BIOLOGICAL AND CHEMICAL PROPERTIES OF THE GKI PEST ALLERGEN REPORT I

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Biological and Chemical Properties of the GKI Pest Allergen. Report I.

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(Submitted 27 July 1961)

As is known, attempts were made by a number of investigators (Korobkova, 1955; Zaplatina and Konnova, 1956; Pavlov, 1958; Shmuter, Lopatukhina and Volosivets, 1958; Bakhrakh with coauthors, 1960; Kozlov with coauthors, 1960) at using cutaneous allergic reactions for determining the intensity of antiplague immunity.

In the capacity of allergen for the cutaneous reaction, Korobkova (1955) used a suspension of a 2 day agar culture of P. pestis EV, which was killed by heating at 60° for an hour, in a physiological solution. She named this preparation pestin. However, it must be noted that Korobkova's microbial pestin was actually a compound complex of substances. Therefore it can be supposed that the reaction which emerges in answer to the introduction of this preparation is not strictly specific since it reflects not only the alteration of the immunological state of an organism which is vaccinated against plague, but also a response to the introduction of unnecessary substances. It was necessary to obtain a more purified preparation which possessed the capability of causing a well expressed specific allergic reaction in immune animals.

In the capacity of allergen for an intradermal test, Pavlova (1958) and Bakhrakh with coauthors (1960) proposed the use of a polysaccharide-containing fraction which was obtained from EV plague microbes after their hydrolysis with acetic acid. The non-purified polysaccharide complex was not distinguished by a strict specificity. After the appropriate processing the authors succeeded in obtaining a purified polysaccharide-containing fraction which was capable of causing a specific intradermal reaction only in immune animals.

Since there are indications in literature that bacterial proteins can be specific allergens (Zeybert, 1941; Dubrovskaya with coauthors, 1955; Tsuverkalov, 1961), we decided to test the protein fraction of plague microbes in the capacity of a plague allergen.

With this aim we used a modified method of Baker and coauthors (1952) which was proposed by them for the isolation of antigens from plague microbes. We incubated a culture of P. pestis EV 76 at 37° for three days on Hottinger agar and washed it with a physiological

solution. Then we treated the microbial suspension with 2 volumes of acetone cooled to -70°. The mixture of acetone with microbes was left overnight at room temperature. The microbial bodies were separated out by filtration through a Buchner funnel and the residue rinsed with acetone and dried in a vacuum. The dry mass was extracted with a 2.5% solution of NaCl (pH = 7.2). After extraction the bacteria were separated out by centrifugation and after dialysis were subjected to fractionation with ammonium sulfate between 25-30, 30-33, 33-40, 40-60, and 60-80% saturation. In this manner five fractions were obtained.

These fractions obtained were dialyzed up to the complete removal of the ammonium sulfate after which the preparations were dried by the lyophilic method. All the fractions mentioned above were tested in the capacity of allergens during the staging of an intradermal experiment on guinea pigs immunized with live EV 76 plague vaccine. Two experiments were carried out with similar results.

In the first experiment 24 guinea pigs were inoculated with 1.5 billion microbial cells of plague vaccine. Four weeks after immunization of the animals the reaction to the antigen was checked by means of an intradermal test. In the second experiment 25 guinea pigs were immunized with 1 million microbial cells of the very same vaccine. The intradermal test was staged after six weeks. In the two experiments there were 15 control, non-vaccinated guinea pigs.

During the intradermal tests the fractions obtained were administered in the amount of 0.01 mg in 0.1 ml of a physiological solution. This dose, as was preliminarily determined, ensured the most constant results.

The reactions were considered according to the following system: Negative reaction - no apparent changes at the site where the allergen was administered; weakly positive reaction - the presence of hyperemia and a small induration up to one square centimeter in area; positive reaction - the presence of hyperemia and an induration from one to two square centimeters in area; sharply positive reaction - the presence of hyperemia and an induration with an area greater than two square centimeters.

The allergic reaction was considered up to the third day, starting from 20-24 hours after staging the test.

On the basis of the data in table 1, the conclusions can be made that all the fractions of the plague microbe isolated by the above described method caused a positive allergic reaction in immunized guinea pigs. However, the most active turned out to be fraction 4 which was obtained at a saturation of 40-60% ammonium sulfate. With this fraction, out of the 15 guinea pigs immunized with live plague vaccine, 11 had a sharply positive reaction, two a positive, and two a weakly positive reaction.

Subsequently in all the experiments during the staging of the intradermal test we used just this most active fraction, called by us conditionally the GKI pest-allergen. (GKI = Gosudarstvennyy Kontrol'nyy Institut, State Control Institute.)

After selecting the pest-allergen we decided to check it in an experiment on guinea pigs, immunized with various doses of EV 76 live plague vaccine. All told in the experiment there were 170 immunized and 50 control guinea pigs. We conducted the immunization subcutaneously with the following doses: 1 million, 1.5 million, 15 million, 150 million, and 1.5 billion microbial cells.

As is apparent from the summary data presented in table 2, out of 168 guinea pigs inoculated with plague vaccine, 90% reacted positively to the GKI pest-allergen while there was 100% negative reactions in the control, non-immunized guinea pigs. Thus, with the help of the GKI pest-allergen we were able to determine the presence of an allergic transformation of the organism in 90% of the inoculated animals.

It must be noted that we weren't able to reveal a significant difference in the nature of the allergic reactions in guinea pigs inoculated with various doses of plague vaccine. This is apparently explained by the fact that even the smallest dose used by us - 1 million microbial cells - as is known, protects the animals well against plague.

In the following experiments we studied the degree of specificity of the intradermal test with GKI pest-allergen. With it we set up an intradermal test on guinea pigs immunized with live vaccines against plague, tularemia, brucellosis and tuberculosis; three guinea pigs were infected with a virulent tubercular strain. Intradermal tests with specific and non-specific allergen were applied simultaneously on both sides of each guinea pig used in the experiment.

From table 3 it is apparent that only the guinea pigs immunized against plague reacted positively to the intradermal administration of the pest-allergen. Animals immunized against tularemia, brucellosis, tuberculosis and also guinea pigs sick with tuberculosis reacted positively only to specific allergens. These facts attest to the specificity of the preparation obtained by us.

After the preliminary study of the biological properties of the GKI pest-allergen, we began the study of its chemical nature. In the six series of pest-allergen obtained the nitrogen was checked quantitatively by the micro-Kjeldahl technique, reducing substances according to Hagedorn, and phosphorous according to Fiske-Subbarow. In chemical composition the series obtained were close: Their nitrogen content fluctuated between 9-10%, reducing substances 17-19%, and phosphorous 0.8-1%. Characteristic qualitative reactions to protein (Biuret reaction, reaction with sulfosalicylic acid) and to polysaccharide (Molisch test) were positive. As a result of the chemical

analysis it was established that the GKI pest-allergen is a protein-polysaccharide complex in which protein is the specific allergen. Polysaccharide precipitated by alcohol from this complex after hydrolysis from an 0.1 N solution with acetic acid didn't evoke an allergic reaction in immunized guinea pigs.

With the aim of clarifying the homogeneity of the protein part, the preparation was investigated by means of paper electrophoresis in various buffer solutions. The sharpest separation of protein was obtained in a veronal-medinal buffer with a pH equal to 8.6. The protein wasn't homogeneous and separated into three fractions (see figure). After paper electrophoresis the fractions were eluted and checked in an allergic test on animals. Only the eluate of the 2nd fraction caused a positive allergic reaction. At the present time the department is engaged in the study of the biochemical structure of the 2nd fraction.

Before moving to the study of the pest-allergen in humans it was necessary to check its toxicity on animals. The harmlessness of the preparation was investigated on white mice and guinea pigs. The intravenous administration of 0.02 mg of the pest-allergen to white mice and the endocardial administration of 0.3 mg of the preparation to guinea pigs didn't cause the death of the animals, that is the preparation proved non-toxic in these doses. After establishing the harmlessness of the preparation on animals, it was decided to test it on volunteers. Out of 59 volunteers, 21 were not vaccinated (control), two were vaccinated with plague vaccine one time intradermally, 19 - one time cutaneously, and 17 - numerous times cutaneously.

A dose of 0.01 mg of pest-allergen (in volume, a 0.1 ml physiological solution) was introduced into the skin of the middle third of the palmar surface of the forearm. The reaction was considered after 24 hours. The following was noted at the site of administration of the pest-allergen: the absence of a local reaction in 19 who weren't vaccinated with plague vaccine (two reactions were questionable) and in 12 who were vaccinated cutaneously one time; in the personnel who were vaccinated many times cutaneously and one time intradermally, a positive reaction to the pest-allergen was obtained. The positive reaction was characterized by hyperemia and consolidation of the skin. The sizes of the sector with hyperemia and skin consolidation reached 2 - 5 cm^2 on the average.

The facts presented in this article on the checking of the GKI pest-allergen in humans appear to the authors as requiring, consequently, verification in a wider experiment. It is extremely necessary since an analysis of the facts presented here forces the supposition to be expressed that a single cutaneous immunization is insufficient for creating an antiplague immunity. For solving such an important problem it is necessary to test the pest-allergen in a large contingent of people vaccinated with plague vaccine by various methods and at various times prior to setting up the test.

Conclusions

1. Out of the acetone dried extract of the EV 76 strain of plague microbe, a fraction was isolated by precipitation with a 40-60% saturation of ammonium sulfate. It was conditionally called the GKI pest-allergen.

2. A 0.01 mg dose of pest-allergen (in volume a 0.1 ml physiological solution) caused a positive allergic reaction in immunized guinea pigs and didn't cause a reaction in control non-immunized animals.

3. The pest-allergen is a protein-polysaccharide complex, in which the specific allergen is the protein part.

4. In animals immunized by live vaccines against tularemia, brucellosis, and tuberculosis, the pest-allergen didn't cause a positive reaction which speaks for the specificity of the preparation.

5. Preliminary experiments set up in 59 volunteers showed that the pest-allergen was harmless for humans when administered intradermally. In persons vaccinated with plague vaccine intradermally and many times cutaneously, the pest-allergen caused an expressed allergic reaction.

Figure, page 76: Electrophorogram of the pest-allergen.

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(Following is the English summary which appears with the Russian original.)

Biological and Chemical Properties of the GKI Pest-Allergen. Communication I.

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A fraction called GKI pest-allergen, was isolated from the extract of acetone dried plague microbes (EB strain) by precipitation with ammonium sulfide (40-60% saturation). This preparation injected intradermally in a dose of 0.01 mg provoked a positive reaction in immunized guinea pigs and caused no reaction in control nonimmune animals. The pest-allergen represents a protein-polysaccharide complex, in which the protein part is the specific allergen.

Preliminary experiments staged on 50 volunteers demonstrated that the pest-allergen injected intradermally is harmless for man. Pest-allergen caused a marked allergic reaction in persons inoculated with the plague vaccine intradermally and repeatedly subjected to skin vaccination.

Table 1

Activity of Various Fractions during Their Intradermal Administration to Guinea Pigs Immunized with Live Plague Vaccine

Fraction used as the allergen	% saturation with ammonium sulfate	Dose of vaccine (in number of microbes)	Number of pigs	Evaluation of reaction			
				sharp pos.	pos.	weak pos.	neg.
1	25 - 30	1.5 billion	7	-	-	4	3
		1 million	5	-	1	2	2
2	30 - 33	1.5 billion	5	1	-	3	1
3	33 - 40	1.5 billion	7	-	-	7	-
		1 million	5	-	2	2	1
4	40 - 60	1.5 billion	5	4	-	1	-
		1 million	10	7	2	1	-
5	60 - 80	1 million	5	-	-	3	2
Fractions 1 - 5		Control	15	-	-	-	15

Table 2

Intradermal test with pest-allergen in guinea pigs immunized with various doses of live plague vaccine.

Dose of vaccine (in number of microbial cells)	Number of guinea pigs	Evaluation of reaction				Total	
		sharp pos.	pos.	weak pos.	neg.	pos. (%)	neg.
1 million	78	23	27	20	8	89.8	10.2
1.5 million	14	8	3	1	2	85.7	14.3
15 million	13	3	6	2	2	84.6	15.4
150 million	13	5	4	3	1	92.3	7.7
1.5 billion	50	8	17	16	9	82.0	12.0
Total.....	168	47	57	42	22	87.1	12.9
Control - non-immunized guinea pigs	50	-	-	-	50	-	100.0

Table 3

Results of intradermal test with pest-allergen in guinea pigs immunized with live vaccines against various infections.

Vaccine	Number of pigs	Reaction to specific allergen			Reaction to pest-allergen	
			pos.	neg.	pos.	neg.
Live plague	9	Pest-allergen	8	1	8	1
Tularemia	8	Tularin	8	-	-	8
Brucellosis	10	Brucellin	10	-	-	10
BCG	9	Tuberculin	9	-	-	9
Virulent tuberculosis strain	3	"	3	-	-	3