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Early Resistance to Experimental Plague in Animals Immunized with Recombinant Plague Vaccine

T. A. Gremyakova, V. A. Amel'chenko, and G. A. Anisimov

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The protective properties of recombinant *Salmonella minnesota* R595/pFS1 strain soon after immunization (1-3 days) are studied in a model of experimental mouse plague. Unlike the commercial EV strain *Yersinia pestis* vaccine produced at the Saratov Anti-Plague Institute (Mikrob Research-Manufacturing Conglomerate), the experimental recombinant preparation affords a high level of protection from the 1st day postvaccination, and surpasses the commercial preparation in such parameters as LD_{50}, mean survival time, and percentage of survivors. By the 21st day the protective indexes of both preparations are comparable.

Key Words: *vaccine; plague; capsule antigen; recombinant strain; Salmonella*

The problem of vaccine prevention of plague remains acute. Vaccination with live *Yersinia pestis* strain EV vaccine produced at the Saratov Anti-Plague Institute (Mikrob Research-Manufacturing Conglomerate) ensures a high level of protection lasting for half a year; resistance appears 21 days after vaccination [2], after which the level of resistance gradually decreases. Revaccination is not efficient and, in addition, may cause autoimmune and allergic reactions. In the USA the killed pestis vaccine based on the virulent strain is used [5].

The chemical vaccines containing capsule antigen (FI), the main protection-affording antigen of plague bacteria and the main somatic antigen, are efficient only for revaccination and, moreover, not in all experimental models [2-5].

The use of heterologous avirulent microorganisms as bearers of respective genetic information appears to be one of the most promising approaches to the design of modern vaccines.

In 1982 the use of a vaccine-expressing virus vector used for specific antigen transport was described [8]. Bacterial vector vaccines are cheaper and more stable. They are readily available, they afford long-lasting immunity, and their amount in the organism is easily controlled by antibiotics.

Today the most prospective bacterial vectors are considered to be Mycobacteriae (BCG vaccine) and *Salmonella* [8]. Salmonellas are preferentially used as live vectors due to the following biological properties: resistance to gastric juce, penetration into the intestinal mucosa, attachment to the intestinal epithelium, entry into enterocytes (M cells), survival in macrophages, and a capacity to colonize the liver and spleen.

Anti-plague immunity in different species is known to be associated with different antigenic complexes of the plague microbe. For mice, rats, and baboons the most immunogenic is capsule antigen. After revaccination or administration in complex with Freund's adjuvant, this antigen is

State Research Institute of Applied Microbiology, Obolensk, Moscow Region. (Presented by A. A. Vorob'ev, Member of the Russian Academy of Medical Sciences)

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TABLE 1. Survival of Vaccinated Mice Infected with Experimental Plague at Different Times after Immunization

Preparation	Time between vaccination and infection, days	Number of surviving animals after a given dose of challenge, CFU (*Y. pestis*, strain 231)				
		10	1×10^2	1×10^3	1×10^4	1×10^5
Killed Re–FI vaccine	1	–	6/6	5/6	5/6	2/6
	2	–	6/6	5/6	3/6	2/6
	3	–	6/6	5/6	2/6	5/6
	21	–	8/9	7/9	5/10	6/10
Commercial plague vaccine	1	–	0/7	0/7	0/7	0/7
	2	–	0/7	0/7	0/7	0/7
	3	–	2/7	0/7	4/7	0/7
	21	–	5/6	4/6	3/6	0/6
Control (unvaccinated animals)	1	0/7	0/7	0/7	–	–
	3	0/7	0/7	0/7	–	–
	21	0/5	0/5	0/5	–	–

Note. Here and in Table 3: the numerator gives the number of animals surviving the infection; the denominator gives the total number of animals in the experiment.

able to induce highly efficient resistance, starting from the 7th day postimmunization [3-5]. In guinea pigs immunogenic properties can be shown by strains that do not synthesize capsule antigen, mouse toxin, and V and W antigens. During the immunization of guinea pigs with the major somatic antigen mixed with complete Freund's adjuvant (infecting dose of 20 DCL) some animals acquire resistance as soon as one day later, and 3, 7, or 14 days postvaccination all animals are resistant to the virulent strain [4].

In the vaccine preparation designed by us, two active substances are involved: a) capsule antigen, which possesses very high immunogenicity in the strain used; and b) Re-chemotype glycolipid which provides a maximum level of heterologous, i.e., cross-reactive, resistance, has adjuvant properties, and represents a part of plague microbe endotoxin.

For the elaboration of vaccinal plague preparations, we used *Salmonella minnesota* strain R595. The cells were transfected with recombinant plasmid encoding FI antigen. We studied the secretion of capsule antigen, estimated the expression levels, conducted a comparative study of physicochemical, immunochemical, and biological properties of different protein substances, and demonstrated the high protective efficacy of vaccination with recombinant *S. minnesota* R595 GSA strain 21 days after immunization.

The goal of this work was to study the efficacy of immunization with recombinant plague vaccine in mouse and guinea pig models, as well as the onset of resistance.

MATERIALS AND METHODS

The following bacteria were used in the study: *S. minnesota* R595/pFS1 (Re-FI vaccine), EV lines of *Y. pestis* from the Research Institute of Epidemiology and Hygiene (commercial dry live vaccine), and *Y. pestis* 231 (a highly virulent strain). The cultures were grown on Hottinger agar at 28°C for 36 h.

Live dry plague vaccine produced at the Stavropol Anti-Plague Institute served as a control. The vaccine culture was prepared according to the enclosed instructions. The vaccinating dose calcu-

TABLE 2. Characteristics of the Protective Properties of Vaccines under Study ($M\pm m$)

Preparation	Time between vaccination and infection, days	Mean survival time, days	LD_{50}, microbes	Index of immunity
Killed Re–FI vaccine	1	8.8±0.7	31,622	10,541
	2	12.0±1.5	14,677	4,892
	3	14.0±1.2	31,622	10,541
	21	10.5±1.0	17,250	5,750
Commercial plague vaccine	1	5.8±0.5	44	15
	2	5.9±0.5	105	35
	3	7.0±0.9	244	81
	21	10.0±0.8	24,404	8,134
Control (unvaccinated animals)	–	4.9±0.5	3	1

TABLE 3. Survival of Guinea Pigs Infected with Experimental Plague 3 Days after Immunization

Preparation	Number of surviving animals after a given dose of challenge, CFU (*Y. pestis*, strain 231)			
	10	1×10^3	1×10^3	1×10^4
Killed Re–Fl vaccine	3/4	2/4	0/4	0/4
Commercial plague vaccine	2/4	1/4	0/4	0/4
Control (unvaccinated animals)	3/5	0/5	0/5	0/5

lated per living cell was 5×10^4 CFU. *S. minnesota* strain cultures were grown for 18 h at 37°C. The killed recombinant vaccine was obtained by heating at 56°C for 2 h followed by control for sterility by seeding on agar in Petri dishes.

The immunochemical activity of capsule protein was recorded in the passive hemagglutination reaction using a commercial diagnostic kit. The specific activity of FI antigen was insensitive to heating.

In the assays of active and passive defense we used 275 outbred white mice weighing 19±1 g and 52 guinea pigs.

Mice were immunized one time via the subcutaneous route with killed Re-FI vaccine in a dose of 10^9 CFU or with live vaccine according to the conventional method (*Y. pestis* strain EV of the line produced by the Research Institute of Epidemiology and Hygiene; 5×10^4 CFU). Guinea pigs received 5×10^9 and 5×10^4 CFU, respectively.

One, two, and three days after immunization the animals were challenged with *Y. pestis* 231 bacterial bodies in doses of 10 to 10^5 CFU (5-10 mice and 4-5 guinea pigs per dose). LD_{50} was calculated routinely [1]. The index of immunity was calculated as the ratio of LD_{50} in the vaccinated group to LD_{50} in the control group. The animals were observed for 21 days after challenge. The results were statistically processed after [1].

RESULTS

The results obtained (Tables 1-4) prove that recombinant Re-FI vaccine both affords an extremely high level of protection, and acts quickly, starting from the 1st day postimmunization. While all control mice died from 10 CFU, all Re-FI-immunized animals sustained 10^2 CFU, 5/6 sustained 10^3 CFU, and nearly half of the animals survived after administration of a high dose (10^4-10^5 CFU). On the other hand, EV live vaccine provided practically no protection at early timepoints, and its antiplague effect reached the efficacy of recombinant Re-FI vaccine as late as 21 days postvaccination.

Regarding guinea pigs, Re-FI as well as EV immunization induced in the first 3 days a weaker level of protection than in mice. Thus, challenge with a dose of 10 CFU resulted in the survival of 3/4 animals (Re-FI vaccine), 2/4 animals (EV vaccine), and 3/5 animals (control). An infecting with a dose of 10^2 CFU yielded the survival of 2/4, 1/4, and 0/5 animals, respectively. Neither vaccine provided any protection against 10^3 and 10^4 CFU at this time (3 days postimmunization).

The value of LD_{50} was 27.5 CFU for Re-FI-immunized guinea pigs, 8.7 CFU for EV-immunized animals, and 9.0 CFU for the control group. Immunization indexes were 3.6, 1, and 1, respectively. The mean survival time was maximal after Re-FI vaccination, shorter after EV vaccination, and minimal in the control group. According to WHO recommendations, bacterial strains used as vectors should meet the following requirements: avirulence, immunogenicity, antigen excretion, and transferability of the corresponding vector [8]. There are two approaches to the construction of vector-bearing strains of Salmonella: attenuation of virulent strains [6] and the use of R mutants (Ra, Rb, Rc, Rd, Re) [7]. Attenuation makes for long-persisting and highly invasive vector cells. However, the pathogenicity of these cells may prove too hazardous against the background of an immunodeficiency state. The second ap-

TABLE 4. Characteristics of Protective Properties of Vaccines under Study (Experiments on Guinea Pigs)

Preparation	Mean survival time (days)	LD_{50}, microbial bodies	Index of immunity
Killed Re–Fl vaccine	10.7	27.5	3
Commercial plague vaccine	9.9	8.7	1
Control (unvaccinated animals)	8.5	9.0	1

proach, i.e., the use of avirulent R mutants bearing core oligosaccharide of various length, which serves as an adjuvant for the presented antigen, yields highly immunogeneic strains.

Use of the *S. minnesota* R595 strain, which expresses capsule antigen of plague microbe and meets the above requirements, as a vector made it possible to design an experimental vaccinal preparation which affords a strong protective effect in the very early postimmunization period in the model of experimental plague in mice and a slightly lesser degree of protection in guinea pigs. In all, the results confirm the validity of the theoretical premises in the construction of recombinant vaccines and the fruitfulness of further work in this domain.

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