

Effect of smoking on bronchoalveolar lavage constituents

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Increasing knowledge has accumulated relating to the effect of cigarette smoking on cells and other constituents in bronchoalveolar lavage (BAL) fluid. Such knowledge is important for the correct evaluation of BAL results. When BAL records are interpreted, smoking has to be considered as a major confounding factor. In this issue of the Journal, BURKE *et al.* [1] add a new aspect to the adverse effects of smoking on the alveolar microenvironment. They suggest that the apparent epithelial lining fluid (ELF) volume retrieved from smokers is 100% greater than from nonsmokers. Clearly, this finding would also affect the cell and protein concentrations in studies using the ELF volume as reference value. Comparable differences in ELF volumes among different study groups have not previously been reported. Although the mechanism of this increase remains unclear, the authors suggest that either increased production or decreased clearance of ELF could be involved, but not enhanced capillary permeability, since a parallel increase in albumin was not observed.

Technical difficulties, however, could also account for the observed effect. One limitation lies in the use of urea to estimate the ELF volume [2]. The basic disadvantage of this method is that urea passively diffuses from plasma and the interstitium into the BAL fluid during the lavage dwell-time [3]. In addition, the fluid dynamics during BAL are complex [4]. The hydrostatic pressures induced by the BAL procedure itself may cause unpredictable movements of water and low molecular weight solutes between the blood and the BAL fluid [5].

On these lines, the data by BURKE *et al.* [1] might be interpreted quite differently: smokers may be more susceptible to increased water and urea movement during the BAL procedure; this would give rise to an artificially increased ELF volume with a consequent artificial decrease in albumin due to the dilution of the ELF volume during the BAL procedure. In this context, it is of interest that another study did not show a significant difference in the concentration of albumin expressed as $\text{ng}\cdot\text{ml}^{-1}$ ELF between asymptomatic smokers and normal nonsmokers [6]. The ideal marker for the assessment of the true ELF volume is yet to be found [7]. BURKE *et al.* [1] are correct when stating that their findings should be confirmed using other methods of ELF determination.

What data have been compiled on the effect of smoking on lung cells and solutes in BAL investigations? The following part of this editorial is meant to briefly outline the field. It must remain incomplete, and thus cannot be taken to be an exhaustive review. It will be limited to changes in normal (*i.e.* healthy, asymptomatic) smokers. Smokers with chronic bronchitis, chronic obstructive lung disease or emphysema, and patients with interstitial lung disease, are different populations and will not be addressed.

Early investigators were impressed by the obvious changes in macrophage morphology and function [8]. Macrophages from smokers are more numerous, larger in size, and show pigmented cytoplasmic inclusions ("smokers' inclusion bodies"). Phagocytosed fluorescent material from tobacco smoke is responsible for the characteristic phenomenon of autofluorescence of smokers' macrophages, which may interfere with surface marker analysis of alveolar macrophages when fluorescence techniques are applied [9, 10]. Nevertheless, a recent study indicated a decreased expression of the CD11/CD18 complex of adhesion molecules on smokers' alveolar macrophages, suggesting that these cells have reached their final maturation stage and/or their maximum phagocytosing capacity [11].

Functionally, alveolar macrophages from smokers display increased motility, including enhanced responsiveness to chemotactic stimulants, increased metabolic activity, and increased content and releasability of lysosomal enzymes, such as elastase and other proteases [12-15]. They show enhanced or normal oxygen radical production, depending on the stimulus [8, 14, 16-19]. The potentially tissue-damaging cytotoxic function of alveolar macrophages appeared to be greater in smokers than in nonsmokers in an assay system which used explanted normal lung parenchyma as target cells [20]. In contrast, the phagocytic and microbicidal activity of smokers' alveolar macrophages was found to be either normal [12-14, 21] or decreased [7, 22] and, again, whether a difference may be detected or not, depends on the choice of stimuli and the targets used in the assay system.

Smokers' macrophages have been reported to spontaneously release a chemotactic factor for neutrophils [23]. The true nature of this factor is not yet clear but it does not appear to be predominantly leukotriene B_4 , since alveolar macrophages from smokers have an impaired capacity to secrete this leukotriene as well as other products of the lipoxygenase and cyclooxygenase pathway [24-26].

The accessory cell function of alveolar macrophages is decreased in smokers [27]. In this context, the diminished interleukin-1 production by lipopolysaccharide (LPS)-stimulated alveolar macrophages in smokers [28] may indicate a possible mechanism by which the local immune responses are suppressed and the mitogenic responses of lung lymphocytes are reduced [29].

Changes in the local T-cell system observed in smokers add further support to the concept that smoking has a suppressive effect on the pulmonary immune response. In this regard, even though the total number of T-cells in BAL fluid is not different between smokers and nonsmokers, the CD4/CD8 ratio is significantly decreased in smokers' BAL fluid due to a lower CD4 and a higher CD8 percentage [30–33]. The local depression of cellular immunoregulation and the low natural killer cell activity in BAL fluid [34] may predispose smokers to the development of both infectious diseases and malignancies. On the other hand, these profound changes in T-cell function may be responsible for the fact that sarcoidosis and hypersensitivity pneumonitis are less prevalent in smokers [35, 36].

Smoking is also associated with an influx of neutrophils into the lower respiratory tract [37]. An increase in the total number of this cell type in smokers' BAL fluid has been reported by several investigators [6, 23, 33, 38]. Neutrophils are probably attracted to the lower respiratory tract of smokers by neutrophil chemotactic factors released from alveolar macrophages [23, 24, 26]. Another mechanism accounting for this influx may be a cigarette smoke-induced loss of the functional activity of chemotactic factor inactivator, as recently described in BAL fluid of smokers [39].

Neutrophils may contribute to the development of chronic bronchitis in two ways. Firstly, by increasing the local protease burden through the release of a neutrophil elastase which has convincingly been shown to be elevated in BAL of smokers [40, 41]. Secondly, by the release of oxidants which may inactivate important defensive proteins such as α_1 -proteinase inhibitor. Whether functional antiprotease activity in BAL fluid of smokers is really diminished compared to nonsmokers, however, still remains controversial [42–47]. A recent study even demonstrated an increase of α_1 -proteinase inhibitor in BAL fluid of smokers [47], indicating a mechanism which may compensate for the increased elastase burden of smokers' epithelial lining fluid.

Immunoglobulins G and A (IgG and IgA) were among the early investigated non-cellular compounds in BAL fluid, both having been found to be normal or increased in smokers [48–51]. Later, elevated histamine and tryptase levels were observed in smokers' BAL fluid, suggesting increased mast cell degranulation in the airways of smokers [52]. In the context of the recent interest in the role of extracellular matrix components in interstitial lung disease, healthy smokers and nonsmokers were studied, and no difference

was observed for any substances, such as fibronectin, hyaluronan, procollagen III peptide, or vitronectin [53, 54]. Finally, the influence of smoking on BAL phospholipids should briefly be mentioned. Data on total surfactant phospholipids are controversial since decreased [55, 56] and increased [57] levels have been reported. The analysis of the composition of the major lipid fractions showed an increase in phosphatidylethanolamine, sphingomyelin and phosphatidylglycerol, but a decrease in the levels of cholesterol in smokers [57].

It is important to recognize that the majority of the reported differences between smokers and nonsmokers were based on the use of reference values such as millilitres of recovered BAL fluid, milligrams of total protein or albumin, or ratios with albumin. Before the history of smoking-induced changes in BAL fluid and cells is re-written by taking into account possible differences in the apparent ELF volume, the results of BURKE *et al.* [1] showing a large increase in the ELF volume of smokers need confirmation by other groups, as well as further explanation of the underlying mechanisms. Any responses from readers of the Journal are welcome.

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