

## **PHENOTYPIC ANALYSIS OF MACROPHAGES AND LYMPHOCYTES OF CIGARETTE SMOKERS**

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The influence of tobacco smoke on human health is still an important problem worldwide. Complex inflammatory processes and changes in the immune system are crucial in the pathogenesis of smoking related disorders like chronic obstructive lung disease, lung cancer, asthma, interstitial lung diseases, atherosclerosis. Inflammatory cells are involved in these reactions and the cellular immune response seems to be most affected by cigarette smoke (CS). The objective of this study is to present the alterations in the immune cell populations in tobacco smokers with special account to pulmonary macrophages and lymphocytes. We compared healthy active smokers (n= 50) with healthy never-smoking volunteers (n= 48). Pulmonary macrophages and lymphocytes were harvested from induced sputum, bronchoalveolar lavage fluid (BALF) and peripheral blood (PB). BALF and induced sputum cell profile, morphology and phenotype of cells were assessed. For macrophage phenotyping the immunocytochemistry method with antibodies anti: CD11b, CD14, CD54 and CD71 was used. For lymphocyte subtypes measurement flow cytometry was used. Following subpopulations of lymphocytes were identified: T, B, natural killer (NK), T helper (Th), T suppressor/ cytotoxic (Ts/c), T activated (Ta, HLADR+, CD25+), regulatory (Treg) and cytotoxic (CD3+/CD16/56+). Expression of death receptor - Fas on immune cells was measured. We found a significantly increased macrophage (pigmented cells) count in smokers when compared with non smokers. This count significantly correlated with pack/years smoked. The induced sputum population of macrophages was characterized by an elevated proportion of cells with expression of CD11b, CD14, CD54 and CD71 surface markers. Fas positive macrophages were more numerous in the BALF of smokers when compared with non smokers. The analysis of BALF lymphocytes revealed significant differences between smokers and nonsmokers. There was a higher proportion of T, T c/s, Ta and cytotoxic T cells in smokers when compared with non smokers. The proportion of Th cells and the Th:Tc/s ratio in the BALF of smokers were lower than in non smokers (46.9% vs 62.7%, 0.51 vs 0.69). No significant differences in the proportion of PB cells between smokers and non smokers were found. A significant increase of Fas positive T cells of smokers was found. Proportion of Fas+ T lymphocytes was significantly higher in asymptomatic smokers when compared with non smokers (mean: 71.6% vs 61.0% for Fas+/ CD4+ and 73.8% vs 58.3% for Fas+/CD8+ lymphocytes). Significant correlations of Fas positive cells with pack years smoked were observed. Preliminary data showed a lower proportion of Treg in PB of smokers than in non smokers. The results of our study confirmed significant changes in the proportion of immune cell subtypes in the lung environment of smokers. These changes were dose-dependent. Changes in the proportion of Fas positive lymphocytes and T reg cells in PB indicated a possible influence of CS on the recruitment of lymphocytes.