PROPERTIES OF LIPOSOMAL ALLERGENS OF *Neisseria perflava*

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Phospholipid vesicles or liposomes are finding ever-increasing application nowadays as a means of, on the one hand, increasing the efficiency of transmembrane transport of various substances, including chemotherapeutic agents [3, 6, 9] and, on the other hand, of reducing the toxic properties of these preparations [3, 6, 13].

Improving the immunogenic effectiveness and reducing the side effects of bacterial allergens are matters which are at the center of attention of allergologists. The study of preparations which possess high biological activity is particularly important in this context. The category of "strong" allergens, i.e., those capable of giving rise to severe bronchospastic reactions in patients with noninfectious asthma, as the writers showed previously [1], includes allergens of nonpathogenic neisserias. The high sensitizing activity of these microorganisms and the marked sensitivity of the bronchopulmonary apparatus of patients with bronchial asthma to neisserial allergens necessitate the search for substances reducing their toxic and anaphylactogenic properties.

This paper describes an attempt to incorporate neisserial allergens into phospholipid vesicles and to study their anaphylactogenic properties with the aim of further improving these allergenic preparations.

EXPERIMENTAL METHOD

Strains Nos. 13 and 10a of *Neisseria perflava* were isolated from the bronchi of patients with infectious-allergic bronchial asthma. The neisserial allergens were prepared from disintegrated *N. perflava* cells by the method used for "soluble" allergens [2]. The protein content in the preparation was determined by the method described previously [12]. Liposomes were formed from a mixture of phosphatidylcholine and cholesterol in the ratio of 7:3 by weight [7]. The purity of the phospholipids was tested by the method in [4]. Removal of neisserial allergen not incorporated into liposomes from the mixture was carried out by chromatography on a column with Sephadex G-200. The eluate was checked for its content of protein [12] and lipids [11], The total lipid concentration in the dispersed phase was 14%. Parallel tests were carried out with ^{125}I-labeled neisserial allergens. The radioactivity of the eluates was determined on a gamma counter (Gamma-Set, ICN). Radioactive labeling of the neisserial allergens was carried out by the method in [10]. Guidance during selection of doses of activity was obtained from the recommendations in [8]. The initial activity of the preparations in individual experiments was equalized and did not exceed 0.5 μCi. To sensitize 54 guinea pigs with a living culture of neisserias the method in [2] was used. The sensitizing dose was one billion bacterial cells in 0.1 ml (per animal). The degree of sensitization was estimated by the active cutaneous anaphylaxis test described in [14]. One skin dose in the test was equivalent to 600 μg protein in 0.1 ml. The anaphylactogenic properties of the preparations were estimated by the reproduction of anaphylactic shock in sensitized animals; the dose of the preparation, expressed as protein, in this case was 800 μg/100 g body weight. The intensity of the shock was estimated by means of the usual criterion [15]. The distribution of liposomes containing neisserial allergens, labeled with ^{125}I, in sensitized and intact guinea pigs was studied. The gamma counter used to determine radioactivity was from Wilj (England).

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TABLE 1. Reactions of Active Cutaneous Anaphylaxis and General Anaphylaxis in Sensitized Guinea Pigs (M ± m)

Allergen	Test	
	active cutaneous anaphylaxis (diameter of patch in mm)	reaction of general anaphylaxis (index in points according to [15])
Neisserial	13,1±0,70	3,0±0,25
Neisserial in liposomes	11,8±0,67	0,3±0,14

TABLE 2. Distribution of ^{125}I-Labeled Neisserial Allergens in Organs of Sensitized and Intact Animals (M ± m)

Organ	Sensitized animals				Intact animals	
	neisserial allergens		neisserial allergen in liposomes		neisserial allergen in liposomes	
	cpm	%	cpm	%	cpm	%
Lungs	4515±212,1	100	4380,5±201,5	97	586,5±50,1	13
Liver	1038,4±56,4	23	1119,0±61,4	27	1941,5±103,1	43
Heart	679,3±39,3	15	812,7±51,3	18	135,5±18,4	3
Spleen	451,5±29,1	10	1435,3±78,1	34	632,3±54,3	14
Lymph node	—	—	1625,4±91,3	36	679,4±56,3	15

EXPERIMENTAL RESULTS

Anaphylactic skin reactions are easily produced in guinea pigs. The formation of cutaneous sensitivity to neisserial allergens was studied in experiments on 20 guinea pigs. At the height of sensitization (28th-30th days) neisserial allergen and the same preparation incorporated into liposomes, in doses equal as regards protein, were injected into depilated areas of skin. As control of the specificity of the skin reactions, allergens in liposomes were injected intradermally in the same dose into a parallel group of six intact animals. The appearance of stained patches 13.1 ± 0.7 mm in diameter was observed 15 min after intravenous injection of 0.25 ml of a 5% solution of Evans' blue into each animal. The maximal skin reaction in this case was expressed as a patch 16 mm in diameter. Similar results, as regards mean values of active cutaneous anaphylaxis, were observed in the same animals in response to injection of liposomes containing neisserial allergens. The maximal reaction in tests carried out in this way did not exceed 13 mm and the mean value of 11.8 ± 0.67 mm. No positive reactions were observed in this case in intact animals (Table 1).

Anaphylactic shock was produced in two groups of guinea pigs (10 animals in each group), sensitized with neisserias. Neisserial allergen was injected intravenously into the animals of group 1, whereas the animals of group 2 received neisserial allergen incorporated into phospholipid vesicles. Lethal anaphylactic shock developed in three animals in group 1, anaphylaxis in four animals was assessed as 3 points, and in another three animals as 2 points. The mean index of anaphylaxis for the group was 3 ± 0,25 points. In the animals of group 2 the index of anaphylaxis was much lower. Its mean value was 0.3 ± 0.14 point. All the animals survived, eight guinea pigs did not develop anaphylaxis, in one the manifestations of anaphylaxis were assessed as 2 points, and one animal showed mild features of anaphylaxis (1 point). The difference between the indices in groups 1 and 2 is significant ($P < 0.01$).

True correlation between the observed effect of a decrease in anaphylactogenicity of the preparation and incorporation of the allergen into liposomes could be established by disintegrating the phospholipid vesicles containing neisserial antigen. For this purpose a mixture of acetone and butyl alcohol in a ratio of 3:1 (v/v) was used. On the addition of the mixture to liposomes containing allergen, the phospholipid membranes were dissolved and the allergen left in solution. After removal of the mixture of acetone and butanol from the solution, the phospholipids with allergens were injected intravenously into sensitized guinea pigs (five animals) of group 3. Lethal anaphylactic shock was observed in two of them, anaphylactic manifestations assessed as 2 points in the other three (the mean index

for the group was 2.8 ± 0.5 points). The difference between the indices for groups 2 and 3 is significant ($P < 0.01$).

To study the mechanism of this effect of a decrease in anaphylactogenic activity of neisserial allergens when incorporated into liposomes, the distribution of allergens in sensitized guinea pigs was investigated. At the height of sensitization, *N. perflava* allergens labeled with ^{125}I were injected into two groups of animals. The guinea pigs of group 1 (five animals) were given an intravenous injection of neisserial allergen without liposomes, whereas the four animals of group 2 received allergen in liposomes. Control intact animals (group 3, three guinea pigs), received an injection of ^{125}I-labeled neisserial allergen in liposomes. The distribution of the label was studied in the liver, in the organ exhibiting shock (the lung), and in the heart, spleen, and lymph nodes of the sensitized animals.

A clear difference in the levels of radioactivity in the lung tissue of the sensitized and intact animals could be detected 15 min after intravenous injection of neisserial allergens. The highest level was recorded in the lung tissues of the animals of group 1, namely 4515.5 ± 212.1 cpm (index of anaphylaxis 2.6 ± 0.49), equivalent to 40.5% of the initial activity of the injected preparation. Allergen in liposomes, injected into the animals of group 2, was retained with almost the same intensity by the lung tissue, and the gamma-ray values were 4380.5 ± 201.5 cpm (39% of the initial activity of the preparation). This effect could be due to microcirculatory changes in the lungs accompanying anaphylaxis. The level of radioactivity in the lung tissues of intact animals (no reaction of general anaphylaxis) was found to be 7.4 times lower than in sensitized guinea pigs (Table 2).

Taking the highest radioactivity recorded in the lung tissues as 100%, the ability of the lung, liver, heart, and spleen tissues of the animals of each group to retain specific allergen could be compared by expressing them as percentages. In the guinea pigs of group 1 the ratio was 100:23:15:10, in group 2 it was 97:27:18:34, and in the intact animals (group 3) it was 13:68:3:14.

Comparative analysis of these results shows that the degree of retention of allergen in the lung and heart tissue was greater in the sensitized than in the intact animals ($P < 0.01$).

The results of the active cutaneous anaphylaxis test thus showed that the biological action of neisserial allergens is preserved after their incorporation into liposomes. When allergens of *N. perflava* in liposomes were injected intravenously into guinea pigs sensitized with living neisserias, an effect of a decrease in the anaphylactogenic activity of the allergens was observed. The ability of the lung tissues of sensitized animals to retain the specific allergen was observed to be increased. On the basis of the results of other investigations [3, 6, 9] into incorporation of drugs into liposomes, it can evidently be postulated that the effect of a reduction in anaphylactogenic activity of liposomal allergens observed in this case, when they accumulated in large quantities in the lung tissues of sensitized animals, is due to the adjuvant properties of lipids or, which also is probable, to limitation of contact between neisserial allergen and the tissues of the target organ (the lungs) on account of the phospholipid membranes formed. These conclusions can be regarded as preliminary.

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