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Differential effects of smoking and COPD upon circulating myeloid derived suppressor cells

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Summary
Background: Chronic obstructive pulmonary disease (COPD) is characterized by an enhanced and persistent innate and acquired immune response to tobacco smoking. Myeloid-derived suppressor cells (MDSCs) modulate T-cell responses by down-modulating the T cell receptor ζ chain (TCR ζ) through the catabolism of L-arginine. The effects of smoking on MDSCs and their potential participation in COPD immunopathogenesis have not been explored so far.
Methods: To investigate it, we compared the level of circulating Lineage-/HLA-DR-/CD33+/CD11b+ MDSCs, the serum concentration of arginase I (ARG I) and the expression of peripheral T-cell receptor ζ chain (TCR ζ) in never smokers, smokers with normal spirometry and COPD patients. Flow cytometry was used to quantify circulating MDSCs and TCR ζ expression. Serum ARG I levels were determined by ELISA.
Results: The main findings of this study were that: (1) current smoking upregulates and activates circulating MDSCs both in smoker controls and COPD patients; and, (2) at variance with

Abbreviations: ARG I, Arginase I; COPD, Chronic obstructive pulmonary disease; MDSCs, Myeloid derived suppressor cells; PBMCs, Peripheral blood mononuclear cells; TCR ζ, T-cell receptor ζ chain.

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the smokers with normal spirometry, in patients with COPD this effect persists after quitting smoking and is accompanied by a significant and specific down-regulation of the TCR ζ chain expression in circulating T lymphocytes.
Conclusion: Smoking modulates circulating MDSCs. Their regulation appears altered in patients with COPD.

Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by an enhanced inflammatory response of the lung to noxious particles or gases [1], where both innate and adaptive immunity participate [2]. Epithelial cells, macrophages and neutrophils are activated by irritants such as cigarette smoke and, as a result, there is a significant increase of dendritic cells subsets, activated T cells (CD4+ and CD8+ T cells), and B cells in the lung parenchyma of COPD patients [3]. This inflammatory cell infiltration persists after smoking cessation, suggesting a self-perpetuating process [4]. All in all, these findings support that the immune response is deregulated in those smokers who develop COPD [3]. Several pieces of evidence support this contention [5–8]. First, abnormalities in forkhead box P3 (FOXP3)+ regulatory T (Treg) cells, which are essential for the maintenance of self-tolerance and immune homeostasis by suppressing T cell effector activity [9], have been reported in COPD patients [5–7]. Second, the γδ CD8+ T lymphocytes, a T cell population with immunoregulatory properties [10], are reduced in both the lungs and peripheral blood of COPD patients as compared to smokers with normal lung function [8].

The so-called myeloid-derived suppressor cells (MDSCs) are a heterogeneous group of myeloid cells that include immature macrophages, granulocytes, dendritic cells (DCs) and other myeloid cells at earlier stages of differentiation with a remarkable ability to repress T-cell responses [11]. MDSCs suppress T-cell function by down-regulating the T-cell receptor ζ chain (TCR ζ), without affecting the rest of TCR subunits (CD3 ε and αß) through the modulation of L-arginine metabolism [12,13]. Human MDSCs release arginase I (ARG I) from cytoplasmic granules into the microenvironment and circulation, where it catabolizes L-arginine and induces T-cell dysfunction [14].

Initially described as one of the mechanisms used by tumor cells to escape the immune system response [15], growing evidence indicates that MDSCs also regulate the immune response in a range of inflammatory non-malignant conditions [11,16]. Although in patients with a variety of malignancies MDSCs are mostly identified as Lin^-/HLA-DR^-/$CD33^+$ $CD11b^+$, this MDSCs phenotype has not been studied in human inflammatory non-malignant settings [17]. Their response to smoking and their potential role in the pathobiology of COPD has not been explored so far. To investigate it, we compared the level of circulating MDSCs in never smokers, smokers with normal spirometry and COPD patients. Besides, we determined the serum levels of ARG I to assess the activation of circulating MDSCs [14,18], and the expression of TCR ζ in peripheral lymphocytes.

Materials and methods

Study design and ethics

This is a cross-sectional, descriptive, comparative and controlled study. All participants signed their informed consent after being aware of the nature and objectives of the study. The project was approved by the Ethics Committee of the Balearic Islands in Spain.

Participants

Participants were selected from individuals referred to the pulmonary function laboratory of our institution or primary care clinics. The diagnosis of COPD was established according to the GOLD recommendations [19]. All COPD patients were clinically stable and had not had any exacerbation or change of therapy for, at least, the last 3 months. Only smokers (with normal spirometry) and COPD patients with a cumulative smoking exposure of, at least, 10 pack-years were selected for the study. Former smokers were defined by having fully quitted smoking for at least 1 year before participating in the study. No participant was receiving oral steroids, immunomodulatory drugs and/or antibiotics. Subjects with atopic diseases, asthma, autoimmune disorders, malignancy, heart failure, kidney diseases and infectious diseases were excluded.

Lung function

Forced spirometry (before and after bronchodilation) was obtained (Micro 6000 Spirometer, Medisoft, France) following international guidelines [20]. The severity of airflow limitation was established on post-bronchodilator results according to the GOLD recommendations [19]. Reference values were those of a Mediterranean population [21].

Peripheral blood sampling and processing

Blood samples were obtained by peripheral venipuncture, and collected in three different tubes: (1) 5 ml in non-heparinized tubes for serum measurements. Blood was allowed to clot and centrifuged 15 min at 3000 rpm at 4 °C. Then, serum was aliquoted and stored at −70 °C until analysis; (2) 10 ml in sodium heparin tubes for isolation of peripheral blood mononuclear cells (PBMCs) as described below; and, (3) 5 ml in EDTA tubes for identification of T cell subpopulations.