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Study of allergic reaction to somatic antigen made from a live plague vaccine of the EV strain

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Abstract

Background and Aim. This article presents the results of experiments conducted to study delayed-type hypersensitivity to somatic plague antigen derived from a live plague vaccine from strain EB. This study aims to investigate delayed-type hypersensitivity to a somatic plague antigen derived from a live plague vaccine of the EV strain.

Materials and Methods. Methods included the following: extraction of the main somatic antigen from the vaccine strain *Yersinia pestis EV*, determination of the Lowry protein concentration by superimposing the results obtained from a spectrophotometer's digital optical density values of the test preparation on a calibration line; testing of the allergen for sterility (on nutrient media), toxicity (on healthy white mice and guinea pigs), pyrogenicity (on rabbits), absence of sensitizing properties and specificity (on healthy guinea pigs); and determination of the allergenic activity of the main somatic antigen by detecting its hyperergic reactivity in in vivo experiments on guinea pigs.

Results. Further experiments conducted to study delayed-type hypersensitivity to somatic plague antigen showed that the obtained preparation is sterile, harmless, apyrogenic, has no sensitizing properties, and is specific.

Additionally, evaluating the allergenic activity of different doses of the investigated preparation revealed that a dose of 50 mcg is optimal for intradermal administration.

Conclusion. The conducted studies will be used for quality control of somatic plague antigen-allergen, which will allow detection of the absence of sensitizing properties or immunological harmlessness of the allergen at the site of injection in healthy people and animals, thereby avoiding positive responses in both infected and immunized individuals, as well as minimizing the occurrence of false reactions.

Key words: Delayed-Type Hypersensitivity; Plague Dry Live Vaccine from EV Strain; Somatic Plague Antigen.

Introduction

Allergic diagnostic tests are biological reactions used to diagnose various diseases, primarily infectious diseases, based on hypersensitivity of the organism caused by an allergen. In the context of infectious diseases, the allergen is typically the causative agent or its toxin. When the corresponding allergen is introduced, the body responds with a local or general reaction, which can be used to diagnose the presence of a specific disease. The diagnostic value of allergic diagnostic tests is determined by their specificity, sensitivity, and safety for humans or animals. Examples of these tests include the Pirke and Mantoux reactions for tuberculosis [1], the Byrne reaction for brucellosis [2], and the Schick reaction for diphtheria [3]. These tests can also detect hypersensitivity to substances causing bronchial asthma attacks [4] and other allergic diseases.

In veterinary medicine, allergic diagnostic tests are used to diagnose diseases such as glanders [5], tuberculosis [6], brucellosis [2], paratuberculosis enteritis [7], tularemia [8], toxoplasmosis [9], and other infectious and invasive diseases. The primary advantage of these tests in veterinary practice is their ease of use for both individual and mass examinations of animals. These tests can identify infected animals even in the absence of clinically expressed disease. Specific allergens used in veterinary diagnostics include mallein for glanders, tuberculin for tuberculosis, abortin, brucellisate, and brucellohydrolizate for brucellosis. The tests are conducted by applying preparations to the conjunctiva of the eye (e.g., mallein and tuberculin) or intradermally.

Scientists at the Kazakh Scientific Research Veterinary Institute, in collaboration with scientists at the Kazakh Center for Quarantine and Zoonotic Infections named after M. Aikimbayev, have developed a method for obtaining somatic plague antigen-allergen from a live plague vaccine derived from the EV strain [10]. Despite the advantages of many recently developed allergodiagnostics, such as precise dosing, ease of application and result recording, and low cost, these preparations often contain a large number of antigens, leading to several disadvantages. The most significant of these is the occurrence of positive responses in both infected and immunized individuals, as well as a certain number of false reactions.

Materials and Methods

A dry live plague vaccine from the Yersinia pestis EV strain, deposited in the museum of live cultures of microorganisms at the State Enterprise "Kazakh Scientific Center for Quarantine and Zoonotic Infections named after M. Aikimbayev," was used as the source material.

Ethical approval

Ethical approval for the animal studies was obtained from the Ethical Commission of the Kazakh Scientific Research Veterinary Institute (Approval No. 2 of 01.02.2005).

The sterility, toxicity, pyrogenicity, absence of sensitizing properties, and specificity of the allergen were tested.

Protein Concentration Determination. After extracting the main somatic antigen from the vaccine strain Yersinia pestis EV, the protein concentration was determined using the Lowry method. This involved superimposing the spectrophotometric results of digital optical density values of the test preparation onto a calibration line, with the abscissa axis representing the protein concentration. The protein content of the sample at pH 7.0-7.2 was 2.7-3.0 mg per ml.

Sterility Testing. Sterility was assessed by culturing the allergen on meat-peptone agar and meat-peptone broth. For each medium (five tubes each), 0.25 ml of the preparation dissolved in physiological solution to a concentration of 1 mg/ ml was sown. The cultures were incubated at 37 °C and 28 °C. After 10 days, the presence or absence of growth in the tubes was checked to determine the sterility of the preparation.

Toxicity Testing. The toxicity of the preparation was studied in healthy white mice (10 individuals) weighing 20-25 g and guinea pigs (6 individuals) weighing 300 g. The allergen was administered to white mice intravenously at a dose of 0.02 mg and to guinea pigs intracardiacally at a dose of 0.3 mg. The animals were monitored for 48 hours and then autopsied.

Apyrogenicity testing. For apyrogenicity testing, the allergen was administered to rabbits at a dose of 1 mg intramuscularly, and body temperature was monitored overnight.

Determination the absence of sensitizing properties. The absence of sensitizing properties or immunological harmlessness of the allergen was tested in healthy guinea pigs (6 individuals). Three guinea pigs were injected three times with 50 mcg of the drug in a volume of 0.1 ml of sterile physiological solution at 5-day intervals. Three guinea pigs served as controls. After 15 days, all animals were injected intradermally with 50 mcg of allergen in 0.1 ml of sterile physiological solution. After 24 hours, the reaction was read by assessing the presence of redness and its size at the injection site.

Specificity testing. The specificity of the intradermal test with the proposed allergen was studied in 10 guinea pigs immunized with live vaccines against plague and tularemia at a dose of 1 million microbial cells. Each guinea pig was simultaneously tested with 0.01 mg of intradermal samples containing specific and nonspecific allergens in a 0.1 ml volume on both flanks.

Determination the allergenic activity. To determine the allergenic activity of the main somatic antigen, its hyperergic reactivity was detected in vivo. Guinea pigs previously immunized with the live plague vaccine EV were used in the experiments. Vaccination was carried out subcutaneously at a dose of 1 million microbial cells. One month after immunization, an intradermal test was performed on the lateral surface of the guinea pigs' torso with a preparation of somatic antigen from the plague microbe containing various doses-30, 50, or 70 mcg-in 0.1 ml of physiological solution. A needleless injector, adjusted for intradermal administration, was used.

The depth of drug penetration was assessed by preliminary experiments involving intradermal injection of physiological solution with a 1% carcass to guinea pigs. After dissection of the tissues at the injection site, the ink infiltrated only the skin if the apparatus was properly adjusted. Allergic reactions were assessed 24 hours after the sample was administered. A positive reaction was characterized by hyperemia and skin thickening. Hyperemia alone was not considered a positive reaction. The reaction was deemed positive if the thickening was at least 10 mm in diameter. In negative reactions, redness and slight thickening disappeared after 24 hours, while in positive reactions, thickening and redness persisted for 3-4 days.

Statistical processing of the obtained data was performed in Statistica 6.0 programme. Student's t-criterion was used to identify the statistical significance of the differences between the results. Differences were considered reliable at $p \leq 0.05$.

Results

Sterility Testing. The results of the study of the sterility of the plague antigen-allergen samples are shown in Table 1.

Table 1 - Results of the Sterility Study of the Plague Antigen-Allergen Samples

Name of Nutrient Medium	Addition of Antigen-Allergen	Test Tube Number	Presence of Growth
Meat-peptone agar	+	1	Absent
	+	2	Absent
	+	3	Absent
	+	4	Absent
	+	5	Absent
Meat-peptone broth	+	1	Absent
	+	2	Absent
	+	3	Absent
	+	4	Absent
	+	5	Absent

As shown in Table 1, after seeding 0.25 ml of the preparation dissolved in physiological solution to a concentration of 1 mg/ml in all 10 tubes, after 10 days of incubation in the thermostat (at 37 °C and 28 °C), no postrenal growth was detected, which indicated the sterility of the preparation.

Toxicity Testing. The results of the toxicity study of the plague antigen-allergen samples are shown in Table 2.

Table 2 - Results of the Toxicity Study of the Plague Antigen-Allergen Samples

Name of Animal	Number of Animals	Introduced Allergen		Death within 48 Hours	Autopsy Results after 48 Hours
		Method	Quantity		
White mouse	1	Intravenously	0.02 mg	No	Hyperaemia of the subcutaneous tissue vessels
	2	Intravenously	0.02 mg	No	Hyperaemia of the subcutaneous tissue vessels
	3	Intravenously	0.02 mg	No	Hyperaemia of the subcutaneous tissue vessels
	4	Intravenously	0.02 mg	No	Hyperaemia of the subcutaneous tissue vessels
	5	Intravenously	0.02 mg	No	No change
	6	Intravenously	0.02 mg	No	Hyperaemia of the subcutaneous tissue vessels
	7	Intravenously	0.02 mg	No	Hyperaemia of the subcutaneous tissue vessels
	8	Intravenously	0.02 mg	No	Hyperaemia of the subcutaneous tissue vessels
	9	Intravenously	0.02 mg	No	Hyperaemia of the subcutaneous tissue vessels
	10	Intravenously	0.02 mg	No	Hyperaemia of the subcutaneous tissue vessels
Guinea pig	1	Intracardiacally	0.3 mg	No	Hyperaemia of the subcutaneous tissue vessels
	2	Intracardiacally	0.3 mg	No	Hyperaemia of the subcutaneous tissue vessels
	3	Intracardiacally	0.3 mg	No	Hyperaemia of the subcutaneous tissue vessels
	4	Intracardiacally	0.3 mg	No	Hyperaemia of the subcutaneous tissue vessels
	5	Intracardiacally	0.3 mg	No	No change
		Intracardiacally	0.3 mg	No	Hyperaemia of the subcutaneous tissue vessels

As shown in Table 2, intravenous injection of the allergen at a dose of 0.02 mg did not cause the death of white mice within 48 hours and at autopsy after the expiry of this period no changes were registered in mouse no. 5, and in the other 9 white mice only hyperaemia of subcutaneous tissue vessels was detected, which indicated that the plague antigen-allergen was harmless. Experimental, intracardiac injection of the allergen at a dose of 0.3 mg did not cause the death of guinea pigs within 48 hours and at autopsy after the expiry of this period no changes were registered in guinea pig no. 5, and in the other 5 guinea pigs only hyperaemia of subcutaneous tissue vessels was detected, which also indicated the harmlessness of the plague antigen-allergen.

Apyrogenicity testing. The apyrogenicity test results of the plague antigen-allergen samples are summarized in Table 3.

Table 3 - Results of Apyrogenicity Tests of Plague Antigen-Allergen Samples

Body Temperature of Rabbits During the Day					
Experimental Rabbits Injected with the Preparation			Control Rabbits Injected with Distilled Water		
Numbers by n/a	Readings		Numbers by n/a	Readings	
	Before Experiment	During Experiment		Before Experiment	During Experiment
1	38.8 °C	38.8 °C	1	38.7 °C	38.7 °C
2	38.9 °C	38.9 °C	2	38.6 °C	38.6 °C
3	38.7 °C	38.7 °C	3	38.9 °C	38.9 °C
4	38.9 °C	38.9 °C	4	38.7 °C	38.7 °C
5	39.0 °C	39.0 °C	5	38.9 °C	38.9 °C
6	38.8 °C	38.8 °C	6	39.0 °C	39.0 °C
7	38.6 °C	38.6 °C	7	38.8 °C	38.8 °C
8	38.7 °C	38.7 °C	8	38.6 °C	38.6 °C
9	38.8 °C	38.8 °C	9	38.7 °C	38.7 °C
10	38.7 °C	38.7 °C	10	38.9 °C	38.9 °C

A test conducted overnight on 10 rabbits injected intramuscularly with the allergen at a dose of 1 mg showed that the body temperature of the experimental animals remained within normal limits (38.6-39.0 °C). No increase in body temperature was detected in control animals administered distilled water (38.6-39.0 °C). The test demonstrated the absence of pyrogenic properties in the plague antigen-allergen samples.

Determination the absence of sensitizing properties. Experiments conducted on six healthy guinea pigs (3 experimental and 3 control) to determine the absence of sensitizing properties or immunological harmlessness of the allergen at the site of administration involved three administrations of the drug at a dose of 50 mcg at 5-day intervals to the experimental animals. A control intradermal injection of the preparation was given to all 6 animals at a dose of 50 mcg after 15 days. The presence of redness no more than 5 mm in size indicated the absence of sensitizing properties of the proposed preparation.

Specificity testing. The study of the specificity of the intradermal test with the proposed allergen, conducted on guinea pigs immunized with live vaccines against plague and tularemia at a dose of 1 million microbial cells, showed that only the pigs immunized against plague reacted positively to the intradermal injection of the allergen. Animals immunized against tularemia reacted positively only to specific allergens. These data confirmed the specificity of the preparation obtained.

The results of the study of the biological properties of plague allergens are summarized in Table 4.

Table 4 - Results of the Study of the Biological Properties of Plague Allergens

Name of Indicators	Characteristics
Appearance, color, solubility	Amorphous powder, light cream color, soluble in distilled water and physiological solution
Indicator pH	7.0-7.2
Sterility	Sterile
Toxicity	Harmless
Pyrogenicity	Apirogenic
Sensitizing properties	None
Specificity	Specific

From the data in Table 4, it follows that the obtained preparation is sterile, harmless, apirogenic, has no sensitizing properties, and is specific.

Determination the allergenic activity. The results of the intensity of the skin reaction in guinea pigs to allergen administration are shown in Table 5.

Table 5 - Intensity of Skin Reactions to Allergen Injection in Guinea Pigs

Immunization Dose	Animal Number	Papule Size after 24 h (mm)
30 mcg	1	3
30 mcg	2	3
30 mcg	3	-
30 mcg	4	3
30 mcg	5	2
30 mcg	6	2.2
50 mcg	1	5
50 mcg	2	6
50 mcg	3	5
50 mcg	4	7
50 mcg	5	6
50 mcg	6	5.8
70 mcg	1	5
70 mcg	2	6
70 mcg	3	7
70 mcg	4	6
70 mcg	5	5
70 mcg	6	5.8

From the data in Table 5, it follows that when the drug was administered at doses of 50 and 70 mcg, the intensity of the skin reaction was similar, averaging 5.8 mm. When a dose of 30 mcg was administered, the size of the papules was approximately half as large.

The results of the activity of recording the allergic state in plague-immune guinea pigs with somatic antigen preparations are summarized in Table 6.

Table 6 - Activity of recording the Allergic State in Plague-Immune Guinea Pigs with Somatic Antigen Preparations

Animal Group		Allergen Dose (mcg)	Effectiveness of Intradermal Test with Allergen
Immunized	1	30	4/5
	2	50	5/5
	3	70	5/5
Controls	4	30	0/5
	5	50	0/5
	6	70	0/5

*Note: The numerator indicates the number of positive responses, and the denominator indicates the number of animals in the experiment.

Table 6 shows that of the guinea pigs inoculated with the plague vaccine, 4 guinea pigs reacted positively to the allergen administered at a dose of 30 mcg, and 5 reacted positively at doses of 50 mcg and 70 mcg. In contrast, the control (unimmunized) guinea pigs showed 100% negative reactions. Evaluating the allergenic activity of different doses of the drug under study, it becomes apparent that the dose of 50 mcg is optimal for intradermal administration.

Discussion and Conclusion

Modern medicine and veterinary medicine have made significant progress in the use of allergic diagnostic tests to detect infectious and invasive diseases in humans and animals, even in the absence of clinically expressed disease. The diagnostic value of allergic diagnostic tests is determined by their specificity, sensitivity, and safety for humans and animals. Experiments conducted at the Kazakh Center of Quarantine and Zoonotic Infections named after M. Aikimbayev and the Kazakh Scientific Research Veterinary Institute led to the development of a method for obtaining somatic plague antigen-allergen from a live plague vaccine of strain EV.

Despite the many advantages of recent allergodiagnostics, such as clear dosing, ease of staging and result recording, and low preparation costs, these preparations have notable disadvantages due to the presence of numerous antigens. The most significant issue is the occurrence of positive responses in both infected and immunized humans and animals, as well as a certain number of false reactions.

Further experiments to study delayed-type hypersensitivity to somatic plague antigen showed that the obtained preparation is sterile (after seeding 0.25 ml of the preparation dissolved in physiological solution in all 10 tubes, after 10 days of incubation in the thermostat (at 37 °C and 28 °C), no postrenal growth was detected), harmless (intravenous and intracardiac injections of the allergen did not cause the death of laboratory animals within 48 hours and at autopsy after the expiry of this period no changes were registered in laboratory animals), apyrogenic (a test conducted on rabbits injected intramuscularly with the allergen showed that the body temperature of the experimental animals remained within normal limits), has no sensitizing properties (the presence of redness no more than 5 mm in size indicated the absence of sensitizing properties of the proposed preparation), and is specific (the study of the specificity of the intradermal test with the proposed allergen, conducted on guinea pigs immunized with live vaccines against plague and tularemia, showed that only the pigs immunized against plague reacted positively to the intradermal injection of the allergen. Animals immunized against tularemia reacted positively only to specific allergens).

The data obtained by us correlate with the data of Gostischev et al. (2018), where the authors report the verification of specificity and allergenic activity of the pestin allergen obtained by them from the vaccine strain of the plague microbe Yersinia pestis EV. In the preparation obtained, the pH and the protein concentration were determined. To check the specificity, the samples were subjected to spectrophotometric and chromatographic analysis. To assess specific activity, blood samples of 17 people immunized with the plague live vaccine from the Yersinia pestis EV strain of the NIIEG line were used for epidemic indications. The contingent was examined before vaccination on days 7, 21 and 3 months after immunization by evaluating the expression intensity of basophils CD63. Biochemical analysis of the obtained by the modified procedure of the pestin and derivatives allowed to judge the qualitative composition, to show the absence of impurities of the protein nature, as well as to determine the carbohydrate profile. The use of the drug as an allergen to assess the formation of antiplague immunity in the vaccinated contingent confirmed its specificity [11].

After the intradermal injection of guinea pigs, previously subcutaneously immunized with live plague vaccine EB or somatic antigen-allergen at doses of 50 and 70 mcg, the intensity of the skin reaction was the same, averaging 5.8 mm. When a dose of 30 mcg was administered, the size of the papules was approximately half as large. Of the laboratory animals inoculated with the plague vaccine, 4 guinea pigs reacted positively to the allergen injected at a dose of 30 mcg, 5 at a dose of 50 mcg, and 5 at a dose of 70 mcg, while the control unimmunized guinea pigs showed 100% negative reactions. Evaluating the allergenic activity of different doses of the drug under study reveals that the dose of 50 mcg is optimal for intradermal administration.

The somatic plague antigen made from the live plague vaccine of strain EV is sterile, harmless, apyrogenic, has no sensitizing properties, and is specific. The optimal dose of somatic plague antigen-allergen for intradermal injection is 50 mcg. The developed preparation is suitable for practical application in the early diagnosis of plague in humans and animals.

Born et al. (2020) point out the need for highly specific, rapid and easy-to-use confirmatory diagnostic methods for reliable pathogen (Yersinia pestis) identification independent of PCR or immunological detection methods based on F1 antigens. The authors used the host specificity provided by phage

receptor-binding (or tail fibre/spike) proteins (RBPs) to develop a specific, rapid and simple method for the detection of Y. pestis based on fluorescence microscopy [12].

The conducted studies will be used for quality control of the somatic plague antigen-allergen, allowing the detection of the absence of sensitizing properties or immunological harmlessness of the allergen at the site of administration in healthy people and animals. This will help avoid positive responses in both infected and immunized individuals, as well as reduce the occurrence of false reactions.

Authors' Contributions

AB and BK: Conceptualized and designed the study, conducted a comprehensive literature search, analyzed the gathered data and drafted the manuscript. BA, FS, BK and MY: Conducted the final revision and proofreading of the manuscript. All authors have read, reviewed, and approved the final manuscript".

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