GLYCOSAMINOGLYCANS AND GLYCOPROTEINS IN BRONCHOALVEOLAR LAVAGE FLUID FROM PATIENTS WITH PULMONARY ALVEOLAR PROTEINOSIS

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Abstract—Bronchoalveolar lavage fluid from two cases of pulmonary alveolar proteinosis were analyzed for glycosaminoglycans and glycoproteins. The clinical courses of the two cases were entirely different. In one patient, signs and symptoms recurred despite repeated therapeutic bronchoalveolar lavages. In the other patient, three successive bronchoalveolar lavages brought about complete recovery. It was found that the bronchoalveolar lavage fluid from the former case contained various subtypes of glycosaminoglycans [hyaluronic acid, chondroitin sulfate A(C), dermatan sulfate, and heparan sulfate] and glycoprotein. On the other hand, the bronchoalveolar lavage fluid from the latter case contained glycoprotein, but no detectable amounts of glycosaminoglycans. There was only a slight qualitative difference in glycoprotein of bronchoalveolar lavage fluid between the two cases. The presence or absence of glycosaminoglycans in bronchoalveolar lavage fluid may be related to the prognosis of pulmonary alveolar proteinosis.

INTRODUCTION

Pulmonary alveolar proteinosis (PAP) is characterized by deposition in alveoli of proteinaceous materials which stain pink with eosin and are strongly positive to periodic acid–Schiff (PAS) stain (1). The patients with PAP usually complain of severe nonproductive cough and shortness of breath on exertion. Removal by means of bronchoalveolar lavage (BAL) of amorphous debris from the alveoli has recently been applied in the treatment of PAP (2).
So far, attention of the majority of biochemists has been directed principally toward analyses of proteins and lipids of the material obtained by BAL. The present report deals with glycoconjugates (glycosaminoglycan and glycoprotein) from two cases of PAP with an entirely different clinical course.

In case 1, radiological findings persisted despite repeated BALs. In case 2, radiological findings disappeared completely after three repeated BALs at intervals of one week. The results of biochemical analyses of BAL fluids from the two cases of PAP are discussed in relation to the clinical courses.

MATERIALS AND METHODS

Case 1. A 36-year-old male had been a cook in a Chinese restaurant for the past 22 years; his chief complaint was shortness of breath. Dyspnea since childhood has become progressively worse. On chest roentgenogram taken on admission (Aug. 24, 1979), bilateral fluffy confluent densities which simulated pulmonary edema were found. Laboratory data were within normal limits except for the higher values of lactate dehydrogenase and serum IgE. In September 1979, an open lung biopsy was carried out and the diagnosis of PAP was established by histological examination of microscopic specimens. He has since been receiving BAL treatments 2-3 times a month. There was a transient improvement in roentgenograms and laboratory data after each BAL. However, the signs and symptoms recurred after varying time intervals. Thus he is still receiving BAL treatment, usually at intervals of 2-3 weeks as of September 1982.

Case 2. A 47-year-old female, an employee in a factory of dairy products, complained of dyspnea and nonproductive cough. Laboratory data on admission in March 1981 were within normal range. An open lung biopsy in October 1981 and histology confirmed the diagnosis of PAP. In this case, three BALs at intervals of one week brought about complete resolution of radiological findings, and respiratory tract signs and symptoms have disappeared.

Method of BAL. Physiological saline was used for BAL. The BAL fluid (usually about 1 liter) was filtered through two layers of gauze. The filtrate, thus freed of mucus and cell components, was centrifuged at 1000 rpm for 10 min. The resulting supernatant was dialyzed against several changes of distilled water, concentrated, lyophilized, and delipidated with a 2:1 mixture of chloroform and methanol.

Preparation of Acid Polysaccharide (APS) Fraction (3). The delipidated material was then digested with pronase P (Kaken Kagaku, Tokyo, Japan) in a 0.1 M Ca(CH3COO)2 solution for 48 h at 37°C. Cold trichloroacetic acid (TCA) was added to a final concentration of 7%. The resulting mixture was centrifuged at 3000 rpm for 10 min. The supernatant after deproteinization was dialyzed against distilled water and concentrated in vacuo as required. Next, four volumes of NaCl-saturated ethanol were added to the condensate. The resulting precipitate was taken up in a small quantity of 0.02 M NaCl. Then a 4% solution of cetylpyridinium chloride (CPC) was added until no more precipitation occurred. After standing overnight at 37°C, the resulting precipitate was taken up in a 2.1 M NaCl solution and was mixed with four volumes of ethanol. The precipitate thus obtained was dialyzed, condensed in vacuo, and lyophilized (crude APS).

Anion-Exchange Chromatography (4). The APS fraction was applied on a column of Dowex-1 × 2 (200-400 mesh, Cl- form). Elution was effected with water and then by a stepwise increase of the concentration of NaCl at 0.25 M intervals from 0.3 to 2.0 M. Finally the column was washed out with a 5.0 M NaCl solution. The eluates at different concentrations of NaCl were pooled separately, dialyzed against water, lyophilized, and used for subsequent studies.

Electrophoresis. Electrophoresis (3, 5) was carried out on a membrane of cellulose acetate (Jeko Sangyo, Tokyo, Japan) for 20 min in a buffer made of formic acid and pyridine (pH 3.0)