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

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The localization of common antigens with tissues of the human bronchopulmonary apparatus was studied in cells of *Neisseria perflava* and *Klebsiella pneumoniae*. Cross reactions of several structures of *N. perflava* and *K. pneumoniae* cells (capsule, cell walls, fractions of cytoplasmic structures, hyaloplasm) were studied in the complement fixation test (CFT) with antilung sera. Antigens cross-reacting with antilung sera were found not only in surface structures (cell walls) of the bacterial cells but also in deep components (cytoplasmic fraction rich in RNP) of the microorganism.

KEY WORDS: *cross-reacting antigens; Neisseria perflava; Klebsiella pneumoniae; human bronchopulmonary apparatus.*

A previous [2] study of the localization of common antigens with cells of *Neisseria perflava* and *Klebsiella pneumoniae* in the tissues of the human bronchopulmonary apparatus showed considerable activity of the microsomal fraction of the lung tissues in cross reactions with antimicrobial sera.

The object of this investigation was to study cross reactions of certain cell structures of *N. perflava* and *K. pneumoniae* (capsule, cell walls, fractions of cytoplasmic structures, hyaloplasm) in the complement fixation test (CFT) with antilung sera.

EXPERIMENTAL METHOD

The same strains of *N. perflava* (Nos. 13 and 10A) and of *K. pneumoniae* (No. 3) were used as in the previous investigations [2].

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

The capsular material of the bacterial cells was isolated as described in [4]. Completeness of separation of the capsule from the bacterial cell was verified by Olt's method [3] and by the phase contrast method.

To obtain cell walls, after removal of the outer layer of the capsule the bacterial cells were broken up in a mechanical disintegrator. The resulting mass was centrifuged at 4000 g for 20 min and undestroyed cells were removed. The cell walls were sedimented from the disintegrated mass of bacteria by centrifugation at 20,000 g for 15 min by the method described in [5]. The residue of cell walls was resuspended in 1 M sodium chloride solution, and the supernatant was regarded as the "coarse" cytoplasmic fraction.

The "coarse" cytoplasmic fraction was separated into hyaloplasm and a fraction conventionally described as the fraction of cytoplasmic structures (CS) by the method of Youmans and Youmans [8] by ultracentrifugation of the cytoplasm at 144,000 g for 3 h. The residue

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TABLE 1. Mean Titers of Antilung Sera in CFT with Separate Components of *Neisseria* and *Klebsiella* Cells (M±m)

Microorganism	Antigens (components of bacterial cell)				
	Walls	Cytoplasm	Hyaloplasm	CS	Capsule
Neisseria perflava	5,4±0,3	6,5±0,21	3,9±0,34	6,3±0,35	Reaction doubtful
Klebsiella pneumoniae	4,5±0,4	6,1±0,24	3,2±0,25	6,1±0,43	Reaction doubtful

containing organoids, inclusions, and submicroscopic cell structures (ribosomes), was separated from the supernatant, which was regarded as hyaloplasm.

Preparation of the tissue extracts and of homogenates of lung and kidney tissues and also of the spleen of a human fetus was carried out as described previously [1]. Two groups of rabbits were immunized: 12 animals with saline extracts of human fetal lung tissues and 5 with kidney extracts from the same donors. Immunization with tissue antigens was carried out by the method described in [6].

The CFT was performed as in [1]. Sera of normal and immune animals were tested in the CFT with tissue and bacterial antigens.

The antibody titer was expressed in $\log_2$ units and the initial dilution of serum was 1:2.

After absorption of the antisera against lung tissue extracts with homogenates of human kidney and spleen [7], reactions of the organ-specific antilung sera with homologous antigen corresponded to a mean titer of 6.8±0.5, but with kidney antigens the reaction was negative. Cross reactions with *Neisseria* were observed in a mean titer of 6.0±0.32, and with *Klebsiella* cells in a titer of 5.3±0.4.

Antisera against kidney tissues reacted with homologous antigen in a titer of 7.3±0.44.

EXPERIMENTAL RESULTS

To study the activity of individual components of the bacterial cell in cross reactions with antilung sera, antisera against human fetal lung tissues were investigated in the CFT with preparations of capsular substance, cell walls, and cytoplasm of *Neisseria* and *Klebsiella* cells.

The observations showed that preparations of the cell walls and cytoplasm were distinguished by high activity in cross reactions with antilung sera. The mean titer of reactions of *Neisseria* cell walls with the antilung sera was 5.4±0.3 and of the cytoplasm 6.5±0.21.

The cytoplasm of the bacterial cell is a complex biological system with many subcellular structures. By differential centrifugation in the WAK-601 ultracentrifuge an attempt was made to separate preparations of *Neisseria* and *Klebsiella* cytoplasm into its components: hyaloplasm and the CS fraction. The components of the cytoplasm were then tested in the CFT with antilung sera.

The tests showed that the reaction between hyaloplasm preparations and antilung sera gave very low titers (Table 1). Whereas the mean titer of the reaction of *Neisseria* cytoplasm with antilung sera was 6.5±0.21 before centrifugation, after ultracentrifugation at 144,000 g for 3 h the cytoplasm, after removal of its organoids and inclusions (hyaloplasm), reacted with the antilung sera in a titer of 3.9±0.34 ($P < 0.01$).

Similar results were obtained with preparations of *Klebsiella* cytoplasm and hyaloplasm. In this case also the reaction titers fell after ultracentrifugation of the cytoplasm from 6.1±0.24 to 3.2±0.25 ($P < 0.05$). The study of the CS fraction of *Neisseria* and *Klebsiella* cells in the CFT with antilung sera revealed high activity of CS in cross reactions with the antilung sera. The mean reaction titers were 6.3±0.35 and 6.1±0.43 respectively. The results of absorption of antilung sera by CS fractions confirmed their antigenic similarity with human lung tissues. Absorption of antilung sera by the CS fraction of *Neisseria* was followed by a decrease in the titers of the reaction with homologous antigen from 6.8±0.50

to 5.3±0.39 ($P < 0.05$). Antikidney sera did not react in these tests with preparations of the cell walls and CS fractions of *Neisseria* and *Klebsiella*.

The results for cross reactions between preparations of the cell walls and CS fractions in the CFT and antilung sera show that these structures of the bacterial cell contain antigenic determinants common with those of the tissues of the human bronchopulmonary apparatus and that a wide variety of antigenic connections exists between the microorganism and the tissue cells of the human bronchopulmonary apparatus, extending both to the surface structures of the bacterial cells and also to the intracellular components of the microorganism.

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