CHEST

Original Research

COPD

Distribution of T-Cell Subsets in BAL Fluid of Patients With Mild to Moderate COPD Depends on Current Smoking Status and Not Airway Obstruction

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Background: **COPD is characterized by chronic inflammation. $CD8^+$ T cells and $CD4^+$ T cells have both been implicated in the inflammatory response. We investigated whether the lymphocyte and T-cell subpopulations in BAL differ between patients with COPD who are current smokers and those who are ex-smokers.**

Methods: **Forty never smokers, 40 smokers with normal lung function, and 38 patients with COPD, GOLD (Global Initiative for Chronic Obstructive Pulmonary Disease) stage I-II (27 smokers and 11 ex-smokers) underwent BAL. Using flow cytometry, cells were analyzed from BAL and blood for T-cell subsets, B cells, natural killer cells, and natural killer T (NKT)-like cells. The differentiation status of $CD4^+$ T cells was also determined.**

Results: **Smokers with or without COPD had higher percentages of $CD8^+$ T cells and NKT-like cells in BAL than did never smokers and ex-smokers with COPD. Most of the NKT-like cells were $CD8^+$. In contrast, the percentages of $CD4^+$ T cells were lower in the smoking than in the nonsmoking groups. In blood, the frequency of $CD4^+$ T cells was increased in the two smoking groups. Current smokers also had increased numbers of activated ($CD69^+$) naive and effector $CD4^+$ T cells in BAL compared with nonsmokers, particularly in patients with COPD. In male smokers with COPD, the percentage of $CD8^+$ T cells in BAL positively correlated with the number of cigarettes per day.**

Conclusions: **Current smoking status has a greater impact than airway obstruction on the distribution of T-cell subsets in BAL of patients with mild to moderate COPD. This fact must be considered when the role of T cells in COPD is evaluated. Our results stress the importance of subgrouping patients with COPD in terms of smoking.** ***CHEST 2014; 145(4):711–722***

Abbreviations: FDR = false discovery rate; GOLD = Global Initiative for Chronic Obstructive Pulmonary Disease; ILC = innate lymphoid cell; NK = natural killer; NKT = natural killer T; PB = peripheral blood

COPD is the fourth-leading cause of death worldwide.[1] The disease is characterized by small airway remodeling and tissue destruction, which are most frequently consequences of an inflammatory response to inhaled smoke. The mechanisms leading to the initiation and persistence of this smoke-induced inflammation are not fully understood but are important to outline to prevent and treat the disease.

Cigarette smoking per se leads to a substantial local inflammation in the lungs,[2] and it is, therefore, essential to distinguish changes induced and maintained by cigarette smoke from those related to disease. When evaluating ex-smokers with COPD, it is also important to consider smoking history, in particular the time elapsed since smoking cessation, because the normalization process after quitting smoking leads to a reduction in the number of inflammatory cells in the lungs[3] but a persistence of airway inflammation in patients with COPD.[4,5]

Recruitment of lymphocytes is believed to play a central role in the pathogenesis of COPD.[6-8] Previous studies have shown that the number of $CD8^+$ T cells is increased in both central and peripheral airways, as well as in the lung parenchyma, in patients with COPD and correlates negatively with the degree of airflow obstruction.[9,10] Altogether, these findings suggest a

key role for $CD8^+$ T cells in COPD-related tissue destruction. $CD4^+$ T cells are also likely to play a role in COPD, particularly in patients with severe disease, because these cells are increased in number in the lung tissue of patients with severe emphysema.[11]

To better understand the role of T cells in relation to smoking and in the pathogenesis of COPD, it is indispensable to map out the different T-cell phenotypes and their local and systemic distribution in COPD compared with healthy subjects. T-cell maturation can be divided into four stages, which can be identified based on their expression of the surface markers CD45RA and CD27. By combining these markers, it is possible to discriminate naive ($CD27^+CD45RA^+$), central memory ($CD27^+CD45RA^-$), effector memory ($CD27^-CD45RA^-$), and effector ($CD27^-CD45RA^+$) subpopulations.[12-15]

In addition to $CD8^+$ and $CD4^+$ T cells, other lymphocyte subsets may be involved in smoke-induced inflammation. Natural killer (NK) cells are large granular lymphocytes that express CD16, CD56, or both. They are capable of destroying target cells without prior sensitization and play an important role in immune surveillance against tumors and resistance against viral infections[16] but may contribute to inflammation in many other settings. T cells can also express the NK markers CD16 and CD56. These $CD3^+/CD16^+$ and/or $CD56^+$ cells are generally referred to as natural killer T (NKT)-like cells and display features of both T cells and NK cells.[17]

In this study, we addressed major lymphocyte subsets and the differentiation status of T cells, both locally and systemically, in relation to smoking. We hypothesized that not only smoking history, expressed as pack-years, but also current smoking status, may determine the distribution of T-cell subsets in the lung, including subpopulations reflecting maturation. To test this hypothesis, we investigated T cells in BAL and blood from both current smokers with COPD and ex-smokers with COPD and compared them with never smokers and smokers with normal lung function.

Materials and Methods

Study Subjects and Patients

The study was carried out on subjects from the Karolinska COPD and Smoking from an OMIC Perspective (COSMIC) cohort at the Karolinska University Hospital Solna, Karolinska Institutet, Stockholm, Sweden. The aim of the COSMIC study is to investigate and integrate several aspects of COPD and smoking through imaging, transcriptomics, proteomics, metabolomics, and lymphocyte profiling in the context of clinical phenotypes.[18-20] A total of 40 never smokers, 40 smokers with normal lung function (hereafter referred to as "smokers"), and 38 patients with COPD were recruited with the intent to collect peripheral blood (PB) and BAL (Table 1). The patients and control subjects were recruited from among individuals performing spirometry during "The World Spirometry Day," through advertisements in the daily press and via primary care centers. The majority of the patients with COPD were smokers who turned out to have an obstructive spirometry on screening. Three patients and one never smoker did not have BAL because of clinical constraints, but PB was still used for analysis. Of the patients with COPD, 27 were current smokers, and 11 were ex-smokers who had quit smoking a minimum of 2 years before entering the study. Ten of the smokers, seven of the smokers with COPD, and two of the ex-smokers with COPD fulfilled the criteria for chronic bronchitis.[21] Spirometry (Jaeger Masterscope-PC; CareFusion) was performed, and the patients with COPD had a postbronchodilator $FEV_1/FVC < 0.7$ and an FEV_1 50% to 100% predicted (ie, GOLD [Global Initiative for Chronic Obstructive Pulmonary Disease] stage I and II).[22] The distribution of patients according to the updated COPD classification[1] is shown in Table 1. None of the subjects had had any exacerbations during the previous 3 months. Patients with a history of allergy or asthma were excluded, as were patients using oral or inhaled corticosteroids. In vitro screenings for the presence of specific IgE antibodies (Phadiatop; Pharmacia Corp) were negative. Reversibility was tested after inhalation of two doses of 0.25 mg terbutaline (Bricanyl; Turbuhaler; AstraZeneca). Patients with COPD had significantly more dyspnea and ex-smokers with COPD had lower physical domain scores, as assessed by the chronic respiratory disease standardized questionnaire addressing quality of life[23] (Table 1). All participants provided written informed consent, and the study was approved by the regional ethics committee in Stockholm on October 26, 2006 (ref: 2006/959-31/1).

BAL Procedure and Processing of BAL Fluid and PB

Bronchoscopy and BAL were performed according to a standard protocol at our clinic. For details, see e-Appendix 1.

Macrophage Depletion

Prior to monoclonal antibody staining, macrophage depletion from the BAL cells was performed as described previously.[24] For details, see e-Appendix 1.

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