Decreased Contents of Surfactant Proteins A and D in BAL Fluids of Healthy Smokers*

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Hydrophilic surfactant proteins, surfactant protein A (SP-A) and surfactant protein D (SP-D), have important roles in modulating the host defense functions in the peripheral airways. It has been reported that cigarette smoke may alter the component and function of pulmonary surfactant. In this study, we determined the contents of SP-A and SP-D in BAL fluids of healthy smokers and nonsmokers by enzyme-linked immunosorbent assay using monoclonal antibodies against each protein. The contents of SP-A and SP-D in BAL fluids were significantly (p<0.05) decreased in smokers compared to those in nonsmokers, although there was no significant difference of total phospholipid content between smokers and nonsmokers. These results suggest that the decreased levels of SP-A and SP-D in smokers may impair the host defense functions of surfactant in the peripheral airways and might have a crucial role in the development of chronic obstructive lung disease.

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Pulmonary surfactant is a complex mixture of lipids and proteins synthesized by alveolar type II cells. The surfactant secreted from these cells lines on the air-liquid interface and prevents the alveoli from collapsing at end of expiration. Four surfactant-specific proteins (SPs) have been characterized, and these proteins promote lung stability by regulating the surface tension-lowering properties, regulate surfactant homeostasis in the alveoli, and modulate host defense functions in the lung. Hydrophilic surfactant proteins, surfactant protein A (SP-A) and surfactant protein D (SP-D), are the members of C-type lectin superfamily along with mannose-binding proteins. We have developed enzyme-linked immunosorbent assays (ELISAs) using monoclonal antibodies against human SP-A and SP-D, and clarified the clinical usefulness of SP-A and SP-D determination in amniotic fluid, pleural fluid, BAL fluid, and sera.

It has been reported that cigarette smoke may alter the surface activity of pulmonary surfactant. Endobronchial washings from long-term cigarette smokers showed a significant rise in minimum surface tension compared with nonsmokers. There have been numerous reports about lavage phospholipids in smokers. Concerning the contents of SPs in BAL fluids of smokers, to our knowledge, there has been only one recent report on the content of SP-A. However, there has been no report on the content of SP-D. In this study, we determined the contents of SP-A, SP-D, and total phospholipid in BAL fluids of healthy smokers and nonsmokers.

The aim of this study was to clarify the effect of cigarette smoking on SPs by determining the contents of SP-A and SP-D in BAL fluids of healthy smokers and nonsmokers.

Materials and Methods

Study Population

BAL fluid samples were collected from 20 healthy volunteers: 8 current smokers (aged 24 to 48 years) and 12 nonsmokers (aged 18 to 35 years). In smokers, the quantity and duration of smoking were 10 to 40 cigarettes a day (mean, 22 cigarettes a day) and 2 to 28 years (mean, 12.5 years), respectively. They had no history of respiratory disease, and none showed evidence of respiratory disease by physical examination, chest radiograph, and pulmonary function tests. Ethical approval was obtained for all lavage studies, and all healthy volunteers gave their informed consent.

Method of BAL

The lavage procedure was performed using a fiberoptic bronchoscope (Olympus) introduced by the transoral route after premedication with atropine and either hydroxyzine hydrochloride or meperidine (Pethidine) hydrochloride under local anesthesia with lidocaine. The bronchoscope was positioned in a subsegmental lobe.
orifice of the right middle lobe and BAL was performed three times with 50-mL aliquots of 37°C sterile 0.9% saline solution. The fluids recovered were pooled and immediately passed through several layers of loose cotton gauze to remove mucus, and then centrifuged at 250g for 10 min at 4°C to sediment the cells. The supernatants were collected and cryopreserved at -30°C until use. These supernatants were used for determination of SP-A, SP-D, and phospholipid.

The recovered cells were analyzed on total and differential cell counts.

**Determination of Human SP-A**

Monoclonal antibodies were prepared against human SP-A and ELISA for its determination was performed using solid-phase monoclonal antibody PC6 and HRP-conjugated monoclonal antibody PE10 as described previously. This assay system was able to detect SP-A at 2.0 to 250 ng/mL. BAL fluid samples were diluted 1:8 to 1:64. All assays were performed in duplicate and the data were expressed as a mean of duplicate samples.

**Determination of Human SP-D**

The human SP-D was also measured by sandwich ELISA using monoclonal antibody 7C6 and HRP-conjugated monoclonal antibody 6B2 as described previously. This assay made it possible to determine the concentration of human SP-D ranging from 3.13 to 200 ng/mL. BAL fluid samples were diluted 1:8 to 1:64. All assays were performed in duplicate and the data were expressed as a mean of duplicate samples.

**Measurement of Phospholipid**

Lipids were extracted by the method of Bligh and Dyer and lipid phosphorus was determined by the method of Bartlett.

**Statistical Analysis**

Data were expressed as mean±SEM. All data were evaluated statistically by analysis of variance. The Mann-Whitney U test was used to compare paired sets of data, and the level of critical significance was assigned at p less than 0.05.

**Results**

The recovery rates of BAL fluids were 65±6% in smokers and 67±6% in nonsmokers. There was no significant difference of the recovery rates between two groups. In differential cell counts in BAL fluids, smokers showed significantly (p<0.01) higher proportion of alveolar macrophages than that in nonsmokers (smokers, 94.2±1.3%; nonsmokers, 82.1±2.7%), with a corresponding decrease in the proportion of lymphocytes. The total number of alveolar macrophages in smokers (2.35±0.48×10⁵/mL) was also significantly (p<0.01) higher than that in nonsmokers (0.68±0.11×10⁵/mL).

The concentrations of SP-A, SP-D, and total phospholipid in BAL fluids from each sample were plotted as shown in Figure 1. The level of SP-A in BAL fluids of smokers (1.8±0.4 µg/mL) was significantly (p<0.05) decreased compared with that in nonsmokers (3.1±0.4 µg/mL). The SP-D concentration in BAL fluids of smokers was also demonstrated to be significantly low (0.5±0.1 µg/mL; p<0.05), while that of nonsmokers was 1.3±0.2 µg/mL. The level of total phospholipid in BAL fluids of smokers (35.4±5.6 nmol/mL) was also lower than that of nonsmokers (44.4±3.2 nmol/mL), although no significant difference was found.

The ratios of SP-A to phospholipid and SP-D to phospholipid were decreased in smokers (52.8±7.4 ng/nmol and 17.9±4.7 ng/nmol, respectively) com-
pared with those in nonsmokers (68.5±8.2 ng/nmol and 23.9±4.2 ng/nmol, respectively) (Fig 2). The ratios of SP-A to phospholipid were threefold higher than those of SP-D to phospholipid in both groups. We analyzed the relationship between the levels of SP-A and those of SP-D in BAL fluids, but we were unable to find a significant correlation between them (r=0.064). The ratio of SP-A to SP-D in smokers (5.4±2.3) was higher than that in nonsmokers (3.8±0.8).

We next compared the alveolar macrophage proportion and its total number in cells obtained by lung lavages with the levels of SP-A, SP-D, and total phospholipid. There were no significant correlations between them (data not shown).

**Discussion**

We demonstrate that the contents of SP-A and SP-D in BAL fluids obtained from smokers show a significant decrease in comparison to those in nonsmokers, while there was no significant difference of total phospholipid contents between smokers and nonsmokers.

There is no clearly confirmed evidence about the contents of phospholipid in BAL fluids of smokers. A lower total phospholipid content has been observed in human smokers by Finley and Ladman. However, Low et al. reported that there was no difference in lavage phospholipid concentration between smokers and nonsmokers, and Hughes and Haslam reported that the smoker had significantly higher levels of total phospholipid compared with nonsmokers. Conflicting results have been published about the influence of tobacco smoking on alveolar phospholipid level. Our result was consistent with those of Low et al. In this study, both levels of BAL fluid SP-A and SP-D were decreased in smokers. This result was not consistent with the report by Hamm et al. that the levels of BAL fluid SP-A were slightly higher in smokers than that in nonsmokers (4.5 µg/mL and 3.4 µg/mL, respectively), although not significant. The apparent discrepancies between the findings in these two studies remain unexplained.

The earliest pathologic abnormalities to develop within the lungs of smokers consists of accumulations of alveolar macrophages within the alveoli. The surfactant in cigarette smokers is being sequestered from the alveolar epithelial surface into alveolar macrophages, reducing the alveolar pool size of surfactant. In this study, we could not find significant correlations between the alveolar macrophage proportion and the level of surfactant components (SP-A, SP-D, and phospholipid), and between the total macrophage number and the level of surfactant components (data not shown). It appears likely that there are mechanisms that reduce surfactant in smokers other than phagocytosis by alveolar macrophages. Linnoila et al. have reported that lung tumor cells with SP-A immunoreactivity were seen more frequently in patients with a lighter smoking history. The production of SPs from alveolar type II cells might be reduced in smokers. It should be clarified in future studies whether cigarette smoking may reduce the production of SPs.

It has been reported that SP-A and SP-D play crucial roles in host defense mechanisms and immunomodulation of the peripheral airways, possibly by enhancing microbial recognition, phagocytosis, and/or killing by resident phagocytic cells. The significant reductions of BAL fluid SP-A and SP-D in smokers might attenuate the host defense functions of surfactant in the peripheral airways of smokers, and may lead to direct toxic injury of the lung and the development of chronic obstructive lung disease. In this study, we demonstrated significantly reduced levels of BAL fluid SP-A and SP-D only in healthy smokers other than smokers who have developed chronic obstructive lung disease. It would be interesting to determine whether patients with chronic obstructive lung diseases have reduced levels of SP-A and SP-D in BAL fluids. Further studies are needed to confirm the possible roles of these hydrophilic SPs in the development of chronic obstructive lung disease in smokers.

We also found that there was no correlation between the contents of SP-A and those of SP-D in BAL fluids. Both SP-A and SP-D are synthesized in alveolar type II cells and nonciliated epithelial cells and secreted into the alveolar spaces. Although the reason of discrepancies between the contents of SP-A and SP-D in each subject remains unclear at present, this may be related to the independent regulation of synthesis and expression of these proteins.

In conclusion, we clarified that the contents of BAL fluids SP-A and SP-D in smokers showed a significant
decrease in comparison to that in nonsmokers, although there was no significant difference of total phospholipid contents between these two groups. This reduction of these hydrophilic SPs in smokers may have an important effect on the host defense systems of the peripheral airways and might be a crucial cause of chronic obstructive lung disease.

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REFERENCES