

THE MECHANISM OF IMMUNITY TO TETANUS TOXIN AT EARLY PERIODS AFTER ACTIVE IMMUNIZATION

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Studies of the dynamics of development of immunity after primary immunization against tetanus by several authors [1, 3, 4, 10] have shown that the resistance of animals to tetanus toxin or to a culture of tetanus spores develops before the appearance of determinate amounts of antitoxin or of its minimum concentration in the blood. In previous work [5], we also showed that the resistance of white rats after immunization with relatively large doses of adsorbed tetanus toxoid (200–500 units) could be demonstrated from the 6th day after immunization, whereas antitoxin could be found in the blood in determinate amounts (0.002 antitoxin units and over) only from the 8th day.

The aim of the present research was to study the factors responsible for creating the resistance of animals to tetanus toxin at early periods after immunization when no trace of antitoxin was present in the blood.

EXPERIMENTAL METHODS AND RESULTS

Experiments were carried out on white rats weighing 160–180 g. For immunization we used concentrated, purified tetanus toxoid, adsorbed on Al(OH)₃, of series No. 57 of the Institute of Epidemiology and Microbiology of the AMN SSSR, containing 1000 units/ml. The immunizing dose was 200 units (0.2 ml) and was injected into the muscles of the left calf.

In the first series of experiments it was found that when 15 M.L.D. of tetanus toxin was injected on the 7th day after immunization, the majority of animals survived (6–7 of the 10 rats), although the antitoxin titer in the blood at this time was below the minimum determinate level (less than 0.002 antitoxin units). All the unimmunized control animals died on the second day after injection of this dose of toxin.

The results obtained suggest that, despite the absence of antitoxin from the blood of the immunized animals, neutralization of the toxin took place in the body of the animals. In order to test this hypothesis, we carried out experiments to study the dynamics of the resorption of toxin into the blood stream after being injected intramuscularly into immunized animals. The dose of toxin was 15 M.L.D., and this was injected into the muscles of the left or right calf. The toxin content of the serum at different times after injection was determined by biological titration in white mice. In the determination of the toxin titer at each period, a mixture of equal volumes of the sera of 4–5 rats was used. The results of the experiments are shown in Table 1.

The results show that the toxin content of the blood of the rats receiving injections of toxin on the 5th day after immunization was almost the same as that of the unimmunized animals. When the toxin was injected on the 6th day after immunization, it appeared in the blood at the same time and in the same titer as in the control

TABLE 1

Content of Toxin in the Blood Serum of Immunized Rats at Different Times after the Intramuscular Injection of 15 M.L.D. of Tetanus Toxin

Day, after immunization, that toxin was injected	Content of toxin (in M.L.D. for mice) in 1 ml of serum			
	interval between injection of toxin and taking of blood for investigation			
	minutes	minutes	hours	hours
5th	0	0.5—1	1	4
6th	0	0.5—1	0.25	<0.25 (traces)
7th	0	0	0	0
Control (unimmunized rats)	0	1	1	4—6

TABLE 2

Determination of the Antitoxic Properties of the Serum of Rats Immunized with 200 units Adsorbed Tetanus Toxoid, 6 Hours after Injection of 5 M.L.D. of Tetanus Toxin

Serum	Result
Of immunized rats receiving 5 M.L.D. tetanus toxin	1 ml of serum neutralized 2 M.L.D. for mice of tetanus toxin
Of immunized rats receiving physiological saline instead of toxin	The serum possessed no neutralizing properties against 2 M.L.D. for mice of toxin
Of immunized rats receiving no injections of any sort	The same
Of normal (unimmunized) rats	The same

animals, but subsequently (after 3 hours and especially after 6 hours) its titer fell considerably below that of the controls. On the 7th day after immunization, toxin, injected intramuscularly, was not generally found in the blood. Nevertheless, the serum of immunized animals, not receiving injections of toxin, possessed no toxin-neutralizing properties on the 6th and 7th day after immunization when investigated in vitro.

The experiments thus showed that, despite the absence of determinate amounts of antitoxin from the blood of animals at early periods after immunization (6th–7th day), neutralization of toxin took place in the animal body. The supposition arose that this neutralization might be due to antitoxin entering the blood after the injection of the toxin. That this was possible was shown by the fact that toxin, when injected into the animals on the 6th day after immunization, was absorbed without hindrance into the blood at the site of injection, although subsequently its content in the blood fell sharply.

In order to test this hypothesis we carried out experiments to determine the antitoxin in the blood of immunized rats after receiving injections of toxin. White rats were immunized with 200 units adsorbed tetanus toxoid. On the 7th day after immunization the rats were given an injection of 5 M.L.D. of tetanus toxin, and 6 hours later the toxin-neutralizing properties of the serum were investigated in vitro. The results of these experiments are shown in Table 2.

It is apparent from the results described that 6 hours after injection of the toxin the serum of the immunized animals possessed antitoxic properties; the serum of immunized animals receiving injections of physiological

TABLE 3

Determination of the Antitoxin in the Serum of Rats Immunized with 200 Units of Adsorbed Tetanus Toxoid, 6 Hours after Injection of Various Substances

Substance injected	Dose	Experimental animal no.	Antitoxin titer (in antitoxin units)
Tetanus toxoid	50 units	1	>0.02
		2	>0.02
		3	0.005
		4	≥ 0.005
		5	$>0.002 < 0.005$
Perfringens toxoid	40 units	6	0.005
		7	$>0.005 < 0.01$
		8	>0.02
Cortisone	0.05 mg	9	$>0.01 < 0.02$
		10	$>0.005 < 0.01$
	5 mg	11	>0.02
		12	>0.02
		13	$>0.02 < 0.03$
		14	$>0.005 < 0.01$
		15	$>0.01 < 0.02$
Physiological saline	0.5 ml	16	<0.002
		17	<0.002
		18	<0.002
		19	<0.002
		20	<0.002

saline instead of toxin, in the same volume, or no injections at all did not possess these properties, which was in agreement with earlier findings [5]. The appearance of antitoxin in the blood of the animals was thus connected with the action of the tetanus toxin injected into the animals.

In a final series of experiments we investigated the possibility of reproduction of the phenomenon of appearance of antitoxin in the blood by the action of various substances. For this purpose white rats were immunized with 200 units of adsorbed tetanus toxoid, and on the 7th day after immunization they received the following by injection: specific antigen — concentrated, purified, unadsorbed tetanus toxoid; nonspecific antigen — perfringens toxoid; cortisone which, according to the literature [11], plays an important role in the mechanism of the stress reaction. The antitoxin content of the serum was determined by the usual method of biological titration in mice. The results obtained are shown in Table 3.

The results of these experiments show that at early periods after immunization (the 7th day) white rats possessed the ability to react in response to the injection of various substances by the rapid appearance of antitoxin in the blood.

The problem of the site of formation of tetanus antitoxin (from whence it presumably enters the blood in response to the action of various factors) has not been specially investigated. In a series of experiments on white rats, however, we showed that, at early periods after immunization (6th-7th day), in the absence of antitoxin from the blood, the regional iliac lymphatic glands corresponding to the site of injection of the toxoid for immunization, possessed the power to neutralize any toxin which reached them — this was evidence of the production of antitoxin in these lymphatic glands. The results of these investigations, in agreement with data in the literature [2, 6, 7, 8, 9] on the site of formation of tetanus antitoxin in the body, suggest that the appearance of antitoxin in the blood in response to the action of various factors is connected with its entry into the blood stream from the regional lymphatic apparatus.

The findings thus show that one of the factors responsible for creating the resistance of white rats to tetanus toxin at early periods after primary immunization, in the absence of any determinate amount of antitoxin in the blood at the time of injection of the toxin, is the rapid delivery of antitoxin to the blood stream from its site of formation as a result of the action of various specific and nonspecific stimuli on the animal body, and especially of the tetanus toxin itself.

SUMMARY

Experiments on white rats have established that after primary immunization with a single, relatively large dose of adsorbed tetanus toxoid the resistance of the animals to tetanus toxin develops prior to the appearance of antitoxin in the blood. However, the administration at that period of various substances, such as tetanus toxin and Bac. perfringens toxoid, or cortisone, makes the animals react by rapid production of antitoxin in the blood in detectable amounts. This phenomenon is regarded as a nonspecific stimulation of the antitoxin-forming apparatus (regional lymph nodes) periods after immunization and in the absence of antitoxin in the blood at the moment of toxin administration.

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*Original Russian pagination. See C. B. translation.