Analysis of the antigen-specific t-cell response in patients with chronic obstructive pulmonary disease.

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Chronic obstructive pulmonary disease (COPD) is a progressive inflammatory lung disease, characterized by the accumulation of activated, oligoclonal, Type 1 committed effector T lymphocytes (particularly CD8+), together with macrophages and neutrophils [1-4]. While cigarette smoke exposure is the major risk factor for COPD, only a minority (about 15 to 20%) of smokers develops the disease. Although genetic and/or phenotypic differences may account for the different susceptibility to COPD, environmental factors might also play a role. In particular, lower respiratory tract infections in childhood are known to be an independent risk factor for adult COPD [5]. On the other hand, COPD patients often experience infectious exacerbations and their airways may be colonized by several pathogens. Therefore, persistent infections might represent an inflammatory stimulus which contribute to COPD development.

Among the pathogens that commonly cause lower respiratory tract infections, Adenoviruses (ADV), Respiratory Syncytial Virus (RSV), Chlamydia Pneumoniae and Influenza virus are thought to be potentially involved in COPD pathogenesis, due to their ability to persist for many years in a latent form and continuously stimulate the immune system [6-9], or because they are associated with disease exacerbations [10] or finally their presence have been detected more frequently in COPD patients than in controls [8, 11].

We hypothesise that in subjects exposed to cigarette smoke, a latent infection could be one of the factors that induce accumulation of antigen-specific T lymphocytes in the lung and amplify the smoke-induced lung inflammation, favouring the pathologic changes of COPD. Therefore, aim of the study is to quantify the frequencies of T cells specific for ADV, RSV, Influenza virus and C. pneumoniae among total mononuclear cells from peripheral blood and lung tissue samples of smokers with COPD compared to smokers without airflow limitation, by detecting the production of IFN-γ in response to specific antigens in
an Enzyme Linked Immunospot (ELISpot) assay. Moreover, the study will evaluate if these antigen-specific responses are mostly restricted by CD4+ or CD8+ T lymphocytes. Live wild type ADV (serotype 5), RSV (serotype A2), Influenza virus (serotype H3N2) and C. pneumoniae are used as antigens.

Patients undergoing lobectomy or pneumonectomy for lung cancer attending the Department of Respiratory Diseases and the Department of Thoracic Surgery of the Policlinico University Hospital in Modena are enrolled. For each patients we obtain 15 mls of venous peripheral blood, in order to separate peripheral blood mononuclear cells (PBMCs). At the time of lung surgery, we obtain up to five lung tissue samples (1 cm³ each) from lung areas far from cancer lesions. These lung samples are processed to obtain single cell suspensions through mechanic technique.

We collected blood and lung samples from 5 patients. A flow cytometry staining for CD3, CD4, CD8, HLA-DR and CXCR3 confirmed that our technique for obtaining lung single cell suspensions allow us to isolate T lymphocytes from lung tissues.

The study is conducted in collaboration with David Lewinsohn at Oregon Health and Science University, Portland, Oregon, USA, where all the experiments are going to be performed. The set up phase has been completed. Stocks of ADV, RSV, Influenza virus and C. pneumoniae have been generated. The use of these live pathogens in an ELISpot assay has been optimized and preliminary experiments on PBMCs from normal donors have been performed in order to define the optimal amount of antigen. Experiments on patients’ samples will be run in the next months.
References