IMMUNOLOGY

ENHANCEMENT OF PROTECTIVE PROPERTIES OF ANTITETANUS SERUM AFTER ITS IRRADIATION WITH X-RAYS

R. V. Petrov (Moscow)

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The possibility of improving the quality of therapeutic and prophylactic preparations by exposure to ionizing radiation is of considerable interest. Quite encouraging results have been obtained by ultraviolet irradiation of such preparations. Vaccines prepared by ultraviolet irradiation of bacteria [3] had more powerful immunogenic properties than those obtained by thermal treatment. The toxicity of quinine may be raised or lowered by ultraviolet irradiation, according to the dosage applied [4].

N. V. Kurilov has shown that the leucocytosis appearing in rabbits after injection of horse serum is more pronounced if it has been previously exposed to irradiation with x-rays [1]. A.Frank [5] has reported that the avidity of serum is raised by x-ray irradiation.

In this paper we present evidence of the enhancement of the protective power of antitoxic antitetanus serum after x-ray irradiation. The protective properties of the serum were tested on white mice infected with B. tetanus.

EXPERIMENTAL METHODS

We used antitetanus serum "Diaferm 3 LEM AMN", Series 1713, which was irradiated with a "Dermomobile" generator in open cuvettes, at 30 kv, 20 ma, and 15,000 r/min, without a filter. The distance from the source of radiation to the middle of the layer of serum, which did not exceed 0.5 cm, was 10 cm. The serum was used during the 24 hours following its irradiation, and was stored under the same conditions as the ordinary serum (in the dark at 12-14°).

Mice weighing 18-22 g were given a certainly fatal dose of B. tetanus, Strain No. 280 (0.05 ml of 10% calcium chloride was injected into a thigh muscle, followed immediately by 0.05 ml of a 10-fold dilution of a 24-hour culture of the pathogen, grown in Kitt-Tarozzi medium), and by 1 minimum protective dose of serum (a single injection of 0.1 ml = 125 units, into the thigh muscles of the opposite leg).

EXPERIMENTAL RESULTS

A typical picture of ascending tetanus developed in mice not given antitetanus serum. A few hours after the inoculation, the mice tended to drag the injected leg, although otherwise their behavior was quite normal. By the following day the inoculated leg could not be moved, the tail was rigid, and the other hind leg showed only limited mobility. By the second day the hind legs, pelvic region, and tail were paralyzed, and the tail was twisted into a spiral; rigors appeared from time to time, and some of the animals died. All the mice were dead on the third day.

A single injection of antitoxic serum did not ensure survival of all the inoculated mice; the survival time of those mice which died was, however, longer than for unprotected ones.

In all, 3 series of experiments were performed; the results were uniform, and are presented in Table 1.

It appears from the data of Table 1 that ordinary serum protected 7% of mice inoculated with tetanus, whereas 35.3% of those given irradiated serum survived, and the survival time of those eventually dying was prolonged.

TABLE 1

Survival Rate of Mice Infected with Tetanus, and Given Irradiated and Ordinary Antitetanus Serum

Serum	Number of mice	Infective dose (ml of 24-hour culture)	Dose of serum (units)	Number of mice dead by the 3rd day	6th day	8th day	14th day	Total number of mice dying	% survival rate
Irradiated with 510,000 r	85	0.005	125	—	6	36	13	55	35.3
Ordinary	85	0.005	125	—	20	46	13	79	7
Not given	25	0.005	—	25	—	—	—	25	0

It may be supposed that the enhancement of the protective action of antitetanus serum after irradiation is due to increase in its content of antibodies. It is, however, also possible that irradiation enhanced its non-specific stimulating effect on the defensive mechanisms of the organism.

In order to elucidate this problem, we performed a number of special experiments, in which we studied the effect of irradiated serum on the intensity of certain factors of natural immunity, from the leucocyte count and phagocytic activity of uninfected mice given irradiated and ordinary serum (Table 2).

TABLE 2

Leucocyte Count and Phagocytic Activity of the Leucocytes in the Peritoneal Cavity of White Mice After Injection on the Previous Day of 0.2 ml of Irradiated and Ordinary Antitetanus Serum

Serum	Number of mice	Leucocyte count			Phagocytary index		
		mean	maximum	minimum	mean	maximum	minimum
Irradiated with 510,000 r	10	14 300	17 800	7 800	28.4	31.0	26.2
Not irradiated	10	9 550	12 750	7 000	24.1	27.9	21.0
Not given	10	8 450	10 100	6 600	19.5	21.6	17.4

It appears from the data of Table 2 that injection of irradiated serum is followed by a more pronounced leucocytosis and by a greater rise in phagocytary activity of the leucocytes than after injection of ordinary serum.

It follows that the stimulating effect of irradiated serum on the non-specific protective mechanisms of the body is greater than is that of ordinary serum. This is particularly striking for phagocytosis, since the mice were given antitetanus serum, whereas the phagocytary activity was measured with respect to staphylococci.

We also examined the protective action of irradiated serum under conditions excluding its specific action. With this object, we injected irradiated antitetanus serum into mice infected with typhoid fever germs, and irradiated antigangrene serum into mice inoculated with tetanus germs (Tables 3 and 4).

As appears from Table 3, introduction of irradiated antitetanus serum doubles the survival rate of mice inoculated with E. typhi, as compared with those receiving ordinary serum. The raising of the resistance of mice to E. typhi infection by previous administration of antitetanus serum can only be a result of enhancement of non-specific resistance of the organism.

The next experiment shows that greater protection is given to mice infected with tetanus by previous injection of irradiated serum, pointing to greater stimulation of non-specific resistance of the organism. In this experiment one group of mice was inoculated with Cl. tetani, and then given 0.1 ml (125 units) of antitetanus serum mixed

with 0.1 ml of anti-gas gangrene serum, while a second group received 0.1 ml of antitetanus serum + 0.1 ml of irradiated (510,000 r) anti-gas gangrene serum.

TABLE 3

Resistance of White Mice to Typhoid Fever Infection After Injection of 0.2 ml of Irradiated or Unirradiated Antitetanus Serum

Serum	No. of mice	Dose of typhoid germs given (millions)	No. of mice dying	No. of mice surviving
Irradiated (510,000 r)	10	500	2	8
Not irradiated	10	500	6	4
Not given	10	500	10	0

TABLE 4

Effect of Irradiated Anti-gas Gangrene Serum on the Protective Action of Antitetanus Serum

Serum	No. of mice	Dose of Cl. tetani (ml of 24 hr culture)	Dose of antitetanus serum (units)	Dose of anti-gas gangrene serum (ml)	No. of mice dying within 14 days	No. of mice surviving
Unirradiated antitetanus serum + irradiated anti-gas gangrene serum	10	0.005	125	0.1	3	7
Unirradiated antitetanus serum + unirradiated anti-gas gangrene serum	10	0.005	125	0.1	7	3
Not given	10	0.005	—	—	10	0

It appears from the data of Table 4 that irradiated anti-gas gangrene serum considerably enhances the protective effect of antitetanus serum, as compared with ordinary anti-gas gangrene serum. Thus a mixture of unirradiated specific serum with irradiated non-specific serum gives an effect equivalent to that of irradiated specific serum alone.

The superior protective power of irradiated antitetanus serum is thus ascribable to enhancement of its non-specific action.

It is not possible, without further experimental study, to discuss the mechanism whereby x-ray irradiation of serum leads to enhancement of its non-specific stimulation of the defensive processes of the organism. It can, however, be said that this action is probably not connected with modification of antitoxins, since globulins are particularly resistant to the action of ionizing radiation.

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