

THE RELATIONSHIP BETWEEN ALLERGY AND TETANUS ANTITOXIN IMMUNITY

L. G. Kovtunovich and E. A. Shablobskaya

L'vov Institute of Epidemiology, Microbiology, and Hygiene

(Director, Candidate of Medical Sciences S. D. Klyuzko,

Scientific Director, Professor L. A. Chernaya)

(Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)

Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*,

Vol. 52, No. 11, pp. 85-88, November, 1961

Original article submitted November 15, 1960

Until now, the problem of the relationship between increased sensitivity and immunity has not been sufficiently studied, and published reports are widely at variance. Thus, Dews and Code [5], and Cohen, Walsh and Dzury [4] have stated that the effect of cortisone, mustard gas, or total x-irradiation suppresses the development of sensitization, allergic shock, and the elaboration of specific antibodies. A. A. Kolchurina [2] showed that in guinea pigs which had received a single injection of purified adsorbed diphtheritic toxoid, sensitization was about twice as high, and the amount of antitoxin 20 times higher than in animals which had received native toxoid. Nevertheless, from their experiments, Baserga and Bergamini [3] concluded that the level of specific antibodies does not play a principal part in sensitization or desensitization. Uhr, Pappenheimer, and Yoneda [6] observed allergic reactions to take place after an intracutaneous injection of diphtheric toxin or toxoid without any specific antibodies appearing in the serum. Therefore, in considering the relationship between increased sensitivity and immunity, L. A. Zil'ber [1] stressed that we do not know "whether these conditions which contrast so strikingly in their appearance are necessarily associated with each other or dependent one upon the other, or whether the production of each may take place independently."

The object of the present investigation has been to compare the strength of antitoxic immunity with the appearance of allergic responses, after immunizing with adsorbed and with native tetanus toxoid.

EXPERIMENTAL METHOD

The experiments were carried out on 30 male and female smooth-haired guinea pigs weighing 350-500 g, and on 24 male and female chinchilla rabbits weighing 2.5-3 kg. The tetanus toxoid was prepared by the N. F. Gamaleya Institute of Experimental Medicine, AMN SSSR. Two batches of purified concentrated tetanus toxoid adsorbed on aluminum hydroxide were used; No. 16 contained 500 binding units (BU) per ml, and the other (No. 74) contained 200 BU. One batch (No. 377) of native tetanus toxoid contained 40 BU.

The animals were immunized with two injections given at an interval of 20 days, and were revaccinated three and a half months later. The purified concentrated and adsorbed toxoid was given as an injection of 0.5 ml, while for the native toxoid, 1 ml was given at the first and 2 ml at the second injection and at the revaccination.

The antitoxin concentration was determined before the injections, at various periods during the primary immunization, and after the revaccination. The estimation was made on white mice by the usual method.

EXPERIMENTAL RESULTS

The first injection of either the native or the adsorbed toxoid caused no local reactions, apart from some small indurations which were formed 3-5 days after the injection of the adsorbed toxoid. After the second injection and revaccination, in most of the guinea pigs there was a dense edema and an infiltration of the subcutaneous tissue; it was either limited to the site of the injection or included a large part of the side and of the abdominal wall. Infiltrations appeared 24 hours after the injection, they were maintained and somewhat increased during the next 24 hours, and then disappeared leaving no trace. It therefore appeared that the edema and infiltration constituted a slow allergic response to the injected antigen. Results on the relationship between the level of antitoxin in the blood and the allergic response caused by repeated injections of toxoid are shown in Table 1.

The results given in Table 1 show that there was a definite relationship between the level of antitoxin in the blood and the occurrence of local responses to repeated injections of toxoid. In the response to the first injection either of adsorbed or of native toxoid, when there was no antitoxin in the blood, there was no allergic reaction. When the concentration of antitoxin was increased, the number of animals responding by an allergic edema to repeated injections of tetanus toxoid increased.

It should be noted that in guinea pigs who were immunized with adsorbed toxoid and in whom the level of antitoxin was always higher, the allergic reactions occurred more frequently than in those which had been injected with native toxoid. The allergic reactions to repeated injections of tetanus toxoid occurred only in guinea pigs, and never in rabbits, despite the fact that they were injected in the same way and showed the same level of antitoxin in the blood.

The relationship between the level of antitoxic immunity and allergy was shown more clearly by intracutaneous injections of 0.1 ml of the same toxoid as was used for immunization into an area of skin from which the hair had been removed. The results were estimated during the subsequent 24-28 hours in terms of the extent of the hyperemia, infiltration, and necrosis. It should be noted that after the first injection of tetanus toxoid, particularly of the purified and adsorbed toxoid, in some animals there was a small degree of induration and hyperemia over an area not greater than 0.5 cm in diameter, and this response was recorded as a negative reaction. A positive reaction was recorded when the hyperemia and infiltration extended over an area having a diameter of 0.5 to 2 cm; a strong positive reaction was the term used to describe a necrosis of the superficial layers of the epidermis, a hy-

TABLE 1. Relationship between the Level of Antitoxin in the Blood and the Local Reactions in Response to Repeated Injections of Tetanus Toxoid

Time of measurement of antitoxin	Titer of antitoxin (in antitoxic units per ml of blood serum)	Animals		Number of animals giving a local response to the injection of tetanus toxoid			
		species	number	adsorbed		native	
				no reaction	edema + infiltration	no reaction	edema + infiltration
After the first injection	< 0.001	Guinea pig	30	20	0	10	0
		Rabbit	24	16	0	8	0
After the second injection	0.15	Guinea pig	10	—	—	6	4
		Rabbit	8	—	—	8	0
After revaccination	0.35	Guinea pig	20	6	14	—	—
		Rabbit	16	16	0	—	—
	0.15	Guinea pig	10	—	—	5	5
		Rabbit	8	—	—	8	0
	7.5	Guinea pig	20	3	17	—	—
		Rabbit	16	16	0	—	—

peremia, and an infiltration greater than 2 cm in diameter. Local cutaneous responses occurred 24 hours after the injection of the antitoxin, they were maintained for the next 24 hours, and then disappeared without trace. They were therefore ascribed to allergic responses of the "tuberculin type."

The results of the cutaneous tests with tetanus toxoid are given in Table 2, and show that there is a definite relationship between the level of antitoxin in the blood, and the allergic response. The results obtained do not however give reason to suppose that the higher the level of antibodies the more frequently allergy occurs, or that there is any definite level of antitoxin at which positive cutaneous reactions occur. Apparently the kind of antitoxin and the number of times it was given were of principal significance. Thus, an amount of antitoxin of from 0.15 to 0.35 antitoxic units per ml caused in all the guinea pigs and rabbits immunized with the adsorbed toxoid to develop posi-

tive allergic reactions in response to intravenous injection of the same antigen. Nevertheless, when the guinea pigs were immunized with native toxoid, of 20 tests, 12 were positive and 8 negative, whereas in rabbits all the cutaneous responses were negative.

TABLE 2. Relationship between the Level of Antitoxin in the Blood and the Occurrence of Allergic Reactions to Samples of Tetanus Toxoid Applied to the Skin

Time of measurement of antitoxin	Titer of antitoxin (in anti-toxic units per ml of blood serum)	Animals		Number of animals responding to cutaneous tests with tetanus toxoid					
		species	number	adsorbed			native		
				no reaction	reaction		no reaction	reaction	
					positive	strongly positive		positive	strongly positive
Before the first injection	< 0.001	Guinea pig	30	20	0	0	10	0	0
		Rabbit	24	16	0	0	8	0	0
On the 20th day after the first injection	0.15	Guinea pig	10	-	-	-	5	5	0
		Rabbit	8	-	-	-	8	0	0
	0.35	Guinea pig	20	0	1	19	-	-	-
		Rabbit	16	1	15	0	-	-	-
3½ months after the second injection	0.15	Guinea pig	10	-	-	-	3	7	0
		Rabbit	8	-	-	-	8	0	0
	7.5	Guinea pig	20	0	8	12	-	-	-
		Rabbit	16	1	15	0	-	-	-
1½ months after revaccination	7.5	Guinea pig	10	-	-	-	2	8	0
		Rabbit	8	-	-	-	8	0	0
	60	Guinea pig	20	0	11	9	-	-	-
		Rabbit	16	0	16	0	-	-	-

The same relationship was observed for the high concentration of antitoxin (7.5 antitoxic units per ml) which occurred after the second injection with adsorbed toxoid, and after revaccination with native toxoid. Then all the guinea pigs and rabbits receiving the adsorbed toxoid gave positive or strongly positive allergic responses. When native toxoid was used, out of 10 guinea pigs, in two the response was negative and in eight it was positive, but there were no strongly positive responses. Further, in all eight rabbits, there was no cutaneous allergic response.

Our researches have therefore shown that although the extent of the allergic changes is associated with the immunogenic properties of the toxoids, there is no complete parallelism between the titer of the antitoxin and the intensity of the allergic response. It was not possible to demonstrate any definite level of antitoxin in the blood at which positive cutaneous reactions occurred. It appears that the most important factor in the development of allergy is not the strength of the antitoxic immunity but the quality of the antigen and the number of times which it is given.

It is essential to note that the sensitizing and immunogenic properties of purified, concentrated, and adsorbed tetanus toxoid are far higher than those of native preparations. When tetanus toxoids are used, both immunity and increased sensitivity appear to be brought about by the same antigen, so that it is not possible to separate the two effects.

SUMMARY

A study of interrelationship between allergy and the blood antitoxin level was carried out in 30 guinea pigs and 24 rabbits immunized with crude, as well as purified concentrated and sorbed tetanus toxoid. The presence of allergic reconstruction was assessed by the appearance of local reactions to repeated tetanus toxoid injections in sub-

cutaneous and intradermal routes of administration. Investigations carried out have demonstrated that although the extent of allergic reconstruction is connected with the immunogenic properties of toxoids, no complete parallelism exists between the antitoxin level and the intensity of allergic reactions. There was also no definite antitoxin titre at which positive skin reactions appear. The main significance for the appearance of allergy may evidently be attached not to the level of antitoxic immunity, but to the quality of the antigen and the number of its administrations. In using tetanus toxoids the immunity and the increased sensitivity are evidently conditioned by the same antigen and it is impossible to separate them from each other.

LITERATURE CITED

1. L. A. Zil'ber, Fundamentals of Immunity [in Russian] (Moscow, 1958).
2. A. A. Kolchurina, Symposium on Problems of Bacterial Toxins and Toxoids. Abstracts of Reports [in Russian] (Moscow, 1959), p. 48.
3. R. Baserga and F. Bergamini, J. Immunol. Vol. 71, No. 6 (1953), p. 397.
4. S. D. Cohen, J. W. Walsh, and D. S. Dzury, Ibid. Vol. 73, No. 2 (1954), p. 100.
5. P. B. Dews and C. F. Code, Ibid. Vol. 70, No. 3 (1953), p. 199.
6. J. W. Uhr, A. M. Pappenheimer, and M. Yoneda, J. Exp. Med. Vol. 105, No. 1 (1957), p. 1.

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.
