THE AUTOIMMUNE PROCESS IN ACUTE RADIATION SICKNESS AND THE EFFECT OF ANTIBIOTICS ON ITS DEVELOPMENT

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The important role of autosensitization in the development of radiation sickness has been shown by several investigations [2, 9, 14]. Autoantibodies were first detected in the blood of irradiated animals by the complement fixation reaction 3-4 weeks [8, 12], and by Wannier's method on the third day [11] after irradiation. However, during the experimental development of methods of combined treatment of radiation sickness, too little use has been made of desensitizing preparations. No investigation has been made of the possible desensitizing effect of preparations and, in particular, antibiotics used for the treatment of radiation sickness. Meanwhile, reports have recently appeared suggesting that the therapeutic effect of antibiotics in radiation sickness may be due to some extent to their action of metabolic processes and on the reactivity of the host [4, 5, 10] quite independently of their antibacterial properties [6, 7]. There are some reports [15, 16] that antibiotics are able to suppress the phenomena of anaphylaxis.

In face of these facts, the authors decided to make further investigation in order to determine more exactly the times of appearance of autoantibodies to various organs of irradiated rabbits, and also to study the effect of antibiotics on autoimmune processes in radiation sickness.

EXPERIMENTAL METHOD

Investigations were conducted on 25 rabbits weighing 2.0-2.5 kg and receiving whole-body x-ray irradiation in a dose of 600 R. The animals were irradiated on the RUM-11 apparatus, with a voltage of 180 kV, current 200 mA, filter 0.5 mm Cu, tube 20 · 20 cm, skin-focus distance 50 cm, and dose rate 46.1 R/min.

Penicillin, streptomycin, and oxytetracycline (in doses of 40,000 units/kg body weight, intramuscularly, in each case) and chloramphenicol (0.05 g by mouth) were used in the experiments. These antibiotics were given 1 h before irradiation, and subsequently once daily for 30 days.

The action of the antibiotics on the autoimmune processes in acute radiation sickness was judged from the titer of autoantibodies against the liver, kidney, spleen, lymph glands, intestine, heart, and blood vessels. Irradiated animals thus receiving antibiotics served as controls.

The autoantibodies were determined before irradiation (rabbits in which no autoantibodies were found were used for the experiment) and on the 2nd, 5th, 10th, 15th, and 30th days after irradiation by Wannier's method as the most sensitive [1, 10], and also by precipitation of the "nonprecipitating" antibody-antigen complex with ammonium sulfate [13]. This last method was developed by two of the authors (A. I. N. and M. Sh. M.) for use in the detection of autoantibodies against organs, and its was used in the following variants. Test antigens were prepared from organs and tissues of a healthy rabbit and a rabbit irradiated in a dose of 600 R (sacrifice on the 5th day after irradiation). Immediately after the animal had been killed the organs were washed free from blood and the intestine from contents with physiological saline. The tissues were then homogenized in a tissue microblender at 500 rpm with physiological saline in the ratio 1:10. The preparations were kept for 18-20 h at 4°, when the residue was separated by centrifugation. The supernatant fluid was poured into a jar with a ground stopper, two volumes of chloroform were added, and the contents of the jar were vigorously mixed for 2 h on a shaker, after which they were centrifuged to give a transparent solution, which was used as the antigen.

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Effect of Antibiotics on Titer of Autoantibodies against Organs of Rabbits Irradiated in a Dose of 600 R

Expt. No.	Antibiotic	Day of test after irradiation	Titers of autoantibodies by reaction with test antigens from organs of healthy animals: liver	kidney	large intestine	small intestine	spleen	lymph gland	heart	aorta
1	Penicillin	2	0	0	4 (5)	0	0	2 (5)	0	0
		7	0	0	2—4 (5)	0	0	2 (5)	0	0
		15	4—8 (5)	2 (5)	16 (5)	2—4 (5)	4 (5)	4—8 (5)	2 (5)	2 (5)
		30	4—8 (5)	2—4 (5)	16 (5)	2 (5)	2 (5)	2 (5)	0	0
2	Streptomycin	2	0	0	2— 4 (5)	0	0	2—4 (5)	0	0
		7	0	0	2 (5)	0	0	2 (5)	0	2 (1)
		15	2 (5)	2 (5)	16 (5)	2—4 (5)	4 (5)	4—8 (5)	2 (5)	2 (5)
		30	2 (5)	2 (5)	8—16 (5)	2 (5)	2 (5)	0	0	0
3	Oxytetracycline	2	0	0	2 (5)	0	0	2—4 (5)	0	0
		7	2 (5)	0	2 (5)	0	0	2 (5)	0	0
		15	2—4 (5)	2 (5)	16 (5)	4 (5)	4 (5)	8 (5)	2 (5)	2 (5)
		30	4 (5)	32 (5)	8—16 (5)	2 (5)	2 (5)	2 (2)	0	0
4	Chloramphenicol	2	0	0	2 (5)	0	0	2—4 (5)	0	0
		7	2 (4)	0	2 (5)	0	0	2 (5)	0	0
		15	4 (5)	2 (5)	16 (5)	2—4 (5)	4 (5)	4—8 (5)	2—4(5)	2 (5)
		30	4 (5)	2 (5)	16 (5)	2 (5)	2 (5)	2 (1)	0	0
5	Physiological saline (control)	2	2 (1)	0	2—8 (5)	2 (5)	0	2—4 (4)	0	0
		7	2—4 (5)	2 (1)	4—8 (5)	2—4 (2)	2 (2)	2—16 (5)	0	0
		15	2—8 (5)	2—4 (5)	16 (5)	2—4 (5)	4 (5)	4—16 (5)	4 (5)	2 (5)
		30	2—8 (5)	2 (5)	8—16 (5)	2 (5)	2 (5)	2 (5)	2 (5)	0

Note. Reciprocals of the titers of the antibodies are given in the table.
The number of animals in whose blood autoantibodies were found is given in parenthesis (of five animals taken in the experiment).

Antigens of the liver, kideny, spleen, lymph glands, heart, aorta, and large and small intestine of the irradiated and unirradiated rabbits were used in the investigation.

The autoantibodies and their titer were determined as follows. To determine the titer of autoantibodies the test sera were diluted with physiological saline (pH 7.2-7.6) in the ratios 1:2, 1:4, 1:8, 1:16, and so on. Next the serum was poured into two cells 3.077 mm in diameter, in volumes of 1 ml each, and the optical turbidity was determined. For this purpose, the cell with physiological saline was placed in the left compartment of the FEK-N57 photoelectric colorimeter, and the optical density of the control and the test serum was determined in relation to it. Next, 0.1 ml of antigen was added to 1 cell, while to the other was added (the control sample) the same volume of horse serum, equal in protein content to the test antigen. The contents of the cells were mixed and determined nephelometrically. Next, to each cell was added 0.1 ml of saturated ammonium sulfate solution, and the contents were mixed and again determined nephelometrically (approximately 2 min after addition of the ammonium sulfate solution). This procedure with ammonium sulfate was repeated until no increase was obtained in the turbidity of the test serum. In the presence of autoantibodies, turbidity appeared sooner in the cell to which the test antigen was added. In the absence of autoantibodies, turbidity appeared simultaneously in both cells and the readings were identical.

The maximal dilution of the serum giving a positive reaction with the test antigens was taken as the titer of the autoantibodies.

By means of Wannier's reaction, autoantibodies were detected on the 3rd day after irradiation of the rabbit against the liver, the large and small intestine, the spleen, and the lymph glands; after 15 days autoantibodies were found against all organs taken in the experiment. The reaction was positive with antigens prepared from the organs of both the irradiated and the healthy animal.

EXPERIMENTAL RESULTS

The results of detection of the autoantibodies by the method of salting out the antigen – antibody complex followed by nephelometric determination, and the effect of the tested antibiotics on their titers are shown in the table.

Since the titers of autoantibodies were basically similar when tissues of the healthy and irradiated animals were used as test antigen, only the results of experiments with test antigens prepared from the organs of the healthy animals are given in the table.

It is clear from the table that the method used enables autoantibodies against the liver, the large and small intestine, the spleen, and the lymph gland to be detected 2-5 days after irradiation.

By the 7th-15th days the titer of the autoantibodies showed a sharp increase. It was observed that the higher titer of antibodies was found in antigens from the liver and the lymph glands, i.e., organs with a protective function. On the 30th day of the experiment the titer of autoantibodies fell. However, it remained comparatively high for the organs with barrier function.

These results possibly extend our information concerning the mechanism of development of infectious complications in irradiated animals.

Consequently, by means of the method of detecting autoantibodies developed by the authors, not only can the early signs of autosensitization be detected, but dynamic observations may also be made on the appearance and the increase in the titer of autoantibodies against different organs in radiation sickness.

Hence, in animals, during the first week after exposure to ionizing radiation, autoantibodies were formed against different organs and their titer increased as the radiation sickness developed.

The view is fully shared that autoantibodies in the irradiated organism play an important role in the pathogenesis of radiation sickness.

It is further evident from the table that the antibiotics, in the doses in which they were given, did not prevent the development of autosensitization arising under the influence of x-rays. At the same time, they appreciably depressed the formation of autoantibodies against various organs of irradiated animals. The most active in this respect were streptomycin and oxytetracycline. For instance, administration of streptomycin lowered the titer of antibodies against the liver and lymph glands several times. The remaining antibiotics had a less marked effect in lowering the titer of autoantibodies. Characteristically, the administration of antibiotics prevents the production of antibodies against normal tissue.

Importance is attached to the fact that the titer of autoantibodies against the liver, spleen, and lymph gland – i.e., against the system of organs performing barrier function, fell considerably. Evidently this may to some extent explain the beneficial effect of antibiotics on the course of radiation sickness.

It also follws from the table that the titer of autoantibodies fell faster in the irradiated animals receiving antibiotics than in the controls. This was seen most clearly on the 30th day of the experiment, especially by the disappearance of autoantibodies against normal organs and tissue.

Hence, antibiotics affect the dynamics of the autoimmune processes arising in acute radiation sickness by depressing the formation of autoantibodies aginst the animal's organs. The mechanism of this phenomenon may evidently be attributed to competitive action of the antibiotics with the autoantigen.

SUMMARY

Materials are presented on the formation of autoantibodies during irradiation of rabbits in a dose of 600 R and the influence produced on their titer by antibiotics (penicillin, streptomycin, oxytetracycline, chloramphenicol).

Methods are described for the detection of autoantibodies by means of sedimenting the antigen-antibody complex with subsequent nephelometry. Autoantibodies to the liver, spleen, kidney, lymphatic glands, aorta, heart, large and small intestine were determined.

It has been found that in rabbits, on the second day after their exposure to ionizing radiation, there form autoantibodies, whose titer increases with the progression of radiation sickness.

Antibiotics inhibit the development of the autoimmune process.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of the first issue of this year.
