THE EFFECT OF TRANSPLANTATION OF BONE MARROW OR LYMPHOCYTES ON THE RESISTANCE OF IRRADIATED ANIMALS TO DIPHTHERIA TOXIN

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The treatment of irradiated animals by injection of bone marrow cells is a method which has been widely and successfully used [2, 4, 5, 9, 10]. In most cases the animals are irradiated with more than the lethal dose, i.e., by an absolute lethal dose (for example, a dose of 900 r for mice). Such irradiation leads to total suppression of the immunological reactivity; consequently, it is possible to transplant homologous bone marrow successfully, the destroyed hemopoietic tissue is replaced and the animal remains alive. Good results may, however, follow injection of marrow cells after irradiation in doses causing death of not more than 50% of animals. This effect is expressed, not by the increased survival rate, but by the earlier recovery of those systems of the body responsible, in particular, for the immunity of the animal. We have previously shown [1], for example, that the injection of homologous bone marrow into guinea pigs, irradiated with a dose of 200 r, increases the efficacy of the seroprophylaxis of diphtheritic toxemia. The work of M. A. Tumanyan and A. V. Izvekova [2] has shown that injection of bone marrow strengthens the natural resistance of the irradiated animal to infection.

The aim of the present investigation was to ascertain whether the injection of cells from the bone marrow and lymph glands of an immune or a nonimmune donor influences the lowered natural resistance of animals to diphtheria toxin.

EXPERIMENTAL

We used guinea pigs weighing 250-300 g. Irradiation was given by a twin RUM-3 apparatus in a dose of 200 r. Conditions of irradiation: 195 kv, 15 ma, 0.5 mm Cu + 1.0 mm Al. About 10-20% of the animals died from this dose of irradiation during the period of observation (14 days). Marrow was taken from the femur and tibia of donors, washed, suspended in physiological saline, and then injected intravenously in a dose of 90-100 million cells. To obtain lymphatic cells, the cervical lymph glands were taken from donors and ground in a glass homogenizer. The cells were separated from the stroma and injected intravenously in a dose of 25 million cells. A proportion of the donors were immunized with three doses (over a period of 20 days) of crude diphtheria toxoid. The bone marrow and lymphatic cells were taken nine days after the last injection of antigen, and when "immune" cells were used they were washed before being injected into the recipient, by double centrifugation. Blood was taken from both donors (before sacrifice) and recipients (for 7 days after receiving the injection of cells), and was tested for the presence of antitoxin. The titration was carried out by Jensen's method as modified by Khalyapina. The plan of the experiment was as follows: the animals were irradiated; immediately thereafter the guinea pigs of the experimental groups were injected intravenously with marrow or lymphocytes from immune or nonimmune donors; for seven days after irradiation the resistance of the animals to diphtheria toxin was measured; at the same time blood was taken for testing for the presence of antibodies. The experimental results were treated statistically; LD_{50} was calculated by the probit method.

RESULTS

Two experiments were performed. Firstly, it must be pointed out that the injection of marrow or lymphatic cells had no effect on the number of leukocytes in the peripheral blood (Table 1) nor, evidently, on the severity of the radiation sickness.

TABLE 1. Leukocyte Count in the Blood of Animals 4-5 Days after Irradiation (Mean of 5 observations)

Series of experiments	Group of animals	Experimental conditions	Leukocyte count
First	First	Control (not irradiated)	18,430
	Second	Irradiation	3,630
	Third	Irradiation + bone marrow	4,330
Second	First	Control (not irradiated)	8,000
	Second	Irradiation	4,440
	Third	Irradiation + immune bone marrow	3,400
	Fourth	Irradiation + immune lymphocytes	3,270
	Fifth	Irradiation + nonimmune bone marrow	4,000
	Sixth	Irradiation + nonimmune lymphocytes	3,830

TABLE 2. The Influence of Bone Marrow on the Resistance of Irradiated Animals

Group of animals	Experimental conditions	No. of animals	LD_{50} of toxin (in MLD)	P_1 by comparison with first group	P_2 by comparison with second group.	IR
First	Control (not irradiated)	30	0.93± 0.24	–	–	1.0
Second	Irradiation	42	0.31± 0.07	< 0.02	–	0.33
Third	Irradiation + bone marrow	42	0.49± 0.13	0.05	0.25	0.53

TABLE 3. The Effect of Bone Marrow and Lymphatic Cells on the Natural Resistance of Irradiated Animals

Group of animals	Experimental conditions	No. of animals	LD_{50} of toxin (in MLD)	P_1 by comparison with first group	P_2 by comparison with second group	IR	Antitoxin titer (in antitoxin units)
First	Control (not irradiated)	50	1.43± 0.08	–	<0.001	1.0	<0.0025
Second	Irradiation	49	0.49± 0.1	< 0.001	–	0.34	–
Third	Irradiation + immune bone marrow	42	1.24± 0.26	0.5	<0.01	0.87	<0.0025
Fourth	Irradiation + nonimmune bone marrow	45	0.99± 0.2	0.05	<0.05	0.69	–
Fifth	Irradiation + immune lymphocytes	37	0.64± 0.1	< 0.01	<0.25	0.45	<0.0025
Sixth	Irradiation + nonimmune lymphatic cells	45	0.28± 0.1	< 0.001	0.1	0.19	<0.0025

The results of the second series of experiments gave the first hint that the injection of bone marrow cells may lead to an increase in the resistance of the animals; this is clear from the index of resistance (IR)* (Table 2), although in this case the difference between the two experimental groups was, admittedly, not significant ($P_2 = 0.25$).

The second series of experiments comprised six groups. The irradiated animals were treated by injection of bone marrow cells and lymphocytes from normal and immunized animals (Table 3). Here it can be seen more clearly that the injection of bone marrow cells leads to an increase in the resistance of the animals to diphtheria toxin after it has been lowered by irradiation; the value of IR for the irradiated animals (second group), for instance, was 0.34, and for those treated with bone marrow (third and fourth groups) it was 0.69 and 0.87. Meanwhile the injection of lymphocytes had no beneficial effect (fifth and sixth groups).

In all cases the degree of accuracy of the method did not permit the detection of antibodies in the blood of the recipient animals (see Table 3); meanwhile in the immunized donors the antibody titer was equal to 0.2 antitoxin units, but no antibodies were found in the unimmunized donors (i.e., < 0.0025 antitoxin units).

The experimental results show firstly, that injection of homologous bone marrow cells has a beneficial effect, as manifested by an increase in the natural resistance of the animals to diphtheria toxin, lowered as a result of irradiation. Whereas in the first experiment (see Table 2) the difference between the treated and untreated irradiated animals in their sensitivity to toxin was not significant ($P_2 = 0.25$), in the second this difference was definitely significant ($P_2 < 0.05$; see Table 3, second and fourth groups). The significance of this difference shows that important changes took place in the group of animals treated with bone marrow by comparison with the animals receiving irradiation alone. The injection of lymphocytes, however, was ineffective. Moreover, the injection of lymphocytes from unimmunized donors actually worsened the state of natural resistance of the irradiated animals.

When the experiment was designed, one of its objects was to discover if the qualitative state of the bone marrow is important, i.e., whether it was taken from immunized or unimmunized donors. The results obtained by M. A. Tumanyan and A. V. Izvekova [3] suggested that this did not matter. A second object was to find out if antibodies are formed by the immune cells of the donors in the body of the recipient. It is easier to give an answer to this question. In the conditions of our experiment, when both bone marrow and lymphatic cells were taken in the reproductive phase (9 days after the last injection of antigen), no antibodies could be found in the recipients' blood; more precisely, antibodies were not detected by the method of titration used. This does not mean, however, that no antibodies whatsoever were formed in the recipient's body; there are no grounds for denying that traces of antitoxin might be present. May this be the reason why the injection of "immune cells" was apparently more effective than the injection of the analogous "nonimmune cells"? In fact, after treatment with "immune bone marrow" there was no significant difference between the resistance of the control unirradiated animals and the treated irradiated animals ($P_1 = 0.5$; see Table 3, first and third groups). At the same time the difference between the control animals and the animals treated with "nonimmune bone marrow" was at the limit of significance** ($P_1 = 0.05$; see Table 3, first and fourth groups).

The study of antibody formation by the transplanted cells in the recipient's body may give an important clue to the mechanism of production of antitoxins. Several researchers have shown that antibodies may be formed by cells of immunized donors in vitro [11] and in vivo [6, 8]. What is the situation with regard to antitoxins? In the first place positive results may be expected from the transplantation of lymphoid cells. In the experiment under discussion, no antitoxin was found after transplantation of lymphocytes, although after transplantation of "immune cells" the resistance of the animals was higher than after transplantation of "nonimmune cells" (fifth and sixth groups). Working in our laboratory, M. A. Tumanyan was also unable to detect the formation of agglutinins in a similar experiment***. It may be that more delicate methods are required for such investigations, or perhaps a different method of immunization, or the cells taken from the donors at a different time. These problems must be the subject of future study.

A few main conclusions may be drawn from the experimental results described above. We have found that transplantation of bone marrow cells into irradiated animals significantly increases their natural resistance to diph-

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* The IR is a ratio showing the degree of lowering of the resistance of animals compared to that of control animals, the LD_{50} of which is expressed as unity.

** The difference is significant if $P < 0.05$.

*** Personal communication.

theria toxin, lowered as a result of the irradiation. Injection of cells from lymph glands has no perceptible beneficial effect. In our experimental conditions no antitoxin formation was found in the recipients' blood as a result of transplantation of cells from immunized donors.

SUMMARY

Two experiments were staged on guinea pigs to investigate the effect produced by transplantation of the cells of homologous bone marrow and lymph nodes, in order to restore the natural resistance of the animals to diphteria toxin, reduced as a result of irradiation (200 r.). Both immunized and nonimmunized guinea pigs served as donors of the cells. Bone marrow was administered intravenously in a dose of $90-100.10^6$ cells, lymphocytes -25.10^6 cells. As established, bone marrow cells raised the resistance of the animals to diphtheria toxin, reduced as a result of irradiation. The resistance index (I. R.) in the group of animals treated with «immune bone marrow» equals 0.87 and with the «nonimmune one» -0.69; in irradiated control animals it equalled 0.34; the resistance index of the normal animals was accepted as 1.0. Transplantation of lymphatic cells proved ineffective. It was impossible to detect any antitoxin in the recipients' blood, i.e. the transplanted cells produced no detectable amounts of the toxin.

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