THE STUDY OF THE IMMUNOLOGICAL EFFICACY OF COMBINED IMMUNIZATION AGAINST GAS GANGRENE, TETANUS, AND BOTULISM IN EXPERIMENTS ON MONKEYS

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The efficacy of combined immunization of man against anaerobic infections (gas gangrene, botulism, tetanus) has received little study, and therefore the immunization of animals closely related to man (monkeys) is a matter of interest.

In 1950 the first experiments to immunize monkeys with triple toxoid (perfringens, oedematiens, and tetanus toxoids), pentatoxoid (triple toxoid + types A and B botulinum toxoids), and ditoxoid (types A and B botulinum toxoid) to be undertaken in the USSR were performed by workers in the anaerobic division of the N. F. Gamaleya Institute of Epidemiology and Microbiology (S. A. Zelevinskaya and T. I. Bulatova). During the four years from 1950 until 1953 experiments were performed on 30 monkeys (green marmoset, Macacus rhesus, Papio hamadryas), aged from 3 to 5 years and weighing 3600-6700 g.

Several series of concentrated triple toxoid and pentatoxoid (unadsorbed) were prepared for immunization of the monkeys, in which the antitoxin-fixing power of the perfringens toxoid was 10-20 units, the oedematiens toxoid 24-40 units, and the tetanus toxoid 33 units.

In 1950, 10 monkeys were immunized with 3 doses (1, 2, and 2 ml) of triple toxoid, and re-immunized 9 months later with a dose of 1.7 ml triple toxoid, and again 2 years after the first revaccination with 1 ml of triple toxoid.

After the third injection, the perfringens antitoxin titer was $> 0.2 < 0.5$ antitoxin unit in one monkey, and < 0.05 antitoxin unit in the remainder, the oedematiens titer was 0.02-0.1 unit or < 0.02 antitoxin unit, and the tetanus titer was > 0.1 antitoxin unit.

After revaccination in 1951 the perfringens antitoxin titer was 0.1-1.5 unit, the oedematiens titer 1-10 units, and the tetanus titer 1-3 units. In 1953, before revaccination the perfringens and oedematiens antitoxin titers were < 0.1 unit, and after revaccination the perfringens antitoxin titer was 0.1-2 units, the oedematiens titer 2-15 units, i.e., the titers were the same as after the first revaccination in 1951.

In 1951, 20 monkeys were immunized with 3 doses of pentatoxoid and also with ditoxoid. The immunization gave no perceptible increase in the titers of types A and B botulinum antitoxins, and the titers of perfringens and oedematiens antitoxins were just the same as in the monkeys of the first group after triple immunization.

In 1960, i.e., 9 years after immunization, the 6 surviving monkeys of the second group were inoculated with pentatoxoid in a dose of 1 ml, containing 40 fixation units of perfringens toxoid, 60 units oedematiens toxoid, 250 units tetanus toxoid, and 50 units each of botulinum toxoids A and B. The toxoid was adsorbed on aluminum hydroxide.

It should be noted that in the case of some of these monkeys (Koldun, Tsitron, Erdzhis) this procedure was a revaccination in respect of all 5 antigens, whereas in 3 monkeys (Chernogolovyi, Angar, Dyuimovochka) it was revaccination only in relation to botulinum toxoids; in relation to perfringens, oedematiens, and tetanus toxoids the procedure was a primary immunization (see Table 1).

Revaccination of Monkeys with Pentatoxoid in 1960

Monkey	Dose of toxoid in 3 injections in 1951	Antitoxin titer (in antitoxin units)										
		before revaccination					14 days after revaccination					
		perfringens	oedematiens	tetanus	botulinum A	botulinum B	perfringens	oedematiens	tetanus	botulinum A	botulinum B
Koldun	3.7 ml triple toxoid + 1.5 ml botulinum A toxoid + 1 ml botulinum B toxoid	<0.1	<0.1	<0.1	<0.05	<0.05	<0.1	> 0.5 <1	> 2	> 0.5 <1	> 8 <15
Tsitron	4 ml triple toxoid + 1.5 ml botulinum A toxoid + 1 ml botulinum B toxoid	<0.1	<0.1	<0.1	<0.05	<0.05	<0.1	> 1 <3	> 2	> 80	> 20 <40
Erdzhis	6 ml triple toxoid + 0.6 ml botulinum A toxoid + 0.3 ml botulinum B toxoid	<0.1	<0.1	> 0.01 <0.1	<0.05	<0.05	<0.1	> 0.1 <0.5	2	> 8 <15	<1 <4
Dyuimovochka	1 ml botulinum A toxoid + 1 ml botulinum B toxoid	—	—	—	<0.05	<0.05	<0.1	=0.1	<0.1	> 8 <15	> 8 <15
Chernogolovyi	1.5 ml botulinum A toxoid	—	—	—	<0.05	—	<0.1	<0.1	<0.1	> 80	—
Angar	1 ml botulinum B toxoid	—	—	—	—	<0.05	<0.1	<0.1	<0.1	—	> 40

The antitoxin titers before immunization were <0.1 antitoxinunit in every case. Fourteen days after immunization the perfringens antitoxin titer in all the monkeys was <0.1 unit, and the oedematiens antitoxin titer between 0.1 and 0.3 units in the 3 revaccinated monkeys and 0.1 unit or less in the 3 monkeys receiving oedematiens toxoid for the first time. The titer of tetanus antitoxin in the revaccinated monkeys was 2-5 units, and in the other 3 monkeys it was not determined.

It is interesting to note that the titers of botulinum antitoxins in the monkeys previously immunized with botulinum toxoids alone and in the monkeys previously immunized with pentatoxoid were roughly the same in 1960 after revaccination with pentatoxoid.

The experiments on monkeys thus showed that perfringens toxoid in doses of 10-20 units/ml, when administered in the form of compositite vaccines (triple toxoid and pentatoxoid), possessed weak immunizing properties even if three immunizing doses were given, followed by revaccination. The work of I. A. Larina and co-workers [1] has shown that in monkeys immunized with triple toxoid containing 30 fixation units/ml, adsorbed on aluminum hydroxide, the perfringens antitoxin titer after immunization with two doses (each of 1 ml at an interval of 30 days) was 0.2-2 units. It is evident that in this case, besides the higher concentration and greater purification of the antigen, its adsorption on aluminum hydroxide was also important.

In our investigations late revaccination after primary immunization was more effective as regards formation of perfringens and oedematiens antitoxins if it was carried out 9 months or 2 years after primary immunization. Revaccination 9 years after primary immunization was ineffective from the point of view of formation of perfringens antitoxin. Triple immunization of monkeys with pentatoxoid did not give any increase in the titers of botulinum antitoxins, although it was not without effect.

Late revaccination was highly effective as regards the formation of types A and B botulinum antitoxins even if carried out 9 years after immunization.

LITERATURE CITED

1. I. A. Larina, E. K. Dzhikidze, and A. S. Aksenova, Byull. Éksper. Biol., 9, 88 (1961).