DEVELOPMENT OF RESISTANCE TO PLAGUE TOXIN IN ALBINO MICE AFTER REPEATED INJECTION

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A. A. Kanchukh, N. N. Novosel'tsev, and V. I. Nikolaeva

Rostov-on-Don Plague Research Institute
(Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)
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The role of plague toxin in the pathogenesis of plague and in the formation of immunity to this infection has not been adequately explained. Plague toxin occupies an intermediate position between the exo- and endotoxins. Like the exotoxins, it is detoxicated by formalin, but in contrast to them it is not neutralized by antiserum in multiple proportions and does not exhibit the phenomenon of summation when injected in sublethal doses at short intervals [3]. Some authors [7] consider that immunity cannot be created to the toxin if it is injected in an unattenuated form. However, as long ago as in 1898 it was shown [9] that if a filtrate of a 20-day broth culture was injected into albino mice, at first in a sublethal dose, and then at intervals of 8-10 days in doses 5-10 times larger than the first dose, the animals became resistant to 100 lethal doses of the original preparation or more. Some investigators [2] observed an increase in resistance to the toxin after the repeated injection of toxic fractions of the plague microorganism. Others [8] failed to obtain any marked degree of resistance to plague toxin experimentally.

The object of the present investigation was to study the changes in the susceptibility of albino mice to the repeated injection of increasing doses of plague toxin.

METHOD AND RESULTS

Preparations of partially purified toxin were used in the investigation, with an LD_{50} value of about 5 μg (when injected intraperitoneally).

Albino mice weighing 18-20 g received an intraperitoneal injection of 0.5 ml of a 1:2 dilution of toxin. Four animals were taken for each dose. The results were read after 24 h. The value of LD_{50} was calculated by Ashmarin's method [1]. Antibodies to the toxin were determined by the hemagglutination reaction [4].

Preliminary experiments showed that titration of the toxin preparation in animals surviving one or two trials of toxicity had a considerable effect on the value of LD_{50}. The value of this index, obtained in experiments on a group of mice surviving a single toxicity trial was twice as high, and in experiments on mice surviving two toxicity trials, five times as high, as the value of LD_{50} obtained in experiments on intact mice. It must be remembered, of course, that the results of titration were also affected by the individual susceptibility of the mice, and that among the mice surviving the toxicity trials there may have been animals with a naturally increased resistance to toxin.

The next step was to investigate the susceptibility of mice to the toxin during the repeated injection of a series of progressively doubled doses. Experiments were carried out on 42 mice. All the animals received toxin daily, starting with a dose of 0.5 LD_{50} and then progressively doubled. After 5 injections the surviving mice were divided into two groups: one group continued with the daily injections of doubled doses of toxin, the other at intervals of 3 days. The results in Table 1 show that at the end of the experiment 12 mice survived injection of the maximal dose, amounting to 512 units of toxin, and in the course of the 11 injections they had received 1020 units of toxin; 17 of the 42 mice survived the previous dose.

The length of the interval had no significant effect on the result of the experiment, although in the group in which, starting with the 6th dose, the mice received toxin at intervals of 3 days, resistance to the toxin was evidently higher than is shown by the results of the injection of the 7th, 8th, and 9th doses.

TABLE 1. Sensitivity of Albino Mice to Plague Toxin Following Injection of Gradually Increasing Doses and with Different Intervals between Injections

Index	Toxin injected	Number of injections of toxin											No. of surviving mice
		1	2	3	4	5	6	7	8	9	10	11	
		Dose of toxin injected in LD_{50}											
		0.5	1	2	4	8	16	32	64	128	256	512	
No. of dying animals	Daily						1(14)	1(13)	2(12)	2(10)	0(8)	2(8)	6
	At intervals of 3 days	−(42)*	−(42)	5(42)	2(37)	7(35)	3(14)	−(11)	−(11)	−(11)	−(10)	3(9)	6
Coefficient of mortality	Daily						0.07	0.08	0.16	0.2	0	0.25	
	At intervals of 3 days	0	0	0.12	0.05	0.2	0.24	0	0	0	0.1	0.33	

*Here and in Table 2 the number of animals receiving toxin is given in parentheses.

The 12 surviving mice receiving a total of 1020 LD_{50} were exsanguinated, and their sera were tested in the passive hemagglutination reaction to detect antibodies to plague toxin. In the two mice with the smallest dilution of serum (1:20) absolutely no antibodies to the toxin were found, while the rest the titers varied within wide limits (from 1:80 to 1:10,240).

The wide range of antitoxin titers, extending down to negative results, showed that the antitoxin level did not play an important role in the manifestation of resistance to the toxin. This is also clear from the fact that plague toxin is not neutralized in vivo in multiple proportions [11]. It has been shown [11] that toxin–antitoxin complex is unstable and dissociates under the influence of blood serum with liberation of the toxin. These findings have been confirmed by other workers [6] who have shown that if a mixture of toxin and antitoxin is injected intravenously into mice only a few LD_{50} units of toxin are neutralized.

Our own results also demonstrated the instability of the toxin–antitoxin complex. The experiment was modified as follows: 2 ml of whole agglutinating plague antiserum was used to exhaust 1.25 mg of a preparation of plague toxin, LD_{50} of which was equal to 2 μg; the precipitate which formed was separated by centrifugation and washed with a small volume of physiological saline (pH 7.2). The original centrifugate and the washings from the precipitate proved to be nontoxic, but the precipitate caused death of mice, although the value of LD_{50} rose to 11.8 μg, indicating that liberation of toxin from the toxin–antitoxin complex took place even after intraperitoneal injection.

In the next series of experiments an attempt was made to discover whether the resistance to plague toxin developing in the mice was specific and whether it persisted after injection of the toxin ceased. Mice with different levels of resistance to plague toxin were injected at different periods after the last injection of toxin with test doses of plague toxin (8, 50, and 100 LD_{50}) and perfringens toxin (4 LD_{50}). Equivalent and smaller doses of toxin also were injected into control groups of animals, including, besides mice not receiving a single dose of toxin (intact mice), mice surviving a single injection of a sublethal dose of plague toxin.

In the course of development of resistance to plague toxin at various times (after 2, 3, 5, and 9 injections of toxin) and 30, 50, and 60 days after the last injection of toxin some of the mice were sacrificed for histological investigation, which was also carried out on the mice dying during this period.

It is clear from Table 2 that mice which were resistant to plague toxin had no resistance to perfringens toxin. Hence, resistance to plague toxin was not the result of a lowering of the reactivity of the mice, but the result of specific adaptation to plague toxin. The experiment showed that the increased resistance persisted for a long time in the animals (period of

TABLE 2. Resistance of Mice to Plague Toxin at Different Times of Immunization

Group of mice	Time elapsing after cessation of immunization with plague toxin (in days)									
	5		31		50				60	
	Number of units of toxins									
	Plague	Perfringens	Plague	Perfringens	Plague	Perfringens			Plague	
	8	4	8	4	50	5	4	1	100	5
	Number of dying mice									
Resistant to 8 units plague toxin	−(5)	5(5)	−(4)	4(4)	−	−	−	−	−	−
Resistant to 128 units plague toxin	−	−	−	−	−(5)	−	5(5)	−	−(3)	−
Surviving a single injection of a sublethal dose of plague toxin	−	−	5(5)	5(5)	5(5)	5(5)	5(5)	4(5)	−	3(3)
Intact	5(5)	(5)	5(5)	5(5)	5(5)	5(5)	5(5)	4(5)	−	3(3)

observation 2 months), and this also was evidence that the resistance could not be attributed to a reactivity. Meanwhile, in the animals sacrificed at the end of the experiment, i.e., highly resistant to the toxin, no antibodies to the toxin were found.

The results of the histological investigation were as follows. In the intact animals of the control group, dying from a few doses of plague toxin, marked vascular changes were noted: paralytic dilatation of the vessels, increased permeability, perivascular tissue edema, escape of blood by diapedesis. The severity of the vascular disturbances was dependent on the dose of the toxin (5-50 LD_{50}). In the mice of the control group receiving a large dose of toxin (50 LD_{50}) preceded by the injection of a sublethal dose, besides disturbances of vascular permeability, the formation of red thrombi was observed in the veins of the leptomeninges.

In the mice dying after repeated injections of increasing doses of toxin, the same disturbances were found as in the animals of the control groups, and in individual cases red thrombi also were observed in the veins of the leptomeninges. In the liver, besides disturbances of vascular permeability, marked regressive changes were seen in the cells of the parenchyma, with focal areas of necrobiosis. In the animals sacrificed at the same times and surviving injection of the corresponding number of lethal doses of toxin, the vascular disorders and regressive changes in the cells of the liver parenchyma were less intensive.

In the animals surviving 5, 9, and 10 injections of toxin, at various periods after the last injection (5-50 days) considerable necrobiotic changes were found in the liver (in the parenchymal cells); Desse's spaces were widened, and along the layers of Glisson's capsule there were groups of histiocytes with small numbers of eosinophilic leukocytes and solitary plasma cells. In the myocardium the loosening and edema of the interstitial tissue were ill defined; in places the muscle fibers were swollen and irregularly stained with eosin. In this group of animals hyperplasia of the lymphadenoid tissue of the follicles and medullary cords of the lymph glands was seen for the first time; in the spleen congestion of the pulp and an increase in the size of the follicles were found, but no plasma cells appeared.

The results demonstrate that after the repeated injection of plague toxin in its active form into albino mice, the animals develop resistance to high doses of toxin of the order of several hundreds of LD_{50}. This resistance to the toxin is much greater than that obtained by Ajl and Rust [6] by the immunization of mice with formalinized toxin; in their experiments the maximal resistance to toxin was 60-80 LD_{50}.

The absence of a parallel between resistance to toxin and the titer of antibodies to the toxin, and also the demonstration of the instability of the toxin–antitoxin complex, indicate that the mechanism of development of resistance to the toxin cannot be explained simply by neutralization of the toxin by antibodies.

The low level of antibodies to the toxin, or even their complete absence in some animals, agreed with the results of the histological investigation, showing the absence of reaction of the plasma cells, evidently a sign of the depression of immunogenesis as a whole. Meanwhile, the susceptibility of the animals towards the injection of a nonspecific toxin (perfringens) remained.

A fact deserving attention was that the vascular disturbances and degenerative changes observed in the parenchymatous organs, corresponding in their general features to those described by other authors [5, 12], developed not only in mice that were susceptible to the toxin, but also in mice with a high level of resistance to it. It is important to note that these changes were more marked in the liver and less so in the heart and kidneys.

Clearly the tolerance of albino mice to large doses of plague toxin observed in experimental conditions has nothing to do with the phenomena of immunity.

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