Induced sputum CD8+ T-lymphocyte subpopulations in chronic obstructive pulmonary disease

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Summary Background: Previous studies have shown that the inflammatory response to cigarette smoking differs between smokers who develop chronic obstructive pulmonary disease (COPD) and those who do not and that the CD8+ T-lymphocytes have been identified as a key player in this process. The aim of this study was to investigate further the role of CD8+ cells and their subtypes in sputum cells.

Methods: Sputum induction was performed in 36 COPD patients, 25 smokers without COPD and 10 non-smoking healthy controls. After stimulation of sputum lymphocytes with phorbol-myristate-acetate, we used double immunocytochemical methods to identify CD4+, CD8+ cells and CD8+ INFγ or IL4 cells (Tc1; Tc2).

Results: COPD patients had an increased number of CD8+ cells in sputum as compared with smokers without COPD (P = 0.0001) and control subjects (P = 0.001). CD8+ -IL4 cells were reduced both in COPD and in smokers without COPD compared to controls (P = 0.0001), while CD8+ -INFγ cells were significantly reduced only in COPD (P = 0.001) as compared with controls. A significant (P = 0.02) relationship between the CD8+ -IL4/CD8+ -INFγ ratio and FEV1 (% pred) was found only in COPD patients.

Conclusion: These findings suggest that an imbalance both in T-lymphocyte subpopulation (CD4/CD8) and in CD8+ cell subsets (Tc1/Tc2) characterizes the inflammatory responses of smokers with established COPD.

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Introduction

It is widely accepted that abnormal inflammatory responses to several noxious agents (smoking) play an important role in the pathogenesis of chronic obstructive pulmonary disease (COPD).1 Previous studies have provided evidence for an inflammatory process in the large airways of subjects with COPD with symptoms of chronic bronchitis, consisting predominantly of activated T-cells.2 In comparison with smokers who did not develop airflow limitation, patients with COPD have increased numbers of CD8+ cells present in the small3 and large airways.4,5 Although some reports have linked CD8+ T-cells with the inflammation induced by smoking itself,6,7 a significant correlation between CD8+ numbers and FEV1 in COPD patients has been reported.5 On the other hand, de Jong and

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colleagues have shown that CD8\(^+\) T-cells in peripheral blood were increased in non-smoking COPD patients, while the CD4/CD8\(^+\) ratio was significantly correlated with lung function and serum IgE.\(^8\) In addition, Majori and colleagues\(^9\) examined T-cell cytokine profile and reported a Th1-like immune response of peripheral blood CD4\(^+\) T-cells in subjects with COPD. Mattoli et al.\(^10\) expanded these data to BAL samples of COPD individuals with mainly chronic bronchitis symptoms and showed that the CD8\(^+\) T-cell Th1Th2 phenotype of smokers with COPD is different from the CD8\(^+\) T-cell clones of smokers without COPD. The predominance of CD8\(^+\) T-cells in peripheral blood, bronchial biopsies and in BAL seems to display a clear difference between smokers with COPD and without airflow limitation.\(^3\)–\(^5\) However, the precise function of the CD8\(^+\) T-cells in pathogenesis of COPD has not been established. In the current study, we assessed the cellular populations, and in particular, that of CD8\(^+\) T-cells and their subtype in induced sputum in order to investigate the role of CD8\(^+\) T-cells in the pathogenesis of COPD. We compared a group of current smokers who had already developed COPD and a group of current smokers without COPD. We recruited smokers with the same smoking history but with and without COPD. In addition, measurements were made in healthy non-smokers.

**Material and methods**

**Subjects**

Seventy-one males were studied: (a) 36 current smokers with COPD; (b) 25 non-COPD current smokers with a similar smoking history to the COPD patients (Table 1); and (c) 10 healthy non-smokers. The diagnosis of COPD was based on the European consensus criteria including obstructive spirometry (FEV\(_1/VC\) ratio lower than 88% of that predicted for men).\(^11\) All COPD patients had been free of an acute exacerbation for at least 4 weeks preceding the study and none had received antibiotics or corticosteroids (oral and inhaled) or theophylline over the same period. Neither smokers nor healthy volunteers had a history of cardiopulmonary disease and all had normal lung function. All the subjects were non-atopic (i.e. they had negative skin tests for common allergen extracts) and had no history of asthma or allergic rhinitis. The selection of only males was not included in the design of this study but was the consequence of the rarity of the female COPD patients in Greece. We failed to recruit a mixed population because, two screened female COPD individuals refused to participate and three other had concomitant disease. The Hospital Ethical Committee approved the protocol and all subjects gave their consent.

**Spirometry and sputum induction**

Spirometry with bronchodilation test was performed with a computerized system (MasterLab; 2.12, Jaeger, Wuerzburg, Germany) according to standardized guidelines.\(^12\) Subjects did not use short or long acting bronchodilators during 12 h prior to the measurements and did not smoke or drink tea or coffee in the morning of the spirometry and induced sputum. Spirometry and induced sputum session always took place between 8.00–11.00 a.m.

Sputum was induced via inhalation of a hypertonic saline aerosol, generated by an ultrasonic nebulizer (Ultraneb 2000; DeVilbiss, Somerset, PA, USA) according to standard method\(^13\) and was processed within 15 min after termination of the induction. The viscid portions of the expectorated sample were separated from the sputum as previously described.\(^13\)

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Demographics and spirometric values of the studied groups.</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>COPD</td>
</tr>
<tr>
<td>N</td>
<td>36</td>
</tr>
<tr>
<td>Sex M/F</td>
<td>36/0</td>
</tr>
<tr>
<td>Age years (x±SD)</td>
<td>61±6.5</td>
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<tr>
<td>Smoking habit (pack years (x±SD))</td>
<td>50±10</td>
</tr>
<tr>
<td>FEV(_1/VC)</td>
<td>56±11</td>
</tr>
<tr>
<td>FEV(_1) (% pred.)</td>
<td>42.7±18.9</td>
</tr>
<tr>
<td>ΔFEV(_1) (% baseline) (post-bronchodilation)</td>
<td>3.4±1.1</td>
</tr>
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</table>

*Statistical significance between COPD and non-COPD smokers (P<0.01),
**Statistical significance between COPD and normal smokers (P<0.001).
Sputum processing, total (TCC) and differential cell counting (DCC)

The weight of the plugs was determined, and dithiothreitol 0.1% (Sputolysin; Calbiochem, La Jolla, CA, USA) twice the weight of the plugs was added. The samples were agitated on a vortex mixer in a wide-bore plastic test tube and placed in a shaking water bath for 15 min at 37°C to ensure complete homogenization. Subsequently, RPMI-1640 plus 10% FCS (fetal calf serum) (GIBCO BRL, Cheshire, UK) was added in a volume twice that of the homogenized sample. The samples were then filtered through a 48 μm nylon gauze (Thompson, Ontario, Canada) and agitated on a vortex mixer (suspension A). A total cell count (TCC) of the filtered sample was performed and viability was tested by means of the trypsin blue exclusion method. The suspension was then centrifuged at 400g for 5 min and the pellet was resuspended with 500 μl RPMI-1640 + 10% FCS. The supernatant was aspirated and stored in Eppendorf cups at −80°C.

The samples were adjusted to a concentration of 0.35 × 10⁶ cells/ml (suspension B). Cytospins were made by putting 50 μl of this cell suspension in the funnels of an aerospray cytocentrifuge (Wescor, Claremont, Ont., Canada) at 300 rpm with low deceleration for 5 min. Two slides were stained with May–Giemsa–Grunwald (MGG) stain for the differential cell counts (DCCs). Five hundred non-squamous cells in each coded MGG cytospin were counted in a blinded fashion by two independent investigators and averaged. Cell differential counts were expressed as percentage of non-squamous cells and as absolute number of cells per gram of selected sputum sample. Absolute cell numbers were calculated by multiplying the cell percentage by the total (non-squamous) cell number in the sputum, divided by the weight of the selected sputum sample. The variability of the lymphocyte DCCs per slide was examined and found no more of 5%.

Immunocytochemical analysis and CD4 + ve and CD8 + ve cells measurement

The immunocytochemical analysis and T-cell determination were performed in sputum cytopsins. Briefly, sputum lymphocytes were stimulated by incubating suspension B in 24-well plates at a concentration of 2 × 10⁶ cells/μl for 5 h, under 5% CO₂, at 37°C, in RPMI-1640 at 10% FCS in the presence of phorbol 12-myristate 13-acetate (PMA) 25 ng/ml, ionomycin 1 μmol and brebeldin A 10 μg/ml (Sigma-AldrichCorp. St. Louis, MO, USA). Cytospins were made using cytocentrifugation of 50 μl of the stimulated suspension and were stored at −80°C for immunocytochemical analysis later. Approximately 175,000 cells were cytospined on each slide (3500 cells/μl × 50 μl = 175,000 cells), among which there was a sufficient number of lymphocytes to stain.

After defrosting the slides, they were fixed in acetone for 10 min and rehydrated in Tris-Buffered Saline (TBS). The double immunocytochemical method was performed in two steps as previously described. Briefly, the specimens were first incubated for 30 min with bovine serum to block the unspecific binding and then were exposed to the first primary antibody at a dilution of 1:50 for 30 min at room temperature. After washing in TBS three times they were exposed to the first secondary antibody, rabbit anti-mouse immunoglobulin fluorescein isothiocyanate (FITC)-conjugated for 15 min (Immunotech Marseille, France) at a dilution of 1:50. After washing in TBS three times they were exposed to the second primary antibody at a dilution of 1:50 for 30 min at room temperature. After washing in TBS three times they were exposed to the second secondary antibody, rabbit anti-mouse immunoglobulin phycoerythrin-conjugated (IgG-PE) for 15 min (Immunotech Marseille, France) at a dilution of 1:50. After washing in TBS three times the slides were mounted with 30% glycerol in TBS. Two investigators examined the slides under ultraviolet microscope and their results were averaged. Three replicate measurements were performed by each observer in 10 slides. Both intra- and inter-observer coefficient of variation were <15%. The CD4/CD8 and CD8-INF-ve/CD8-IL4+ve cell ratio were calculated. For the estimation of each ratio, 500 T-cells were counted, with more than one cytospin stained if necessary, because this number was sufficient to obtain a mean value per subject that remained constant after further increasing the number of cells counted. Results were also expressed as percentage of lymphocytes by dividing the number of lymphocytes stained per slide by total the number of lymphocytes per slide. Negative controls were obtained by the use of mouse-anti-mouse immunoglobulin to estimate the non-specific binding. Positive controls were obtained by the use of T-lymphoma cells cytopsined on slides. Macrophages were excluded from counting by morphology.

Measurement of CD4 + ve and CD8 + ve T-cells

The primary anti-CD4 mouse anti-human monoclonal antibody (Caltag Burlingame, CA, USA) with secondary rabbit anti-mouse IgG-FITC antibody and the primary anti-CD8 mouse anti-human
monoclonal antibody (Caltag Burlingame, CA, USA) with secondary rabbit anti-mouse IgG-phycoerythrin-conjugated (IgG-PE) antibody were used. At least 500 CD4+ ve and CD8+ cells were counted to estimate CD4/CD8 ratio.

Measurement of CD8+ -IFNγ producing and CD8+ -IL4 producing T-cells
The primary anti-CD8 mouse anti-human monoclonal antibody with secondary rabbit anti-mouse IgG-FITC antibody and the primary anti-INFγ mouse anti-human monoclonal antibody (Caltag Burlingame, CA, USA) with secondary rabbit anti-mouse IgG-phycoerythrin-conjugated (IgG-PE) antibody were used. At least 500 CD8+ cells were counted to estimate the number of CD8-IFNγ+ ve cells. The same method was applied for staining CD8+ cells with anti-IL4 antibody (Caltag Burlingame, CA, USA).

Statistics
Clinical characteristics are presented as mean ± SD and cell characteristics as median (range) unless stated otherwise. Normality was detected using the Kolmogorov–Smirnov test. Differences among the three groups of subjects were tested using the Kruskal–Wallis test for non-normal and ANOVA for normal distributed variables. Once it had been determined that differences existed between the means, post hoc pairwise multiple comparisons were made by Bonferroni test for parametric and by Conover–Inman method15 for non-parametric comparisons to determine which pair was statistically significantly different. Correlation between cell parameters and physiological variables were analyzed using Pearson’s correlation coefficient. The statistical software StatsDirect (Camcode; Cambridge, UK) was used for the entire analysis. A P value of <0.05 was considered statistically significant.

Results

Subjects

Demographic and spirometric data of the three groups of subjects are shown in Table 1. The COPD patients exhibited significantly lower values of FEV1 (% pred) and FEV1/VC (%) than non-COPD smokers, though the smoking history was similar. There was an age difference among the studied groups although non-statistically significant. There were no significant differences in FEV1/VC (%) and FEV1 (% pred) between non-COPD smokers and healthy subjects. The postbroncodilation change in FEV1 (inhalation of 400 mcg salbutamol) was similar in the three groups (Table 1). The induced sputum procedure was well tolerated by all subjects.

Cellular composition of induced sputum

The viability of the cells (% total) in induced sputum did not differ between COPD, non-COPD smokers and healthy controls: (median range) 84 (93–76), 86 (95–77) and 81 (88–72), respectively, (P = 0.23). More than 90% of the cells were non-squamous cells. The TCC (gr−1) was higher in patients with COPD than in non-COPD smokers (P = 0.001) and controls (P = 0.0001) (Table 2). The TCC was also higher in non-COPD smokers than in healthy individuals (P = 0.05) (Table 2). The significant differences in cellular parameters among three groups are presented in Table 2. The most significant differences between the three groups were: neutrophil percentages were higher in COPD than in the other two groups, whereas macrophage percentages were lower in COPD than in the other two groups (Table 2). Lymphocyte percentages were lower in COPD than in the non-COPD smokers and higher than in the healthy group (Table 2).

Lymphocyte and CD4+ , CD8+ subpopulations

The total number of CD8+ cells and their percentage (% of lymphocytes) were significantly higher in COPD patients compared with both non-COPD smokers (P = 0.0001) and healthy individuals (P = 0.0001) (Table 3). However, the comparison of CD8+ cells (total numbers and percentage) between non-COPD smokers and healthy group showed no significant difference (Table 3). Significantly lower percentages of CD4+ cells were found in COPD patients compared with non-COPD smokers (P = 0.0001) and with healthy controls (P = 0.0001) (Table 3). In contrast, no significant difference in CD4+ cells, percentages and total numbers, was found between non-COPD smokers and healthy controls. Patients with COPD had a significant lower CD4/CD8 ratio compared with both non-COPD smokers (P = 0.001) and controls (P = 0.002) (Table 3). In contrast, the CD4/CD8 ratio did not differ between non-COPD smokers and healthy (P = 0.8) (Table 3).

CD8+ subpopulations

The percentage of CD8-IFNγ cells (Tc1) (% of CD8+) in sputum was significantly (P = 0.001) lower in patients suffering from COPD than in smokers.
without COPD: 31 (11–80) vs. 46 (23–85), or healthy controls 31 (11–80) vs. 54 (40–67) (P = 0.001) (Fig. 1). However, no significant difference was found between smokers without COPD and healthy controls (P = 0.4) (Fig. 1). CD8-IL4 cells (Tc2) (% of CD8+) in sputum did not differ between COPD and non-COPD smokers: 3.5 (1–11) vs. 4 (1–15), (Fig. 2). Patients with COPD had significantly lower Tc2 cells (% of CD8+ cells) than healthy controls: 3.5 (1–11) vs. 23.6 (14–40), P = 0.001 (Fig. 2). The same was true concerning the comparison between smokers without COPD and healthy group: 4 (1–15) vs. 23.6 (14–40), (Fig. 2).

CD8-IL4 cells to CD8-IL4 cells ratio (Tc1/Tc2) was significantly lower in COPD than in non-COPD smokers: 9 (1–27) vs. 13.6 (4–55), (Fig. 3). In addition, smokers with COPD had a significantly higher Tc1/Tc2 ratio than healthy controls: 9 (1–27) vs. 2.6 (1–4), (Fig. 3). A significantly higher Tc1/Tc2 ratio was also found in smokers without COPD than in healthy individuals: 13.6 (4–55) vs. 2.6 (1–4), (Fig. 3). Only in COPD patients, there was a weak but statistically significant (r = 0.401, P = 0.02) correlation between the Tc1/Tc2 ratio and FEV1 (% pred) (Fig. 4).

**Table 2** Cellular populations in sputum of COPD, non-COPD smokers and, healthy subjects (median, ranges).

<table>
<thead>
<tr>
<th></th>
<th>COPD smokers (Group A, n = 36)</th>
<th>Non-COPD smokers (Group B, n = 25)</th>
<th>Healthy (Group C, n = 10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCC, × 10⁷ cells/g</td>
<td>8.15 (1.1–18)</td>
<td>4.4 (0.7–8.0)</td>
<td>1.13 (0.2–2.0)</td>
<td>A vs. B: 0.001, B vs. C: 0.05, A vs. C: 0.0001</td>
</tr>
<tr>
<td>Neutrophils, × 10⁷ cells/g</td>
<td>6.35 (0.3–17.0)</td>
<td>1.73 (0.3–4.9)</td>
<td>0.41 (0.1–1.2)</td>
<td>A vs. B: 0.0001, B vs. C: NS, A vs. C: 0.0001</td>
</tr>
<tr>
<td>Macrophages, × 10⁷ cells/g</td>
<td>0.87 (0.09–3.4)</td>
<td>1.36 (0.05–4.0)</td>
<td>0.60 (0.09–1.5)</td>
<td>A vs. B: NS, B vs. C: 0.008, A vs. C: NS</td>
</tr>
<tr>
<td>Lymphocytes, × 10⁷ cells/g</td>
<td>0.13 (0.002–1.2)</td>
<td>0.11 (0.003–0.8)</td>
<td>0.07 (0.007–0.15)</td>
<td>A vs. B: NS, B vs. C: NS, A vs. C: 0.05</td>
</tr>
<tr>
<td>Eosinophils, × 10⁷ cells/g</td>
<td>0.09 (0–2.0)</td>
<td>0.08 (0–1.4)</td>
<td>0.006 (0–0.02)</td>
<td>A vs. B: NS, B vs. C: NS, A vs. C: NS</td>
</tr>
<tr>
<td>Neutrophils, %</td>
<td>80.7 (30–96)</td>
<td>54.7 (17–96)</td>
<td>37.9 (27–53)</td>
<td>A vs. B: 0.0001, B vs. C: 0.02, A vs. C: 0.0001</td>
</tr>
<tr>
<td>Macrophages, %</td>
<td>14.5 (10–70)</td>
<td>38.4 (20–80)</td>
<td>53.5 (39–68)</td>
<td>A vs. B: 0.0001, B vs. C: 0.01, A vs. C: 0.0001</td>
</tr>
<tr>
<td>Lymphocytes, %</td>
<td>1.6 (0.6–9.4)</td>
<td>3.1 (1.3–10)</td>
<td>1.1 (0.4–4)</td>
<td>A vs. B: NS, B vs. C: 0.002, A vs. C: 0.05</td>
</tr>
<tr>
<td>Eosinophils, %</td>
<td>1.2 (0–18)</td>
<td>2.2 (0–38)</td>
<td>0.85 (0–2.2)</td>
<td>A vs. B: NS, B vs. C: 0.01, A vs. C: NS</td>
</tr>
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</table>

**Table 3** T-lymphocyte subpopulations in sputum between COPD, non-COPD smokers and, healthy subjects (median, ranges).

<table>
<thead>
<tr>
<th></th>
<th>COPD smokers (Group A, n = 36)</th>
<th>Non-COPD smokers (Group B, n = 25)</th>
<th>Healthy (Group C, n = 10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD8, × 10⁴ cells/g</td>
<td>40.9 (2–322)</td>
<td>13.9 (1–82)</td>
<td>16.9 (1.6–29)</td>
<td>A vs. B: 0.005, B vs. C: NS, A vs. C: 0.01</td>
</tr>
<tr>
<td>CD4, × 10⁴ cells/g</td>
<td>44 (2–483)</td>
<td>38.9 (4–415)</td>
<td>32.4 (5–94)</td>
<td>A vs. B: NS, B vs. C: NS, A vs. C: NS</td>
</tr>
<tr>
<td>CD8, % of lymphocytes</td>
<td>46 (24–58)</td>
<td>31 (4–50)</td>
<td>27.4 (24–41)</td>
<td>A vs. B: 0.0001, B vs. C: NS, A vs. C: 0.0001</td>
</tr>
<tr>
<td>CD4, % of lymphocytes</td>
<td>52 (40–71)</td>
<td>64 (3–86)</td>
<td>71 (42–77)</td>
<td>A vs. B: 0.0001, B vs. C: NS, A vs. C: 0.0001</td>
</tr>
<tr>
<td>CD4/CD8 ratio</td>
<td>1.1 (0.7–2.5)</td>
<td>2.01 (1–7.4)</td>
<td>2.44 (1.4–3.3)</td>
<td>A vs. B: 0.0001, B vs. C: NS, A vs. C: 0.008</td>
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**Discussion**

The main findings of the present study were that COPD patients have increased numbers of CD8+...
T-lymphocytes in sputum samples with a concurrent imbalance between CD4$^+$ and CD8$^+$ (decreased CD4$^+$/CD8$^+$ ratio). In addition, our results showed that there are significant differences in CD8$^+$ subtypes CD8$^+$-INF$_g$ (T$c_1$) and CD8$^+$-IL4 (T$c_2$) of sputum cells between current smokers with established COPD, smokers with an equivalent smoking history but without COPD and non-smoking healthy individuals. Although cigarette smoking has been identified as the prime cause of COPD, it remains unclear why only a small fraction of smokers develop clinically relevant disease. It is therefore of critical importance to study smokers who develop COPD and to compare them with smokers having a similar smoking history but without COPD. The studied population was all male. Although there is lack of data on gender differences in T-cell immunity, this could be a limitation of the study as our data cannot be precisely extrapolated to women with COPD. Differences in the cellular composition between smokers who develop COPD and those without COPD have been reported in bronchial biopsies and bronchoalveolar lavage fluid. From these studies, it became apparent that CD8$^+$ lymphocytes could have a central role in the inflammation of airways related to the pathogenesis of the disease.

In the present study, the method of induced sputum was used to investigate T-lymphocytes in COPD patients and to compare them with those in smokers without COPD and in healthy, non-smoking individuals. The method involves stimulation of lymphocytes with PMA and ionomycin. Following this procedure, immunocytochemical methods were used to detect surface antigen and intracellular cytokines. We demonstrate that sputum...
CD8\(^+\) cells and their immunological profile can be studied by this method. Under these experimental conditions of culturing and stimulating lymphocytes with PMA the intracellular cytokine expression can be affected. PMA is a potent stimulator of CD8\(^+\) cells, which enhances their ability to express IL4 or IFN\(\gamma\). It has been used to study human CD8\(^+\) cells and their intracellular cytokine production in asthma and in sarcoidosis. In the above reports, PMA stimulation augmented the percentage of IFN\(\gamma\)- and IL4-producing T-suppressor/cytotoxic lymphocytes, showing important effector differences in cytokine regulation not detectable otherwise. We employed the same stimulation procedure for the CD8\(^+\) subsets in all three groups of subjects. Our findings although non-quantitative demonstrated an important different intracellular cytokine expression in smokers with established COPD only after sufficient in vitro stimulation with PMA.

Our results showed that the percentage of CD8\(^+\) cell in COPD patients was significantly higher than in smokers (\(P = 0.0001\)) and in controls (\(P = 0.0001\)) (Table 3). About half of the lymphocytes in the sputum of COPD patients were CD8\(^+\) cells. These results are in line with findings of increased CD8\(^+\) in lung parenchyma, pulmonary arteries\(^1\) and in the peripheral airways.\(^3\) It seems that arteries, tissues, and airway lumen have a similar CD8\(^+\) lymphocyte infiltration, suggesting a CD8\(^+\) inflammation of the entire respiratory system in smokers with COPD. There are several ways in which the increased numbers of CD8\(^+\) cells might be associated with inflammation in COPD. The increased accumulation and activation could be triggered by the smoking\(^5\) or by repeated virus infections, a common cause of acute exacerbations.\(^2\) On the other hand, CD8\(^+\) cells, because of their potent cytotoxic abilities, may induce airway and lung damage, especially when they are present in excess and in activation.\(^2\) Even more, they may contribute to inflammatory responses in lungs through the release of cytokines although this observation has been reported in animal studies.\(^2\) The findings of increased CD8\(^+\) cells in sputum, beyond the confirmation of the findings of previous studies of bronchial and lung biopsies, may be clinically useful, since sputum induction is a safe procedure and could be used to monitor inflammatory changes in the lungs of COPD patients over time.

In addition, the ratio of CD4\(^+\) to CD8\(^+\) cells was found to be statistically significantly lower in COPD patients than in smokers (\(P = 0.0001\)) or controls (\(P = 0.008\)). The CD4\(^+\)/CD8\(^+\) ratio did not differ between non-COPD smokers and controls. This finding suggests that the "abnormal" inflammation in COPD could be the result of an imbalance between the CD8\(^+\) and the CD4\(^+\) T-lymphocytes, which was observed only in those smokers who developed COPD. When these observations are linked with the study of Amadori et al.,\(^2\) who reported a genetic control of the ratio between CD4\(^+\) and CD8\(^+\) cells with a small (5%) percentage of population having a CD4/CD8 ratio of lower than 1, they also provide some evidence as to why a minority of smoker will develop COPD. It is possible that individuals with a genetically determined increase of the CD8\(^+\) population might be more susceptible to further CD8\(^+\) cell abnormalities.\(^2\) This genetically determined increase of CD8\(^+\) cells might be enhanced by smoking\(^5\) or repeated virus infections,\(^2\) inducing an abnormal inflammatory response related to the pathogenesis of COPD. This hypothesis is not new. It has been suggested by O' Shaughnessy and colleagues,\(^5\) who reported higher counts of CD8\(^+\) cells in bronchial biopsies of smokers with COPD compared to the smokers without COPD. Thus, the differences in CD8\(^+\) cells between COPD and non-COPD smokers found in this study could mean that smoking is more likely to induce the development of airflow limitation in individuals who have a genetically determined tendency to a low CD4/CD8 ratio.

In this study, using a immunocytochemical method after sufficient stimulation with PMA, we found fewer sputum interferon-\(\gamma\) producing CD8\(^+\) cells (Tc1) in patients with COPD in comparison with those in smokers and controls (Fig. 1). The mean values of interleukin-4 expressing CD8\(^+\) cells were similar in COPD and in smokers without COPD. However, both were significantly lower than normal (Fig. 2). It is obvious from Figs. 1 and 2 that a significant proportion of CD8\(^+\) cells remain of unknown cytokine profile (TcD). The data of this study are not sufficient to explore the cytokine expression of the whole CD8\(^+\) subset. However, the major difference found between smokers without and with COPD is that seen in Tc1 cells (Figs. 1 and 2). This finding is also illustrated in Fig. 3, where the Tc1/Tc2 ratio is presented. Figure 3 shows that the Tc1/Tc2 ratio is low (2.6) in normal subjects, increased in (13.6) in smokers without COPD and to a lesser extent (9.5) in COPD patients. From these results (Figs. 1–3), we could conclude that smoking induces a dramatic drop in Tc2 in smokers both with and without COPD.

However, smokers without COPD showed a capacity of high Tc1 numbers, almost similar to that found in controls (Fig. 1). We could submit that the inflammation induced by smoking is different
in "normal" smokers and in smokers with COPD at the level of CD8+ subtypes. Thus, an imbalance between Tc1/Tc2 may also be involved in the pathogenesis of COPD. The CD8+ cells produce a variety of cytokines, which in some instances exceeds that of CD4Th subsets.27,28 The polarization of T-cell responses to either T1-type responses (characterized by IFN-γ production and related with cell-mediated immunity) or T2-type responses (characterized by IL4 and IL5 production and associated mainly with humoral immunity) provides a better basis for understanding how the T-cells promote their interactions with other immune cells. It is possible that the T-cell cytokine imbalance seen in COPD promotes macrophage activation. On activation, macrophages might enhance functions such as the generation of reactive oxygen species and acute inflammation through the secretion of short-lived non-specific inflammatory mediators, such as eicosanoids and nitric oxide.29 In addition, activated macrophages become more efficient antigen-presenting cells; while CD4+ -derived and CD8+ -derived IFN-γ stimulate alveolar macrophages to secrete cytokines, including IL12, which feeds back into the cell lymphocyte line promoting T-cells to differentiate into cytotoxic T-lymphocyte differentiation.30

Both the CD4/CD8 and Tc1/Tc2 imbalance seen in smokers with COPD may contribute to the abnormal inflammatory relation to the development of COPD. The observation in this study, that only in COPD patients the FEV1 (% pred) was weakly but statistically significantly related with the Tc1/Tc2 ratio (Fig. 4), provides further evidence for the above hypothesis. The non-quantitative method used in this study is an explanation of the quite weak correlation observed between Tc1/Tc2 and FEV1 in COPD patients. Although a correlation does not always imply a cause–effect relationship, we believe that the imbalance of CD8+ T-lymphocyte subpopulation, seen primarily in COPD patients and related to the expiratory airflow limitation (degree of obstruction) (FEV1), provides sufficient evidence for further investigation of its role in the pathogenesis of COPD.

In conclusion, our findings demonstrated a different inflammatory response to cigarette smoking at the level of T-cell lymphocytes between COPD and current smoker. A predominance of CD8+ cells in sputum and the consequent imbalance of the CD4/CD8 ratio was shown in smokers with COPD. We report an imbalance of sputum CD8+ subsets, such as the CD8+ -INFγ/CD8+ -IL4 ratio that may provide new insight into the pathogenesis of COPD. However, the precise role of these cells (Tc1, Tc2) remains to be investigated.

References


