EFFECT OF IONIZING RADIATION ON THE DEVELOPMENT OF IMMUNITY BY IMMUNIZATION WITH LIVE TULAREMIA VACCINE

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In a previous communication we have already demonstrated that in conditions of severe radiation sickness an acute exarcerbation of the vaccinal tularemia infection occurs in mice and that this is associated with the residual virulence of the vaccine strain B. tularense [1]. In the case of guinea pigs a different picture was observed x-ray irradiation had no exacerbating effect on the vaccinal tularemia infection [2].

The present study is concerned with the action of ionizing radiation on the development of immunity by immunization with live tularemia vaccine.

EXPERIMENTAL METHOD

White mice with an average weight of 15-20 g were irradiated with a dose of 400 r, 504, 360, 192, and 24 hr before being subcutaneously injected with 50 bacterial cells of a 48-hr culture of the vaccine strain B. tularense isolated from dry, live tularemia vaccine (F. N. Gamaley Institute of Epidemiology and Microbiology of the Academy of Medical Sciences of the USSR, batch No. 459, control No. 8209), as well as 48 and 168 hr after infection with a similar dose of the vaccine strain. 480 hr after the injection, the animals were infected with a virulent strain B. tularense No.188 in a dose of 100 DCLM. At the same time the nonirradiated, vaccinated and unvaccinated control mice were also infected. The number of bacterial cells was determined by the intestinal optical standard.

Guinea pigs used weighed an average of 400-500 g. In the first test they were vaccinated subcutaneously with the vaccine strain and in the other 2 tests dermally with live tularemia vaccine (F. N. Gamaley Institute of Epidemiology and Microbiology of the Academy of

TABLE 1. Effect of Irradiation on the Course of Vaccinal Infection and the Development of Immunity in Guinea Pigs Vaccinated with Live Tularemia Vaccine (test No. 2)

Vaccination	Radiation dose (in r)	Number of guinea pigs	Type of vaccinal infection				Number of guinea pigs	Immunity tests	
			death rate of guinea pigs (after 720 hr)	average titre of agglutinins	vaccination				
					av infiltrate diam (mm) 192 hr after vaccination	av duration of inflammatory reaction		dose of virulent strain of B. tularense (number of bacterial cells)	number of guinea pigs dying from tularemia
24 hr before irradiation	180	10	1	1:95	18,3	8,6	8	10 000	0
24 hr after irradiation	180	10	2	1:62	9,2	6,3	8	10 000	1
168 hr after irradiation	180	10	1	1:88	9,6	7,7	9	10 000	1
Control	—	10	0	1:144	13,1	9,2	10	10 000	0
"	—	—	—	—	—	—	3	1	3
"	—	—	—	—	—	—	3	10	3

Medical Sciences, USSR, batch No. 459, control No. 8209). Three scratches, each 2 cm long, were made in the depilated stomach surfaces of the guinea pigs and 3 drops of tularemia vaccine were rubbed in at different periods before and 24 hrs after irradiation. The radiation dose was in the range 180 r [LD-10–20(30)] to 224 r [DL-50–60(30)]. The guinea pigs were then studied for the intensity and duration of the inflammatory reaction at the site of vaccination, the allergic reaction to the injection of tularin and the titre of specific agglutinins.

696-744 hr after vaccination the guinea pigs were subcutaneously infected in the right haunch with a virulent strain B. tularense No. 188. Those that died within 720 hr after testing for immunity were examined for pathological-anatomical lesions and sowings were made on a hard yolk medium, a bacterioscopy was carried out of the prints of the organs stained by the Romanovskii-Gimza method, and white mice were infected with the suspension of the organs. In the last test, all the surviving guinea pigs were killed by phlebotomy 720 hr after infection with the virulent strain, the titre of agglutinins in the serum was determined, sowings of the organs were made on a hard yolk medium, and white mice were infected with the suspension of the organs. Organs of the white mice that died were then sown on a hard yolk medium and used for the thermal precipitation reaction with antitularin serum.

The animals were irradiated in an x-ray therapeutic apparatus RUM-3. Conditions of irradiation were as follows: 180 kv, 10 ma filters 1 mm Al and 0.5 mm Cu, distance from the anode tube 40 cm, filed 20 · 20 cm, dosage 26-27 r/min.

Three tests were conducted on guinea pigs (using a total of 144 animals). In the first test (27 guinea pigs) the animals were subcutaneously injected with 10,000 bacterial cells of the vaccine strain over a period of 24 hr or 24 hr before irradiation with a large dose of x-rays (224 r) which caused death in 50-60% of the animals. In guinea pigs vaccinated 24 hr after irradiation a severe inhibition of the allergic reaction was observed and upon testing for immunity (infection 1000 DLM of virulent strain B. tularense) both the surviving guinea pigs of this group died from tularemia. Irradiation carried out 24 hr after vaccination did not have any definite effect on the development of immunity in this test.

Results of the second and third tests are given in Tables 1 and 2.

In these tests vaccinations were made dermally. As will be observed from the data in Tables 1 and 2, irradiation 24 hr before vaccination produced a slight inhibition of the immunological reactivity only in one of the 2 tests, whereas irradiation 336 and 504 hr before vaccination had no inhibiting effect on the intensity of immunity. Irradiation 24 hr after vaccination was

TABLE 2. Effect of Irradiation on Vaccinal Infection and the Development of Immunity in Guinea Pigs Vaccinated with Live Tularemia Vaccine (test No. 3)

Vaccination	Radiation dose (in r)	Type of vaccinal infection				vaccination		Immunity tests			killed after 720 hr					
														number of positive results		
		number of guinea pigs	death rate of guinea pigs (after 720 hr)	av titre of agglutinins 600 hr after vaccination	allergic reaction (av diameter of the infiltrate in mm)	av duration of the reaction (in hr)	av infiltrate diam (mm) at reaction height	number of guinea pigs	dose of the virulent strain B. tularense (number of bacterial cells)	number of guinea pigs dying from tularemia	total	due to festering of regional lymph nodes	from specific changes in internal organs	biological tests	sowings	av titre of agglutinins
24 hr before irradiation . .	205	10	5	1:108	1,0	10	17,0	5	100	2	1	1	0	1	1	1:20
After irradiation:																
24 hr after	205	10	2	1:52	2,6	6,8	13,4	8	100	2	6	6	0	1	1	1:113
144 hr after	205	10	4	1:113	1,3	6,0	10,7	6	100	0	6	6	0	0	0	1:120
296 hr after.	205	10	8	1:120	5,0	2,2	3,8	2	100	0	2	1	0	0	0	1:120
504 hr after	205	9	6	1:160	—	9,0	12,6	3	100	0	3	3	0	0	0	1:106
Control	—	8	0	1:80	4,0	9,4	19,0	8	100	0	8	5	1	4	4	1:75
"	—	—	—	—	—	—	—	14	1	14	—	—	—	—	—	—

TABLE 3. Effect of Radiation on the Course of Vaccinal Tularemia Infection and on the Development of Immunity in White Mice

Mice	Before immunity tests						After immunity testing		
	number of mice	radiation dose (in r)	B. tularense vaccine strain dose (number of bacterial cells)	number of dead animals			no. mice infected with vir. strain B. tularense	B. tularense virulent strain dose (no. of bacterial cells)	no. of tularemia fatalities
				from tularemia	from radiation sickness and other causes	total			
Irradiation before the injection of the vaccine strain:									
504 hr before	20	400	50	4	10	14	6	100	0
360 hr before	20	400	50	1	8	9	11	100	4
192 hr before	30	400	50	8	8	16	14	100	1
24 hr before	30	400	50	11	14	25	5	100	1
Irradiation after the injection of the vaccine strain:									
48 hr after	20	400	50	8	5	13	7	100	2
168 hr after	20	400	50	2	7	9	11	100	6
Control	20	—	50	1	1	2	18	100	4
"	—	—	—	—	—	—	3	1	3
"	—	—	—	—	—	—	3	0,1	1

followed by inhibition of the development of immunity in one of the 2 tests (see Table 2).

Thus, dermal vaccination creates a very intense immunity and the process of its development is extremely radioresistant. The x-ray doses which produced an acute radiation sickness (with a decrease in the white cell count, 700-900 per mm^3), and caused death to a large number of animals, produced only a very slight inhibition of the development of immunity in infection with a massive dose of virulent tularemia microorganisms (1000-10,000 DCLM).

As will be seen from Tables 1 and 2 the degree of inhibition of the development of immunity in irradiated animals did not coincide with the degree of distinctness of the inflammatory reaction at the site of vaccination. Thus, irradiation 24 hr after vaccination did not weaken the inflammatory reaction at the site of vaccination but reduced the intensity of postvaccinal immunity.

From Tables 1 and 2 it will also be seen that in the presence of a radiation syndrome there is no definite parallelism between the intensity of the synthesis of specific antibodies as a result of vaccinal infection and the intensity of postvaccinal immunity except in guinea pigs vaccinated 24 hr after irradiation; in these, the antibody titre as compared with the control showed a certain fall and was accompanied by a decrease in the intensity of immunity. At the same time the inhibiting action of ionizing radiation on the development of postvaccinal immunity was associated with the inhibition of the allergic reorganization of the organism (see Table 2).

In all the experimental and control guinea pigs which had survived the immunity tests and were killed 720 hr after infection with the virulent strain B. tularense, no pathological lesions characteristic of tularemia in guinea pigs were observed in the internal organs. Nevertheless, in all the irradiated animals (with the exception of one guinea pig vaccinated 336 hr after irradiation) severe inflammation and purulent melting of regional lymphatic nodes characteristic of tularemia were found whereas among the vaccinated control guinea pigs these lesions were found only in 5 of the 8 animals.

Biological tests on mice showed that the organs of 4 of the 8 vaccinated control guinea pigs 720 hr after undergoing immunity tests, contained virulent tularemia bacteria; moreover, of 18 vaccinated guinea pigs which had been subjected to irradiation, virulent bacteria in the internal organs were found in only 2 animals (see Table 2). From these findings we may assume that inhibition of postvaccinal immunity provoked by the action of ionizing radiation is associated not with the suppression of the ability of the organism to render harmless virulent tularemia bacteria but with some other factors.

Table 3 embodies the results of the test carried out to study the action of x-ray irradiation on the development of immunity in white mice.

As will be observed from the data in Table 3, a radiation dose of 400 r which provoked death in 40-50% of the animals produced an acute exacerbation of the

vaccinal tularemia infection; this was particularly noticeable in mice irradiated 24 and 48 hr after the injection of the vaccine strain. On testing the immunity from a dose of virulent tularemia bacteria 100 DCLM the death rate from tularemia in mice irradiated at different periods before vaccination was no greater than in the vaccinated control animals which had not been irradiated.

Similar results were obtained for mice irradiated 48 hr after the injection of the vaccine strain. A distinct reduction in the immunity was observed only in animals irradiated 168 hr after injection of the vaccine strain.

Thus, our findings show that the process of the development of postvaccinal immunity by immunization with live tularemia vaccine has considerable radioresistance. These results likewise prove the absence of a definite parallelism between the ability of x-rays to provoke inhibition of immunity and their effect on the intensity of a number of immunobiological reactions (antibody synthesis, inflammatory reaction at site of vaccine injection). A more exact indicator of the degree of inhibition of immunity in irradiated animals is the allergic reaction to tularin injection.

SUMMARY

The process of formation of postvaccinal immunity against tularemia after immunization with the living tularemia vaccine is very radioresistant. Experiments were performed on guinea pigs. These animals were irradiated with various x-ray doses, ranging from 10-20 (30) LD to 50-60 (30) LD, at different periods before the vaccination and 24 hr after it. Such irradiation caused only a moderate reduction in intensity of the postvaccinal immunity. The process of postvaccinal immunity formation was found to be even more radioresistant in white mice, in which irradiation provoked an acute exacerbation of the vaccinal infection. The authors compare the ability of the irradiated and normal organisms to form immunity and the intensity of immunobiological reactions (antibody synthesis, inflammatory reaction at the site of vaccine injection, the ability of the tissues to render the virulent Past. tularensis harmless, allergic reaction to tularin).

LITERATURE CITED

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* Original Russian pagination. See C.B. translation.