EXPERIMENTAL STUDY OF THE SENSITIZING ACTIVITY OF Neisseria perflava

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The sensitizing activity of Neisseria perflava isolated from the bronchial mucosa of patients with infectious asthma was studied in experiments on guinea pigs. It was shown that Ovary's passive cutaneous anaphylaxis test and the tracheal chain contraction test can be reproduced with Neisseria antigens. High sensitizing activity of N. perflava was found compared with two other microorganisms: Klebsiella pneumoniae and Staphylococcus aureus, inhabiting the bronchi of patients with infectious asthma.

KEY WORDS: Neisseria perflava; active cutaneous anaphylaxis; tracheal chain contraction test.

The writers showed previously [3] that pigmented species of Neisseria – Neisseria perflava, Neisseria flava, and Neisseria subflava – inhabit the mucous membranes of the bronchi of patients with infectious-allergic bronchial asthma. When skin and inhalation tests with Neisseria allergens are carried out on such patients, increased sensitivity to N. perflava is found in more than 90% of cases. Later observations showed [1] that strains of N. perflava to whose allergens strongly positive cutaneous and provocation tests were obtained possess common antigenic determinants with human lung tissues.

In the investigation described below an attempt was accordingly made to study under experimental conditions the sensitizing activity of strains of N. perflava whose allergens have been found to be active in clinical tests.

EXPERIMENTAL METHOD

Strains N. perflava Nos. 13 and 10a, Klebsiella pneumoniae strain No. 3, and Staphylococcus aureus strain D-1, isolated from the bronchial mucosa of patients with infectious asthma were studied. Previous investigations [2] showed that strains of N. perflava possess common antigenic determinants with human lung tissues and exhibit high activity in skin and inhalation tests of patients with infectious asthma.

Bacterial antigens were used in the form of disintegrated bacterial cells grown as described in [6]. The soluble antigen for the Ovary's skin tests were made up as in [4].

Experiments were carried out on 50 guinea pigs. The animals were sensitized by the scheme taken from a paper by Sukhodoeva et al. [4] and the result was assessed by Ovary's active cutaneous anaphylaxis test (ACA, [7]) and the tracheal chain contraction test. Ovary's skin tests were carried out on the 21st day after the second injection of the antigen whereas the tracheal chain test was set up on different groups of animals on the 21st day and 2, 3, 4, and 5 months after the end of the sensitization cycle.

Albino guinea pigs, sensitized with a clarified preparation of disintegrated neisserias, were given an intradermal injection of inactive doses of antigens previously tested on intact animals. For example, injection

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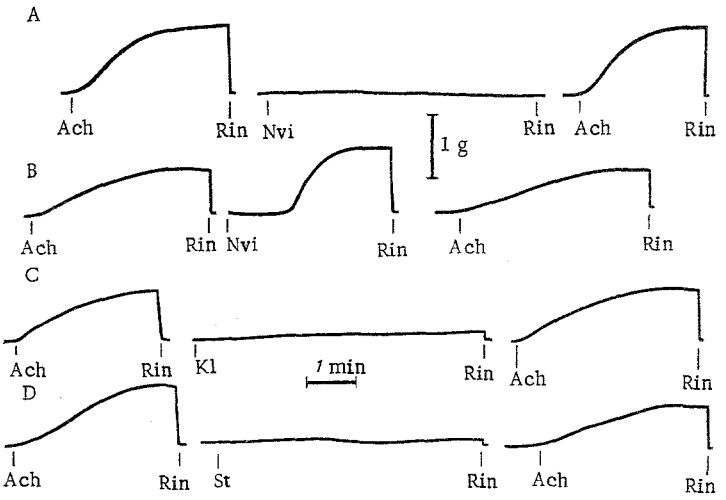

Fig. 1. Action of different microbial antigens on TC of guinea pig sensitized with neisserias. A) TC of normal guinea pig; B, C, D) tracheal preparations of guinea pig sensitized with disintegrated N. perflava cells (Ach – acetylcholine in concentration of 0.5 mg/ml, N_{vi} – antigen of N. perflava, Kl – antigen of K. pneumoniae, St – antigen of S. aureus, Rin – rinsed with Kreb's solution.

of N. perflava antigen in a dose of 600 μg in 0.1 ml protein into the skin of intact animals produced no infiltrative changes in the skin, and the corresponding dose for Staphylococcus and Klebsiella antigens was 200 μg.

Tracheal chain (TC) preparations were made by the method of Castillo and de Beer [8]. The concentration of the preparations causing threshold contraction of the chain was determined. In the present experiments the sensitivity of TC to antigens of N. perflava, K. pneumoniae, and S. aureus was equivalent to a dose of 100 μg protein/ml. Two preparations of TC were obtained from the bronchi of one animal, and different solutions of the microbial allergens were tested on them alternately.

EXPERIMENTAL RESULTS

The response of the skin of guinea pigs sensitized with neisserias to injection of homologous antigen, in the form of disintegrated bacterial cells and capsular material of neisserias, was studied. After injection of the disintegrated neisserias in a dose of 600 μg protein in 0.1 ml a stain from 6 to 12 mm in diameter (mean diameter 10.3 ± 2.68 mm) appeared on the animal's skin. As the control, parallel with the injections of the microbial preparations, physiological saline was injected into the skin of the sensitized animal, and the reaction to this injection was negative.

Considering the results of the writers' previous investigation [5], which showed the possibility of antigenic affinity between neisserias and two other microorganisms inhabiting the respiratory tract, K. pneumoniae and S. aureus, a group of animals sensitized with N. perflava was given injections of allergen preparations from these two species. Crossed reactions were observed in these animals to allergens of the two microorganisms: The mean diameter of the stain to K. pneumonia allergen was 7.3 ± 2.65 mm and to S. aureus allergen 6.4 ± 2.62 mm.

Tracheal Chain Tests. Before starting the experiments on the sensitized animals, the response of TC of intact guinea pigs to contact with N. perflava antigens was assessed; no response to this allergen was recorded (Fig. 1A).

The absence of a response of TC of the unsensitized animals to contact with N. perflava allergens was used as the control for specificity of the response of TC of the sensitized animal to the same preparations. The response of TC to acetylcholine in a working dose of 0.5 μg/ml was taken as 100%. Contraction of TC of guinea pigs sensitized with neisserias to homologous antigen (Fig. 1B) was found to range from 222 to 125% (M ± m = 162.8 ± 25.8%; Fig. 2A).

Sensitization to N. perflava lasted 6 months from the day of the last injection of the antigen into the guinea pigs. The character of contraction of TC observed in response to the N. perflava antigen was intensive and relaxation of TC after rinsing developed slowly.

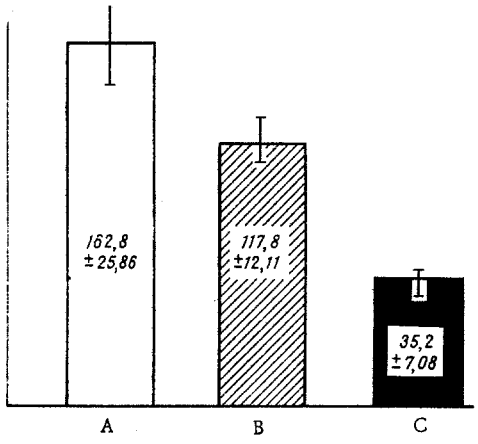

Fig. 2. Comparative sensitizing ability of N. perflava (A), K. pneumoniae (B), and S. aureus (C). Ordinate, response of contraction of TC (in %).

Considering the antigenic relationship between Neisseria, Staphylococcus, and Klebsiella, contraction of TC preparations of animals sensitized with N. perflava to allergens of K. pneumoniae and S. aureus was studied. TC of these animals was found not to respond to injection of K. pneumoniae or S. aureus allergens (Fig. 1C, D). These results point to the high selectivity of the bronchial tissues relative to the reacting agent.

In the course of the study of Neisseria allergens an attempt was made to compare the sensitizing activity of N. perflava with that of S. aureus and K. pneumoniae. Contraction of TC of guinea pigs sensitized with K. pneumoniae to homologous antigen were studied. The mean percentage of response of TC of this group of animals was 117.8 ± 12.11 (Fig. 2B). TC of animals sensitized with S. aureus (Fig. 2C) responded less actively to the homologous antigen: The intensity of the response in this case was 35.2 ± 7.08% ($P < 0.001$).

Intensive skin reactions and reactions of contraction of TC of guinea pigs sensitized with neisserias to homologous antigen thus indicate the high allergenic activity of N. perflava. Meanwhile the absence of a response of the TC of animals sensitized with neisserias to S. aureus allergen can perhaps be taken as evidence of the activity of specific Neisseria antigens.

The possibility cannot be ruled out that these facts may be explained by different mechanisms of the response of the host to contact with bacterial allergens.

A comparative study of the allergenic activity of three microorganisms which sensitize the human bronchopulmonary system showed that the highest sensitizing activity was possessed by N. perflava.

LITERATURE CITED

1. A. D. Ado, N. V. Adrianova, and V. P. Dovzhik, Sov. Med., No. 9, 12 (1968).
2. A. D. Ado and V. N. Fedoseeva, Zh. Mikrobiol., No. 8, 123 (1974).
3. V. V. Kovaleva, Z. M. Andreeva, V. N. Fedoseeva, et al., Zh. Mikrobiol., No. 8, 59 (1975).
4. G. S. Sukhodoeva, N. D. Beklemishev, Ya. L. Bul'vakhter, et al., Trudy Nauch. Issled. Inst. Kraev. Patol. Kazakh. SSR, 21, 81 (1971).
5. V. G. Chitaeva and V. N. Fedoseeva, in: Bronchial Asthma. Abstracts of Proceedings of an All-Union Conference [in Russian], Moscow (1974), pp. 126-127.
6. L. Bechounkova, H. Malota, and E. Marsalek, Allergie Asthma, 5, 386 (1959).
7. J. Castillo and E. de Beer, J. Pharmacol. Exp. Ther., 90, 105 (1947).
8. Z. Ovary, Acta Allerg. (Copenhagen), 6, Suppl. 3, 170 (1953).