

Inflammatory cell profiles and T-lymphocyte subsets in chronic obstructive pulmonary disease and severe persistent asthma

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Summary

Background Severe persistent asthma (SPA) and chronic obstructive pulmonary disease (COPD) are both associated with non-reversible airflow limitation and airway neutrophilia.

Objective To compare inflammatory cell profiles and T lymphocyte subsets between SPA and COPD patients with similar severity of airflow limitation.

Methods Sputum induction and lung function tests were performed in 15 COPD patients aged (mean $\pm$ SD) 68 ± 8 years, ex-smokers, mean forced expiratory volume in 1 s (FEV₁) 45% of predicted (%pred) and 13 SPA aged 55 ± 10 years, non-smokers, mean FEV₁ 49%pred. All patients were on inhaled steroid treatment. Eight asthmatics exhibited irreversible airflow limitation. Differential cell count, metachromatic cell count and double immunocytochemistry for the analysis of T lymphocyte subsets were performed on sputum slides.

Results COPD patients had increased sputum neutrophils in comparison with SPA ($P < 0.03$), but similar to SPA with fixed obstruction. In COPD sputum neutrophils negatively correlated with the lung transfer factor for carbon monoxide (K_{CO}) ($r = -0.462$, $P = 0.04$). SPA showed significantly increased eosinophils and metachromatic cells vs. COPD patients ($P < 0.04$, $P < 0.007$, respectively). Increased CD4/CD8 and decreased CD4-IFN- γ /CD4-IL4⁺ cell ratio ($P < 0.001$) were found in SPA vs. COPD. In SPA, CD4/CD8⁺ cell ratio correlated with sputum eosinophils ($r = 0.567$, $P = 0.04$).

Conclusion In spite of treatment with inhaled steroids, SPA and COPD exhibit distinct sputum inflammatory cell patterns, although SPA with fixed airflow limitation and COPD patients have similar numbers of neutrophils.

Keywords CD4, CD8, COPD, lymphocytes, severe asthma, sputum, Tc1, Tc2, Th1, Th2

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Introduction

Asthma and chronic obstructive pulmonary disease (COPD) are the two most common pulmonary diseases, with continuously increasing morbidity, mortality and prevalence rates [1, 2]. Although considerable progress has been made in understanding the underlying pathology of both diseases, the complex interactions between inflammatory cells and mediators involved in each disease are not fully understood.

COPD is characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases [1]. Similar cell profiles have been found in the various pulmonary compartments, but the bronchioles and parenchyma are primarily affected [3, 4]. In the airway lumen there is evidence of neutrophilic inflammation, while in the mucosa there is predominance of macrophages and CD8⁺ lymphocytes [5–7]. CD8⁺ lympho-

cytes may produce IFN- γ (Tc1), IL4 (Tc2) or both (Tc0) [8]. Recent evidence suggests that CD8⁺ lymphocytes of type 1 (Tc1) are implicated in COPD [9].

Asthma is also an inflammatory disorder of the airways, associated with increased airway hyper-responsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing [2]. These episodes are associated with variable airway obstruction that is generally reversible. However, a subgroup of asthmatics, defined as severe persistent asthmatics (SPA) in the classification of the Global Initiative for Asthma (GINA) [2], often exhibit irreversible obstruction, with a concurrent steep rate of decline in forced expiratory volume in 1 s (FEV₁) [10]. In contrast to COPD, inflammation in asthma affects mainly the proximal airways [4]. Eosinophils and mast cells are increased, while lymphocytes of the CD4⁺ rather than the CD8⁺ subset orchestrate the inflammatory process by the local release of regulatory cytokines [11]. CD4⁺ lymphocytes are further phenotyped according to their levels of production of IFN- γ (Th1), IL4 (Th2) or both (Th0) [8]. Evidence suggests that Th2 cells lead the asthmatic disorder [12].

Although different inflammatory mechanisms are involved in asthma and in COPD, some patients share features of both

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diseases [13]. High numbers of sputum eosinophils have been reported in COPD patients with partial reversibility of airflow limitation or with steroid response [14, 15] and increased mastocytes have been found in bronchial biopsies of COPD patients [16]. On the other hand, SPA has high concentrations of neutrophils in bronchoalveolar lavage fluid (BALF) and sputum [17, 18] and patients with non-infectious status asthmaticus exhibit prominent neutrophilia [19].

These findings indicate that SPA is a distinct disease state resembling COPD functionally (increased prevalence of irreversible airflow limitation) and pathologically (increased neutrophil numbers). The aim of this study was to compare airway inflammation between SPA and COPD patients with a similar severity of airway obstruction. We especially focused on T lymphocytes and their subsets because the asthmatic inflammation is driven by the Th2 subset of CD4⁺ cells, while the Tc1 subset of CD8⁺ cells is implicated in the pathogenesis of COPD. Our results showed significant differences in sputum T cell subsets between patients with SPA and COPD.

Materials and methods

Subjects

Thirteen patients with SPA, non-smokers and 15 with COPD, ex-smokers, were recruited from the pulmonary outpatient department of the University Hospital of Heraklion.

The diagnosis of COPD was made according to the European Respiratory Society criteria [20]. All COPD patients had irreversible airflow limitation as defined by $\Delta FEV_1 < 15\%$ of predicted (%pred) baseline FEV₁ after inhalation of 400 mcg salbutamol. All had negative skin prick reaction and RAST and normal IgE count.

The diagnosis of SPA was made according to the GINA classification [2]. All asthmatics were atopic. Eight exhibited irreversible airflow limitation.

All studied patients were treated with inhaled steroids (>800 mg/day fluticasone or >1200 mg/day budesonide) and long-acting β_2 -agonists. No subject was receiving other drugs or suffered from another pulmonary or systemic disease. No subject had a respiratory infection at least 6 weeks preceding the study.

The Hospital Ethical Committee approved the protocol and subjects gave their consent.

Study design

Day 1: medical history, skin prick tests, spirometry and the single breath method for measurement of the transfer factor for carbon monoxide (K_{CO}) were performed (MasterLab, Jaeger 2.12, Wuerzburg, Germany) [21]. Day 2: total serum IgE count, RASTs and sputum induction.

Sputum induction and processing

Sputum was induced and processed based on the method of Pin et al. [22] as previously described [23]. Lymphocytes were stimulated by incubating the cell suspension in 24-well plates at a concentration of 2×10^6 cells/mL for 5 h, under 5% CO₂, at 37 °C, in RPMI 1640 (HyClone, South Logan, UT, USA) at 10% FCS in the presence of phorbol 12-myristate 13-

acetate 25 ng/mL (PMA), ionomycin 1 μ mol/L and brebeldin A 10 μ g/mL (Sigma, St Louis, MO, USA). Cytospins were made using cytocentrifugation of 50 μ L of a 3500 cells/ μ L cell suspension and were stored at -80 °C for immunocytochemical analysis later. Approximately 175 000 cells were cytopinned on each slide, among which there was a mean of 1500 T lymphocytes per slide to stain.

Two slides were stained with May-Grunwald-Giemsa for differential cell count and two others with toluidine-blue for metachromatic cell count. The results from the two slides were averaged.

Immunocytochemical analysis

After defrosting, the cytopins were fixed in acetone and rehydrated in TBS. The double immunocytochemical method was performed as previously described [24]. Two investigators examined the slides under double visual and ultraviolet microscope and their results were averaged. The visual filter was used to differentiate cells by morphology and the UV filter to count fluorescent lymphocytes in the same optical field. Three replicate measurements were performed by each observer in 10 slides. Both intra- and inter-observer coefficient of variation were <15%. The results were expressed as CD4/CD8, CD4-IFN- γ /CD4-IL4 and CD8-IFN- γ /CD8-IL4⁺ cell ratio. For the estimation of each ratio 500 T cells were counted, with more than one cytopin 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.

Measurement of CD4⁺ and CD8⁺ T cells The primary anti-CD4 mouse anti-human monoclonal antibody (Caltag, Burlingame, CA, USA) with secondary rabbit anti-mouse IgG-fluorescein isothiocyanate (FITC) antibody (Immunotech, Marseille, France) and the primary anti-CD8 mouse anti-human monoclonal antibody (Caltag) with secondary rabbit anti-mouse IgG-phycoerythrin (IgG-PE)-conjugated antibody (Marseille) were used. Five hundred CD4⁺ and CD8⁺ cells were counted for each patient to estimate CD4/CD8 ratio.

Measurement of CD8⁺-IFN- γ -producing, CD8⁺-IL4-producing, CD4⁺-IFN- γ -producing and CD4⁺-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-IFN- γ mouse anti-human monoclonal antibody (Caltag) with secondary rabbit anti-mouse IgG-PE-conjugated antibody were used to detect CD8⁺-IFN- γ -producing T cells. Five hundred CD8⁺ cells were counted for each patient to estimate the number of CD8-IFN- γ ⁺ cells. The same method was applied for staining CD8⁺ cells with anti-IL4 antibody (Immunotech) and CD4⁺ cells with anti-IFN- γ or anti-IL4 antibody.

Negative controls were obtained by the use of mouse-anti-mouse Ig to estimate the non-specific binding. Positive controls were obtained by the use of T lymphoma cells cytopinned on slides.

Statistical analysis

The differences among three groups were tested using the Kruskal-Wallis test for non-normally and the ANOVA test for

normally distributed variables. The differences between two groups were tested using the Mann–Whitney test for non-normally and the *t*-test for normally distributed variables. Correlations were analysed using the Spearman's–Rho correlation coefficient. The software StatsDirect (Camcode, Cambridge, UK) was used. A $P < 0.05$ was considered significant.

Results

Subjects

Patient characteristics and the mean lung function data are shown in Table 1. Subjects in the two groups had similar age and treatment regimes. All patients showed airflow limitation, as indicated by low FEV₁ and FEV₁/FVC (forced vital capacity) ratio. Baseline FEV₁ (%pred) was comparable between the two groups ($P = 0.2$). Asthmatics could be further divided into two subgroups according to the reversibility of their airway obstruction (reversible – Δ FEV₁ = 15%, irreversible – Δ FEV₁ < 15%). The lung transfer factor for CO corrected for alveolar ventilation (TL_{CO}/VA) was significantly higher in asthma (within normal limits), than in COPD ($P < 0.04$).

An adequate sputum sample was obtained from all subjects. The sputum induction procedure was well tolerated by all COPD subjects, while four SPA reported mild troublesome symptoms (31%) and in one the FEV₁ value fell more than 20% of baseline predicted. In these patients the procedure was terminated. All subjects responded well to bronchodilator treatment. There were no exacerbations after the induction in either group.

Inflammatory indices

Data are expressed as mean $\pm$ SD for normally distributed variables and median (range) for non-normally distributed variables. The viability of cells recovered by sputum induction was $79 \pm 15\%$ and $83 \pm 16\%$ in the asthmatic and COPD

group, respectively. The percentage of squamous epithelial cells on differential counting was $7.5 \pm 7\%$ in asthma and $4.6 \pm 5.5\%$ in COPD. No significant difference between asthmatics and COPD patients was observed in total cell count [median (range) 8 190 000 (331 200, 33 982 600) vs. 1 872 000 (656 500, 65 320 000) cells/g sputum], percentages of macrophages [median (range) 39 (1, 59)% vs. 27 (10, 41)% non-squamous cells] or lymphocytes [median (range) 3 (0.4, 5)% vs. 1 (0.4, 6)% non-squamous cells]. Neutrophil percentages were significantly higher in the COPD group than in the asthma group ($P < 0.03$) (Fig. 1). However, the neutrophil percentages did not differ between the subgroup of eight asthmatic patients with irreversible obstruction and COPD patients. Eosinophil percentages were significantly higher in the overall asthma group and in the subgroup of asthmatics with irreversible obstruction than in the COPD group ($P < 0.04$ and $P = 0.05$, respectively) (Fig. 1). Sputum eosinophilia, as defined by sputum eosinophils $\geq 3\%$ of non-squamous cells, was found in eight asthmatics, irreversible and reversible.

Metachromatic cells were significantly higher in asthmatics than in COPD patients ($P < 0.007$). When only irreversible asthmatics were compared with COPD patients, the difference between the two groups became marginally significant ($P = 0.05$).

Figure 2 shows sputum CD4/CD8⁺ cell ratios. Asthmatics showed a higher CD4/CD8⁺ cell ratio than COPD. In Fig. 3, sputum CD4-IFN- γ /CD4-IL4⁺ and CD8-IFN- γ /CD8-IL4⁺ cell ratios are shown. Asthmatics showed a lower CD4-IFN- γ /CD4-IL4⁺ cell ratio. The two groups did not differ as far as the CD8-IFN- γ /CD8-IL4⁺ cell ratio was concerned. When only asthmatics with irreversible airway obstruction were compared with COPD, similar statistical differences were observed in sputum CD4/CD8⁺ cell ratio and CD4-IFN- γ /CD4-IL4⁺ cell ratio ($P < 0.01$ and $P < 0.001$, respectively).

In COPD sputum neutrophils showed a negative correlation to K_{CO} ($r = -0.462$, $P = 0.04$) and in the asthmatic group CD4/CD8⁺ cell ratio positively correlated to sputum eosinophils ($r = 0.567$, $P = 0.04$).

Table 1. Patient characteristics and lung function data

	SPA Total (irreversible)*	COPD	<i>P</i>
Number	13 (8)	15	
Age (years)	55 $\pm$ 10 (57 $\pm$ 12)	68 $\pm$ 8	
Sex	3M/10F (2M/6F)	15M/0F	†
Atopy	13/13 (8/8)	0/15	†
Smoking (p/year)	0 $\pm$ 0 (0 $\pm$ 0)	65 $\pm$ 26	†
Duration of disease (years)	20 $\pm$ 7 (21 $\pm$ 8)	15 $\pm$ 9	
FEV ₁ /FVC (%pred)	58 $\pm$ 12 (4 $\pm$ 10)	53 $\pm$ 10	
FEV ₁ (%pred)	49 $\pm$ 10 (47 $\pm$ 8)	45 $\pm$ 7	
Δ FEV ₁ (%)	13 $\pm$ 10 (6 $\pm$ 4)	3 $\pm$ 3	†
TL _{CO} /VA (K_{CO}) (%pred)	103 $\pm$ 10 (107 $\pm$ 13)	76 $\pm$ 10	†

Data are expressed as mean $\pm$ SD; Δ FEV₁ is change in FEV₁ (%pred baseline) after inhalation of 400 mcg salbutamol. SPA, severe persistent asthma; COPD, chronic obstructive pulmonary disease; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; TL_{CO}/VA, lung transfer factor for CO/alveolar ventilation; M, male; F, female; %pred, % of predicted. *Subgroup of SPA with irreversible airflow limitation (Δ FEV₁ < 15%). † $P < 0.05$.

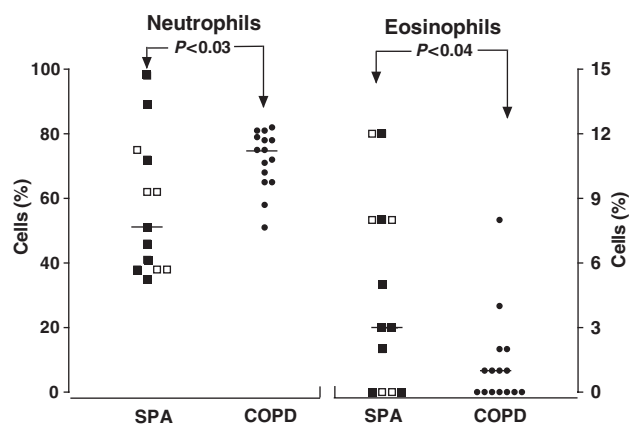


Fig. 1. Sputum neutrophilic and eosinophilic percentages (% of total non-squamous cell count) in severe persistent asthma (SPA) patients (with reversible (□) or with irreversible airway obstruction (■)) and in chronic obstructive pulmonary disease (COPD) (•).

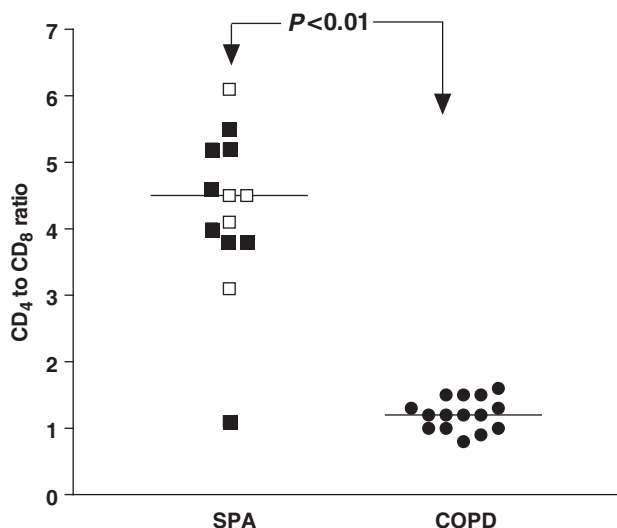


Fig 2. Sputum CD4/CD8⁺ cell ratios in severe persistent asthma (SPA) patients (with reversible (□) or with irreversible airway obstruction (■)) and in chronic obstructive pulmonary disease (COPD) (●).

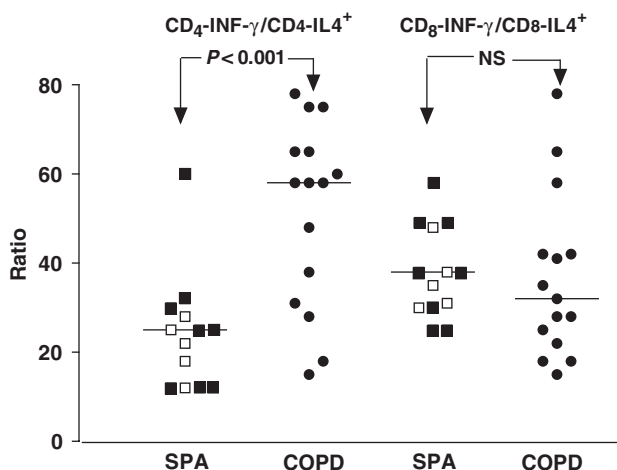


Fig 3. Sputum CD4-INF- γ /CD4-IL4⁺ cell ratios and CD8-INF- γ /CD8-IL4⁺ cell ratios in severe persistent asthma (SPA) patients (with reversible (□) or with irreversible airway obstruction (■)) and in chronic obstructive pulmonary disease (COPD) (●).

Discussion

This study showed that sputum samples from patients with COPD and SPA with similar degrees of airflow limitation and similar steroid intake have different inflammatory cell profiles. High eosinophils, metachromatic cells, CD4/CD8⁺ cell ratios, low neutrophils and CD4-INF- γ /CD4-IL4⁺ cell ratios were found in asthma in comparison with COPD. These findings suggest that two different inflammatory processes, driven by T lymphocytes, are taking place in the lungs of SPA and COPD patients and may support a role for sputum induction in the differential diagnosis of asthma and COPD. However, SPA with irreversible airway obstruction exhibits similar neutrophil numbers with COPD.

It is well known that corticosteroid treatment significantly alters sputum inflammatory markers in asthmatic patients [25]. On the other hand, there is conflicting evidence regarding the effect of inhaled steroids on sputum-inflammatory indices

in COPD patients [25, 26]. Admittedly, steroids do not act in the same way in the two diseases and they may have differently influenced the results in SPA and COPD. However, we decided to investigate both groups under the same anti-inflammatory treatment in order to avoid the confounding effect of treatment as much as possible, as well as for obvious ethical reasons.

Because of the well-known overlap between asthma and COPD, we tried carefully to exclude subjects with both disorders. Our COPD patients were unlikely to have asthma, because they were all non-allergic, non-atopic, with fixed bronchoconstriction. Furthermore, the values of K_{CO} were below the normal predicted in COPD patients. We are also certain that our asthmatics, even those with irreversible airflow limitation, did not have COPD, because they were all non-smokers, allergic, atopic, with a history of asthma for a minimum of 13 years.

Our study confirms that sputum induction is a safe method to investigate airway inflammation in SPA [18]. Only one asthmatic patient exhibited a fall in FEV₁ > 20% during the inhalation of hypertonic saline, which was readily reversed by bronchodilator administration. We also provide evidence that lymphocyte subpopulations and subtypes in sputum can be studied by immunocytochemical methods. Before immunocytochemistry, T lymphocytes were stimulated with PMA and ionomycin. Theoretically, this intervention may alter the cytokine profile of the cells. However, we speculate that the differences found in T cell subsets *in vitro* reflect real differences *in vivo*, since the samples from both diseases were stimulated in the same way.

Considering it important to achieve high repeatability of our results, we tried as much as possible to eliminate variability in sputum sampling. Differences in induction time or in saline concentration could alter sputum inflammatory pattern [27]. To exclude these factors the induction time was set up to 25 min in all patients (except four patients who reported troublesome symptoms before the end of time) and a standard saline concentration was always used (N/S). Moreover, all sputum induction and processing were performed from the same investigator. By using a relatively high cell count per slide and by minimizing variability in sputum sampling, we achieved good agreement in the analysis of different sputum aliquots. Ten different slides from the same sputum sample of five patients were stained for T lymphocyte subpopulations and counted. The intra-sample coefficient of variation was < 7%. Although the repeatability of our results in one patient over a long time was not tested, repeatability within 2 days was > 90% and previous studies have shown high repeatability of sputum differential cell count and inflammatory mediator measurements under standard experimental conditions [27].

In the present study, significant differences were found in sputum T lymphocytes between SPA and COPD patients. SPA exhibited an increase in CD4/CD8⁺ cell ratio in comparison with COPD patients (Fig. 2) ($P < 0.001$). The significantly higher ratio of CD4/CD8⁺ cells in SPA confirms the enhancement of CD4⁺ cells' response in asthma and CD8⁺ cells' response in COPD, as it has been already suggested from peripheral blood, BALF and lung biopsy studies [12, 28]. Accordingly, the recent study of Fabbri et al. [29] compared severe asthmatics with fixed airflow obstruction

tion with COPD patients and found a higher CD4/CD8⁺ cell ratio of T cells infiltrating the airway mucosa of the first. On the contrary, Leckie et al. [30] failed to find any difference in the number of sputum CD4⁺ and CD8⁺ cell populations of patients with asthma, COPD or healthy subjects. This discrepancy could be attributed to the fact that we and Fabbri et al. have studied only SPA.

A significantly lower CD4-IFN- γ /CD4-IL4⁺ cell ratio was also found in sputum cells from SPA vs. COPD patients (Fig. 3). Since Th0 cells are IFN- γ as well as IL-4⁺, the lower CD4-IFN- γ /CD4-IL4⁺ cell ratio means a lower Th1/Th2 ratio. Therefore, the Th2-type reaction is enhanced in sputum samples from SPA vs. COPD patients.

CD4⁺ cells of type 2 have also been found increased in blood, biopsies and BALF of asthmatic patients [12]. They express regulatory cytokines, like IL-5 that promotes eosinophil differentiation, activation and survival, as well as IL-4 and IL-13, which induce IgE production and eosinophil recruitment via vascular cell adhesion molecule-1 expression on the vascular endothelium [11]. Indeed, the sputum CD4/CD8 ratio in our asthmatic group positively correlated to the number and percentages of eosinophils. Although a correlation does not always imply a cause-and-effect relationship, this finding could provide an explanation why eosinophilia persists despite steroid treatment in some asthmatics. It has been previously shown that in patients with corticosteroid-resistant asthma, there is resistance to IL-5 gene repression by steroids [31]. Possibly, the higher CD4-Th2 cell numbers observed in our study among some SPA also reflect a persisting release of IL-5 from the above cells, which both support higher eosinophil levels, despite steroid treatment.

An increase in CD8⁺ cells in the airway lumen and lung parenchyma of COPD patients has been previously reported [6, 7]. This study demonstrated an increase in CD8⁺ cells relative to CD4⁺ in COPD vs. SPA (Fig. 2). CD8⁺ cells are also known as cytotoxic cells, which act through the release of perforins, granzymes or through the Fas ligand pathway [32]. Interesting evidence suggests that they can cause a TNF- α -mediated lung injury [33]. Although previous studies have shown a negative correlation between CD8⁺ cells and FEV₁ value in COPD patients [6, 7, 28]; such a correlation was not confirmed by our results. This is attributed to the fact that due to the design of the study, our COPD patients all had similar FEV₁ values, so that a correlation could not be established between FEV₁ and any other parameter.

Recent evidence suggests that the CD8⁺ cells implicated in COPD are of type 1. Saetta et al. [9] have reported high Tc1 numbers in peripheral airways of COPD patients when compared with healthy smokers or non-smokers, while a type 1 cytokine phenotype has been demonstrated in peripheral blood [34]. In the present study, no difference was found in the CD8-IFN- γ /CD8-IL4⁺ cell ratio between COPD patients and SPA, but in both groups CD8-IFN- γ ⁺ cells were more than CD8-IL4⁺ cells (Fig. 3). Since Tc0 cells are IFN- γ as well as IL4⁺, increased CD8-IFN- γ ⁺ cells means increased Tc1 cells. Tc1 cells may be involved in the inflammatory processes taking place in the airway lumen of both COPD patients and SPA. Type 1 T lymphocytes are crucial in the response to viral infections and viral infections are frequent in patients with COPD or asthma [35–37]. Tc1 cells express IFN- γ , which can induce emphysema in animal models and

are also implicated in the asthmatic disorder through the induction of airway responsiveness [38].

Previous studies have suggested that neutrophils are involved in the pathogenesis of COPD and severe asthma. Increased sputum neutrophils were found in COPD patients and SPA included in the present study. While steroids may increase sputum neutrophils, long-acting β_2 -agonists may reduce them [39]. Although steroid and long-acting β_2 -agonist treatment was similar in both our groups, SPA had less neutrophils than COPD patients ($P < 0.03$) (Fig. 1). Recently, Fabbri et al. [29] found fewer neutrophils in the sputum of severe asthmatics vs. COPD patients with a similar severity of fixed airflow obstruction [29]. However, in our study the subgroup of asthmatics with irreversible airway obstruction showed no statistical difference in the percentage of sputum neutrophils with COPD patients. Although this observation must be carefully interpreted, due to the low number of patients with irreversible SPA and to the large scatter of values, it may implicate neutrophils in irreversible obstruction. Evidence supporting such a hypothesis has already been given by Lacoste et al. [40], who found greater percentages of neutrophils in patients with COPD than in those with chronic bronchitis. Neutrophils could contribute to the development of irreversible obstruction in both COPD and in asthma by the release of proteolytic enzymes during the processes of airway wall and parenchymal remodelling [41]. However, their effect could alter different locations of the respiratory system (parenchyma in COPD vs. airways in asthma). Our finding that K_{CO} , a marker of emphysema severity and neutrophil numbers are negatively correlated in COPD patients is in agreement with this hypothesis.

The role of eosinophils in SPA is not clear either. Wenzel et al. [42] have demonstrated that there are two subtypes of severe asthma, depending on the absence or presence of eosinophils in endobronchial biopsies. Sputum analysis of severe asthmatics has provided evidence for persistent eosinophilic inflammation [18, 29]. Similarly, our overall asthmatic group had significantly higher eosinophil percentages than the COPD group ($P < 0.045$). However, some asthmatics had low eosinophil numbers (<3%) (Fig. 1). Gibson et al. [43] have already reported heterogeneity regarding sputum eosinophil numbers in severe asthmatics. This suggests that there are two phenotypes in SPA depending on the presence or absence of eosinophils in the airways.

In the present study, higher metachromatic cell numbers (mast cells and basophils) were found in SPA compared with COPD ($P < 0.007$). Previously, increased metachromatic cell numbers were found in sputum from asthmatics [22, 44] and increased mast cell numbers in biopsies from COPD patients, but not in BALF [45]. Although our results also suggest that metachromatic cells are rare in the airway lumen of COPD patients, their role in COPD pathogenesis remains to be determined.

In summary, evidence of different sputum inflammatory cell profiles between SPA and COPD suggests that two distinct inflammatory mechanisms are involved in the pathogenesis of these two obstructive disorders. The enhancement of the CD4-Th2 cell response in asthma and CD8 response in COPD was confirmed in sputum samples. Apparent differences were also detected in eosinophil,

neutrophil percentages and metachromatic cells, although similar neutrophil numbers were found between SPA with irreversible airway obstruction and COPD patients. Interestingly, all differences were demonstrated in spite of corticosteroid treatment. These findings support the important role of sputum induction in the research field of obstructive lung diseases and indicate the potential utility of this method in the differential diagnosis of SPA and COPD.

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