume in 1 second

FRC : functional residual capacity

FVC : forced vital capacity

LI : labelling index

MOD : mean optical density

TLC : total lung capacity

RV : residual volume

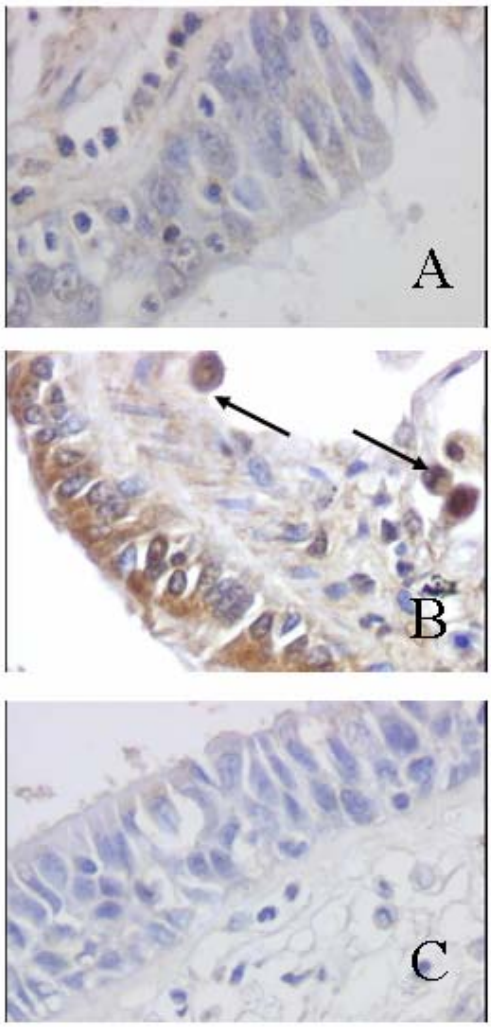


Figure 1

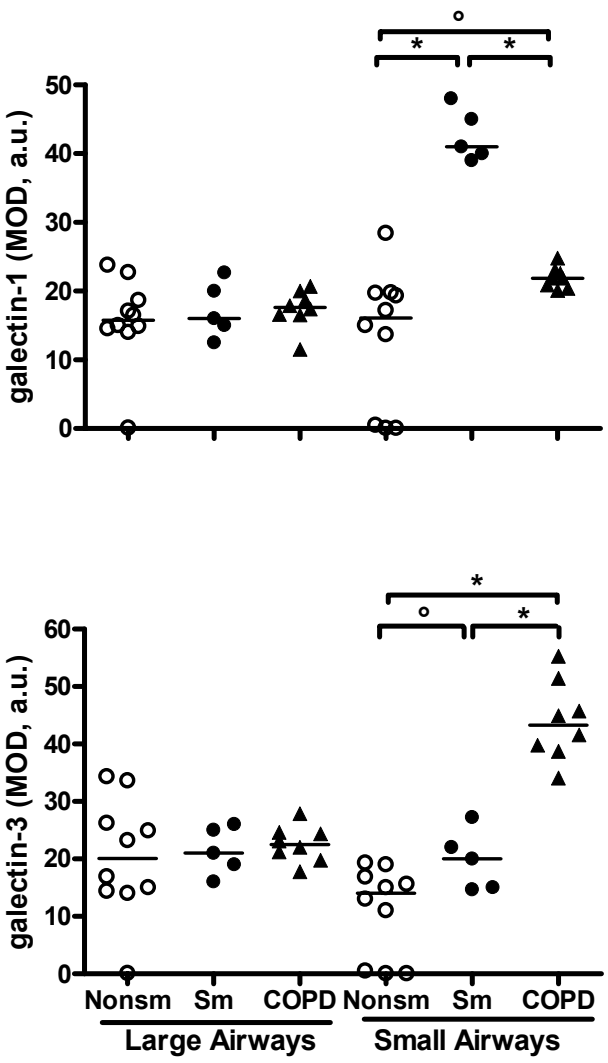

galectin-1 (MOD, a.u.)
50
40
30
20
10
0
°
*
*
galectin-3 (MOD, a.u.)
60
50
40
30
20
10
0
*
°
*
Nonsm
Sm
COPD
Nonsm
Sm
COPD
Large Airways
Small Airways

Figure 2

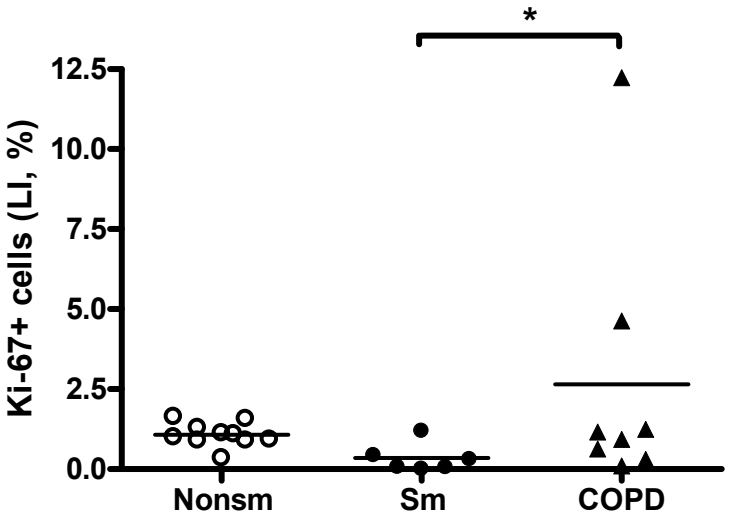


Figure 3

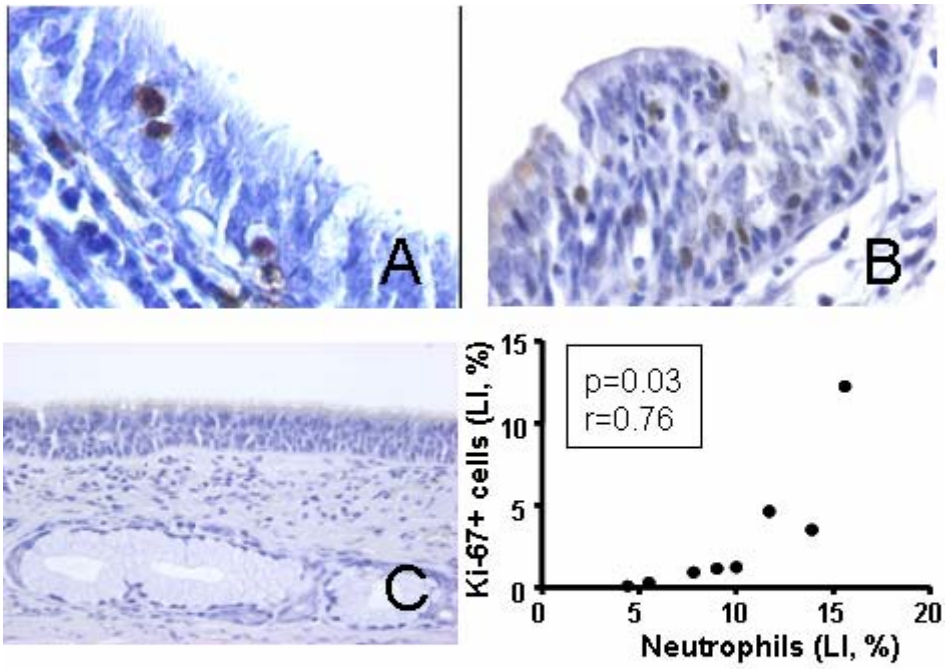
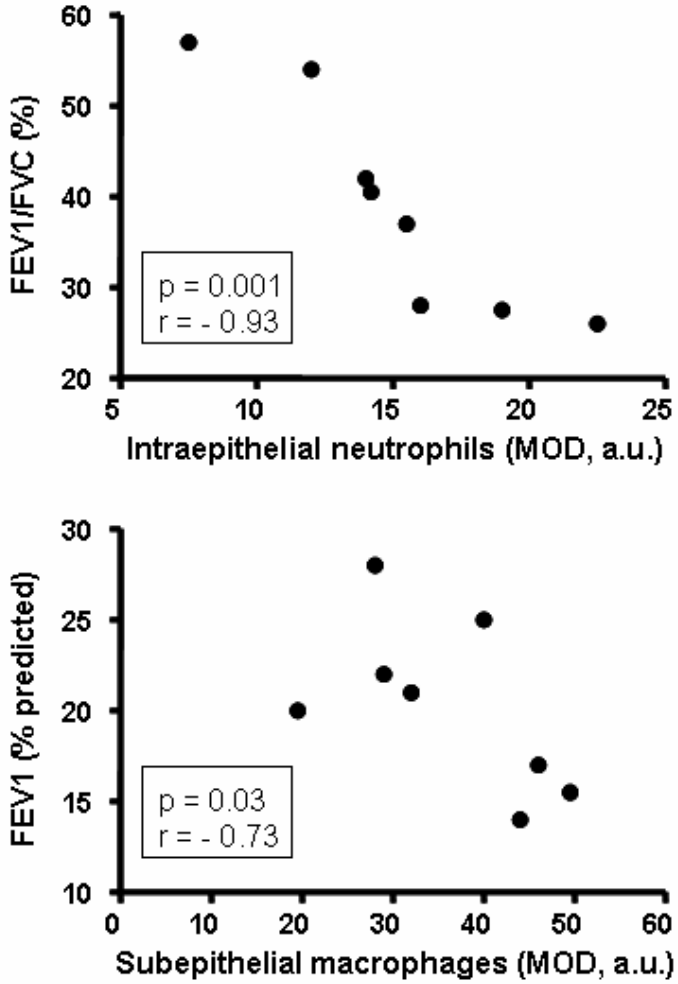


Figure 4


FEV1/FVC (%)
60
50
40
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p = 0.001
r = - 0.93
5
10
15
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25
Intraepithelial neutrophils (MOD, a.u.)
FEV1 (% predicted)
30
25
20
15
10
p = 0.03
r = - 0.73
0
10
20
30
40
50
60
Subepithelial macrophages (MOD, a.u.)

Figure 5