ANTIGEN RELATIONSHIPS IN BOTULINUS PENTATOXOID AS SHOWN BY THE STUDY OF IMMUNITY INDICES IN ANIMALS

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At the present time there is no longer any doubt about the necessity and the possibility of vaccination with composite preparations, although the problem of immunological competition between antigens cannot be regarded as finally settled. Both synergistic or competitive relationships may be present between the antigens of a composite preparation [7].

Special interest in this respect has been shown in sorbed (or, in the wider sense, depot) composite preparations, the use of which may lead to distinctive patterns of immunogenesis, differing from those obtained by the use of complex preparations composed of corpuscular vaccines or crude toxoids, because of their different physico-chemical state [2, 5, 13, 14].

The information in the literature concerning the interaction between antigens in composite sorbed toxoids is highly contradictory. Some writers indicate a lowering of immunity to individual components in sorbed polytoxoids [3, 8, 9, 11, 15], while others deny that there is competition between antigens in this case, and even report a stimulating action if immunization is carried out with a mixture of sorbed antigens [10, 12].

The importance of both a quantitative and a qualitative selection of antigens has rightly been recognized in this field [1, 4].

It must be pointed out that in most of these articles the writer has drawn his conclusion regarding the antigen interrelationships in sorbed composite preparations on the basis of only one test of immunity: either the level of specific antibodies in the blood or the resistance of animals to the corresponding toxins or to a culture of the agent, and made no attempt to compare these indices.

In our opinion such a comparison is essential, for in the first case we are studying the action of the composite vaccine on only the humoral factor of immunity, and in the second case – on the immunity of the organism as a whole, with all its protective mechanisms.

Because of these considerations, in experiments on the same guinea pigs (weighing 300-400 g) we studied the resistance to toxins and the level of the blood antitoxins after immunization with a pentatoxoid, consisting of botulinum toxoids types A, B, C, D, and E, and monotoxoids, sorbed on aluminum hydroxide, combined into one composite vaccine.

EXPERIMENTAL METHOD

The animals were immunized with a single subcutaneous injection of pentatoxoid and monotoxoids in the same doses (in terms of fixation units* of antigens) as pentatoxoid. In all cases sorption was complete, and the volume of the dose for vaccination was 0.5 ml. On the 25th day after immunization the antitoxin level of the guinea pigs was determined (by titration of pooled serum from the animals of each group in albino mice), and 5 days after taking the blood, the degree of immunity of these same guinea pigs to the corresponding toxins, injected subcutaneously, was investigated.

EXPERIMENTAL RESULTS

Three experiments were carried out on the guinea pigs and gave identical results. The combined results of the 3 experiments are given in Table 1.

*The fixation unit, corresponding to 1 antitoxin unit of specific serum, characterizes the antigenic power of the toxoid.

TABLE 1. Blood Antitoxin Level and Degree of Immunity to Toxins in Guinea Pigs Immunized Once With Botulinum Mono- and Pentatoxoids, Sorbed on $Al(OH)_3$

Preparation	Type of botulinum toxoid	Vaccination dose: fixation units (to 1 antitoxin unit)	Vaccination dose: $Al(OH)_3$ mg	Antitoxin titers: No. of guinea pigs	Antitoxin titers: antitoxin units in 1 ml	Degree of immunity: No. of guinea pigs	Degree of immunity: No. of MLDs of toxin injected	Degree of immunity: Results after observation for 7 days
Pentatoxoid	A	100	2,0—3,88	75	0,3 (0,1—0,4)	1 4 4	2 000 4 000 8 000	O O O O O O O O O
	B	50			0,4 (0,3—0,5)	4 5 3	1 000 2 000 4 000	O O t+ O O O t+ O O t
	C	50			0,8 (0,5—1)	4 4 2	1 000 2 000 4 000	O O O O O O t t O O
	D	50			0,07 (0,05—0,1)	2 6 6	25 50 100	Ot O O O O t t O O O O t t
	E	25			0,08 (0,05—0,1)	1 3 5	250 500 1 000	O O O O O O O O O
Monotoxoid	A	100	0,5	15	0,4 (0,3—0,5)	5 3 2	2 000 4 000 8 000	O O O O O O O O O O
	B	50	0,14—0,23	15	1,1 (0,5—2)	4 3 1	1 000 2 000 4 000	t t++ t++ t
	C	50	0,22—0,25	15	1,6 (1—3)	5 3 2	1 000 2 000 4 000	O O t ++ t++ t ++
	D	50	0,22—0,32	15	0,06 (0,05—0,1)	3 4 2	25 50 100	O O t O O t t t t
	E	25	0,75—2,8	15	1,16 (0,5—2)	3 4 2	250 500 1 000	t ++ t t + ++

Note. The antitoxin titers are mean values, the maximal and minimal figures being given in parentheses.

Legend: O) healthy; t) developed specific toxic manifestations; +) died.

It follows from Table 1 that after receiving pentatoxoid injections guinea pigs produce antitoxins to all five types of antigens. The lowest antitoxin titers were observed in respect of types D and E.

Immunization with monotoxoids, with the same doses of antigens but a lower content of sorbent in the vaccination dose, produced antitoxins in the same titers (types A and D) or substantially higher titers (types B, C, and E) than after immunization with pentatoxoid.

The study of the resistance of guinea pigs to specific toxins showed that most animals immunized with pentatoxoid were resistant to type A toxin up to a dose of 8000 MLD, types B and C to 4000 MLD, type D to 100 MLD, and type E to 1000 MLD.

TABLE 2. Immunizing Power of Botulinum Monotoxoids with an Increased Content of Sorbent (combined results of 2 experiments)

Type of botulinum toxoid	In vaccination dose		Antitoxin titers		Degree of immunity		
	Fixation units (to 1 anti-toxin unit)	Al(OH)$_3$ (in mg)	No. of guinea pigs	Antitoxin units in 1 ml	No. of guinea pigs	No. of MLDs of toxin injected	Results after observation for 7 days
A	100	10	10	1,25 (0,5—2)	2 2 2	2 000 4 000 8 000	O O O O O O
B	50	10	10	3	3 4 2	1 000 2 000 4 000	t t + t t + + + +
C	50	10	10	(3,4—5)	1 2 4 2	250 500 1 000 2 000	t t t t t t + t t
D	50	10	10	0,75 (0,5—1)	3 4 2	25 50 100	O O O t t t t t t
E	25	10	10	0,75 (0,5—1)	1 4 3	250 500 1 000	+ + + + + + + +

Note and legend as in Table 1.

In the case of immunization with monotoxoids, the resistance of the animals to toxins was lower: they were resistant to less than 1000 MLD of toxins of types B and C, to 50 MLD of type D toxin, and to less than 250 MLD of type E toxin.

Consequently, in spite of the fact that the blood antitoxin titers of the guinea pigs immunized with monotoxoids were equal to or higher than the corresponding antitoxin titers of the animals immunized with pentatoxoid, the latter animals were more resistant to toxins. This was especially noticeable when the ratios between the numbers of MLDs of toxins to which the guinea pigs were resistant and 0.1 antitoxin unit of the corresponding antitoxins determined in the blood were compared in the animals immunized with pentatoxoid and monotoxoids. In the first case (pentatoxoid), for type B this ratio was 1000:1, in the second (monotoxoid) it was less than 100:1, and the corresponding ratios for type C were 500:1 and less than 62:1, for type D 1430:1 and 84:1, and for type E 1250:1 and less than 22:1.

The fact that the blood antibody level did not fully reflect the actual resistance of the animals to toxins was also demonstrated by experiments in which the animals were immunized with monotoxoids to which additional sorbent had been added. We have previously shown [6] that the addition of $Al(OH)_3$ to botulinum toxoids in amounts exceeding that necessary to ensure complete sorption of antigens stimulates antitoxin formation in guinea pigs.

In Table 2 we give the antitoxin titers and figures showing the resistance to toxins of the guinea pigs immunized with botulinum monotoxoids (with the same preparations in the same doses as in Table 1) with an increased content of sorbent.

Comparison of the data in Tables 1 and 2 shows that where excess of sorbent was given the resistance of the animals to toxins did not increase despite the higher level of blood antitoxins. Thus when the guinea pigs were immunized with sorbed botulinum pentatoxoid, the titers of antibodies produced against all the components were sometimes lower, but the resistance of the animals to toxins was considerably higher than when the analogous monotoxoids were injected.

The variation in antitoxic immunity, as expressed by the antibody level determined in the blood, was also shown by the results of experiments in which guinea pigs were immunized with toxoids to which additional sorbent had been added. In these experiments the animals with a higher antitoxin level were not resistant to doses of toxin which were survived by animals with a low antitoxin level in the case of vaccination with pentatoxoid.

It is evident that during immunization with sorbed botulin pentatoxoid, in contrast to immunization with monotoxoids, tissue immunity is manifested to a higher degree than antitoxic immunity. The lower antitoxin titers during immunization with pentatoxoid than with monotoxoids are under no circumstances evidence of the presence of competitive relationships between the antigens in the pentatoxoid, for the resistance of the animals to toxins, i.e., the insusceptibility of the organism as a whole, was much higher in this case. These facts show that in order to give a correct interpretation of the relationships between the antigens in composite sorbed vaccines attention must be directed not so much to the humoral factors of immunity as to the resulting insusceptibility to infections or to the action of toxins.

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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.