EFFECT OF ANTISERA SPECIFIC FOR IRRADIATED MALIGNANT TISSUES ON THE GROWTH OF EXPERIMENTAL TUMORS IN ANIMALS SUBJECTED TO IRRADIATION

COMMUNICATION I. CAPACITY OF IRRADIATED ANTIGENS TO PROVOKE THE FORMATION OF ANTIBODIES IN ANIMALS OF VARIOUS SPECIES

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It is known that during the irradiation of animals the resistance of the organism decreases, changes occur in the metabolism, etc. [1-7, 11-19]. Earlier, we reported on changes in the antigenic and biological properties of Ehrlich's mouse malignancy and the Brown-Pearce rabbit tumor under the influence of roentgen ray irradiation in vitro [8-10].

Taking into account these data, as well as the widespread use of penetrating radiation in oncology, we decided to fully study the capactiy of antigens from malignant tissue, subjected to the action of roentgen rays, to provoke the formation of antitumoral antibodies, and to clarify the effect of the resultant serum on the growth of the tumor in the irradiated animals.

In this report we present the results of investigations designed to obtain antiserum against irradiated antigens from malignant tissues in various species of animals. In the work we used tissues from Ehrlich's mouse tumor, the Brown-Pearce rabbit carcinoma, and human stomach cancer.

METHOD

The experiments were carried out on rabbits, sheep and horses. All the animals were immunized with aqueous saline extracts of the tumor tissues indicated above. Dosage of the antigens was regulated on the basis of the protein content; the amount of protein was determined by Conway's nitrogen method. Half of the antigens used for the immunizations were subjected to the action of roentgen rays in vitro in a dose of 2000 r, while the other half were not irradiated and were used as the control. The irradiation was performed with the aid of the RUT-260-20 (RUM-7) apparatus, with a voltage of 60 kv, current intensity of 5 ma, a 0.1 mm Al filter, focal length of 75 mm, and duration of radiation of 15 sec.

The rabbits were immunized with antigens from the cells of Ehrlich's mouse carcinoma (ascitic form), the sheep with antigens from the Brown-Pearce rabbit carcinoma, and the horses with antigens from the tissues from human stomach cancer. Immunization of the rabbits was carried out intravenously, 4 times a day, in a dose of 12 mg of protein per course of immunization. Two groups of rabbits were set up in the experiments, 6 animals in each. The first group received the irradiated antigens, the second—unirradiated.

TABLE 1

Results of the CF Reaction of Sera from Rabbits Immunized with Irradiated Ascitic Ehrlich Cells

Serum dilution	Serum no.	Immunization	Reaction with antigens of ascitic Ehrlich cells: irradiated	Reaction with antigens of ascitic Ehrlich cells: unirradiated	Reaction with antigens: mouse spleen	Serum No.	Immunization	Reaction with antigens of ascitic Ehrlich cells: irradiated	Reaction with antigens of ascitic Ehrlich cells: unirradiated	Reaction with antigens: mouse spleen
1:100	97	with antigen from irradiated ascitic Ehrlich cells	++++	++++	++++	84	with antigen from unirradiated ascitic Ehrlich cells	++++	++++	++++
1:200	97		+++	++++	+++	84		+++	+++	++++
1:400	97		+++	+++	++	84		++++	+++	+++
1:800	97		++	+++	+	84		+++	++	++
1:1000	97		±	++	H	84		++	+	+
1:100	79		++++	++++	++++	87		++++	++++	++++
1:200	79		+++	+++	+++	87		+++	+++	+++
1:400	79		++	+++	++	87		+++	++	++
1:800	79		+	++	±	87		++	+	+
1:1000	79		±	+	H	87		+	H	H
1:100	88		++++	++++	++++	1 602		++++	+++	+++
1:200	88		+++	+++	++	1 602		+++	++	++
1:400	88		++	++	+	1 602		++	±	+
1:800	88		+	++	H	1 602		+	H	H
1:1000	88			+	H	1 602			H	H
1:100	3 659		++++	++++	+++	2 562		++++	++++	++++
1:200	3 659		+++	+++	++	2 562		+++	++++	+++
1:400	3 659		++	+++	+	2 562		++	++	+++
1:800	3 659		+	++	H	2 562		++	+	++
1:1000	3 659		H	+	H	2 562		+	+	+
1:100	2 395		++++	++++	++++	3 305		++++	++++	++++
1:200	2 395		+++	++++	+++	3 305		+++	+++	+++
1:400	2 395		++	+++	++	3 305		+++	++	+++
1:800	2 395		+	++	±	3 305		++	+	++
1:1000	2 395		+	++	H	3 305		++	+	+
1:100	2 648		+++	++++	++++	89		+++	++++	++++
1:200	2 648		++	+++	+++	89		+++	+++	+++
1:400	2 648		+	+	+	89		++	++	++
1:800	2 648		H	±	H	89		+	±	+
1:1000	2 648		H	H	H	89		+	H	H

The sheep were immunized intramuscularly with five injections of antigens—every 6th day, in an increasing dose based on the following scale: 1st injection—1.5 mg of protein per kg of weight of the sheep, 2nd—3 mg per kg of weight, 3rd—4.5 mg per kg of weight, 4th—6 mg per kg and 5th injection—7.5 mg per kg of weight. Two of the sheep were immunized with irradiated antigens and two with unirradiated antigens.

The horses were immunized subcutaneously with an injection of antigens according to the same schema as for the sheep, but in different dosages: for the 1st injection we used antigens on the basis of 0.5 mg of protein per kg of weight of the horse, for the 2nd—1 mg per kg of weight, for the 3rd—2 mg per kg of weight, for the 4th—2.5 mg per kg of weight, and for the 5th injection—3 mg per kg of weight. One horse was immunized with irradiated antigens, and the second with untreated antigens.

Prior to immunization, as well as during and after it, blood was drawn from all the animals involved in the experiment and serum prepared. In the rabbits the blood was investigated every third day over the course of 2.5 mo, in the sheep and horses—every 6th day for 2 mo. The sera obtained were tested for complement fixation (CF) at 37°. Irradiation of the antigens for setting up the CF was performed in the same manner as for the immunization. In determining specificity, each serum in the CF reaction was tested with two antigens—from irradiated and unirradiated tumor tissue. As a control for the specificity the reaction was also set up with antigens from normal splenic tissue.

RESULTS

One of the typical protocols is presented in Table 1, showing the data on titration of the rabbit sera obtained on the 9th day following completion of the immunization.

TABLE 2

Results of the CF Reaction of Sera from Sheep Immunized with Irradiated and Unirradiated Brown-Pearce Tumor Tissue

Serum dilution	Serum No.	Immunization	Reaction with tissue antigens: from the Brown-Pearce tumor, irradiated	Reaction with tissue antigens: from the Brown-Pearce tumor, unirradiated	Reaction with tissue antigens: from rabbit spleen	Serum no.	Immunization	Reaction with tissue antigens: from the Brown-Pearce tumor, irradiated	Reaction with tissue antigens: from the Brown-Pearce tumor, unirradiated	Reaction with tissue antigens: from rabbit spleen
1:20	2	with antigen from irradiated Brown Pearce tumor tissue	++++	++++	+++	4	with antigen from unirradiated Brown Pearce tumor tissue	++++	++++	++++
1:40	2		++	+++	+++	4		++++	+++	+++
1:100	2		++	++	++	4		+++	++	++
1:200	2		±	++	+	4		++	±	+
1:400	2		H	+	H	4		+	H	±

TABLE 3

Results of the CF Reaction of Sera from Horses Immunized with Irradiated and Unirradiated Tissues from Human Stomach Cancer

Serum dilution	Serum no.	Immunization	Reaction with tissue antigens: from human stomach cancer, irradiated	Reaction with tissue antigens: from human stomach cancer, unirradiated	Reaction with tissue antigens: from human spleen	Serum no.	Immunization	Reaction with tissue antigens: from human stomach cancer, unirradiated	Reaction with tissue antigens: from human stomach cancer, irradiated	Reaction with tissue antigens: from human spleen
1:20	9	with antigen from irradiated human stomach cancer tissue	+++	++++	+++	12	with antigen from unirradiated human stomach cancer tissue	+++	++	+
1:40	9		++	+++	+	12		++	+	+
1:100	9		±	++	±	12		++	±	±
1:200	9		H	+	H	12		+	H	H

From this table it can be seen that in all cases the sera obtained from the rabbits that had been immunized with antigens from irradiated tissues were not strictly specific and reacted in the CF test with analogous antigens in lower titer than with the natural (unirradiated) antigen. Thus, for example, the serum from rabbit No. 3659 reacted with ++ to a titer of 1:400 of the antigens from the irradiated tissues, while with antigens from the unirradiated tissues a reading of ++ was noted with a dilution of 1: 800. It also follows from Table 1 that sera obtained by immunization with antigens from unirradiated tissues possessed relatively great specificity in the CF test, reacting with urirradiated tumor antigens in higher titers than with irradiated. We acquired comparable data in the other experiments.

The results from titrating the sera of the sheep and horses were seen to be similar to those described above. In Table 2 we show one of the typical protocols for the titration of serum from the sheep.

From Table 2 it can be seen that the sera obtained from immunization with irradiated antigens reacted with a ++ in the CF test when combined with irradiated antigen in a titer of 1:100, while with unirradiated antigen the reaction was ++ in a titer of 1:200. It should be noted that in these experiments, too, the sera from the animals immunized with unirradiated tumor antigens were more specific and reacted with the unirradiated analogous antigens in higher titers.

In titrating the sera from the horses we also obtained less specific sera in the case of immunization of the animals with irradiated tumor tissues (Table 3).

Studies of the dynamics of the formation of antitumoral antibodies showed that throughout the entire course of the experiments the titer of the sera with the irradiated antigens was lower than with the unirradiated ones. Only in isolated cases, at later intervals following the completion of immunization (36th - 40th day), were we able to observe a higher titer of the sera in the CF reaction with antigens from irradiated tissues.

Thus, the results of the investigation using the CF reaction of the immune sera obtained from rabbits, sheep and horses by immunizing the animals with irradiated and unirradiated tissues from various tumors showed that under the influence of ionizing radiation cancer antigens partially lose their immunizing properties and diminish their capacity to react in the CF test with analogous antigens. It is rather probable that this phenomenon is related to a change in the antigenic properties of tumor cells subsequent to the action of radiation, and to the formation of new antigenic structures possessing lower immunogenetic potential.

SUMMARY

The work was devoted to the study of the effect of penetrating radiation, particularly of x-rays, on the ability of the cells of mice Ehrich's cancer, rabbit Brown-Pearce carcinoma and human cancer of the stomach to produce antibody formation in various species of animals. The data obtained point to the fact that x-rays acting in vitro upon the cells of these tumors change their immunological properties. This is manifested in a reduced ability of antisera (obtained by immunization of animals with irradiated tumor cells) to interact with analogous antigens.

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