Pulmonary Alveolar Proteinosis*  
Abnormal in Vitro Function of Alveolar Macrophages  
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Pulmonary alveolar proteinosis is characterized by the accumulation of granular proteinaceous material within the alveoli of the lung. It is well established that patients with pulmonary alveolar proteinosis have a high incidence of complicating pulmonary infections, which suggests that the function of the alveolar macrophages is abnormal. To investigate the function of these cells, they were obtained from two patients by pulmonary lavage

Pulmonary alveolar proteinosis is a pulmonary disorder of unknown cause that is characterized by the accumulation of lipid-rich granular material within alveoli. This process of alveolar filling generally is not associated with a significant interstitial or intra-alveolar inflammatory response. Mononuclear cells may be increased in the rather normal-appearing alveolar septa; and in some instances, interstitial fibrosis is present. Free cells within alveoli are reported to be chiefly type-2 granular pneumonocytes in various stages of degeneration and alveolar macrophages, which are not greatly increased in number.1,2

Pulmonary infections due to Nocardi a, Candida, and Cryptococcus, as well as the usual bacterial pathogens such as Streptococcus pneumoniae and Staphylococcus aureus, occur with high incidence in patients with alveolar proteinosis.1,3,4 The high incidence of complicating pulmonary infections and the report of Golde and colleagues5 that alveolar macrophages from patients with alveolar proteinosis were defective in their ability to kill ingested Candida organisms suggest that the antimicrobial clearance mechanism of alveolar macrophages may be defective. This report presents observations on the functional status of alveolar macrophages obtained by bronchoalveolar lavage from two patients with pulmonary alveolar proteinosis.

Materials and Methods

Patients

Two patients with pulmonary alveolar proteinosis diagnosed by open lung biopsy underwent bronchopulmonary lavage as therapy for their pulmonary disorder. Patient 1 had had an abnormal chest x-ray film and progressive symptoms of cough and dyspnea for three years. At the time of study, he had extensive pulmonary infiltrates, severe dyspnea, and a pulmonary abscess caused by infection with Nocardia asteroides. Clinical data for this patient and the beneficial effect of bronchoalveolar lavage on his respiratory abnormalities have been published.6 Copious quantities of granular proteinaceous material were obtained by lavage.

Patient 2 had had a mild cough and dyspnea for ten years and a stable chest x-ray film and data on pulmonary function over that period. At the time of bronchoalveolar lavage, data from tests of pulmonary function were mildly abnormal, with spirometric data and pulmonary volumes in the low normal range and the steady-state diffusing capacity of the lung at 75 percent of normal. Lavage of the lobe that roentgenographically appeared to be most involved yielded only 5 ml of granular proteinaceous material, and the patient’s condition has not symptomatically improved.

Methods

The material from bronchoalveolar lavage was centrifuged at 250 g for ten minutes at 4°C. This produced two visible layers of sediment, with cells pelleted in the bottom of the tube and the granular proteinaceous material on top. After most of the top layer was aspirated, the layer of cells was washed three to five times with Hanks’ balanced salt solution to remove additional granular proteinaceous material. The cells were resuspended in Hanks’ solution and were counted with a hemacytometer.

Portions of the total sediment from lavage, as well as the cellular and granular proteinaceous layer were placed in a 2.5 percent solution of phosphate-buffered glutaraldehyde (pH

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were tested with each experiment, in order to demonstrate flasks containing serum and bacteria but no macrophages were tested with each experiment, in order to demonstrate

were postfixed in a 2 percent solution of osmium in phosphate buffer (pH 7.2) and was embedded in epoxy resin (Epon). Thin sections for examination with the transmission electron microscope were cut with a diamond knife and were stained with uranyl acetate and lead citrate.

In vitro phagocytic activity of the alveolar macrophages obtained from these patients was determined by a method similar to that of Green and Carolin. After the macrophages were washed and counted as described, flasks were prepared by adding to each flask $2.5 \times 10^6$ macrophages, 10 percent autologous serum, and $5 \times 10^6$ *S. aureus* organisms in a total volume of 2.0 ml of Hanks' balanced salt solution. Control flasks containing serum and bacteria but no macrophages were tested with each experiment, in order to demonstrate that the organisms were in a lag phase of growth. Contents of the flasks were sampled initially and at one, two, and three hours after preparation, and the number of viable staphylococcal organisms in the culture medium were measured by the standard pour-plate technique. Results were expressed as a percentage of the initial bacterial count.

**RESULTS**

The phagocytic phase of the antibacterial ability of alveolar macrophages obtained from the patients with pulmonary alveolar proteinosis is compared with this function in alveolar macrophages obtained from normal subjects in Figure 1. The data points for the patients with pulmonary alveolar proteinosis are derived from three separate experiments with macrophages from patient 1 and two experiments with macrophages from patient 2. The control group consisted of 18 normal volunteers. This group included cigarette smokers and nonsmokers because one patient with pulmonary alveolar proteinosis was a smoker and the other had not smoked for 10 to 12 years. The differences in numbers of *S. aureus* organisms recovered from the culture at one hour is not significant, but at two and three hours, a significantly larger number of organisms remained in the cultures of alveolar macrophages from patients with pulmonary alveolar proteinosis (*P* < 0.02). The curve representing the decrease in *S. aureus* organisms in flasks containing macrophages from patients with pulmonary alveolar proteinosis suggests that in this system the cells initially remove a nearly normal number of organisms from the culture but do not continue to do so after one hour. The bacteria are in a lag phase of growth, and the numbers of bacteria remaining at the second and third hour are not significantly different from the number at one hour. This is consistent with the variation of ± 10 percent seen in control flasks which contain bacteria, serum, and Hanks' balanced salt solution but no macrophages. The lack of further phagocytosis is not explained by a decreased viability of alveolar macrophages from patients with pulmonary alveolar proteinosis or by detachment of cells from the flask, which would decrease phagocytic activity, because there was no change in these factors over the three-hour period of observation. These data strongly suggest that alveolar macrophages from patients with pulmonary alveolar proteinosis are functionally abnormal, and this assay suggests that there is a defect in phagocytosis of *S. aureus*.

Examination of the cells in the sediment and of the granular proteinaceous material revealed that almost all cells obtained by lavage were mononuclear cells. The majority of these mononuclear cells were macrophages, with lymphocytes accounting for 10 to 20 percent of the cells in most sections. An occasional polymorphonuclear leukocyte was observed, as well as a few cells that resembled but could not be positively identified as type-2 pneumocytes. Figure 2 is a representative field from a specimen containing both cells and granular proteinaceous material. There are two very large cells that contain many inclusions. Much of this inclusive material is identical to the granular proteinaceous ma-
FIGURE 2. Free alveolar cells obtained by pulmonary lavage from patient with pulmonary alveolar proteinosis who smoked cigarettes. Cells 1 and 2 do not differ markedly from normal alveolar macrophages in Figure 3. Cells similar to cells 3 and 4 were more commonly encountered and are characterized by many inclusions (I) and vacuoles (V). Compared to normal cells, these are also more rounded and have fewer cytoplasmic extensions (arrows). Many of these inclusions are identical to extracellular granular proteinaceous material (GPM). Cells 3 and 4 have fewer mitochondria, compared to cell 1 and normal cells in Figure 3. Bar represents 10 μ (uranyl acetate and lead citrate, original magnification × 1,650).

Material outside the cell and is the most striking difference between the appearance of the macrophages of patients with pulmonary alveolar proteinosis and that of normal alveolar macrophages (Fig 3). The portion of the cell seen in the right lower corner of Figure 2 demonstrates the large vacuoles that are the second most prominent feature of these abnormal cells. The smaller cell on the left in Figure 2 is similar in appearance to normal human alveolar macrophages.

DISCUSSION

These data strongly suggest that alveolar macrophages from patients with pulmonary alveolar proteinosis are functionally abnormal, compared with normal alveolar macrophages. The decrease in viable organisms at one hour in Figure 1 illustrates that these abnormal macrophages are capable of an initial burst of phagocytic activity in vitro but apparently do not continue to ingest organisms after this time. Phagocytosis is an energy-requiring process that consists of movement of the phagocyte to the phagocytic particle, encasing the particle in cell membrane, and internalization of this phagocytic vesicle. The ingestion of many phagocytic particles by alveolar macrophages requires a high level of oxidative metabolism and utilizes substantial quantities of cell membrane. Utilization of cell membrane during a massive phagocytic challenge can result in reduced phagocytic reserve and morphologic changes consisting of rounding of the cells, loss of folds of cell membrane, and smoothing of the surface of the cell. The morphology of the cells from a patient with pulmonary alveolar proteinosis in Figure 2 reveals numerous phagocytic inclusions, and the cytoplasmic extensions are fewer in number and more rounded, when compared to normal alveolar macrophages in Figure 3. Therefore the behavior of the macrophages from the patients with pulmonary alveolar proteinosis in these experiments could be explained on the basis that the macrophages reach maximum phagocytic capacity early after exposure to bacteria, because the phagocytic load that they have acquired in vivo has decreased the reserve of cell membrane needed for continued phagocytic activity. The presence of many phagocytic inclusions in the alveolar macrophages from patients with pulmonary alveolar proteinosis indicates that these macrophages do phagocytize in vivo, which suggests that the cells are not defective when they enter the alveoli and leads one to speculate that the defect in phagocytic function observed in this study was probably acquired after the macrophages became resident in the alveoli. An alternate hypothesis for the decreased phagocytic activity observed in these cells would be metabolic inhibition or disruption of microtubular or microfilamentous function, possibly by material ingested by these cells.

Golde et al reported that alveolar macrophages from patients with pulmonary alveolar proteinosis had defective adherence to glass, chemotaxis, and intracellular killing of Candida pseudotropicalis. These investigators did not note differences in phagocytosis of these organisms between macrophages from patients with pulmonary alveolar proteinosis and normal human alveolar macrophages over a 60-minute period of observation. This is the
same observation that I made at one hour; but over a longer period of observation, defective phagocytosis was observed. Although the methods were different, the study by Golde et al and the present report give good evidence that alveolar macrophages from patients with pulmonary alveolar proteinosis are functionally defective in antimicrobial activity.

Although the two patients in this report were clinically dissimilar (one was severely symptomatic, with copious amounts of alveolar filling material, and the other was only mildly symptomatic, with minimal physiologic and radiologic derangement and scant alveolar filling material), the same type and degree of defective function was observed in their alveolar macrophages. This lends support for the hypothesis that abnormal alveolar clearance has a role in this disease but also suggests that the rate of production of granular proteinaceous material is important in determining the degree of disability.

REFERENCES