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Increased intraepithelial (CD103+) CD8+ T cells in the airways of smokers with and without chronic obstructive pulmonary disease

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ABSTRACT

T cells are accumulated in the lungs of chronic obstructive pulmonary disease (COPD) patients. Intraepithelial T cells, expressing the integrin αE (CD103) β7, and regulatory T cells have been implicated in pathogenesis of the disease. We asked whether COPD patients and smokers have altered frequencies of these T cells and if their phenotypes differ.

A total of 40 never-smokers, 40 smokers with normal lung function and 38 COPD patients (GOLD I and II), of which 11 were ex-smokers, were included. T cells in bronchoalveolar lavage (BAL) fluid and peripheral blood were analysed for the expression of CD103, FOXP3 and markers of activation and differentiation using multi-colour flow cytometry.

Smokers, regardless of airway obstruction, had significantly more CD8+CD103+ cells in their BAL fluid compared to never-smokers but less of those cells were CD27+CD69−. Smokers, in particular those with chronic bronchitis, had a higher percentage of CD4+FOXP3+ T-regulatory BAL cells compared to never-smokers and COPD ex-smokers.

Chronic cigarette smoking leads to an accumulation of CD8+ T cells with an altered phenotype in the airway epithelium. The increased frequency of regulatory T cells may influence the ability to regulate smoke-induced inflammation which could be decisive for disease development. Our results further indicate a reversibility of smoke-induced changes.

Introduction

Cigarette smoking is the major risk factor for chronic obstructive pulmonary disease (COPD). The incidence of the disease, causing significant morbidity and mortality worldwide, is estimated to increase over the next decades (Rabe et al., 2007). The noxious particles and gases in the cigarette smoke induce an inflammatory response in the lungs (Celli and MacNee, 2004), which in predisposed individuals may lead to structural changes, limitation of expiratory airflow and development of disease. In contrast to smokers with normal lung function, much of the inflammatory response in COPD persists after smoking cessation (Willemse et al., 2005; Skold et al., 1992).

T cells have been implicated in the pathogenesis of COPD. CD8+ T cells are increased in the lungs of COPD patients, and their numbers correlate with decline in lung function (O'Shaughnessy et al., 1997; Saetta et al., 1998). Recent studies have suggested a role also for CD4+ T cells, both as effector cells, especially in severe disease (Sullivan et al., 2005), and as regulatory cells (Lane, 2010). Impairment in T-regulatory cell functions has been associated with several autoimmune and chronic inflammatory diseases (Smyth et al., 2010; Buckner, 2010).

The first line of defence against inhaled particles is made up of airway epithelial cells, expressing the adhesion molecule E-cadherin which can bind to the integrin αE (CD103) β7 on T cells. Expression of CD103 has traditionally been associated with epithelial T cell recruitment and retention. Consequently, the proportion of CD4+ and CD8+ T cells expressing CD103 is higher in T cells from bronchoalveolar lavage (BAL) than in T cells from peripheral blood (PB) (Rihs et al., 1996; Glader et al., 2005; Lohmeyer et al., 1999; Wikén et al., 2012). Recent findings suggest further functions of CD103, such as promoting migration through epithelial cell layers (Schlikum et al., 2008). In interstitial lung diseases, the frequency of CD4+CD103+ T cells in BAL is reported to be increased compared to healthy, whereas no alterations have been shown in the CD8+CD103+ subset (Rihs et al., 1996; Lohmeyer et al., 1999; Braun

Abbreviations: BAL, bronchoalveolar lavage; COPD, chronic obstructive pulmonary disease; DLCO, carbon monoxide diffusing capacity; FEV_1, forced expiratory volume in 1 s; FOXP3, forkhead box P3; FVC, forced vital capacity; MFI, median fluorescence intensity; RV, residual volume.

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et al., 2003). In COPD, an accumulation of both CD4+ and CD8+ T cells in the airway epithelium has previously been demonstrated (Lofdahl et al., 2008), but it is unknown whether these cells also express CD103.

When T cells are activated there is a shift in the expression of surface markers, e.g. CD27 is gradually down-regulated whereas CD69, CD25 and HLA-DR are upregulated at different stages of maturity. By analysing combinations of certain T cell markers it is thus feasible to characterise these cells in terms of activation and possibly also function (Chattopadhyay and Roederer, 2010).

In the present study, we hypothesised that cigarette smoke alters both the frequency and state of activation of CD103+ T cells as well as the frequency of T-regulatory cells in the lung, and that these changes also reflect development to COPD. To test this hypothesis, we investigated lung and blood T cells from smokers, never-smokers and COPD patients using multi-colour flow cytometry.

Materials and methods

Study subjects

Forty never-smokers (20 males/20 females aged median 60 (range 45–66) years), 40 smokers with normal lung function (20 males/20 females aged 53 (44–65) years) and 38 patients with COPD (20 males/18 females aged 61 (47–66) years) participated. All of them underwent a clinical examination, chest X-ray and spirometry (Jaeger Masterscope PC, CareFusion, Hong Kong, China). Never-smokers had a post-bronchodilator FEV_1/FVC of 0.80 (0.70–0.93) (median (range)) and an FEV_1 of 119% (89–147) of predicted using the European Coal and Steel Community (ECSC) normal values (Stocks and Quanjer, 1995). The smokers lung functions were FEV_1/FVC 0.78 (0.71–0.88) and FEV_1 109% (91–140) of predicted. The patients with COPD had FEV_1/FVC 0.62 (0.43–0.69) and FEV_1 79% (51–99) of predicted corresponding to GOLD stage I and II. The smokers had a smoking history of 34 (15–84) pack years. The COPD group consisted of both current smokers ($n = 27$) and ex-smokers ($n = 11$) with a smoking history of 42 (17–94) and 30 (17–41) pack years respectively. The smoking controls and the smoking COPD patients had a self-reported cigarette consumption of 20 (10–40) (median (range)) and 20 (3–25) cigarettes/day for the last 6 months respectively. Eleven COPD patients were ex-smokers, and they had stopped smoking 7 (2–19) years before entering the study. Nine COPD patients (8 current smokers and 1 ex-smoker) and 10 smokers fulfilled the criteria for chronic bronchitis (Stuart-Hariss et al., 1965). None of the participants were being treated with corticosteroids, but six of the COPD patients were using bronchodilators. Individuals with a history of allergy or asthma were excluded from the study. *In vitro* screenings for presence of specific IgE antibodies (Phadiatop, Pharmacia, Uppsala, Sweden) were negative. Smokers and COPD patients with symptom worsening (exacerbation) during the last three months were excluded.

Each participant gave informed consent and the study was approved by the Regional Ethical Review Board, Stockholm, Sweden.

Bronchoscopy, BAL and processing of BAL cells

Bronchoscopy and BAL were performed in the morning as previously described (Löfdahl et al., 2005). Due to clinical constraints, BAL was not performed on one of the never-smokers and two of the COPD smokers. All smokers refrained from smoking for at least 8 h prior to bronchoscopy. The BAL-fluid was strained through a Dacron net (Millipore, Cork, Ireland), centrifuged at $400 \times g$ for 10 min at 4 °C and the cell pellet was resuspended in PBS. Cell concentration was then adjusted for flow cytometric analysis as described below. Cell viability, total BAL cell differentials and frequencies of CD4+ and CD8+ T cells are included in another manuscript (Forsslund et al., manuscript in preparation).

Table 1
Two panels of monoclonal antibodies used to characterise lymphocytes in BAL and PB.

Antigen	Function	Fluorochrome
CD3	T cell	Pacific Blue
CD4	T-helper cell	APC-H7
CD8	T-killer cell	AmCyan
CD103	Adhesion	FITC
HLA-DR	Late activation	PE
CD25	Activation	PE-Cy5
CD69	Early activation	PE-Cy7
CD27	Co-stimulatory	APC
CD3	T cell	Pacific Blue
CD4	T-helper cell	APC-H7
CD8	T-killer cell	AmCyan
CD69	Early activation	FITC
FOXP3	Regulatory T cell	PE
CD25	Activation	PE-Cy5
CD27	Co-stimulatory	APC

All antibodies were purchased from BD except FOXP3 from eBioscience. Additional isotype controls with immunoglobulin G (IgG1 and IgG2) were used. FITC, fluorescein isothiocyanate; PE, phycoerythrin; Cy, cyanine dye; APC, allophycocyanin.

Macrophage depletion

The BAL cells were washed twice with cell wash (BD, Franklin Lakes, NJ, USA) and resuspended to a concentration of 1×10^6 cells/100 μL. Macrophage depletion was performed as described elsewhere (Wong and Varesio, 1984) and in online supplementary Material and Methods. Since macrophage-depleted BAL cells were also used for other projects, this procedure was generally performed on a total of $5–10 \times 10^6$ cells. Macrophage-depleted cells were equally distributed in aliquots corresponding to approximately 1×10^6 BAL cells pre-depletion, and two such aliquots were used for the two different antibody panels (Table 1). The cells were incubated at 4 °C in darkness for 25 min and washed twice before flow cytometric analysis.

Peripheral blood

Peripheral blood was obtained by venipuncture in the morning prior to bronchoscopy. The blood was added in 100 μL aliquots to each mixture of antibodies (Table 1). The cells were incubated at room temperature in darkness for 25 min, whereafter erythrocytes were removed by incubating with lysing solution (BD) for 8 min. The cells were washed twice before flow cytometric analysis.

Intracellular staining of FOXP3 T-regulatory cells

BAL and blood cells were stained with surface markers as described, followed by intracellular staining (see online supplementary Materials and methods) and analysis by flow cytometry.

Flow cytometry analysis of T cells

An eight-colour flow cytometer (FACSCanto II, BD) was used to analyse T cell surface markers. Data was processed in FACSDiva 6.1.2 (BD). At least 50 detected events in the final gates were required for analysis. Due to technical difficulties, the data obtained from some subjects were excluded. Therefore, the number of