THE EFFECT OF HOMOLOGOUS HEMOGENETIC CELL TRANSPLANTATION ON ANTIBODY SYNTHESIS IN IRRADIATED ANIMALS

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A. S. Shevelev

Departments of Microbiology and Roentgenology and Radiology, Smolensk Medical Institute
(Presented by Active Member, Academy of Medical Sciences, USSR, V. V. Parin)
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One of the most important tasks of contemporary noninfectious immunology is the search for methods of suppressing the immunological reactivity of an organism, in particular, transplantation immunity and autoimmune reactions. Various methods of both specific [4, 5, 9, 10, 12, 15] and nonspecific inhibition of immunological reactivity have been suggested. Among the latter, one of the most successful is the use of ionizing radiation [1, 3, 11, 13]. However, a defect of this and other similar methods is the brevity of the effect and the necessity of using ionizing radiation or chemical preparations (cortisone, 6-mercaptopurine, etc.) in large, nearly lethal, doses.

New approaches to the study of this problem were outlined during a study of the mechanism of the therapeutic effect of hemogenetic tissue during acute radiation sickness. It was established that during transplantation of hemogenetic cells of animals irradiated with a lethal dose, there is observed a prolonged nonspecific depression of immunological reactions (6-8, 14, 15), particularly during transplantation of homologous cells. However, uniformly fatal doses of radiation were employed in the experiments and while these produced radiochimera they prevented the use of an adequate control and did not permit any conclusions regarding the real influence of transplanted hemopoetic tissue on the synthesis of antibodies in the irradiated animals.

The purpose of this investigation was to explain how the transplantation of homologous hemogenetic tissue (bone marrow and spleen) affects the synthesis of antibodies by animals irradiated with doses causing the death of less than 100% of the irradiated individuals in 30 days and which permitted using as controls not only nonirradiated but also irradiated animals which did not receive injections of hemogenetic tissue.

EXPERIMENTAL METHOD

The majority of the experiments were done on non-inbred white mice (average weight 20-25 g). In one experiment non-inbred white rats (average weight 130-150 g) were used. Only females were used as hemogenetic tissue donors. The recipients were, as a rule, males.

Irradiation was carried out in the RUM-11 X-ray treatment apparatus. Irradiation conditions: voltage 180 kV, current strength 15 mA, 0.5 mm Cu and 1 mm Al filters, focal distance 50 cm, field 20 × 20 cm, dose rate 30 r/min.

A bone marrow cell suspension was obtained by washing femoral, humeral and tibial bones 3-4 times with ice-cold Hank's solution. The spleen cell suspension was prepared by mechanical pulverizing of the tissue with shears with subsequent repeated passage through a syringe without a needle. The obtained suspension was filtered through nylon mesh, after which a determination of the nucleated cells was made and the necessary amount of Hank's solution added. Freshly prepared suspensions which were kept on ice were used for intravenous injection (the first eight hours after irradiation).

Formalin suspensions of typhoid (strain TV_2) or dysentery (Flexner's bacillus) bacteria or sheep erythrocytes were used as antigens. The antigens were injected intraperitoneally within valuous period after irradiation and transplantation. The dose of bacteria was 100-200 × 10^6, sheep erythrocytes-0.2 ml of 20% suspension in physiological solution. Blood for agglutination reactions (from bacterial injections) or lysis (from sheep erythrocyte injections)

Effect of Transplantation of Homologous Spleen Cells on the Synthesis of Antibody by Sublethally Irradiated Rats

Character of spleen cells	Number of rats	Cell dose × 10^6	Radiation dose (in r)	Antibody titer: to typhoid bacteria — 7 days after 1st injection: Me	$\bar{X}$	P	to typhoid bacteria — 7 days after 2nd injection: Me	$\bar{X}$	P	to sheep erythrocytes — 7 days after 1st injection: Me	$\bar{X}$	P
From 5 donors	4	50	300	100	0	>0,05	480	—2,77	<0,05	60	—0,79	>0,05
From 1 donor	5	50	300	20	—3,31[1]	<0,01	320	—2,96[1]	<0,05	10	—3,31	<0,01
From 5 donors after freezing and thawing	5	50	300	40	—0,06	>0,05	Was not determined			40	—0,34	>0,05
From 5 donors	5	200	300	80	—1,4	>0,05	Was not determined			10	—1,23	>0,05
Irradiation control	5	—	300	40	—1,96	>0,05	Was not determined			40	—1,17[1]	>0,05
Immunization control	5	—	—	320	—	—	3 840	—	—	160	—	—

Notes. 1. First injection of dead S. typhosa made immediately after irradiation and transplantation, reinjection—94 days after first. Sheep erythrocytes injected 26 days after transplantation. 2. $\bar{X}$-value of the ordinal criterion $\bar{X}$ with respect to the radiation control. 3. Me—median (presented values, inverted titer). 4. P—degree of reliability.

*Value of X with respect to immunization control.

was taken from the tail vein. Most frequently the reactions were carried out with a mixture of sera, but in some experiments with individual sera. Statistical treatment of the results obtained was carried out using the ordinal criterion $\bar{X}$ (2).

EXPERIMENTAL RESULTS

Transplantation of bone marrow in mice irradiated with a dose causing death of 50-60% of the animals within 30 days, as a rule, prevented death in the first three weeks after irradiation and caused a sharp inhibition of antibody synthesis down to complete areactivity.

In the first experiment, starting with the 4th week, a secondary illness developed in the treated mice, which was manifested in a loss of weight, diarrhea, ruffled fur, emaciation and, finally, death in the period up to 98 days after irradiation. Sharp inhibition of antibody synthesis was observed upon injection of antigen 3 (typhoid bacteria), 22 (sheep erythrocytes) and 66 (Flexner's bacillus) days after irradiation. Similar results were obtained in the second experiment in which mice were subjected to irradiation with the same dose. In this experiment injection of 50×10^6 bone marrow cells prevented early death of the animals in the first three weeks after the radiation effect with subsequent loss of 80% of the mice from secondary disease in the next three months. With injection of typhoid bacteria immediately after transplantation, agglutinins were not found in the treated animals 20 days after immunization, while in the control irradiated animals they were found in a titer of 1 :320, and in the control immunized nonirradiated mice—in a titer of 1 :640. Similar results were obtained with injection of sheep erythrocytes 33 days after irradiation. In the third experiment, using the same dose of radiation, injection of bone marrow in a dose 30×10^6 nucleated cells did not show any effect on the death rate and caused only moderate inhibition of antibody production.

In two of the above experiments some of the irradiated animals were injected with spleen cells ($41\text{-}50 \times 10^6$) which caused a sharp increase in the death rate in the first 30 days and strongly inhibited antibody synthesis.

In the next two experiments the effect of transplantation of bone marrow and spleen cells on antibody synthesis in mice irradiated with a dose causing death of 9-10% of the animals in 30 days was studied. In both experiments transplantation caused an increase in death rate and inhibition of antibody synthesis. In the first experiments we were able to establish that the increase in death rate as a result of transplantation of donor spleen fragments was accompanied by a greater inhibition of antibody synthesis than with the injection

of bone marrow. It was established also that irradiation of the donors with a sublethal dose (200 r) decreased the subsequent death rate in the recipients and counteracted the inhibitory effect of the hemogenetic cells on antibody production during the injection of antigen 54 (sheep erythrocytes) and 83 (typhoid bacteria) days after irradiation. In the second experiment it was found that with injection of typhoid bacteria 4 days after irradiation, the injection of bone marrow cells (24×10^6) of nonirradiated donors, in the same way as the combined intravenous injection of bone marrow (13.6×10^6) and intraperitoneal injection of spleen cells (2.4×10^6) of irradiated donors, caused similar inhibition of antibody synthesis. However, after the second injection of antigen, 72 days after the first injection, the arising (as a result) immunological reaction in all the periods studied was most sharply inhibited in mice receiving the combined injection of bone marrow and spleen cells. The same results were obtained with injection of sheep erythrocytes 90 days after irradiation. With injection of dysentery bacteria 150 days after the beginning of the experiment inhibition of antibody production was the same in both experimental groups.

From the examples presented it follows that the injection of homologous hemogenetic cells in mice irradiated with a dose causing death in from 9 to 60% of the animals in 30 days not only does not aid normalization of antibody production but causes clear inhibition of antibody synthesis compared with control irradiated mice. This effect is nonspecific and is exhibited both in relation to bacterial and nonbacterial antigens. This nonspecific immunological inertness is very stable and can be retained under certain conditions for several months.

The data presented does not permit at this time the elucidation the mechanism which induces the given condition. However, it gives a basis for the hypothesis that this condition arises as the result of an immunological reaction between the injected cells of donor hemogenetic tissue and immunologically competent host cells weakened as a result of irradiation. In the experiment presented we injected recipients with a mixture of cells from several donors, in connection with which there arose the question of the possible role of the reaction of transplantate in inducing the given areactivity. To answer this question, sublethally irradiated (300 r) rats were injected intravenously with spleen cells (50×10^6) of nonirradiated rats. As seen from the table, under these conditions the injection of homologous spleen cells caused a distinct inhibition of antibody synthesis upon injection of typhoid bacteria immediately after transplantation only in the case where the irradiated recipient was injected with spleen cells taken from one donor. The injection of a mixture of spleen cells from 5 donors did not have this effect even with increased doses of cells up to 200×10^6. This regularity was even clearer in the case of injection of the antigen (sheep erythrocytes) 33 days after transplantation. Together with this, inhibition of the secondary immunological reaction in response to repeated injection of typhoid bacteria developed upon transplantation of cells taken both from one and from five donors. This data gives a basis for considering that at least in regard to the primary immunological response, the reaction of transplantate against transplantate not only does not provoke nonspecific immunological areactivity, but, on the contrary, prevents development of this condition. This fact, that irradiation of the donors decreases the ability of the transplanted cells to cause inhibition of antibody production, permits one to express a hypothesis concerning the possible role of the reaction of transplantate against the host [16] in the pathogenesis of the given phenomenon. However, a satisfactory resolution of this question is possible only in work with inbred animals, particularly with the system parental strain—F_1-hybrid. We are carrying out this research and the results will be presented in a separate report.

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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 this issue.**