THE IMMUNOGENIC PROPERTIES OF SOME FRACTIONS OF *Pasteurella pestis*

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The most effective specific prophylactic preparation at present available against plague is the living vaccine. This has been widely used in the USSR, largely as a result of the work of Soviet microbiologists [2-10] and of the discovery that it can be made in the form of a stable, dried preparation, in a disaccharide-colloidal drying medium [8, 9]. However, the living anti-plague vaccine has a number of disadvantages: it produces a comparatively brief immunity and insufficiently high resistance against the pneumonic form. For this reason, both in the Soviet Union and elsewhere, a search has been undertaken for new methods of use of the vaccine and for new vaccine preparations, with the object of increasing the efficacy of anti-plague vaccination [1, 4, 11-14]. Mention must be made of research showing that it is possible, in principle, to create active immunity against plague by means of various chemical preparations, notably fraction I [1, 11, 14]. These researches are of theoretical as well as practical importance, for they indicate ways of studying the mechanism of formation of immunity to plague infection.

From this point of view, attention is drawn to the investigation of the antigenic properties of the various chemical fractions of *Pasteurella pestis* and to the study of their effect on immunogenesis. In particular, considerable interest has been aroused by the nucleic acids of this microorganism, for neither their antigenic properties nor their role and importance in immunogenesis have yet been studied.

Our purpose was to study the effect of nucleic acids, nucleoprotein and fraction I of *P. pestis* on the formation of immunity when injected into animals along with living vaccine from strain EB, and also to investigate the immunogenic properties of the nucleic acids and of fraction I.

EXPERIMENTAL METHOD

To prepare the vaccine we used strain EB, variant NIIÉG. Antigenic fraction I (FI) was obtained by the method described by Baker and co-workers [11] from a culture of strain EB grown at 37° for 3 days. Extraction with 2.5% sodium chloride solution was carried out for 24 hr, and the FI was precipitated with ammonium sulfate. The fraction obtained with saturation of 0.3 to 0.4 was dialyzed and stored in a dried state.

The nucleic acids (NA) and nucleoprotein (NP) of *P. pestis* were prepared (by N. L. Kulichkov and V. I. Nozdrin) from a broth culture of a virulent strain, grown by a submerged method at 28° for 48 hr. The culture was inactivated with alcohol, and after separation of the latter, it was extracted with 1M NaCl. NP was precipitated from the extract by addition of two volumes of 96% ethyl alcohol; the preparation was then separated, washed, and redissolved in 1M NaCl. The protein and NA were separated by precipitation of the former by addition of a mixture of chloroform and octyl alcohol. On the subsequent addition of one volume of 96% ethyl alcohol, the nucleic acids were precipitated. The NA were kept in a mixture of 1 M NaCl with alcohol (1:1).

The immunogenic properties of these preparations were studied in guinea pigs, albino mice, and monkeys (*Macacus rhesus*, light grey with a black or brown back). The immunized animals were infected with a virulent strain of *P. pestis*, the LD_{50} of which for guinea pigs was 2-3 living microorganisms when inoculated subcutaneously, while the CLD was 10-15 living cells.

In the experiments on guinea pigs, the animals received a subcutaneous injection of NA in buffered physiological saline, in a dose of 3 mg, together with living vaccine in doses of 1000, 10,000, and 100,000 microorganisms according to an optical standard. At the same time, other animals received living vaccine without NA, in the same doses, and a third group received 3 mg NA without living vaccine. Thirty days after vaccination, all the animals

TABLE 1. Immunogenic Activity of Living Vaccine from Strain EB, Injected Subcutaneously into Guinea Pigs along with NA and FI

Dose of vaccine (No. of microorganisms) and of prepration	Infecting dose (in CLD) given subcutaneously after 30 days	No. of animals	
		experimental	surviving
1,000 + 3 mg NA	200	10	10
10,000 + 3 mg NA	200	11	11
100,000 + 3 mg NA	200	12	12
1,000 + 0.1 mg FI	200	10	3
10,000 + 0.1 mg FI	200	12	4
100,000 + 0.1 mg FI	200	12	8
1,000 without preparation	200	9	5
10,000 without preparation	200	11	7
100,000 without preparation	200	10	7
3 mg NA	200	10	0
0.0005 mg FI	10	5	0
0.005 mg FI	10	4	0
0.05 mg FI	10	5	0
0.1 mg FI	10	11	0
0.5 mg FI	10	5	0
Unimmunized	200	12	0
Unimmunized	10	11	0

were inoculated subcutaneously with 200 CLD of a virulent culture. In the control group, unimmunized guinea pigs were innoculated with the same doses of the virulent culture.

EXPERIMENTAL RESULTS

The results given in Table 1 show that the combined administration of very small doses of living vaccine with 3 mg of NA to guinea pigs protected all the experimental animals against subcutaneous injection of a lethal dose of a virulent culture. When living vaccine was given alone, it protected roughly only half the animals. In the dose we used, NA given without vaccine had no immunogenic activity. All the unimmunized animals died from the specific infection.

Simultaneous tests were made of FI, injected subcutaneously into guinea pigs in a dose of 0.1 mg along with the living vaccine, or alone in doses from 0.0005 to 0.5 mg. These showed that FI did not cause immunity in the guinea pigs and did not have a stimulating action on its development after administration together with the living vaccine (see Table 1).

In a second series of experiments on guinea pigs we studied the effect of NA from P. pestis, NA from Brucella melitensis, sodium nucleate (a pharmaceutical preparation obtained from yeast, series No. RZh 1059), and a culture of strain EB killed by heating to 60° for 1 hr, on immunogenesis. All these preparations in various doses were injected subcutaneously into guinea pigs together with living EB vaccine (10,000 microorganisms). The controls were guinea pigs vaccinated with living vaccine (10,000 microorganisms) alone, and unvaccinated animals.

It will be clear from the results given in Table 2 that a stimulating effect on the development of immunity of guinea pigs vaccinated with EB vaccine together with the various preparations was observed only in those animals which received NA from P. pestis in conjunction with the living vaccine; the most effective dose was 3 mg. The remaining preparations (NP from P. pestis, killed culture of strain EB, sodium nucleate, and NA from Br. melitensis) had no stimulating effect on immunogenesis in the guinea pigs inoculated with living vaccine. On the contrary, NP from P. pestis and sodium nucleate had an inhibiting action on the development of immunity.

TABLE 2. Immunogenic Activity of Living Vaccine from Strain EB, Injected Once, Subcutaneously into Guinea Pigs Along with Various Preparations (inoculated subcutaneously with a dose of 200 CLD 30 days later)

Dose of vaccine (No. of microorganisms) and of preparation	No. of animals	
	experimental	surviving
10,000 + 3 mg NA	10	9
10,000 + 1 mg NA	12	10
10,000 + 0.5 mg NA	11	7
10,000 + 5 mg NP	12	4
10,000 + 2.5 mg NP	10	3
10,000 + 1 mg NP	11	8
10,000 + 10^{10} of killed EB vaccine	12	8
10,000 + 5 · 10^9 of killed EB vaccine	12	8
10,000 + 30 mg sodium nucleate	12	4
10,000 + 3 mg sodium nucleate	12	7
10,000 + 0.5 mg sodium nucleate	12	6
10,000 + 3 mg NA from Br. melitensis	12	8
10,000 without preparation	12	8
Unimmunized	6	0

The experiments on monkeys also confirmed the stimulating action of NA on the development of immunity as found in guinea pigs. For instance, all monkeys immunized by two subcutaneous injections of living vaccine (in doses of 1 · 10^9 and 1.5 · 10^9) together with NA (3 mg) survived after aerial infection. Of the six monkeys immunized with the same dose of vaccine but without NA, two became infected and one died. A single immunizing injection of living vaccine in a dose of 1 · 10^9 together with 5 mg NA from P. pestis proved less effective, but the stimulating action of NA was quite obvious. For instance, of the four monkeys immunized with living vaccine plus NA, two survived after infection, whereas of the five animals immunized with vaccine alone, without NA, all died. All the unimmunized monkeys in these experiments died.

Finally, in experiments on albino mice, carried out in order to establish the immunogenic properties of NA and FI from P. pestis, these properties were found to be present in FI (13 of the 14 animals survived), but almost completely absent in the NA preparation (2 of 18 survived.).

Hence, the experiments showed the specific property of NA of Pasteurella pestis to stimulate the formation of immunity in animals against bubonic (in guinea pigs) and pneumonic (in monkeys) plague. It may be concluded from the results that NA of P. pestis may be used to enhance the immunogenic properties of the living vaccine. Further studies of the specific properties of NA of P. pestis will contribute to our understanding of the nature and mechanism of the development of immunity to plague. In our opinion, the stimulating effect on the formation of immunity must be attributed to the DNA in the composition of the NA. We know that DNA plays an important part in the metabolic and synthetic processes of the organism. It is possible that in this particular case this acid influences the synthesis of immune bodies and the process of phagocytosis.

SUMMARY

It was established that nucleic acids (NA) of P. pestis possess a specific property of stimulating the immunity in guinea pigs and monkeys in conjoint administration with the live EB vaccine. Conjoint immunization with the live vaccine and NA protected the animals against experimental bubonic (guinea pigs) and pulmonary plague (monkeys). NA of P. pestis causes no marked immunity in the animals without the live vaccine. Nucleoprotein (NP) and fraction I of P. pestis, as well as killed microbes of the EB strain, sodium nucleate, and NA of Br. melitensis, administered together with the live vaccine caused no stimulating effect on the formation of plague immunity in guinea pigs. Fraction I and NA of P. pestis, as well as sodium nucleate, obtained from yeast in doses and ratios to the live vaccine, depressed the development of immunity to this injection in guinea pigs.

LITERATURE CITED

1. N. I. Aleksandrov, N. E. Gefen, et al., Active Specific Prophylaxis of Infectious Diseases and Ways of Improving It [in Russian], Moscow, 1962.
2. N. N. Zhukov-Verezhnikov, Transactions of Rostov-on-Don Plague Research Institute [in Russian], vol. 1, p. 3, 1939.
3. N. N. Zhukov-Verezhnikov, Zh. mikrobiol., 4-5, 34 (1945).
4. E. I. Korobkova, Zh. mikrobiol, 20, 2, 31 (1938).
5. E. I. Korobkova, A Living Plague Vaccine [in Russian], Moscow, 1956.
6. M. P. Pokrovskaya, Vestnik mikrobiologii, épidemiologii i parazitologii, 1, 3 (1934).
7. M. P. Pokrovskaya, Vestn. mikrobiol., épidemiol. i parazitol., 4, 12 (1934).
8. M. M. Faibich, Zh. mikrobiol., 6, 32 (1946).

9. M. M. Faibich and R. V. Korneev, Collected Works of the Research Institute of Epidemiology and Hygiene [in Russian], No. 2, p. 58, Moscow, 1947.
10. I. A. Chalisov and A. S. Grudenkov, Collected Works of the Research Institute of Epidemiology and Hygiene [in Russian], No. 2, p. 92, Moscow, 1947.
11. E. E. Baker, H. Sommer, et al., J. Immunol., 1952, v. 68, p. 131.
12. N. I. Ehrenkanz and F. K. Meyer, J. infect. Dis., 1955, v. 96, p. 138.
13. W. D. Lawton, G. M. Fukui, and M. J. Surgalla, J. Immunol., 1960, v. 84, p. 475.
14. M. L. Spivack, L. Foster, A. Larson, et al., Ibid., 1958, v. 80, p. 132.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.