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SENSITIZING ACTIVITY OF THE DIPHTHERIAL TOXOID COMPONENTS

by

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А а	*А а*	A, a	Р р	*Р р*	R, r
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В в	*В в*	V, v	Т т	*Т т*	T, t
Г г	*Г г*	G, g	У у	*У у*	U, u
Д д	*Д д*	D, d	Ф ф	*Ф ф*	F, f
Е е	*Е е*	Ye, ye; E, e*	Х х	*Х х*	Kh, kh
Ж ж	*Ж ж*	Zh, zh	Ц ц	*Ц ц*	Ts, ts
З з	*З з*	Z, z	Ч ч	*Ч ч*	Ch, ch
И и	*И и*	I, i	Ш ш	*Ш ш*	Sh, sh
Й й	*Й й*	Y, y	Щ щ	*Щ щ*	Shch, shch
К к	*К к*	K, k	Ъ ъ	*Ъ ъ*	"
Л л	*Л л*	L, l	Ы ы	*Ы ы*	Y, y
М м	*М м*	M, m	Ь ь	*Ь ь*	'
Н н	*Н н*	N, n	Э э	*Э э*	E, e
О о	*О о*	O, o	Ю ю	*Ю ю*	Yu, yu
П п	*П п*	P, p	Я я	*Я я*	Ya, ya

* ye initially, after vowels, and after ъ, ь; e elsewhere. When written as ё in Russian, transliterate as yё or ë. The use of diacritical marks is preferred, but such marks may be omitted when expediency dictates.

SENSITIZING ACTIVITY OF THE DIPHTHERIAL TOXOID COMPONENTS

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Immunization with diphtherial toxoid often causes increased sensitivity which is manifested by repeated injections of this preparation (Roshkovskaya, 1949; Pappenheimer, 1955; Apanashchenko and Nekhotenova 1955; Frolova, 1966, et al.). The diphtherial preparations used in practice are a complex mixture of heterogeneous components with respect to the antigenic and physicochemical properties of the components. In examining the extensive literature pertaining to the so-called sensitizing activity of diphtherial toxoid we did not find any data dealing with the relative participation of the various components of this preparation during the development of increased sensitivity to it. As is known, during the purification of the vaccine preparation, researchers strive to eliminate the "ballast" impurities in order to increase the immunogenic property and decrease the allergen property of the vaccine.

Earlier, we experimentally analyzed the immunogenic property of various components of the diphtherial toxoid (Perepechkina, 1971). In this work we present the study results for the sensitizing activity of the same components.

Purified concentrated diphtherial toxoid (235 Lf/ml) of the No. 127 series was used as the basic preparation (Institute of Vaccines and Sera im. Mechnikov), obtained from a culture of C. diphtheria of the PW-8 strain, Massachusetts, on the Pop-Lingud media and toxoid in the phase of partial detoxification. The molecularly homogeneous toxoid components were separated by two-stage gel-chromatography on the G-75 and G-200 sephadexes. Gel-filtration on the G-75 sephadex enabled us to separate the flocculating toxoid components (peak I, G-75) from the nonflocculating (peak II, G-75). The flocculating component on the G-200 sephadex was divided into 3 fractions with different physicochemical characteristics (Table 1).

Table 1. Characteristic of the diphtherial fractions.

(a) Препарат	(b) Выход (в %)	(c) Коэффициент седиментации	(d) Иммунодиффузионный анализ с антисыворотками: (e) флоккулирующей	(f) монозональной	(g) антимикробной	(h) Общий азот (в %)	(i) Степень очистки (в Lf на 1 мг азота)	(j) Иммуногенность (в МЕ на 1 мг азота)
(k) Исходный	100		+	+	+	15,8	1 470	1 000
I пик Г-75	80		+	+	+	14	2 000	1 890
II пик Г-75	20	0,7	—	—	—	3		
Фракции Г-200								
1-я	14	7,7	+	+	+	14	475	1 111
2-я	15	5,2	+	+	+	11	2 200	3 333
3-я	20	4,2	+	+	—	16,5	2 500	2 500

KEY: (a) Preparation;(b) Yield, in %;(c) Sedimentation coefficient;(d) Immunodiffusion analysis with antisera; (e) flocculating; (f) monozonal; (g) anti-microbial; (h) Total nitrogen (in %);(i) Degree of purification (in Lf per 1 mg of nitrogen);(j) Immunological level (in IU/1 mg of nitrogen);(k) Initial Peak I - G-75, Peak II - G-75, Fractions G-200 1st, 2nd, 3rd.

Immunodiffusion was accomplished by means of the horse antitoxin antidiphtheric flocculating sera produced by the Moscow Institute of Vaccines and Sera, im. Mechnikov, monozonal antitoxin sera produced by the Paris Institute of Sera and Vaccines and Sofiyskiy Institute of Microbiology and Epidemiology and by the

antisera against whole microbe cells of C. diphtheria, given to us with compliments by doctor N. I. Apanashchenko. The total nitrogen content was determined using the Keldal micromethod. The immunogenic level of the fractions, as was reported earlier (Poperechkina 1971), was determined in the tests on active defense of guinea pigs as compared with the reference preparation of the State Control Institute and the content of immunological units (IU) per 1 mg of nitrogen was determined. Guinea pigs weighing 250-300 g and white mice weighing 12-14 g were sensitized with these preparations.

To study the increased sensitivity of the immediate type, the preparations were introduced once under the skin, having sorbed them on aluminum hydroxide. Different groups of guinea pigs received this and its fractions in doses which varied in their nitrogen content (0.5-0.6, 0.8-1, 2.6-3 and 4 mg and the quantity of immunization units (1.2 and 4 IU). The mice were sensitized with individual fractions in doses of 0.125, 1.25 and 12.5 mg of nitrogen. The test guinea pigs received the initial toxoid intraveneously in the amount of 40 mg of nitrogen. The intensity of the anaphylactic shock was determined by the six-point system, deriving the average index according to Weigle (1960). To evaluate the intensity of reaction of the Arthus type, on the 7th and 18th day after sensitization the mice received the basic preparation in a dose of 20 µg of nitrogen in the amount of 0.05 ml in the right half of the upper lip (inter-lip test) and the intensity of the reaction was determined after 1, 6, 24, and 48 hours using the six-point system (Freund and Stone, 1956).

In studying the increased sensitivity of the delayed type the guinea pigs were immunized in the pulvilli of the extremities with different fractions of the toxoid in a dose of 0.5-1 µg of nitrogen in the total Freund's adjuvant, skin reactions were marked for the 5, 7, 10, and 14th days. When evaluating the reaction the diameter of the skin redness was multiplied by the thickness of the skin fold (in millimeters). The reaction to a physiological solution was used as the control. In a group of animals the average reaction index showed by how many times the response to the toxoid exceeded

that to physiological solution. Maximum skin reactions were observed on the 7-10th day after being sensitized with 1 μg of nitrogen of the initial preparation (see the figure); thus, during this period an autopsy was performed on some of the animals to determine the migration inhibition response of the cells of the peritoneal exudate. The increased sensitivity of the delayed type was determined by this response according to the method of David and co-authors (1964) modified by Protasova and co-authors (1966). The guinea pigs were sensitized with 1 μg of nitrogen of the initial toxoid and its fractions in a complete Freund's adjuvant. Each group consisted of 6-7 animals weighing 290-300 g.

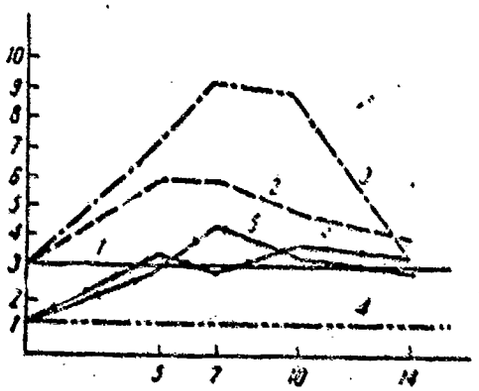

Fig. Dynamics of hypersensitivity of the delayed type (skin test) developed in guinea pigs in response to the injection of the initial 24 hours after the resolution. Conditional units are plotted along the axis of ordinates; the day after the sensitization - along the axis of abscissas. Response to 16 μg of nitrogen of the resolution antigen: 1 - intact animals; 2 - sensitized with 1 μg of nitrogen; 3 - sensitized with 0.5 μg of nitrogen. Response to 1.6 μg of nitrogen of the resolution antigen; 4 - intact animals; 5 - sensitized to 1 μg of nitrogen; 6 - sensitized with 0.1 μg of nitrogen.

The exudate was washed out with Hank's solution containing heparin 72 h after the intraperitoneal 10 ml injection of 2% peptone with glycogen (1 mg/ml). It consisted of macrophages (70-80%), lymphocytes (4-5%), and neutrophilic granulocytes (20-25%). Glass capillaries were filled with this cell suspension and placed into the No. 199 medium with varied toxoid content. These were cultured for 24 h at 37°. The ratio of the area of cell migration in the test cultures (with the toxoid) to that of cell migration in the control cultures (without the toxoid) multiplied by 100 served as the percent index of the migration inhibition, which we accepted as the quantitative characteristic of increased sensitivity of the delayed type.

Mean anaphylactic shock indices in guinea pigs of the various groups which were sensitized with various doses of preparations which varied in their content of nitrogen and immunization units have shown (Table 2) that the sensitization of the animals with the toxoid preparations balanced with respect to nitrogen content of the 1, 2, 3, and 4 series have caused the development of increased hypersensibility of the immediate type of the similar level independently of the physicochemical properties of the preparation. When the sensitizing dose was balanced with respect to the immunogenic level (series 5, 6, and 7) the animals received different doses of toxoid preparations with respect to nitrogen. From the determination results of antitoxic activity (after Jensen) of sera of the animals immunized with the same doses of toxoid preparations in parallel tests it follows (see Table 2) that among the guinea pigs sensitized with 2 IU of the toxoid preparations (series 6) the animals which received the 3nd fraction of G-200 had a low antiphylactic index of 2.2 (the deviation from the other groups of this series is reliable when $P < 0.01$) during a high antitoxic response (0.06-0.12 AU/ml) [AU = antitoxic units]. Consequently we can assume that in the tests on this series the G-200 fraction has a lower sensitizing effect during the immunogenic level which was on the par with other prenarations.

Table 2. Average indices of anaphylactic shock in guinea pigs sensitized with various preparations of diphtheria toxin.

(a) Серия опыта	(b) Сенсибилизирующая доза	(c) Число животных	(d) Средний индекс при иммунизации препаратами		(e) по фракциям G-200			Антитоксическая активность сывороток крови иммунизированных свинок, АЕ/мл (h)
			начальный (e)	пиковый G-75 (f)	1-я	2-я	3-я (g)	
1	0,5—0,6 мг азота	10		3,7		2,2		
2	0,8—1,0 " "	23	3,5	5	4	4,4	3,5	
3	2,6—3,0 " "	23	4,9	4,7	5	5	4,0	
4	4 " "	23	5	4,5	4	5	5	
5	1 МЕ	23	3,7	2,6	4	2	3,5	0,05—0,1
6	2 МЕ	23	4,9	5	5	2,2	4,7	0,06—0,12
7	4 МЕ	23	5	5	4,4	4,4	5	0,1—0,3

KEY: (a) Test series;(b) Sensitizing dose;(c) Number of animals;(d) Average index during the immunization with the preparations;(e) by the initial;(f) by peak I, G-75,(g) By the fractions G-200 1st, 2nd, 3rd; (h) antitoxic activity of sera of the immunized pigs (in AU/ml).

The Arthus-type response of similar intensity was observed in mice immunized with these preparations of toxoid in doses balanced with respect to nitrogen content. On the 18th day of sensitization the intralip test was more intense than on the 7th and occurred quite rapidly with maximum edema 1-6 h after the injection of the test antigen.

The results of quantitative analysis of the hypersensibility of the delayed type to the various diptherial toxoid components with the aid of the specific inhibition response of migration of the peritoneal exudate cell from the sensitized guinea pigs (Table 3) have shown the following: when comparing peak I (flocculating component) with the initial toxoid with respect to the response of the cells to the dilution of the toxoid (1:20,000) the guinea pigs sensitized with the preparation of peak I proved to be less sensitive to the antigen than the animals which received the initial toxoid (the difference is valid when $P < 0.01$). Cells of guinea pigs sensitized with more immunogenic fractions proved to be insensitive to the toxoid (the difference from those not sensitized is unreliable: $P > 0.05$). Consequently, the delayed-type increased response to the diphtherial toxoid revealed by us called for the injection of the initial preparation and its flocculating component, at the time when the fractions characterized by the immunogenic state did not demonstrate any sensitizing activity. The second fraction of the G-200 (see Table 1) had a sedimentation coefficient of 5.2 S, contained a mixture of antigens of the microbial cell, and concentrated within itself a high immunogenic activity (3333 IU per 1 mg of N_2); this fraction did not cause any considerable sensitivity in the tests for determining the hypersensibility of the immediate delayed types. Evidently, this fraction was a fortunate combination of the defense and adjuvant (microbial) factors which, by stimulating the formation of the toxicity-neutralizing antibodies, did not elicit an intense synthesis of "allergic" antibodies. On the contrary, the initial toxoid and its peak I contained considerable amounts of microbial antigens which, in our opinion, act as the

adjuvants with the development of an increased response to the diphtherial toxoid. This point of view is in agreement with the data on the sensitizing activity of the toxigenic diphtherial bacteria themselves (Uhr and co-authors, 1957) and the adjuvant effect of their cell walls (Bulk, 1969). Large-molecular admixtures of bacterial origin in the preparations of diphtherial toxoid can facilitate the development of hypersensibility by interacting with the membrane receptors which are responsive to the lymphocyte antigen. Such interaction of large molecules with the receptor, according to the Smithies and Fisher hypothesis (in: Wallach and Fisher, 1970) occurs at a lower energy level than the bonding of the low-molecule antigens.

Table 3. Inhibition in the migration of exudate cells of guinea pigs sensitized with different preparations of the diptherial toxoid.

(a) Препарат, использованный для сенсибилизации	Средний процент миграции по сравнению с контролем при различных разведениях исходного анатоксина (b)		
	1:200	1:2000	1:20 000
(c) Исходный анатоксин	59,0	70	68
I пик Г-75	64,0	82	104
Фракции Г-200			
1-я	51,0	76	70
2-я	99,5	102	120
3-я	100,0	115	107
Контроль — неиммунизированные	99,0	103	107

KEY: (a) Preparation used for sensitization; (b) Mean percent of migration as compared to the control group with varied dilutions of the initial toxoid; (c) Initial toxoid Peak I of G-75 Fractions G-200 1st 2nd 3rd Control Group (not immunized).

Conclusions:

1. All protein components of the diphtherial toxoid are capable of causing hypersensibility of the immediate type.

2. Among the diphtherial toxoid components the 2nd fraction of G-200 with equal immunogenic level had the lowest sensitizing activity.

3. Admixtures of corynebacterial origin facilitated the development of the delayed-type increased response to the diphtherial toxoid.

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