ROLE OF DONORS' CELLS AND ANTIBODIES IN RESISTANCE OF RADIATION CHIMERAS TO DIPHTHERIA TOXIN

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Experimental proof has been obtained of the ability of lymphoid cells of an immunized donor, when transplanted into an irradiated recipient, to continue to form antibodies [3, 5, 6, 7, 9] and to give a secondary immunological response [4, 8]. In the latter case, the titers of antibodies in radio chimeras may even exceed the corresponding titers in control unirradiated animals [4]. Hence, antibody formation can be restored by transplanting foreign hemopoietic tissue into an animal irradiated with a lethal dose. However, the presence of antibodies, even of antitoxins, does not determine the resistance of a sublethally irradiated animal to infection or to the action of toxins [2].

The object of the present investigation was to study the resistance of radio-chimeras to the action of diphtheria toxin.

EXPERIMENTAL METHOD

Experiments were carried out on 280 guinea pigs of both sexes weighing 300-400 g. The animals were irradiated with γ-rays in a dose of 500-600 R (absolutely lethal dose). The following antigens were used in the investigation: adsorbed (Batch No. 7, 60 Lf/ml) and crude (Batch No. 236, 400 Lf/ml) diphtheria toxoids and diphtheria toxin (Batch No. 65, 1 MLD—0.0036 ml).

On the day after irradiation, the recipients were treated by transplantation of hemopoietic tissue from normal or twice immunized donors. Cells for transplantation were taken from the donors 10-12 days after the end of immunization and were injected intravenously: $200 \cdot 10^6$ bone marrow cells, $300 \cdot 10^6$ cells of a mixture of bone marrow and spleen. The technique of obtaining the cells was described earlier [3, 4]. The recipients were then immunized twice with crude (200 Lf—400 Lf at intervals of 20 days) or once with adsorbed (1 Lf or 2 Lf) toxoid. Resistance to toxin was tested 25-30 days after the beginning of immunization. Before injection of the toxin, blood was taken for estimation of antibodies.

EXPERIMENTAL RESULTS

The study of the resistance of radio-chimeras to diphtheria toxin has the object of elucidating the role of the donor's hemopoietic tissue in this process and of determining the presence of antibodies in the blood stream.

In the first series (two experiments) irradiated guinea pigs were injected with cells of normal, nonimmunized donors (Table 1). The results showed that a single injection of toxoid did not produce intensive immunity in the radio-chimera animals. No antitoxin was found in the blood. Some degree of resistance was obtained when the immunization began 15 days after transplantation. This was very probably associated with restoration of the receipient's own immunological mechanism, for the additional injection of spleen cells had no effect on the development of immunity (Groups 1 and 2).

TABLE 1. Resistance of Radio-Chimeras Treated with Normal Bone Marrow to Diphtheria Toxin

Group	Dose of irradiation (in R)	Transplantation of cells from bone marrow	Transplantation of cells from spleen	Interval before immunization (in days)	Dose of toxoid (Lf)	Dose of toxin (MLD)	No. of animals	Result: died	Result: survived	Result: percent mortality	antitoxin (in A.U.)
1-я	500	+	+	15	2	50	9	8	1	89	<0,005
2-я	500	+	—	15	2	50	7	6	1	86	<0,005
3-я	500	+	+	1	2	50	13	13	0	100	<0,005
4-я	600	+	—	1	2	50	7	7	0	100	<0,005
5-я	—	—	—	—	2	50	19	4	15	21	0,15

TABLE 2. Titers of Antitoxin and Resistance of Irradiated Animals (recipient of "immune" cells) to 50 MLD Toxin 30 Days after a Single Immunization

	Control, unirradiated animals		Irradiated, treated animals	
	titer (in A.U.)	result	titer (in A.U.)	result
	< 0,005	Died	< 0,005	Died
	< 0,005	"	< 0,005	"
	< 0,005	"	0,1	Survived
	< 0,005	"	0,15	"
	< 0,005	"	0,22	Died
	0,03	"	0,37	Survived
	0,05	Survived	0,87	"
	0,075	"		
	0,15	"		
	0,22	"		
	0,4	"		
	0,5	"		
Mean titer and percent mortality	0,162	50	0,162	43

The role of the donor's cells was clearly revealed when the bone marrow and spleen of an immunized donor were transplanted. In conditions analogous to the first series of experiments, after immunization with 1 Lf 24 h after transplantation, quite different results were obtained (Table 2). The titers of antitoxin were the same in both groups of animals, and correspondingly their resistance was identical.

In the next experiment the radio-chimeras (recipients of "immune" cells) were twice immunized with crude toxoid (Table 3). Although the mortality rate among the irradiated animals was significantly higher than among the controls, analysis of the results obtained in each animal showed that the majority of dying irradiated guinea pigs were animals in which no antibodies had formed, and if they are excluded, the mortality rate in this group was 11%. Moreover, a dose of 50 MLD caused death of a control guinea pig in which the titer was 0.3 A.U , while radio-chimeras with titers of 0.15 and 0.33 A.U. survived. No radio-chimera guinea pigs died after a dose of 100 MLD with a much lower titer (0.19, 0.33 A.U.) than the dying control animal (1.25 A.U.).

Hence, in the experimental conditions described, high resistance of the radio-chimeras was produced, depending on the presence of antitoxin in the blood. It was in fact noted that the radio-chimeras had higher resistance than the unirradiated animals with the same level of antibodies. This is contrary to the author's earlier findings [2], showing that after sublethal irradiation the presence of antitoxin does not determine the resistance of the animal. It is probable that a very important role in the resistance of radio-chimeras is played by the foreign bone marrow. This is confirmed by results [1] showing the partial restoration of the resistance of a sublethally irradiated animal by transplantation of bone marrow.

The results show that the resistance of radio-chimeras is a function of the transplanted homologous donors' cells. However, only the transplantation of "immune" cells enables radio-chimeras to produce antibodies in

TABLE 3. Titers of Antitoxin and Resistance of Irradiated Animals (recipients of "immune" cells) to Toxin 10 Days after a Second Injection of Toxoid

Group	Dose of toxin (in (MLD)	Control, unirradiated animals		Irradiated, treated animals	
		titer (in A.U.)	result	titer (in A.U.)	result
1	50	1,3 1,75 4,0	Died Survived "	0,15 0,33 1,92 3,33	Survived " " "
2	100	1,25 1,92 10,0	Died Survived "	< 0,01 0,19 0,33	Died Survived "
3	200	1,92 15,0 15,0	Survived " "	< 0,01 < 0,01 < 0,01	Died " "
4	400	1,05 1,7 4,2	Survived " "	< 0,01 < 0,01 0,7	Died " Survived
5	800	2,0 2,1 20,0 20,0	Died Survived " "	< 0,01 1,25 7,0	Died " Survived
Mean titer and percent mortality		5,39	18	1,6	50

response to immunization and to develop resistance. Very probably antitoxin formation can be used to judge the creation of a true chimerism (following transplantation of "immune" cells). Certain other authors have reached the same conclusion [10].

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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.