A Novel Insight into Adaptive Immunity in Chronic Obstructive Pulmonary Disease

B Cell Activating Factor Belonging to the Tumor Necrosis Factor Family

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***Rationale*: Chronic obstructive pulmonary disease (COPD) is a disorder characterized by an abnormal inflammatory response that persists even after smoking cessation, yet the underlying mechanisms are not fully understood.**

***Objectives*: To investigate the expression of B-cell activating factor of tumor necrosis factor family (BAFF), a crucial mediator in the cross-talk between innate and adaptive immune responses, in patients with COPD and to explore its correlation with disease severity.**

***Methods*: Using immunohistochemistry, expression of BAFF was examined in lung specimens from 21 smokers with COPD (FEV_1 = 57 ± 5% predicted), 14 control smokers (FEV_1 = 99 ± 2% predicted) and 8 nonsmokers (FEV_1 = 104 ± 4% predicted). BAFF was quantified in alveolar macrophages and alveolar walls, in bronchiolar and parenchymal lymphoid follicles, and in peripheral airways and pulmonary arterioles.**

***Measurements and Main Results*: In alveolar macrophages and parenchymal lymphoid follicles, BAFF expression was increased in smokers with COPD compared with control smokers and nonsmokers ($P < 0.05$ for all comparisons). In both compartments, BAFF was also up-regulated in control smokers as compared with nonsmokers ($P = 0.03$ and $P = 0.01$). Moreover, BAFF was overexpressed in bronchiolar lymphoid follicles, alveolar walls, peripheral airways, and pulmonary arterioles from smokers with COPD compared with nonsmokers ($P < 0.05$ for all). Among patients with COPD, $BAFF^+$ macrophages were inversely related to FEV_1 ($P = 0.03$, Spearman's rho [r_S] = −0.48), FEV_1/FVC ($P = 0.02$, $r_S = -0.50$), and Pa_{O_2} values ($P = 0.01$, $r_S = -0.55$).**

***Conclusions*: This study demonstrated overexpression of BAFF in peripheral lung of patients with COPD, mainly in alveolar macrophages and lymphoid follicles. Moreover, BAFF expression was correlated to the degree of lung function impairment and hypoxia, suggesting that it may have a possible impact on disease severity.**

Keywords: inflammatory cells; lymphoid follicles; cigarette smoking; airflow limitation

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Recent studies suggest that, beyond the elastase/antielastase imbalance, activation of adaptive immune responses may be a key component in chronic obstructive pulmonary disease (COPD) pathogenesis. B-cell activating factor of tumor necrosis factor family (BAFF) is a crucial mediator responsible for persistent immune activation, particularly in autoimmune conditions.

What This Study Adds to the Field

This study shows that BAFF is up-regulated in the lung of patients with COPD, mostly in alveolar macrophages and lymphoid follicles, and correlates with disease severity.

COPD is characterized by a chronic inflammation that increases progressively with the severity of the disease and persists long after smoking cessation (1). The mechanisms leading to the initiation and persistence of this inflammatory response are not fully understood, but lymphocytes seem to play a crucial role in disease pathogenesis. T lymphocytes, especially of the CD8 subset, are increased in the lungs of smokers with COPD, are activated and shifted toward a type 1 profile, with production of IFN-γ, and are potentially harmful because they release perforins and granzyme (2–5).

More recently, the involvement of B lymphocytes, either scattered in the airway wall or arranged in lymphoid follicles, has been appreciated (6–8). In particular, lymphoid follicles are reported in smokers with COPD both in the small airways and in the lung parenchyma (8–10). Most importantly, oligoclonal rearrangement of the immunoglobulin genes has been observed in B cells isolated from these follicles, suggesting that specific antigenic stimulation is driving B-cell proliferation (9). The antigen responsible for this induction is yet unknown, but it was first hypothesized that this was a response to chronic infections and airway colonization that occur frequently in patients with severe disease. However, because this oligoclonal response occurs in the absence of bacterial or viral antigens (9), it was proposed that antigen-specific responses could arise against self-epitopes (11), partly because of impaired tolerance (12, 13).

This hypothesis is supported by the recent observations of circulating antibodies against products derived from the pulmonary epithelium, endothelium, and extracellular matrix, such as elastin (11, 14). Moreover, lymphocytes cultured from the lungs of patients with emphysema respond to elastin by secreting IFN-γ, and this response can be blocked by MHC class II antibodies, which indicates that antigen presentation is required (11). The picture emerging from these data, together

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with the observation that lung inflammation persists for years after smoking cessation, suggest that self-perpetuating mechanisms, similar to those occurring in autoimmune diseases, may contribute to the pathogenesis of COPD.

We therefore focused on B-cell activating factor belonging to the tumor necrosis factor (TNF) family (BAFF), a molecule that was originally described as a factor responsible for B-cell survival and maturation (15–19) and has been associated with autoimmune diseases. BAFF is expressed by monocytes, macrophages, dendritic cells, and T cells, where it is up-regulated on cellular activation in both CD4 and CD8 subsets (20–23). Moreover, T cells stimulated in the presence of BAFF secrete more IFN-γ (and less IL-4 and IL-5), suggesting that BAFF may be a Th1 response-promoting cytokine (24–26). BAFF signal is mediated by binding to three different receptors, of which the most specific is BAFF receptor (BAFF-R) (16).

So far, the function of BAFF has been studied mainly in autoimmune diseases. In fact, elevated serum and tissue levels of BAFF have been reported in several autoimmune disorders, such as Sjögren syndrome, systemic lupus erythematosus, and rheumatoid arthritis. Moreover, BAFF expression is correlated with clinical and immunological parameters reflecting disease severity, and BAFF transgenic mice develop lupus-like disease (27–31).

In the present study, we quantified the expression of BAFF in peripheral lung tissue of patients with COPD, compared with age-matched smoking and nonsmoking subjects with normal lung function, focusing on the correlation with disease severity. The results of this study were previously presented in abstract form (32, 33).

METHODS

Subject Characteristics

To quantify the expression of BAFF, we collected lung tissue from 43 subjects undergoing lung volume reduction surgery for the treatment of severe emphysema or lung resection for a solitary peripheral carcinoma. The subjects were categorized into the following three groups: smokers with COPD (Global Initiative for Chronic Obstructive Lung Disease stage I–IV; n = 21); smokers without symptoms of chronic bronchitis or airflow obstruction (control smokers; n = 14), and nonsmokers without symptoms of chronic bronchitis or airflow obstruction (nonsmokers; n = 8). Each patient, in the week before surgery, underwent: interview, electrocardiography, routine blood tests, pulmonary function tests, and chest radiographs. Patients with COPD did not experience any exacerbation, and all recruited subjects had been free of acute upper respiratory tract infections during the month preceding the study. The subjects were nonatopic and had no history of asthma or allergic rhinitis. In subjects with normal lung function, inhalation challenge with methacholine was performed, and all subjects had reactivity within the normal range (provocative dose of methacholine causing a 20% fall in FEV_1 >1.44 mg).

The study conformed to the Declaration of Helsinki and was approved by the Local Ethics Committee; informed written consent was obtained for each subject undergoing surgery.

Immunohistochemistry and Morphometric Analysis

Randomly selected tissue blocks were taken from the subpleural parenchyma (avoiding areas affected by tumor in patients who underwent lung resection for nodules). Samples were fixed in formalin, embedded in paraffin wax, and processed for immunohistochemical analysis of BAFF.

To quantify BAFF expression in alveolar macrophages, at least 20 nonconsecutive high-power fields (hpf) and at least 100 macrophages inside the alveolar spaces were evaluated for each subject; results were expressed as percentage of $BAFF^+$ macrophages over the total number of macrophages examined and as number of macrophages/hpf. For evaluation of BAFF in alveolar walls, 10 nonconsecutive hpf were evaluated for each subject and the results were expressed as the number of positive cells/mm of alveolar wall. BAFF expression was also evaluated in peripheral airways and pulmonary arterioles using a semiquantitative score (0: no staining; 1: weak staining; 2: moderate staining; 3: strong staining).

Furthermore, BAFF expression was quantified in lymphoid follicles. In both parenchyma and peripheral airways, we evaluated all aggregates containing more than 40 contiguous mononuclear cells that demonstrated the characteristic topographical arrangement of B cells ($CD20^+$) and T cells ($CD4^+$ and $CD8^+$). In follicles considered to be positive, the majority of lymphocytes showed a diffuse BAFF staining. In the lung parenchyma, data were expressed as number of positive parenchymal follicles/cm^2 of tissue examined and in the peripheral airways as percentage of airways containing $BAFF^+$ lymphoid follicles over the total number of airways examined.

In a subset of patients (14 subjects with COPD, 6 control smokers, and 3 nonsmokers) the expression of BAFF receptor (BAFF-R) was also investigated. Moreover, to characterize which cells express BAFF, we performed immunohistochemistry for $CD4^+$ and $CD8^+$ T lymphocytes, $CD20^+$ B lymphocytes, CD31 (endothelial cells), and TTF1 (type II pneumocytes) in sequential serial sections. Confocal microscopy was applied to evaluate colocalization of BAFF and the dendritic cell marker CD1a (details of immunohistochemical analyses are included in the online supplement). Furthermore, BAFF expression was stratified according to bacterial colonization as detected either by BAL cultures (when available) or by Gram tissue staining (in all subjects), as described in the online supplement.

Tissue samples were analyzed by a single observer blinded to clinical characteristics using an image-analysis system (Image-Pro Plus, Silver Spring, MD). One-third of the sections were evaluated independently by two additional observers in preliminary analyses to assess the interobserver variability and by the same observer in three different occasions to assess intraobserver variability. The mean coefficients of variation for inter- and intraobserver variability ranged from 9% for quantification of BAFF in lymphoid follicles to 11% for that in alveolar macrophages.

Statistical Analysis

Group data are expressed as mean ± SEM or as median (range). Differences among groups were analyzed using the nonparametric Kruskal-Wallis U test for morphological data, and the analysis of variance for clinical data. The Mann-Whitney U test was performed after Kruskal-Wallis when appropriate. Correlation coefficients were calculated using Spearman rank method. Probability values of $P < 0.05$ were accepted as significant.

RESULTS

Clinical Characteristics of Study Subjects

The clinical characteristics of the subjects examined are shown in Table 1. Demographic analysis revealed that age was not significantly different among the three groups of subjects, but there were more women among nonsmoking control subjects than among smokers with COPD and control smokers. All patients with COPD were smokers (12 current, 9 exsmokers) and the majority had symptoms of chronic bronchitis (15 out of 20, data missing in 1 subject). The cumulative smoking exposure (pack-years) was similar in smokers with COPD and control smokers. As expected from the selection criteria, subjects with COPD had significantly lower values of FEV_1% predicted and FEV_1/FVC (%) as compared with control smokers and nonsmokers.

Among patients with COPD, 9 had severe/very severe COPD and 12 had mild/moderate disease. In smokers with COPD, the values of Pa_{O_2} were significantly reduced and those of Pa_{CO_2} were significantly increased compared with the other two groups of subjects examined. Smokers with COPD had signs of lung hyperinflation (increased residual volume) and impaired carbon monoxide diffusion capacity (decreased D_{LCO})

TABLE 1. CLINICAL CHARACTERISTICS

	Smokers with COPD (GOLD stage I–IV)	Control Smokers	Nonsmokers
Men/women, n	18/3	14/0	4/4*
Age, yr	64 ± 2	63 ± 2	57 ± 5
Smoking history, pack-years	47 ± 5	44 ± 6	—
Current/exsmokers	12/9	5/9	—
FEV_1, % predicted	57 ± 5†‡	99 ± 2	104 ± 4
FEV_1/FVC, %	53 ± 4†‡	78 ± 1	80 ± 1
Pa_{O_2}, mm Hg	72 ± 3†‡	86 ± 2	82 ± 3
Pa_{CO_2}, mm Hg	41 ± 1†‡	37 ± 1	38 ± 2
RV, % predicted	145 ± 13‡	91 ± 12	—
D_{LCO}, % predicted	55 ± 10‡	74 ± 5	—

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; D_{LCO} = diffusing capacity of carbon monoxide; GOLD = Global Initiative for Chronic Obstructive Lung Disease; RV = residual volume.

Values are expressed as mean ± SEM. Measurements of RV were performed in only 23 subjects (16 with COPD and 7 control smokers). Measurements of D_{Lco} were performed in only 16 subjects (11 with COPD and 5 control smokers).

* Significantly different from COPD subjects and control smokers ($P < 0.05$).

† Significantly different from nonsmokers ($P < 0.05$).

‡ Significantly different from control smokers ($P < 0.05$).

as compared with control smokers. Smokers with mild/moderate COPD, control smokers, and nonsmokers did not receive any antiinflammatory therapy (e.g., oral or inhaled corticosteroids) or antibiotics within the month preceding surgery, or bronchodilators within the previous 48 hours. All patients with severe/very severe COPD were treated with inhaled anticholinergics and/or β_2-agonists/inhaled corticosteroids, but none of them with oral steroids.

Reliable information on comorbidities was obtained in 25 out of 43 patients included in our study (12 patients with COPD, 8 control smokers, and 5 nonsmokers). The most common comorbidities in these patients were cardiovascular (myocardial infarction [n = 3], transient ischemic attack [n = 2], angina [n = 1], arterial hypertension [n = 8]) and metabolic (diabetes [n = 3], glucose intolerance [n = 1], gout [n = 1]) morbidities. As for treatment, antihypertensive drugs (calcium antagonists/angiotensin-converting enzyme inhibitors) were recorded in eight patients (four patients with COPD, three control smokers, and one nonsmoker), whereas statins were not much used in this historical population. Peroxisome proliferator–activated receptor (PPAR)-γ agonists and nonsteroidal antiinflammatory drugs were recorded in two patients, respectively (one COPD and one control smoker each).

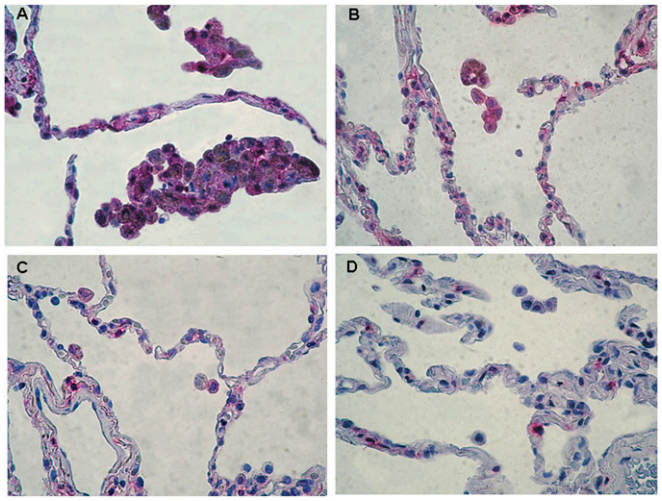

Figure 1. Immunohistochemistry for B-cell activating factor of tumor necrosis factor family in lung tissue from (*A*) a smoker with severe chronic obstructive pulmonary disease (COPD), (*B*) a smoker with moderate COPD, (*C*) a control smoker, and (*D*) a nonsmoker. (*A*) Strong cytoplasmic positivity was seen in all macrophages and in cells of alveolar wall. Weakly positive alveolar macrophages can be observed in the (*C*) smoking and (*D*) nonsmoking control subjects.

Immunohistochemical Findings

BAFF immunoreactivity was mainly detected in the cytoplasm, whereas in some cases a more intense perinuclear staining was observed. Alveolar macrophages and infiltrating lymphoid cells, including those arranged in follicular pattern, were strongly stained (Figures 1 and 2). BAFF positivity was also detected in the cytoplasm of structural cells as the epithelium of peripheral airways and blood vessel endothelium and muscle cells (Figure 2). No staining was present in negative control experiments using isotype-matched control subjects at the same concentrations (*see* Figure E1 in the online supplement).

Comparison by means of Kruskal-Wallis revealed that the three groups of subjects differed significantly with regard to BAFF expression in alveolar macrophages ($P = 0.001$), alveolar walls ($P = 0.04$), peripheral airways ($P = 0.01$), and pulmonary arterioles ($P = 0.002$), as well as in both parenchymal and bronchiolar lymphoid follicles ($P = 0.002$ and $P = 0.01$, respectively). In particular, $BAFF^+$ alveolar macrophages were increased in patients with COPD compared with control

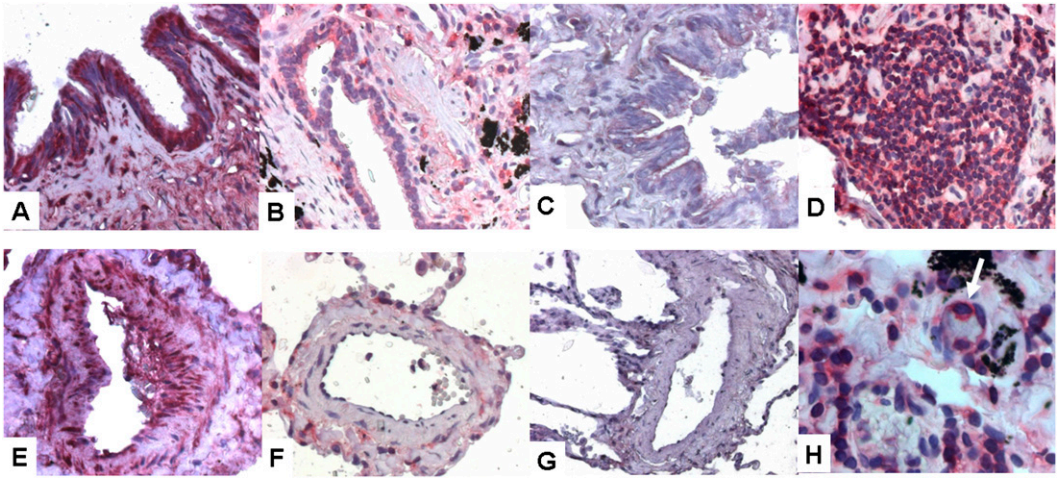

Figure 2. Immunohistochemistry for B-cell activating factor of tumor necrosis factor family in lung sections from (*A, E, D, H*) a patient with chronic obstructive pulmonary disease, (*B, F*) a control smoker, and (*C, G*) a nonsmoker. Strong cytoplasmic positivity was observed in peripheral airways, particularly in (*A*) the epithelium, and (*E*) pulmonary arterioles, but staining was also prominent in (*D*) lymphoid follicles and (*H*) capillary endothelial cells (*arrow*).

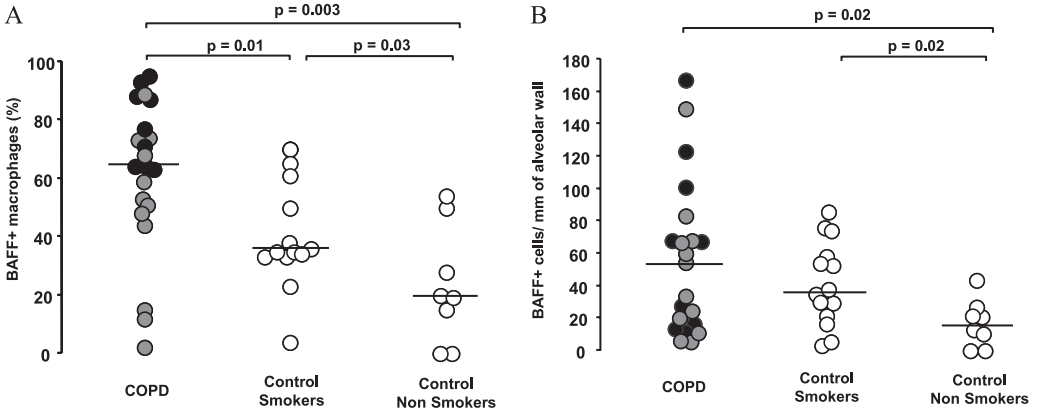

Figure 3. Individual counts for (*A*) percentage of B-cell activating factor of tumor necrosis factor family (BAFF)$^+$ macrophages and (*B*) number of BAFF$^+$ cells in alveolar walls. *Solid circles* represent patients with severe/very severe chronic obstructive pulmonary disease (COPD); *shaded circles* represent those with mild/moderate disease. *Horizontal bars* represent median values. *P* values in figure represent Mann-Whitney U test analyses.

smokers ($P = 0.01$) and nonsmokers ($P = 0.003$) (Figures 1 and 3A). BAFF$^+$ macrophages were also increased in smokers with normal lung function compared with nonsmoking control subjects ($P = 0.03$) (Figure 3A). Similar findings were obtained when the results were expressed as number of BAFF$^+$ macrophages/hpf (Table 2). Moreover, BAFF expression was also increased in alveolar walls of patients with COPD as compared with nonsmoking control subjects ($P = 0.02$), but the difference with smoking control subjects did not reach the levels of statistical significance (Figure 3B). Once more, a significant difference was observed between smokers with normal lung function and nonsmokers ($P = 0.02$).

The extent of BAFF expression in peripheral airways and pulmonary arterioles, as reflected by a semiquantitative score, was also increased in COPD when compared with nonsmoking control subjects ($P = 0.003$ and $P = 0.001$, respectively) (Table 2). Again, the numerical increase observed in smokers with COPD compared with smoking control subjects did not reach the levels of statistical significance. BAFF expression was also different between smoking and nonsmoking control subjects ($P = 0.04$ and $P = 0.01$) (Table 2). Considering patients with severe/very severe and mild/moderate COPD separately, only BAFF$^+$ alveolar macrophages differed significantly between smokers with severe/very severe COPD (median, 77%; range, 63–95%) and those with mild/moderate disease (median, 53%; range, 2–89%; $P = 0.01$). In none of the examined compartments did BAFF expression differ significantly between current and exsmokers.

Given the importance of BAFF in B-cell proliferation, we extended our analysis to lymphoid follicles in both lung parenchyma and peripheral airways. We confirmed previous observations that parenchymal lymphoid follicles were increased in smokers with COPD (median, 3 follicles/cm^2; range, 0–12 follicles/cm^2) as compared with both smoking (median, 1 follicle/cm^2; range, 0–6 follicles/cm^2; $P = 0.009$) and nonsmoking control subjects (median, 0 follicles/cm^2; range, 0–2 follicles/cm^2; $P = 0.002$). Similarly, the percentage of airways containing lymphoid follicles was increased in smokers with COPD (median, 20%; range, 0–100%) as compared with both smoking (median, 0; range, 0–100%; $P = 0.05$) and nonsmoking control subjects (median, 0; range, 0–20%; $P = 0.03$). Of interest, we observed that these lymphoid follicles showed a prominent BAFF staining. In fact, when BAFF was quantified in parenchymal lymphoid follicles, it was increased in smokers with COPD as compared with smoking and nonsmoking control subjects ($P = 0.04$ and $P = 0.002$) (Figure 4A). Moreover, BAFF$^+$ parenchymal lymphoid follicles were increased in smokers with normal lung function compared with nonsmoking control subjects ($P = 0.01$). A similar pattern was observed in peripheral airways but the difference with smoking control subjects did not reach the levels of statistical significance (Figure 4B).

When we examined the relation between BAFF expression and comorbidities, we found no differences in BAFF expression between subjects with cardiovascular/metabolic conditions (n = 14) and those without these conditions (n = 11), either in alveolar macrophages (median, 51%; range, 2–95% vs. median, 44%; range, 0–93%), in alveolar walls (median, 28 cells/mm; range, 3–101 cells/mm; vs. median, 29 cells/mm; range, 0–167 cells/mm) or in lymphoid follicles (median, 2; range, 0–6 follicles/cm^2; vs. median, 1; range, 0–7 follicles/cm^2). The same was true when only patients with COPD were considered:

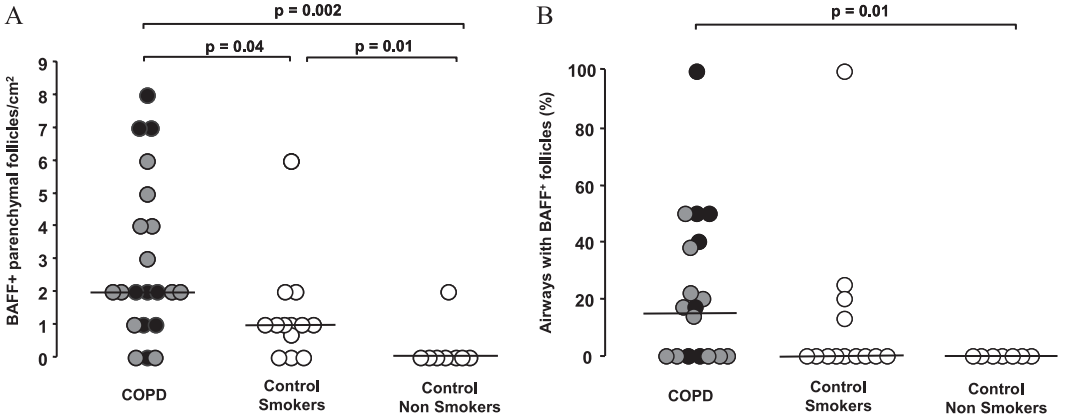

Figure 4. Individual counts for (*A*) number of B-cell activating factor of tumor necrosis factor family (BAFF)$^+$ lymphoid follicles in lung parenchyma and (*B*) percentage of peripheral airways with BAFF$^+$ lymphoid follicles. *Solid circles* represent patients with severe/very severe chronic obstructive pulmonary disease (COPD); *shaded circles* represent those with mild/moderate disease. *Horizontal bars* represent median values. *P* values in figure represent Mann-Whitney U test analyses.

TABLE 2. ADDITIONAL EVALUATION OF B-CELL ACTIVATING FACTOR OF TUMOR NECROSIS FACTOR FAMILY EXPRESSION

	Smokers with COPD	Control Smokers	Nonsmokers
Positive macrophages/hpf	4.5 (0.1–14)*†	3.1 (0.2–9.9)	1.7 (0–5)
BAFF bronchiolar wall score, %	60 (17–100)*	38 (0–83)*	33 (5–42)
BAFF pulmonary arterioles score, %	33 (0–87)*	16 (1–76)*	4 (0–16)

Definition of abbreviations: BAFF = B-cell activating factor of tumor necrosis factor family; COPD = chronic obstructive pulmonary disease; hpf = high-power field.

Values are expressed as median (range).

* Significantly different from nonsmokers ($P < 0.05$).

† Significantly different from control smokers ($P < 0.05$).

indeed BAFF expression did not differ between patients with COPD with (n = 7) or without (n = 5) cardiovascular/metabolic comorbidities.

Furthermore, we examined the relation between BAFF expression and bacterial colonization (*see* online supplement for details), and found no difference between patients with evidence of bacterial colonization (n = 10) and those without (n = 33), either in $BAFF^+$ alveolar macrophages (median, 51%; range, 34–71%; vs. median, 50%; range, 0–95%), $BAFF^+$ cells in alveolar walls (median, 48 cells/mm; range, 16–83 cells/mm; vs. median, 27; range, 0–167 cells/mm) or $BAFF^+$ lymphoid follicles (median, 1 follicle/cm^2; range, 0–6; vs. median, 1 follicle/cm^2; range, 0–8 follicles/cm^2).

We then analyzed the lymphocyte composition of $BAFF^+$ lymphoid follicles by examining sequential serial sections stained with BAFF, CD20, CD4, and CD8. We observed that $CD20^+$ B lymphocytes were the predominant cell types in $BAFF^+$ follicles, surrounded by some $CD8^+$ and $CD4^+$ T cells (Figure 5). Furthermore, confocal microscopy analysis in lymphoid follicles revealed coexpression of BAFF and the dendritic cell marker CD1a (Figure 5). To characterize which cells do express BAFF, besides those of the immune system, sequential serial sections were stained for BAFF and either the type II-pneumocyte marker TTF1 or the endothelial marker CD31. This analysis showed that $BAFF^+$ cells included type II pneumocytes and endothelial cells (Figure E2).

Finally, we also evaluated the expression of the most specific receptor for BAFF, BAFF-R. The receptor was almost absent in lung tissue of nonsmoking subjects and hardly detectable even in smoking control subjects. Conversely, BAFF-R was observed in lung tissue of smokers with COPD, where it was expressed mainly by lymphoid follicles both in the lung parenchyma and associated with peripheral airways (Figure 6) and only occasionally in alveolar macrophages. Quantitative analysis of BAFF-R was performed in parenchymal lymphoid follicles, where the expression of the receptor was significantly increased in COPD as compared with control subjects (median, 0.50 follicles/cm^2; range, 0–4.8 follicles/cm^2; vs. median, 0 follicles/cm^2; range, 0–0.7 follicles/cm^2; $P = 0.03$).

Correlations

When all subjects were considered together, several significant correlations were observed between the different morphometric measurements and functional parameters. All details of these analyses are reported in the online supplement. When we limited the analysis to all smoking subjects, negative correlations were observed between the number of $BAFF^+$ macrophages and the values of FEV_1% predicted ($P = 0.01$, $r_S = -0.43$), FEV_1/FVC ratio ($P = 0.0003$; $r_S = -0.57$) and Pa_{O_2} ($P = 0.01$; Spearman's rho [r_S] $= -0.44$). Moreover, the expression of BAFF in alveolar macrophages was positively related to that in peripheral airways ($P = 0.01$; $r_S = 0.43$) and to the values of phospho-p38 mitogen-activated protein kinases (MAPK) in alveolar macrophages, as obtained from a previous report (34) ($P = 0.01$; $r_S = 0.53$). Finally, the expression of the receptor, BAFF-R, in lymphoid follicles was negatively related to FEV_1/FVC ratio ($P = 0.05$; $r_S = -0.43$), Pa_{O_2} ($P = 0.03$; $r_S = -0.50$) and $D_{L_{CO}}$ values ($P = 0.05$; $r_S = -0.62$).

Of note, some of these correlations were also maintained when only patients with COPD were considered. In particular, among smokers with COPD, the number of $BAFF^+$ macrophages was inversely related to FEV_1% predicted ($P = 0.03$; $r_S = -0.48$) (Figure 7A), FEV_1/FVC ratio ($P = 0.02$; $r_S = -0.50$), and Pa_{O_2} values ($P = 0.01$; $r_S = -0.55$) (Figure 7B). Furthermore, in patients with COPD, there was a positive correlation between $BAFF^+$ alveolar macrophages and phospho-$p38^+$ macrophages ($P = 0.05$; $r_S = 0.53$).

DISCUSSION

This is the first study to demonstrate increased BAFF expression in peripheral lung of patients with COPD, particularly in alveolar macrophages and lymphoid follicles. The expression of BAFF was related to the degree of lung function impairment and hypoxia, suggesting that it may have a possible impact on disease severity.

It is widely accepted that an abnormal inflammatory response plays an important role in the pathogenesis of COPD (2–7, 35, 36). This has been extensively documented for cells of the innate response, such as neutrophils and macrophages, which can cause proteolytic damage to the extracellular matrix and have been historically ascribed as responsible for emphysema (37, 38). More recently, activation of an adaptive immune response in the lung, mediated by the cooperation of dendritic cells and T and B lymphocytes, has been identified as a key component in disease pathogenesis (8–11, 39–42).

Members of the TNF family, by binding to their receptors, collectively play a central role in regulating normal immune function. In this context, BAFF has been originally identified as a factor responsible for B-cell survival and maturation (15–20), but it is important to highlight that it also affects T-cell functions (24–26). In healthy individuals, BAFF is overproduced during infections by cells of the innate system (i.e., neutrophils and macrophages, together with dendritic cells). Of interest, in our study, macrophages were found to be a prominent source of BAFF and it is well known that BAFF is a key mediator through which macrophages can directly regulate B-cell proliferation (22, 23).

BAFF produced at inflammatory sites is cleaved from the cell surface, possibly by the convertase furin, and its release is dependent on p38 MAPK and c-Jun N-terminal kinases (JNK) (15). Soluble BAFF circulates as a homotrimer that activates B and memory effector T cells, promoting effective pathogen clearance (43). Infections and bacterial colonization are a frequent occurrence in patients with COPD and it is plausible that chronic stimulation of macrophages by pathogens may promote

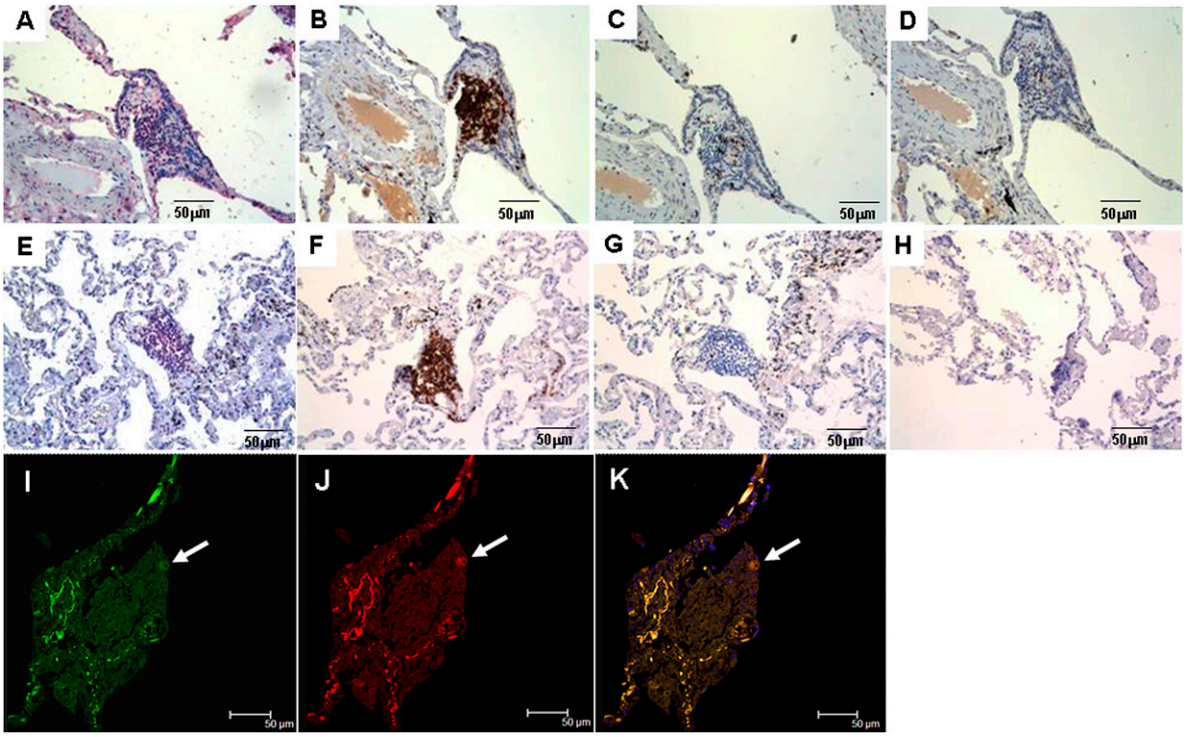

Figure 5. Sequential serial sections representing parenchymal lymphoid follicles in smokers with chronic obstructive pulmonary disease. Immunohistochemical staining with monoclonal antibodies for detection of (*A*, *E*) B-cell activating factor of tumor necrosis factor family (BAFF), (*B*, *F*) CD20, (*C*, *G*) CD4, and (*D*, *H*) CD8. (*I–K*) Confocal analysis in a lymphoid follicle from a smoker with COPD showing costaining of BAFF (*green*) and the dendritic cell marker CD1a (*red*), as indicated by the *arrow*.

deregulated B- and T-cell responses. However, in our study there was no evidence that BAFF expression was associated with the presence of bacterial pathogens. Of note, BAFF activation has been described not only during infections but also in autoimmune and lymphoproliferative disorders. In humans, high levels of BAFF were detected in the blood of patients with rheumatoid arthritis, systemic lupus erythematosus, and Sjögren syndrome (27–31). In animal models, overexpression of BAFF in transgenic mice boosts the number of mature B and effector T cells, and promotes autoimmune-like manifestations, such as high levels of rheumatoid factors, circulating immune complexes, and anti-DNA autoantibodies (26, 29, 44, 45). Moreover, blockade of BAFF inhibits the inflammatory response in a mouse model of rheumatoid arthritis (46).

Of interest, the overexpression of BAFF observed in our study, although it does not provide direct evidence, is in keeping with the recent proposal that autoimmunity may have a role in the pathogenesis of COPD (13, 47). Indeed, the inflammatory process in COPD persists for years after cessation of the offending agent (48) (i.e., cigarette smoke) suggesting activation of self-perpetuating mechanisms involving both humoral and cell-mediated responses. Pioneer studies reported organization of immune cells in lymphoid follicles and demonstration of their oligoclonality (8–10, 41). Our study confirms and extends these observations, demonstrating, for the first time, that the majority of these follicles express BAFF together with its most specific receptor, BAFF-R. In fact, previous results indicate an involvement of BAFF, in cooperation with Notch signaling, in formation of splenic germinal centers, where affinity maturation occurs and memory B cells are generated (17–20). Of interest, in the lung, BAFF is known to be up-regulated in inducible bronchus-associated lymphoid tissue (iBALT) of patients with pulmonary complications of autoimmune diseases, such as rheumatoid arthritis and Sjögren syndrome (49).

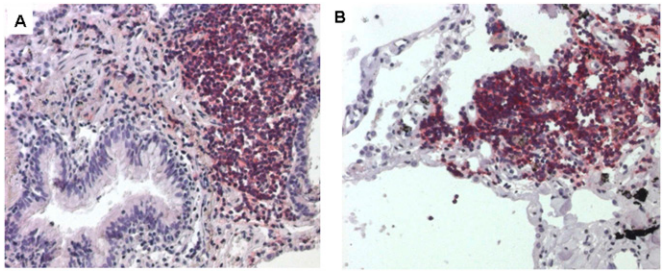

Figure 6. Immunohistochemistry for B-cell activating factor of tumor necrosis factor family receptor in lung sections from patients with chronic obstructive pulmonary disease. Strong cytoplasmic positivity was observed in lymphoid follicles, either (*A*) associated with the peripheral airways, or (*B*) in the lung parenchyma.

We should highlight that in our study BAFF expression was detected not only in cells of the immune response, such as alveolar macrophages and lymphocytes, but also in structural cells, such as the airway and alveolar epithelium and endothelial cells. The observation that BAFF was up-regulated in airway epithelial cells *in vivo* extends previous reports *in vitro* (50) and suggests that lung structural cells may substantially contribute to the local activation of immune responses. Of interest, there is accumulating evidence that structural cells in the lung may produce a wide array of molecules with important immune functions, such as surfactants, defensins, and complement proteins, that have the potential to regulate immune responses in both physiologic (leading to immune tolerance) and pathologic (promoting immune activation) conditions (51).

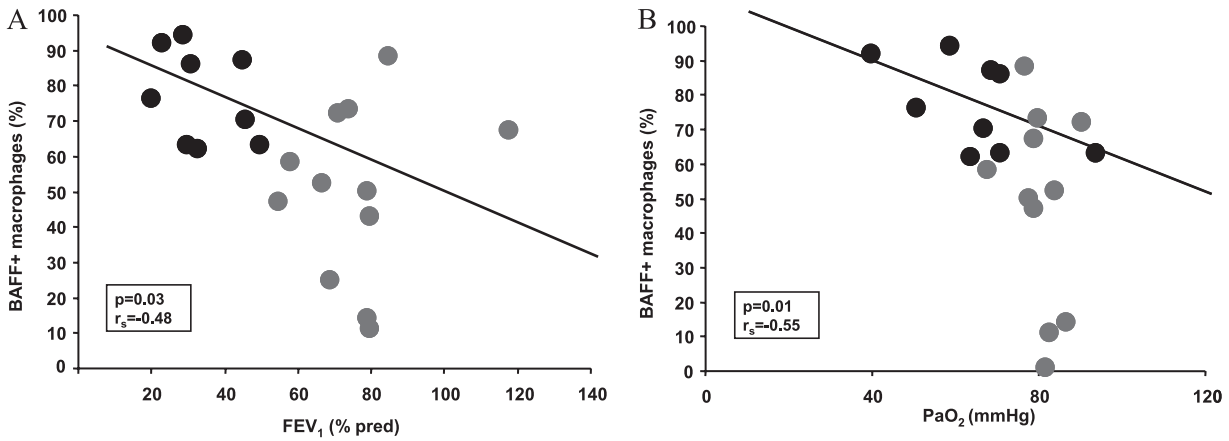

Figure 7. (*A*) Relationship between the values of FEV_1 (% predicted) and the percentage of B-cell activating factor of tumor necrosis factor family $(BAFF)^+$ macrophages in smokers with chronic obstructive pulmonary disease (COPD). (*B*) Relationship between the values of Pa_{O_2} (mm Hg) and the percentage of $BAFF^+$ macrophages in smokers with COPD. *Solid circles* represent patients with severe/very severe COPD; *shaded circles* represent those with mild/moderate disease. rs = Spearman's rho.

Although ours is an observational study, it is worthwhile to note that overexpression of BAFF was associated with that of its specific receptor, BAFF-R, and with the phosphorylation of p38 MAPK, which is involved in BAFF release, suggesting that BAFF signaling is indeed activated in patients with COPD. Undoubtedly, properly designed functional studies would be required to unravel the mechanisms responsible for BAFF overexpression and the consequences of its activation; nevertheless, studies like ours are important, because they may provide the clinical framework for proper functional investigations.

We should acknowledge that a great proportion of subjects, among smokers with mild/moderate COPD and control subjects, had lung cancer, and the presence of cancer itself may have influenced the results by enhancing inflammation and BAFF expression (52, 53). However, smokers with severe COPD who did not have lung cancer had the greatest levels of BAFF expression, at least in macrophages. If a bias due to cancer was present in our study, the up-regulation of BAFF in COPD would be underestimated rather than overestimated. Furthermore, by stratifying our population on the basis of lung function only, we may have overlooked the influence of other important factors, such as chronic comorbidities, which contribute significantly to the heterogeneity observed in patients with COPD. Nevertheless, when we compared BAFF expression between patients with and those without cardiovascular/metabolic comorbidities, we could not detect any difference.

Finally, although we did special tissue staining for detection of bacteria, we could not perform a more specific molecular analysis, and it remains undetermined whether immune activation occurs in response to self-epitopes or rather to exogenous antigens. Indeed, BAFF is crucial for early host defense against pathogens and therefore occupies a place between innate and adaptive immune responses. Enhancement of the adaptive response by the innate system is life saving in the case of infections but is not without risk because it may predispose to the development of autoimmune diseases (43). At present, we cannot definitely conclude on the pathological role, if any, of the immune response in COPD. Indeed, lymphoid follicles and the ensuing local immune responses might be regarded as either protective against colonization or harmful when self-directed, leading to perpetuation of ongoing inflammation and pulmonary damage (54). Evaluation of BAFF could be particularly informative in this context: indeed, because it is a soluble mediator, it can be repeatedly assessed in peripheral blood and therefore evaluated in longitudinal studies as a marker to monitor disease progression.

In conclusion, this study demonstrated increased BAFF expression in peripheral lung of patients with COPD, which was correlated to disease severity. The expression was particularly prominent in macrophages and lymphoid follicles, suggesting that BAFF may be implicated in the crosstalk between cells of the innate and adaptive immunity in COPD.

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