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Characterization of the Inflammatory Reaction in the Peripheral Airways of Cigarette Smokers Using Immunocytochemistry[1-3]

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SUMMARY Lung tissue from 20 patients undergoing resection for a peripheral carcinoma was studied using monoclonal antibodies to identify inflammatory cell types in the peripheral airways and to determine the location of the bronchial-associated lymphoid tissue (BALT). The patients were grouped according to their percent predicted FEV, (%FEV,) into obstructed (%FEV, < 80%) and control (%FEV, > 80%). The resected lungs were filled with dilute cryoembedding media (Tissue-Tek R), frozen over liquid nitrogen, sliced into 2-cm sagittal slices using a band saw, and sampled using a cork bore. Ten serial histologic sections cut from these samples were stained with monoclonal antibodies for specific inflammatory cell types, which were counted and expressed per square millimeter of airway wall area. The results showed that the patients with airway obstruction had more B-lymphocytes in the airway adventitia than did the control subjects ($p < 0.001$) and that the number of submucosal polymorphonuclear leukocytes is related to the amount smoked ($p < 0.02$). They also show the BALT has a different distribution in human than in rodent lungs in that the lymphoid collections are found in the outer walls of the airway rather than in the submucosa.

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Introduction

Cigarette smoking causes an inflammatory reaction in the airways (1) and produces airway obstruction in approximately 15 to 20% of heavy smokers (2). This obstruction is located in the peripheral airways (3), and it is thought to be due to an inflammatory process (4–6). Morphometric studies of the chronic airway inflammation in smokers have been based on classic histologic examination and have provided quantitative information about the airway wall and lumen as well as qualitative information about the nature of the inflammatory response (5, 6). However, these methods do not allow distinction between small monocytes and lymphocytes, and they provide no information about lymphocyte subtypes. Although recent studies of human airways (7) used monoclonal antibodies to obtain information about inflammatory cell types in the epithelium and submucosa of larger airways, they did not sample the small bronchi and bronchioles that are responsible for the airway obstruction (3).

The present studies were designed to compare the number and type of inflammatory cells, expression of histocompatibility antigens, and the location of the bronchial-associated lymphoid tissue (BALT) in the lungs of human smokers with airway obstruction with those without obstruction. They were carried out on lung tissue resected from patients with peripheral lung tumors, which was rapidly frozen shortly after it was resected and studied using monoclonal antibodies to quantitate specific inflammatory cell subtypes and the expression of HLA Class I and Class II antigens.

Methods

Our laboratory is conducting an ongoing study of lung structure and function in which all patients requiring lung resection have their lung function measured preoperatively and the lung specimen fixed in inflation with either formalin or glutaraldehyde. The present study is based on 20 lungs from this series, which were rapidly frozen rather than fixed in order to make them more suitable for immunocytochemical study. Ten of these patients who had airway obstruction (%FEV, < 80%) were compared with 10 without (%FEV, > 80%). All of the patients had smoked cigarettes at some time during their lives, but five of the control subjects and four of the obstructed patients had stopped smoking at least 1 yr prior to resection. Because previous work (8) has shown that the total cell counts do not change after smoking cessation, we did not attempt to separate current and ex-smokers. All patients had a British Medical Council questionnaire administered to obtain occupational and exposure histories, and pulmonary function tests were performed less than 1 wk prior to surgery.

Spirometry was performed on a Collins computerized spirometer (Warren E. Collins, Braintree, MA). The percent predicted forced vital capacity (%FVC), %FEV,, and midmaximal expiratory flow rate (%MMFR) were calculated according to the formula of Crapo and colleagues (9). Lung volumes and pressure-volume curves of the lung were performed in a constant-pressure variable-volume body plethysmograph. The percent predicted residual volume (%RV) was calculated according to the formula of Goldman and Becklake (10), and the %TLC was calculated according to Bates and coworkers (11). The pressure-volume curves of the lung were constructed according to the method of Mead and Wittenberger (12), and the pleural pressure at maximal inflation (Pl,max) was recorded as percent predicted (%Pl,max) according to the formula of Colebach (13). Diffusing capacity (D_{LCO}) was performed by the single-breath method of Ogilvie and coworkers (14) on a PK Morgan automated diffusing capacity analyzer (P.K. Morgan, Chatham, Kent, UK) using the predicted values from Crapo and Morris (15).

Pathology

All specimens were obtained directly from the operating room and inflated with optimal cutting temperature cryoembedding material (OCT tissue Tek R [Miles Inc., Elkhart, IN]), which was diluted 50% with normal saline. The inflated lobe was cradled in aluminum foil and suspended above liquid nitrogen in a covered styrofoam box for 15 min. If after 15 min the medial portion of the lung was not frozen, the lung was turned 180 degrees (hilus down) and allowed to sit above the liquid nitrogen for another 10 min. The frozen lobe was sliced into 2-cm sagittal slices using

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