VACCINAL TULAREMIC INFECTION IN GUINEA PIGS AFFECTED BY RADIATION SICKNESS

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(Received May 17, 1958. Presented by Active Member AMN SSSR N.N. Zhukov-Verezhnikov)

It is known that ionizing radiation causes a lowering of the resistance of the body to pathogenic microorganisms. There are also indications that during irradiation the virulence of the pathogenic and nonpathogenic organisms present in the body may be increased [1, 2]. The problem accordingly arises of the influence of ionizing radiation on the course of infections caused by the administration of live vaccines: may not aggravation of a vaccinal infection take place during radiation sickness and, in particular, may reversion of a vaccinal strain occur? This question is one of great urgency.

For the experimental study of this problem, a suitable model is vaccinal tularemic infection. A vaccinal strain of B. tularense, injected into white mice, is known to posses residual virulence, whereas in guinea pigs and rabbits, as in man, it causes a benign infectious disease. We have previously shown [3] that irradiation of white mice with x-rays causes a sharp lowering of their resistance to the vaccinal strain of B. tularense. A similar lowering is observed as a result of the action of even sublethal doses of x-rays, which becomes more marked with increase of the dose of radiation, and this effect may be noted, moreover, whether the animals are irradiated before or after vaccination.

In the present investigation, we attempted to discover if the development of radiation sickness causes aggravation of a vaccinal tularemic infection in guinea pigs, which are less susceptible to the vaccine strain of B. tularense.

We made parallel studies of the characteristics of vaccinal tularemic infection in response to the action of different doses of ionizing radiation.

EXPERIMENTAL METHOD

Guinea pigs were given a single total irradiation from an RUM-3 x-ray apparatus. Conditions of irradiation: voltage 190 kv, current 10 ma; filtres 0.5 mm Cu and 1 mm Al; distance from the anode of the tube 40 cm; dosage rate of radiation on the body surface of the animals 33-34 r/ min.

Altogether, 4 series of experiments were carried out. In the different series, the dose of radiation varied from 170 to 380 r, and the dose of the vaccinal strain from 10,000 to 10^9 organisms. Irradiation was carried out either 24 hours before the subcutaneous or intradermal (4th experiment) injection of the bacteria or 24 hours after injection. The vaccinal strain of B. tularense was isolated from dry tularemia vaccine from the N. F. Gamaleya Institute of Epidemiology and Microbiology of the AMN SSSR (series No. 458, control No. 732). In experiment No. 3, the guinea pigs were immunized with a restored vaccinal strain Gaiskii No. 15, generously made available to us by Prof. N. G. Olsuf'ev.

TABLE 1

The Effect of Irradiation on the Mortality and Immunological Reaction of Guinea Pigs Inoculated with a Vaccine Strain of B. tularense

Conditions of the experiment	Experiment No. 1									Experiment No. 2								Experiment No.3						
	dose of bacteria	dose of radiation, r	Total number of animals	number of animals dying	allergic reaction (mean diameter, in mm) days after inoculation			agglutinin titer days after inoculation		dose of bacteria	dose of radiation, r	total number of animals	number of animals dying	allergic reaction (mean diameter, in mm) days after inoculation				agglutinin titer	dose of bacteria	dose of radiation, r	total number of animals	number of animals dying	allergic reaction (mean diameter, in mm) days after inoculation		agglutinin titer
					13	20	21	13	20					14	19	26	32						9	20	30	
Irradiation 24 h. before inoculation	10 000	220	6	3	4	2	2	1 : 12	1 : 25	100 000	380	8	6	—*	—	—	—		10^8	290	6	3	0,5	8	1 : 133
Irradiation 24 hours after inoculation	10 000	220	12	6	6	6	7	1 : 63	1: 110	100 000	380	8	4	3,5	5,5	1:35	1 : 20		10^8	290	8	5	0,7	7	1 : 107
Control-inoculation	10 000	—	6	0	8	10,5	11	1 : 90	1: 146	100 000	—	8	0	9,0	9,5	1:50	1 : 45		10^8	—	3	0	2,5	7,2	1 : 120
Control-irradiation	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		—	290	6	2	—	—	—
Irradiation 24 h. before inoculation	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		—	170	5	0	2,7	6,4	1 : 113
Control-irradiation	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		10^8	170	6	0	—	—	—

*Of the 8 experimental guinea pigs, six died and two were killed in the course of the first 10 days after irradiation.

Studies were made of the mortality of the animals, the white blood changes, the specific agglutinin titer, the allergic reaction to tularin, the reaction of the regional lymphatic glands and the weight changes. All animals which died were examined post mortem, including morbid anatomical studies of the cadaver, microscopic examination if impressions of organs for the presence of bacteria, and cultures of the spleen on agar and on solid yolk medium.

EXPERIMENTAL RESULTS

Irradiation did not aggravate the vaccinal infection in any experiment. In subcultures from the dying animals on a solid yolk medium, no strains of B. tularense could be isolated; at autopsy no changes characteristic of tularemia were found. Hence, none of the doses of radiation given (from 170 to 380 r) gave rise to aggravation of the vaccinal tularemic infection resulting from injection of various doses of the vaccinal strain. The results of the three experiments are summarized in Table 1.

The animals died as a rule from radiation sickness. With a dose of radiation of 380 r, causing death of 50-70% of guinea pigs, and after injection of 100,000 organisms of a vaccinal strain of B. tularense, nearly all the animals died on the 8th day after irradiation, showing the characteristic picture: the peritoneal cavity was engorged with blood, multiple hemorrhages were observed in the mucous and serous membranes, and in the surviving animals, focal or diffuse epilation was observed.

TABLE 2

The Effect of Vaccinal Tularemic Infection on the Leucocyte Count of Guinea Pigs during Radiation Sickness (experiment No. 3; dose of vaccinal strain, 10^8 organisms)

Conditions of the experiments	Number of guinea pigs	Mean leucocyte count							
		before irradiation	after irradiation, 3-5 hours	1 day	7 days	10 days	14 days	21 days	31 days
Irradiation with 290 r 24 hours after inoculation	8	7,312	8,577	5,200	2,400	2,550	5,250	8,700	7,750
Irradiation with 290 r 24 hours before inoculation	6	8,175	6,162	3,800	2,875	3,683	4,450	8,175	6,875
Irradiation with 290 r (control)	6	7,375	6,712	4,175	1,980	3,700	4,575	8,300	9,800
Irradiation with 170 r	6	8,075	8,950	6,675	5,610	6,987	6,000	9,525	8,475
Irradiation with 170 r (control)	5	8,250	9,325	5,612	3,900	5,475	4,715	7,188	9,500
Inoculation without irradiation (control)	3	7,900	10,025	9,438	10,250	10,025	10,075	9,582	7,950

Note. In the vaccinated unirradiated guinea pigs, the results were determined on days corresponding to those for the irradiated animals.

With a radiation dose of 290 r, causing the death of 20-30% of animals, and after injection of 10^8 organisms of the vaccine strain, hemorrhagic manifestations were also observed at autopsy of the majority of the dying animals; in 2 guinea pigs, enlargement of the spleen was found, together with necrotic foci in the liver and hepatization of the lungs, which was evidently associated with aggravation of some form of latent infection as a result of the irradiation.

In the case of irradiation with a dose of 220 r and an infecting dose of 10,000 organisms, the guinea pigs also died as a rule showing marked hemorrhagic features (blood-stained fluid in the peritoneal cavity, massive hemorrhages in the subcutaneous cellular tissue, and the mucous and serous membranes).

In the 4th series of experiments, the guinea pigs were inoculated intradermally with 10^9 organisms of the vaccinal strain 24 hours after irradiation with a dose of 290 r. As a control, animals were used which received the same infecting dose of bacteria but were not irradiated, and also noninfected animals irradiated with a dose of 290 r. 5 guinea pigs were killed on the 2nd, 4th, 6th, 8th, 11th, 15th, 22nd and 32nd days after irradiation (one irradiated but not infected, and two each from the remaining two groups). In this experiment, no morbid anatomical signs characteristic of tularemia in guinea pigs were found in a single dying or killed animal. It was not possible to isolate a strain of B. tularense from the spleen of these animals.

Inoculation with the vaccinal strain had no marked effect on the leucocyte count of the irradiated animals. In both the control irradiated and the inoculated irradiated animals, an obious leukopenia was observed (Table 2). A less marked leukopenia as a result of inoculation of the irradiated animals, was observed only on the 7th day after irradiation in case of inoculation 24 hours after irradiation with a dose of 290 and 170 r.

The character of the action of the radiation on the immunological reactions after inoculation with a vaccine strain of B. tularense, differed according to the dose of radiation and of infecting material used. As may be seen from Table 1, an obvious depression of agglutinin formation was observed only when minimal doses of the vaccinal strain (10,000 organisms) and a large dose of radiation – of the order of 50-70/30 LD. Even under these conditions, however, clear depression of antibody formation was observed only in animals inoculated 24 hours after irradiation. In the guinea pigs inoculated with vaccinal strain 24 hours before irradiation, obvious depression of agglutinin formation was not observed in any experiment.

The process of allergic reorganization of the animal was found to be more labile. Some degree of depression and retardation of this process as a result of irradiation was observed in all three experiments, most clearly after inoculation with the minimum dose of infecting material used (10,000 organisms).

Some degree of depression of the allergic reaction to tularin was also found in the guinea pigs inoculated 24 hours before irradiation; this process was, however, most clearly seen in case of inoculation 24 hours after irradiation (see Table 1).

Irradiation caused delay in the reaction of the regional lymphatic glands to injection of the vaccinal strain, which we detected by the method of palpation.

From 33 to 37 days after injection of the vaccinal strain, when the agglutin in titer fell to 1:20-1:40, irradiation with a dose of 380 r failed to elicit any anamnestic increase in antibody titer (the agglutinin titer was determined 6 hours and 1, 2, 3, 6 and 7 days after irradiation); it either was unchanged or slightly decreased.

The results obtained showed that, during inoculation with living bacteria of a vaccinal strain of B. tularense, irradiation has a more demonstrable action on the allergic reorganization of the animal than on atibody production. Evidently after inoculation with living bacteria, the mechanisms responsible for the allergic reorganization are more radiosensitive than those responsible for the production of antibodies. Although depression of the process of development of infective allergy is more marked in case of irradiation given 24 hours before inoculation, it is, nevertheless, observed also when irradiation is given 24 hours after inoculation with the vaccinal strain. At the same time, ionizing radiation has absolutely no effect on antibody formation when irradiation is given 24 hours after inoculation, and only in special conditions does it cause a slight depression of antibody formation when irradiation is given 24 hours before inoculation.

SUMMARY

Different doses of x-rays (from sublethal to 50-70 LD) administered 24 hours before the vaccination or 24 hours after it do not cause exacerbation of the vaccinal tularemic infection in guinea pigs immunized with live microbes of vaccinal B. tularense strain. As shown, no reversion of the vaccinal B. tularense strain is possible in these conditions. In our experiments irradiation had a more pronounced effect on the allergic reconstruction of the body than on the formation of the antibodies, and this is probably due to a more pronounced radiosensitivity of the mechanisms effecting the process of allergization of the body as compared to those of antibody formation.

LITERATURE CITED

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