SENSITIZING PROPERTIES OF FRACTIONS OF TETANUS TOXOID ISOLATED BY ION-EXCHANGE CHROMATOGRAPHY

G. S. Neprina, N. V. Medunitsyn, and V. K. Golshmid

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The ability of fractions of tetanus toxoid isolated by ion-exchange chromatography on DEAE-cellulose to induce allergic reactions of immediate and delayed types was studied in guinea-pigs. The fractions differed in their specific activity, the size of the specific antigen molecule, and also in their protective properties. Strict correlation was absent between the protective and sensitizing properties of the fractions. The allergenic activity of the fractions was mainly determined by the amount of protein injected at sensitization. The fraction purified to the greatest degree from ballast substances possessed reduced sensitizing properties.

The need to study the sensitizing properties of tetanus toxoid arises from the demands of medical practice. During immunization with tetanus toxoid postvaccinal allergic complications may arise, and under experimental conditions tetanus toxoid induces the development of increased sensitivity of immediate and delayed types [1, 3-7].

The object of the investigation described below was to obtain a fraction of tetanus toxoid which would combine high immunogenicity with reduced allergenic activity.

EXPERIMENTAL METHOD

The fractions were isolated by ion-exchange chromatography on a column with DEAE-cellulose (working size 250 × 50 mm). The tetanus toxoid used for fractionation was purified by the method adopted at the N. F. Gamaleya Institute of Epidemiology and Microbiology. The toxin was eluted stepwise with 0.005 M phosphate buffer solution, pH 7.0, with sodium chloride in an increasing molar concentration: 0.1, 0.2, 0.3, 0.5, and 2.0 M; the resulting fractions were designated 1, 2, 3, 4, and 5, respectively. The antitoxin-binding activity of the fractions was determined in albino mice in binding units (BU) per ml and the total and protein nitrogen contents were determined by the Kjeldahl method. The sedimentation coefficient and the homogeneity of the proteins comprising the fractions were determined on a Spinco (model E) ultracentrifuge at 56,100 rpm.

To obtain an allergic reaction of immediate type (anaphylactic) the guinea-pigs were sensitized with the test preparations adsorbed on alumina by a single subcutaneous injection in a dose of 2 BU. On the 30th day all the animals received an intravenous injection of 1 ml of the original toxoid containing 40 BU. The severity of the anaphylactic reaction for each group was characterized by the percentage of severe and lethal shock. To obtain delayed allergy the guinea-pigs were sensitized with the preparations in a dose of 1 BU mixed with Freund's adjuvant in the hind paws. On the 30th day all the animals were injected intradermally with the original toxoid and the fraction used for sensitization in a dose of 6 μg in a volume of 0.1 ml. The mean logarithm of the "volume" of the reaction was calculated for each group of animals. The

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TABLE 1. Characteristics of Original Tetanus Toxoid and of Its Fractions Isolated on DEAE-Cellulose (M ± m)

Preparation	Antitoxin-binding activity (in BU/ml)	Specific antitoxin-binding activity (in BU/μg protein nitrogen)	Sedimentation coefficient	Immunogenic activity	
				ED_{50} (in BU)	P
Original toxoid	225±35	895±53		0,95 (1,27÷0,75) n=187	—
Fraction					
1	6±4	228±97	1,08	2,50 (3,5÷1,78) n=171	<0,05
2	212±74	1 512±122	6,8[1]	1,00 (1,32÷0,76) n=168	>0,05
3	29±23	427±17	7,35[1]	0,66 (0,86÷0,51) n=221	>0,05
4	1,6±1	102±21	3,5	Inactive	—
5	0,7±0,9	123±104		Inactive	—

Legend: Here and in Tables 2 and 3, n denotes number of animals, P the criterion of significance of differences between the original toxoid and fractions.

[1]Sedimentation coefficient of the main peak.

TABLE 2. Anaphylactic Shock in Guinea Pigs Sensitized with Original Tetanus Toxoid and Its Fractions (M ± m)

Preparation	Dose		No. of animals	Number of animals with lethal and severe shock		P
	BU	μg protein		abs.	% ± m	
Original toxoid	2,0	13	32	27	71±7	—
Fraction						
1	2,0	17	13	7	54±14	>0,05
2	2,0	7	24	9	37±10	<0,01
2	3,7	13	11	6	54±15	>0,05
3	2,0	28	37	22	59±8	>0,05
4	0,3	13	33	15	45±9	<0,02
5	0,2	13	37	14	38±8	<0,001

"volume" of the reaction is the term conventionally given to the product of two diameters of the patch of hyperemia and the thickness of the area of infiltration, measured 24 h after the reacting injection of the antigen [2]. Because of their low content of specific antigen fractions 4 and 5 were tested in a dose equal in its protein content to the original toxoid.

EXPERIMENTAL RESULTS

The experimental results showed that the isolated fractions of tetanus toxoid differed both in their specific activity and in the size of their specific antigen molecule (Table 1).

Earlier experiments to study active protection of albino mice against a constant dose of tetanus toxin showed that fractions with different molecular weights also possess different protective activity. Fraction 1, evidently a low-molecular-weight fragment of the specific antigen, possessed lower protective power than the original toxoid and fractions 2 and 3.

The results of further investigations showed that the molecular composition of the fraction has no decisive influence on their allergenic activity (Tables 2 and 3). Fractions 1 and 3, containing proteins differing sharply in their molecular composition, created different levels of sensitization in the animals. Fraction 2 was found to evoke less severe skin reactions and anaphylactic shock than the original toxoid, whereas fraction 3, consisting of proteins of similar molecular weight to the proteins of fraction 2, did not possess this advantage. Incidentally, these fractions were not homogeneous and they contained several impurities, which may have affected the total allergenic activity. Since fraction 2 had the highest specific antigenic activity of all the preparations studied, the decrease in its sensitizing properties could be attributed to a lower content of nitrogenous substances injected into the animals during sensitization. In fact, if the sensitizing doses of the original toxoid and fraction 2 were equalized as regards their protein content, the differences between the intensities of the allergic reactions evoked by them disappeared.

TABLE 3. Intensity of Skin Reactions in Guinea Pigs Sensitized with Original Tetanus Toxoid and Its Fractions (M ± m)

Preparation	Dose		Size of skin reaction (mean logarithm of "volume" ±m)			
	BU	μg protein	to original toxoid	P	to sensitizing preparation	P
Original toxoid	1,0	6—8	2,96±0,06 n=27		2,96±0,06 n=27	
Fraction:						
1	1,0	8—50	3,03±0,08 n=14	>0,05	3,04±0,1 n=10	>0,05
2	1,0	3—5	2,75±0,06 n=29	<0,02	2,69±0,05 n=34	<0,01
2	1,70—2,00	6—8	2,85±0,06 n=19	>0,05	2,87±0,08 n=18	>0,05
3	1,0	14—16	2,96±0,07 n=16	>0,05	2,84±0,06 n=17	>0,05
4	0,10—0,15	6—8	2,27±0,27 n=10	<0,02	2,38±0,07 n=13	<0,001
5	0,02—0,10	6—8	0,76±0,4 n=6	<0,01	0,97±0,36 n=13	<0,001

The results of these experiments show that no direct correlation exists between the protective and sensitizing properties of the fractions of tetanus toxoid. The fraction 1, with weak immunogenicity, was indistinguishable from the original preparation in the intensity of its allergic reactions of immediate and delayed types.

The nonimmunogenic ballast fractions 4 and 5 possessed well-marked anaphylactogenic activity.

It can thus be concluded from the analysis of these experiments that all fractions of tetanus toxoid obtained by ion-exchange chromatography can evoke allergic reactions of immediate and delayed types in guinea pigs. The intensity of the allergic manifestations arising during sensitization with a given fraction is largely dependent on the quantity of protein injected. It thus follows that the attempt to attain higher degrees of purification of the tetanus toxoid used on a large scale in practice is justified and desirable.

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