CROSS-REACTING ANTIGENS OF BACTERIA OF THE HUMAN BRONCHOPULMONARY SYSTEM

V. G. Chitaeva

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Antigenic kinship of certain strains of Neisseria perflava, Klebsiella pneumoniae, and Staphylococcus aureas, active sensitizers of the human bronchopulmonary system, was studied. The complement fixation test, Ouchterlony's precipitation test, and immunoelectrophoresis revealed the presence of a series of similar antigenic determinants common to all three bacteria and for the determinants responsible for cross-reactions between only two of these microorganisms.

KEY WORDS: cross-reacting antigens; capsular preparation; polyallergic reactions.

In the specific diagnosis of infectious-allergic bronchial asthma in the majority of cases polyallergic skin reactions are recorded to preparations of Neisseria, Klebsiella, and Staphylococcus [1]. So far, however, the mechanism of these polyvalent allergic reactions has received little study. Some workers consider that common antigenic determinants are concerned in the pathogenesis of this phenomenon [2, 4].

The object of this investigation was to study the possibility of antigenic kinship between certain strains of Neisseria perflava, Klebsiella pneumoniae, and Staphylococcus aureus, possessing high allergenic activity during skin tests on patients with infectious asthma.

EXPERIMENTAL METHOD

Strains Nos. 10a and 13 of N. perflava, No. 3 of K. pneumoniae, and D-1 of S. aureus were used. All strains were isolated from the mucous membrane of the bronchi during bronchoscopy on patients with infectious-allergic bronchial asthma and were identified at the L. A. Tarasevich State Serologic Control Institute.

The bacterial suspensions obtained by the method described in [6] were disintegrated in a Mickle's apparatus with glass beads at 300 rpm for 40 min. To obtain the soluble antigen the material was centrifuged at 8000g for 40 min. The capsular substance was isolated by the method described in [5] by treating the bacterial suspension in a mechanical disintegrator. Absence of contamination of the cells in the preparation was verified by the phase-contrast method.

Three groups of rabbits were immunized: 15 with N. perflava, 15 with K. pneumoniae, and eight with S. aureus. A $2 \cdot 10^9$ cell suspension of the culture was used as the antigen. The scheme of immunization was: the antigen in a volume of 1 ml was injected subcutaneously, intramuscularly, and intravenously in turn at intervals of 2 days. After a gap of 1 week three further injections were given: initially 1 ml of antigen was injected intramuscularly, and 2 days later the same volume of suspension was injected intramuscularly, and 0.5 ml was injected intravenously after 1 h. On the seventh day the limiting titers were determined in the reaction with homologous antigen.

Absorption of the immune sera was carried out by the method described in [3].

The antibacterial sera were tested in the complement fixation test (CFT), Ouchterlony's precipitation test, and by immunoelectrophoresis in agar.

The CFT was set up in the usual way after preliminary titration of the antigens and controls for anticomplementarity of the antigens and sera. Ouchterlony's precipitation test was carried out in 1% agar gel made up in veronal–medinal buffer, pH 8.2.

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TABLE 1. Mean Titers of Cross Reactions with Antibacterial Sera

Antigen	Mean titer in CFT		
	anti-Neisseria serum	anti-Klebsiella serum	anti-Staphylococcus serum
Neisseria perflava	1 : 467 (8,9±0,34)	1 : 40 (5,3±0,25)	1 : 40 (5,3±0,25)
Staphylococcus aureus	1 : 39 (5,2±0,30)	1 : 38 (5,0±0,26)	1 : 178 (7,5±0,49)
Klebsiella pneumoniae	1 : 40 (5,3±0,28)	1 : 562 (9,16±0,26)	1 : 40 (5,3±0,25)

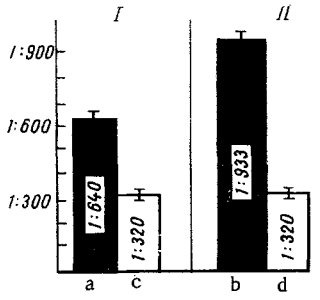

Fig. 1

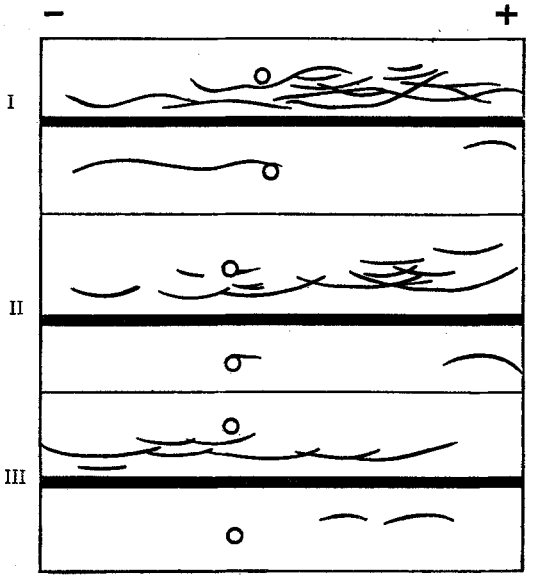

Fig. 2

Fig. 1. Mean titers of anti-Neisseria sera in CFT with homologous antigen before (a, b) and after (c, d) absorption by Klebsiella (I) and Staphylococcus (II) cells. Ordinate, titer of antibodies.

Fig. 2. Scheme of crossed immunoelectrophoretic reactions. I: top well contains disintegrated neisserias, bottom well disintegrated klebsiellas, gutter contains anti-Neisseria serum; II) top well contains disintegrated staphylococci, bottom well disintegrated neisserias, gutter anti-Staphylococcus serum; III) top well contains disintegrated klebsiellas, bottom well disintegrated staphylococci, gutter anti-Klebsiella serum.

Immunoelectrophoresis was performed on slides in 1% agar gel dissolved in veronal–medinal buffer, pH 8.2, ionic strength 0.05. The conditions of electrophoresis were: current 50 mA, voltage 240 V, duration 2.5 h.

EXPERIMENTAL RESULTS

As Table 1 shows, the antibacterial sera reacted in the crossed CFT with heterologous bacteria in mean titers of 1:38-1:40. After complete absorption of the anti-Neisseria sera by bacterial suspensions of Klebsiella or Staphylococcus (Fig. 1), the mean titers of the reaction with homologous antigen were reduced by 50-67% compared with initially. These results indicate the presence of cross-reacting antigens in the composition of the strains of N. perflava, K. pneumoniae, and S. aureus tested.

It was shown by immunodiffusion methods that the antigenic similarity of the three species of bacteria is due to a series of common antigenic determinants. Disintegrated staphylococci and klebsiellas gave cross-reactions with anti-Neisseria sera, with the formation of at least two precipitation lines. In turn, disintegrated neisserias and staphylococci precipitated anti-Klebsiella sera, whereas Klebsiella and Neisseria antigens reacted with the sera of rabbits immunized with Staphylococcus. Complete absorption of the group of anti-Neisseria sera by Klebsiella cells led to the disappearance of one or two lines from the antigenic spectrum of

Neisseria. Meanwhile complete exhaustion of the anti-Neisseria sera by Staphylococcus cells reduced the precipitation spectrum of the homologous antigen by three lines. The results point to the presence of several immunologically identical antigens in the composition of the strains of N. perflava, K. pneumoniae, and S. aureus.

Immunoelectrophoretic investigation of disintegrated neisserias, klebsiellas, and staphylococci in the reaction with heterologous immune sera gave the exact number and location of the cross-reacting antigens in the composition of the antigenic spectrum of the bacteria. The fraction common to all three bacteria possessed high anodal mobility. At the same time, components responsible for crossed reactions of only two bacteria were found: a common antigen of neisserias and klebsiellas was located in the cathodal part of the antigenic spectra of the tested strains, a fraction immunologically identical in neisserias and staphylococci possessed very weak anodal mobility and was located near the point where the antigens were applied, whereas a cross-reacting antigen of klebsiellas and staphylococci possessed lower anodal mobility than the common antigen of all three bacteria (Fig. 2). Crossed immunodiffusion tests with capsular preparations of the strains of Neisseria, Klebsiella, and Staphylococcus showed that the common antigen of these bacteria is located in the surface structures of the cell.

It can be concluded from these results that a series of common antigenic determinants is present in the strains of the three most important sensitizing bacteria of the human bronchopulmonary system tested, namely Neisseria perflava, Klebsiella pneumoniae, and Staphylococcus aureus.

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