METABOLIC ACTIVITY IN HUMAN ALVEOLAR MACROPHAGES INCREASES AFTER CESSION OF SMOKING

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Abstract—Alveolar macrophages (AMs) were recruited by bronchoalveolar lavage (BAL) from human smokers before and one, three, and six months after smoking cessation. The metabolic activity of the AM was quantified as luminol-enhanced chemiluminescence (CL) both at rest and after in vitro stimulation with phorbol myristate acetate (PMA). The resting CL values did not differ before and after smoking cessation. The activity after PMA stimulation was unaltered at one and three months. However, the maximal metabolic response, as well as the rate, were significantly (P < 0.02 and P < 0.01, respectively) higher at six months, compared to prior smoking cessation. In addition, the time to reach the maximal peak was reduced after six smoke-free months, indicating a more rapid cell activation. The cell concentration in the BAL-fluid decreased (P < 0.001) as soon as after one smoke-free month and remained low at the following lavages. The lower metabolic response one and three months after smoking cessation, and the increased response six months after, together with a rapid normalization of the cell concentration in the BAL fluid, may be explained by the persistence of tobacco-smoke particles in the alveolar space, which could influence cell activity.

INTRODUCTION

Macrophages and granulocytes are the main propagators of inflammatory reactions in the lung. These phagocytic cells respond with degranulation and a respiratory burst when stimulated with particulate or soluble agents. This generation and release of highly reactive oxygen-derived metabolites damages both the target as well as adjacent tissues. The consequences are usually of limited importance after an acute inflammation, but may damage the lung beyond reversal if the cells are chronically activated. Thus, the development of fibrosis and emphysema have been suggested to be some of these side effects (1–3).

The alveolar macrophages (AMs) are the main phagocytic cell in the alveoli.
These cells are located in the epithelial lining fluid and are unique, as they can interfere directly with the inhaled air. Hence, the structure and function of AMs depend largely on the environment. As a consequence, chronic exposure to tobacco smoke influences AMs to a great extent (4). For instance, the release of oxygen radicals and proteolytic enzymes from AMs are affected by tobacco smoke exposure (5–9). The cessation of smoking abruptly creates a new environment in the alveolar space in general and for the AMs in particular. We have recently reported (10) that tobacco-smoke particles can remain in the AMs for more than 15 months after smoking cessation, as measured by an increased cell fluorescence.

The aim of the present investigation was to determine if smoking cessation alters the metabolic properties of human AMs. We examined AMs from a group of human smokers before, and one, three, and six months after smoking cessation. Alveolar cells were recruited by bronchoalveolar lavage, and their metabolic activation was determined by chemiluminescence both at rest and after in vitro stimulation.

MATERIALS AND METHODS

Subjects. Eighteen healthy smokers (mean age 41.4 years, range 27–61; 17 women) participated in the study. Their mean cigarette consumption was 22.6 ± 7.8 (mean ± SD) pack years, and the present consumption exceeded 15 cigarettes a day for the last 10 years. Some of the subjects were excluded at different points of time because of relapse in smoking. Thus, all 18 participated in the lavage prior to smoking cessation, while 14, 13, and 10 underwent BAL at one, three, and six months after smoking cessation, respectively.

All subjects had a normal chest X-ray and were free of medication. A routine physical examination showed nothing abnormal. The study had the approval of the local ethics committee, and informed consent was obtained.

Preparation of Alveolar Macrophages. AMs were recruited by bronchoalveolar lavage (BAL), as previously described (11). Briefly, the bronchoscope was wedged in a middle lobe bronchus and sterile saline solution at 37°C were instilled in five aliquots of 50 ml each. The fluid was gently suctioned back and collected in a plastic bottle kept on ice. The BAL fluid was strained through a double layer of Dacron nets (Millipore, Bedford, Ireland) to remove mucus. Cells were pelleted by centrifugation at 400g, 4°C, for 10 min, and the supernatants were poured off. The cells were resuspended in a Hanks’ balanced salt solution with 1% fetal calf serum. The total number of cells was counted in a Bürker chamber. Viability exceeded 85% as tested by the exclusion of trypan blue. Smears for differential counts were prepared by cytocentrifugation at 400 rpm, 4°C, for 10 min, and the supernatants were poured off. The cells were resuspended in a Hanks’ balanced salt solution with 1% fetal calf serum. The total number of cells was counted in a Bürker chamber. Viability exceeded 85% as tested by the exclusion of trypan blue. Smears for differential counts were prepared by cytocentrifugation at 500 rpm for 3 min (Cytospin 2, Shandon, Southern Products, Runcorn, England). Smears were stained with May-Grünwald Giemsa and 500 cells were counted.

Two million cells were washed twice by centrifugation in 1 ml cold 0.15 M phosphate-buffered saline, pH 7.4, supplemented with 10 mM EDTA and 0.02% NaN₃ (PBS-EDTA). The washed cells were then resuspended in 1 ml Krebs-Ringer phosphate buffer supplemented with 10 mM glucose (KRG).

Measurement of Macrophage Activation. The metabolic activation of the AM was measured as luminol-enhanced chemiluminescence (CL) in a LKB Wallac Luminometer 1251 (Wallac Co.,