COMPLEX IMMUNIZATION OF GUINEA PIGS WITH LIVE VACCINES AGAINST PLAGUE, SMALLPOX AND YELLOW FEVER AND WITH KILLED CHOLERA CORPUSCULAR VACCINE IN VARIOUS CONCENTRATIONS

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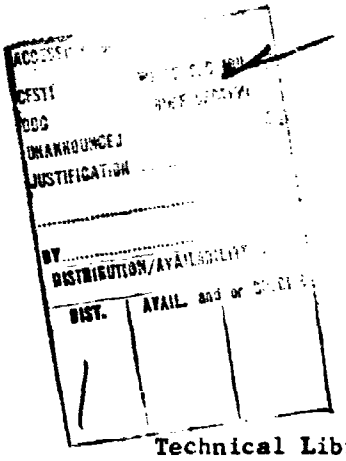

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COMPLEX IMMUNIZATION OF GUINEA PIGS WITH LIVE VACCINES AGAINST PLAGUE, SMALLPOX AND YELLOW FEVER AND WITH KILLED CHOLERA CORPUSCULAR VACCINE IN VARIOUS CONCENTRATIONS

[Following is the translation of an article by M. I. Lyubashevskiy and I. F. Taran, Stavropl Branch of the All-Union Antiplague Institute "Mikrob", published in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology), No 4, 1966, pages 17--21. It was submitted on 14 Oct 1965. Translation performed by Sp/7 Charles T. Ostertag Jr.]

Mass specific prophylaxis has been hampered by the introduction of new vaccines into public health practice and the increase in the number of compulsory inoculations. In connection with this it was important to clear up the feasibility of using associated vaccines in various combinations. Up to the present time general recognition has already been obtained by a number of associated vaccines, the immunological effectiveness of which proved to be no lower than when the corresponding antigens were administered separately. The feasibility of complex vaccination against such particularly dangerous infections as plague, cholera, smallpox and yellow fever has been studied little. We undertook the present investigation for the purpose of clearing up the feasibility of combined vaccination against these infections.

The tests were set up on guinea pigs of both sexes weighing 300--350 grams. They were immunized with the following vaccines: 1) EV native plague vaccine, taken from dried series No 1811 vaccine from the Stavropl Antiplague Institute for the Kavkaz and Zakvkaz; the test guinea pigs were immunized with 3 billion microbial cells (based on the optical turbidity standard); 2) smallpox vaccine series No 83 from the Moscow Institute of Viral Preparations; in our tests one inoculation dose for the animals consisted of 1.5 of the dose intended for the immunization of humans; 3) the vaccine against yellow fever was 17-D of series No 62-2 from the Pasteur Institute in Paris; the vaccine virus 17-D was preliminarily titrated on inbred mice; as a result it was established that the log LD_{50} for the virus of this series of vaccine equaled 3.7 (this satisfied the requirements set forth for the immunization of humans); in our tests one inoculation dose of this vaccine comprised one dose intended for the immunization of humans; 4) killed liquid cholera vaccine series No 47/3 from the Saratov Institute "Mikrob"; the animals were immunized with one inoculation dose equaling 6 billion vibrios.

The compatibility of the vaccines was studied in various combinations. For the immunization of the animals the vaccine was prepared in the laboratory

by means of mixing the individual monovaccines before use. The vaccine against yellow fever was diluted with physiological solution with a pH of 7.2--7.4, containing 10% normal rabbit serum. The vaccine against yellow fever and cholera was administered subcutaneously in the right inguinal area. The plague and smallpox vaccine was diluted with 50% glycerin in physiological solution and administered cutaneously by the generally accepted method.

All told 270 guinea pigs, broken down into 13 groups, were immunized. The animals of 4 groups (10 pigs in each) were immunized with monovaccines, the animals of 5 groups (20 pigs in each) - with divaccine in the following combinations: Smallpox and vaccine 17-D, smallpox and cholera, plague and vaccine 17-D, cholera and vaccine 17-D, smallpox and plague. The animals of 3 groups (30 guinea pigs in each) received trivaccine in the following combinations: Smallpox, vaccine 17-D and cholera; smallpox, vaccine 17-D and plague. Finally 40 pigs received tetravaccine made up of all 4 antigens.

Consideration of the general and local postvaccinal reaction in the inoculated animals was carried out for the first 10--12 days after immunization. For 2--3 days after immunization the temperature of the animals was measured (selectively by groups). During the observations on the intensity and nature of the local reaction we took into consideration the magnitude of the sector of hyperemia and infiltrate, the periods for the formation and casting off of the scabs on the scarifications and the resolution of the infiltrate.

The status of immunological alteration in the inoculated animals in respect to yellow fever and smallpox was judged based on the accumulation of antibodies in the serum. Antibodies to yellow fever were determined by the reaction of hemagglutination inhibition of goose erythrocytes, to smallpox - by hemagglutination inhibition of chick erythrocytes. The smallpox antigen was prepared by the generally accepted method, and the Nagler reaction was used in the modification of Solovyev and Akatovaya. The antigen for the reaction of hemagglutination inhibition in yellow fever was prepared from the neurotropic Dakar strain of the yellow fever virus and the reaction was set up according to Clark and Casals with 4--8 AU of antigen.

The intensity of the immunity against plague was studied by means of infecting the vaccinated animals with 200 Dcl of the No 261 virulent culture of P. pestis; for nonimmunized animals one lethal dose of this strain comprised 50 microbial cells based on the turbidity standard. Immunity against cholera was verified by means of infecting immunized animals with 2 Dcl of a virulent strain of cholera vibrio No 128; the minimum lethal dose of cholera vibrio was determined on nonimmunized animals, and it turned out to be equal to 2 billion vibrios. An expressed local reaction was not observed in the animals inoculated with the yellow fever, plague and cholera vaccines (both separately and in combination). After administering the smallpox vaccine in various combinations with the remaining three antigens a local reaction in the form of hyperemia and an infiltrate on a sector from 0.5 up to 1 x 1.2 cm began to appear in 48 hours after immunization; in 3--4 days after immunization in the majority of

animals vesicles and pustules formed at the site of application; the formation of scabs over the scarifications took place in 3--4 days; resolution of the infiltrate and dropping off of the scabs occurred on the 7--10th day. No significant difference was noted in the course of the local reaction in the animals inoculated with the smallpox vaccine alone and in combination with the remaining three vaccines.

In 60 guinea pigs the intensity of immunity was verified 2 months after immunization by means of infection with 200 Dcl of a virulent culture of P. pestis No 261 (table 1). All the pigs vaccinated with plague monovaccine and this vaccine in combination with the remaining three vaccines survived after being infected with the virulent plague culture. During a bacteriological investigation of these animals 30 days after infection a culture of P. pestis was not isolated. All 15 nonimmunized animals died in 4--8 days after infection, and profuse growth was obtained during seeding from their internal organs.

Verification of the intensity of immunity against cholera was carried out on 6 groups of animals (57 pigs), immunized with cholera monovaccine alone and in combination with the remaining three vaccines (table 2). When applied in combination with the other vaccines, the vaccine against cholera conditioned a more intense immunity in comparison with that caused by the monovaccine. This may possibly be explained by the fact that the resistance of the animals to the cholera causative agent is guaranteed not only by the development of a specific immunity, but also by the increased general reactivity of the organism.

For the purpose of exposing the immune alteration in guinea pigs immunized with smallpox vaccine in various combinations with the remaining three vaccines, the sera of 79 vaccinated and 10 nonvaccinated guinea pigs were studied in the reaction of hemagglutination inhibition (table 3).

It was noted that the antigens in various combinations did not exert an inhibiting influence on the formation of immunity against smallpox; during associated vaccination the immunological activity of the sera from these animals was expressed to the same degree as during immunization with smallpox vaccine alone. During the investigation of sera from the 10 nonimmunized pigs the reaction of hemagglutination inhibition was negative.

The sera of 65 guinea pigs were studied (table 4) for determining the immunological alteration in animals immunized against yellow fever with specific vaccine alone and in combination with the remaining three vaccines.

The immunological activity of the sera from animals immunized against yellow fever with the monovaccine and in various combinations with the remaining three vaccines was expressed to approximately the same degree. The reaction of hemagglutination inhibition was negative with the sera of nonvaccinated animals.

Conclusions

1. During the complex immunization of guinea pigs with live vaccines against plague, smallpox and yellow fever and also killed vaccine against cholera in various combinations, no suppression was noted in the effectiveness of any of the antigens used.

2. During associated vaccination the immunity against plague in the animals was of the same intensity as following inoculation with plague monovaccine; the intensity of immunity against cholera was higher in the animals inoculated with cholera vaccine in combination with other vaccines; following the immunization of guinea pigs with smallpox vaccine and the 17-D vaccine against yellow fever, the reaction of hemagglutination inhibition was the same as that following the application of these vaccines in a complex with plague and cholera vaccines.

Table 1

Condition of immunity against plague in guinea pigs 60 days after vaccination

Group	Vaccine	Dose of vaccine		Method of vaccination	Infection dose (in Dcl)	Number of animals	Result of infection	
		In billions of microbial cells	In human-doses				Died	Survived
1st	Plague	3	-	Cutaneous	200	10	0	10
2nd	Plague + smallpox	3	-	"	200	10	0	10
			1,5	"				
3rd	Plague + 17-D	3		"	200	10	0	10
			1,5	"				
4th	Plague + smallpox + 17-D	3		"	200	8	0	8
			1,5	"				
			1	Subcutaneous				
5th	Plague + smallpox + cholera	3		Cutaneous	200	10	0	10
			1,5	"				
		6		Subcutaneous				
6th	Plague + smallpox + cholera + 17-D	3		Cutaneous	200	10	0	10
			1,5	"				
		6		Subcutaneous				
			1	"				
7th	Nonvaccinated				200	10	10	0
8th	"				1	5	5	0

Table 2

Condition of immunity against cholera in guinea pigs one month after vaccination

Group	Vaccine	Dose of vaccine		Method of vaccination	Infection dose (in Dcl)	Number of animals	Result of infection	
		In billions of microbial cells	In human-doses				Died	Survived
1st	Cholera	6		Subcutaneous	2	10	7	3
2nd	Cholera + smallpox	6	1.5	" Cutaneous	2	10	6	4
3rd	Cholera + 17-D	6	1	Subcutaneous "	2	8	0	8
4th	Cholera + smallpox + 17-D	6	1.5 1	" Cutaneous Subcutaneous	2	10	0	10
5th	Cholera + plague + smallpox	6 3	1.5	" Cutaneous "	2	10	0	10
6th	Cholera + smallpox + plague + 17-D	6 3	1.5 1	Subcutaneous Cutaneous " Subcutaneous	2	9	1	8
7th	Nonvaccinated				2	10	10	0
8th	"				1	5	5	0

Table 3

Indices of the hemagglutination inhibition reaction 23 days after immunization with smallpox vaccine alone and in combination with the three remaining vaccines

Group	Vaccine	Number of animals	Titer of antibodies			
			1:16	1:32 - 1:64	1:128 - 1:256	1:512
1st	Smallpox	10	-	5	3	2
2nd	Smallpox + 17-D	9	-	5	4	-
3rd	Smallpox + plague	10	3	5	2	-
4th	Smallpox + cholera	10	-	5	4	1
5th	Smallpox + cholera + 17-D	10	1	2	7	-
				8.8%	40.5%	50.7%
6th	Smallpox + plague + cholera . . .	10	-	4	5	1
7th	Smallpox + plague + 17-D	10	1	2	7	-
8th	Smallpox + plague + cholera + 17-D	10	2	4	3	1
9th	Nonvaccinated	10	-	-	-	-

Table 4

Hemagglutination inhibition reaction 26 days after vaccination against yellow fever

Group	Vaccine	Number of animals	Titer of antibodies					
			Not exposed	1:120	1:40-1:80	1:160-1:320	1:640-1:1280	1:2560-1:5120
1st	17-D	9	2	-	2	-	3	2
2nd	17-D + plague	10	1	-	1	3	1	4
3rd	17-D + cholera	10	3	1	2	1	1	2
4th	17-D + smallpox . . .	8	2	-	3	-	3	-
5th	17-D + smallpox + cholera	10	2	1	2	3	1	1
6th	17-D + smallpox + plague	9	4	1	1	1	1	1
7th	17-D + smallpox + plague + cholera . .	9	4	-	2	3	-	-
8th	Nonvaccinated	10	10	-	-	-	-	-
9th	Specific serum	1	-	-	1	-	-	-