LOCALIZATION OF COMMON (CROSS-REACTING) ANTIGENS WITH Neisseria perflava AND Klebsiella pneumoniae IN TISSUES OF THE HUMAN BRONCHOPULMONARY APPARATUS

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Antigenic similarity was studied between the microsomal fraction of tissues of the human bronchopulmonary apparatus and bacterial cells living in the respiratory tract: Neisseria perflava and Klebsiella pneumoniae. Cross reactions were studied with antimicrosomal sera in the complement fixation test with N. perflava and K. pneumoniae. Fixation of antibacterial antibodies and antibodies against microsomal fractions of the lung tissues was investigated in tissue sections of the human lungs and bronchi. The presence of antigens cross reacting with the antimicrobial sera was demonstrated in the microsomal fraction of tissues of the human bronchopulmonary apparatus.

KEY WORDS: human bronchopulmonary apparatus; cross-reacting antigens.

The study of cross-reacting tissue antigens of human organs and of microorganisms causing damage to them is now a topical problem in immunology. Previous investigations by the writers showed common antigenic determinants in the tissues of the human bronchopulmonary apparatus and certain strains of Neisseria perflava living in the mucous membranes of the bronchi of patients with infectious asthma. Later, antigenic similarity with human lung tissues was discovered in strains of Klebsiella pneumoniae and Staphyloccus aureus.

Considering the observed [7] organ specificity of the microsomal fraction of animal lung tissues, it was decided to study antigenic similarity between the microsomal fraction of human lung tissues and microorganisms of the respiratory tract: N. perflava and K. pneumoniae.

EXPERIMENTAL METHOD

Strains No. 10A and 13 of N. perflava and No. 3 of K. pneumoniae were used. The strains were isolated in the Allergologic Research Laboratory, Academy of Medical Sciences of the USSR, from the mucous membranes of the bronchi during bronchoscopy on patients with infectious-allergic bronchial asthma and they were identified in the living cultures division (Head, Professor Z. M. Andreeva) of the L. A. Tarasevich State Research Institute of Standardization and Control of Medical-Biological Preparations.

Bacterial antigens were obtained by the method described previously [1].

Homogenates and extracts of lung, kidney, and spleen tissues of a stillborn human fetus, with unexpanded lungs, were prepared by the method published previously [1]. Only sterile tissues were used, and tissues from the same donor were used in one experiment. Separation of the homogenates of the lung tissues (more exactly, tissues of the alveoli, small bronchi, and capillaries) and isolation of the microsomal fraction were carried out as described in [7] by differential ultracentrifugation of the tissue homog-

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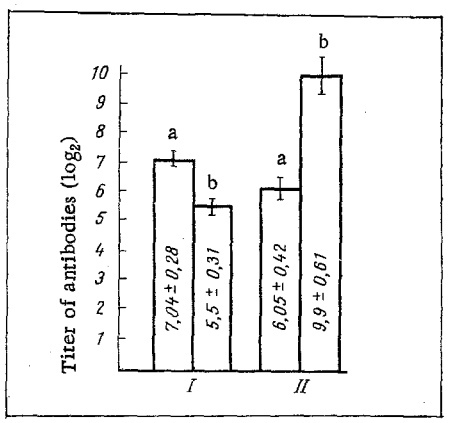

Fig. 1

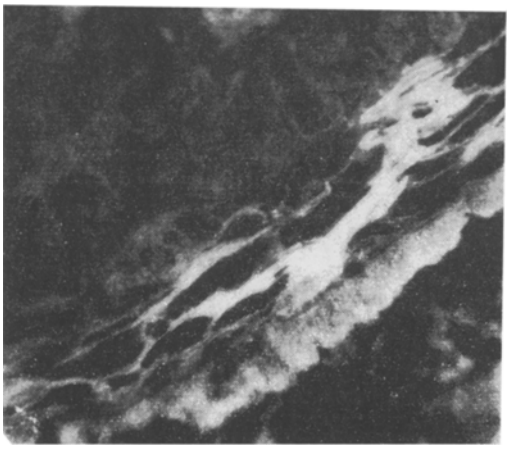

Fig. 2

Fig. 1. Results of CFT of sera against N. perflava and antimicrosomal sera with antigens of N. perflava and microsomes of tissues of the human bronchopulmonary apparatus: a) antilung serum; b) serum against N. perflava. Ordinate, log_2 of CFT titers; abscissa, antigens: I) microsomes of tissues from human bronchi and lungs; II) of N. perflava.

Fig. 2. Fixation of antibodies to microsomal fraction on tissues of human fetal bronchi; 200×.

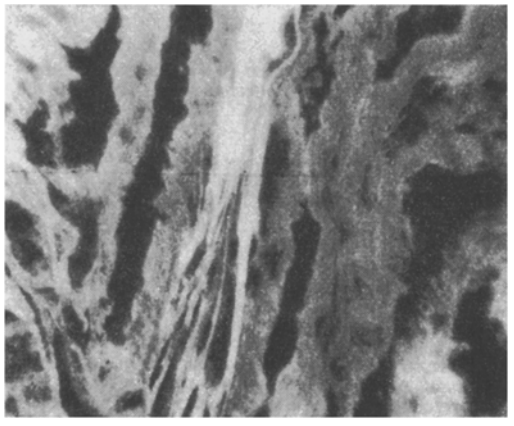

Fig. 3. Fixation of antibodies against N. perflava on tissues of human fetal bronchi; 200×.

enates. Specific activity of the microsomal fraction was tested in the complement fixation test (CFT) in the cold and in Ouchterlony's precipitation test with antilung sera.

The rabbits were immunized with tissue antigens in accordance with the scheme given by Gery and Davies [4]. Twelve animals were immunized with extracts of human fetal lung tissues, eight with the microsomal fraction of human fetal lung tissues, five rabbits with human fetal kidney extracts, and five with cells of N. perflava. The protein concentration in the preparations was 10 mg/ml per injection. Alumina (20%) was used as the adjuvant. The adjuvant was injected as a single dose mixed with antigen in a volume of 1 ml. Immune sera were absorbed with the tissue homogenates by the method of Kaplan and Svec [5].

Immunization of the rabbits with microbial antigens was carried out in accordance with the following scheme. Cells of N. perflava were injected into the animals in a dose of 2 billion cells/ml per injection, subcutaneously, intramuscularly, and intravenously at intervals of 3 days. The concentration of antibodies in the sera was expressed in log_2 units and the initial dilution of the serum was 1:2.

Antisera against extracts of human fetal lung tissues, after absorption with a mixture of homogenates of spleen and kidney tissues from the same donor, reacted in the CFT with homologous antigen in a titer of 6.8 ± 0.5.

Organ-specific antilung sera were used to estimate the activity of cross-reacting antigens of experimental strains of N. perflava and K. pneumoniae. The antilung sera were found to be active in the CFT with the above-mentioned strains and the mean titers of the reaction were 6.0 ± 0.3 and 5.3 ± 0.42, respectively. The reactions of the antimicrosomal sera with homologous antigen corresponded to a mean titer of 7.04 ± 0.28, whereas sera against N. perflava reacted with the homologous antigen in a titer of 9.9 ± 0.61.

The CFT in the cold with sera of normal and immune animals was carried out in the usual way after

preliminary titration of the antigens and with parallel controls for anticomplement activity of the test sera.

The indirect Coons' immunofluorescence test [2] was used to study fixation of antibacterial and anti-lung antibodies in frozen tissue sections from human fetal lungs and bronchi.

EXPERIMENTAL RESULTS

Antigenic similarity between the microsomal fraction of tissues of the human bronchopulmonary apparatus and N. perflava and K. pneumoniae was studied in two directions. First, cross reactions between antimicrosomal sera in the CFT with antigens of N. perflava and K. pneumoniae were investigated, and second, the reactions of antibacterial sera with antigens of the microsomal fraction of the lung tissues were studied.

The mean titer of cross reactions of antisera against the microsomal fraction of human lung tissues with antigens of N. perflava was found to be 6.05 ± 0.42 compared with a titer of 7.04 ± 0.28 in the reaction between antimicrosomal sera and the homologous antigen (Fig. 1). Antigens of K. pneumoniae were less active in cross reactions with antimicrosomal sera and the mean titer in the CFT was 4.55 ± 0.38.

Investigation of sera against N. perflava in the CFT with the microsomal fraction of human lung tissues gave positive reactions with a mean titer of 5.5 ± 0.31. The mean titer in reactions between the test sera and homologous antigen was 9.91 ± 0.61.

The indirect immunofluorescence method was used to study fixation of antibacterial antibodies against the microsomal fraction of human lung tissues on tissue sections from the bronchi and lungs of a stillborn human fetus.

As the control for specificity of the reactions, serial sections were treated with the sera of "normal" animals and with the sera of rabbits immunized with extracts of the kidneys of the donors of the lung tissues. Antikidney sera were used as the control for specificity of fixation of antibodies against the microsomal fraction of lung tissues because of data in the literature [3, 6] that indicate the antigenic similarity of mammalian lung and kidney tissues.

The study of fixation of antimicrosomal antibodies by the immunofluorescence method showed intensive fluorescence of the bronchial epithelium and muscle cells of the bronchus in tissue sections from the human fetal bronchus treated with antimicrosomal serum (Fig. 2). After application of anti-Neisseria sera to the bronchial sections, an intensive reaction also was found in the region of the bronchial muscular structures (Fig. 3). On treatment of the sections with antisera against human kidney tissue extracts no reaction was observed in these bronchial structures.

The results relating to cross reactions of antimicrosomal sera with antigens of N. perflava and K. pneumoniae and between anti-Neisseria sera and antigens of tissue microsomes of the human bronchopulmonary apparatus indicate the presence of common antigens. Fixation of antimicrosomal and anti-Neisseria antibodies on the same tissue structures of the human bronchopulmonary apparatus (muscular structures) indicates that antigenic determinants common with Neisseria may be located in these parts of the lung tissues.

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