EFFECT OF REACTION OF THE GRAFT AGAINST THE HOST ON TRANSPLANTATION IMMUNITY WHEN A STRONG ANTIGENIC DIFFERENCE EXISTS BETWEEN DONOR AND RECIPIENT

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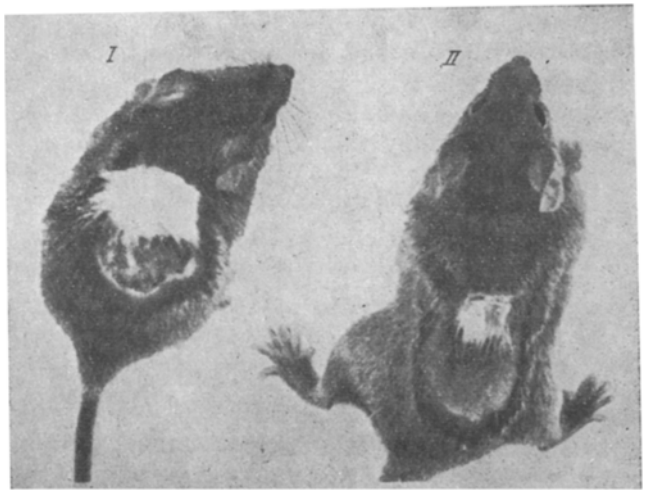
The immunologic nature of rejection of homografts has been confirmed experimentally and clinically [7, 8, 13, 15]. An important task in noninfectious immunology is the search for new and effective methods of suppressing transplantation immunity. Rejection of grafts is brought about by the recipient's lymphoid cells [3, 12, 13, 15, 16, 19]. To overcome tissue incompatability, the ability of the recipient's lymphoid system to destroy homografts must be suppressed. A promising approach to this problem is the study of the effect of transplantation of foreign immunologically competent cells on reactivity of recipients to homografts. Transplanted immunologically competent cells react against the foreign antigens of the host and induce homologous disease in the recipient, who is unable to reject transplanted homologous donor cells [4, 9, 18]. The effect of the reaction of the graft against the host on transplantation immunity has been examined in isolated investigations [2, 11, 20]. However, the detailed study of the effect of immunologically competent cells on reactivity of the recipient toward the homograft is extremely important because the reaction of the graft against the host causes a marked lymphoid atrophy at a later stage in the recipient.

An attempt was made in the present investigation to discover how the reaction of the graft against the host induced by injection of immunologically competent cells of parent strains into F_1 hybrids (C57Bl/6 × CBA) affects transplantation immunity in cases when there is a strong antigenic difference between donors and recipients.



Graft on stunted mouse in good condition (I) and signs of rejection of graft present—loss of hair, necrosis, shrinking—on a healthy mouse (II) at the same times (13th day after transplantation.

EXPERIMENTAL METHOD

Inbred mice of lines C57Bl/6, CBA, F_1 (C57Bl/6 × CBA), and A, obtained from the Nursery of Pure Line Animals, Academy of Sciences of the USSR (Stolbovaya Station) were used in the experiments. The F_1 (C57Bl/6 × CBA) hybrids were used as recipients, CBA and C57Bl/6 mice as spleen donors, and the A mice as donors of skin grafts. The mice weighed 18-19 g and were aged 2.5-3 months. Skin from line A mice was transplanted 1, 7, and 15 days after injection of spleen cells of parent strains. The mouse spleens were minced in a glass homogenizer with the addition of ice-cold Hanks's solution. The cell suspension was filtered through fine mesh silk, kept on ice, and injected into the recipients 1-1.5 h after preparation. In one series of experiments the recipients were irradiated in a sublethal dose 2 h before injection of the spleen cells.

Skin grafting was carried out by the method of Billingham and Medawar [5]. In some experiments

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TABLE 1. Effect of Intraperitoneal Transplantation of Spleen Cells of CBA Mice on Rejection of Skin Graft from Line A Mouse onto Sublethally Irradiated F_1 (C57Bl/6 × CBA) Hybrids. Difference at H-2

Group of mice	No. of mice	Dose of radiation	No. of spleen cells, $\times 10^6$	Survival of graft (in days) when transplanted			
				on 7th day of experiment		on 15th day of experiment	
				M ± m	P	M ± m	P
1st	20	400	50	11 ± 0.42	<0.01	10.3 ± 0.58	<0.05
2nd	20	400	–	9.5 ± 0.44	>0.05	7.9 ± 0.22	>0.05
3rd	20	–	50	8.3 ± 0.22	>0.05	8.6 ± 0.22	>0.05
4th	10	–	–	8.5 ± 0.33	–		

TABLE 2. Effect of Intraperitoneal Injection of Large Doses of Spleen Cells of CBA Mice on Transplantation Immunity of (C57Bl/6 × CBA) Hybrids in Conditions of Strong Antigenic Differences

Group of mice	No. of mice	No. of spleen cells, $\times 10^6$	Survival of first graft (in days)		Survival of second graft (in days)	
			M ± m	P	M ± m	P
1st (first graft applied on day after injection of spleen cells)	10	200	9.5 ± 0.22	<0.01	6.4 ± 0.67	>0.05
2nd (first graft applied on 7th day after injection of spleen cells)	10	200	8.7 ± 0.22	>0.05	6.7 ± 0.56	>0.05
3rd (first graft applied on 15th day after injection of spleen cells)	10	200	9.1 ± 0.33	>0.05	8.3 ± 0.79	<0.05
4th (intact mice)	10	–	8.6 ± 0.22	–	5.8 ± 0.44	–

the reactivity of the recipient to a second graft was studied. The survival of the graft was counted from the moment of transplantation to the appearance of the first signs of rejection.

EXPERIMENTAL RESULTS AND DISCUSSION

In the experiments of series I rejection of the skin graft from line A mice by sublethally irradiated F_1 hybrids (C57Bl/6 × CBA) (differing at H-2) was studied at various times after intraperitoneal injection of small doses of CBA spleen cells (50 million). If the graft was transplanted onto hybrids on the day after irradiation and injection of spleen cells, a statistically significant increase was observed in its length of survival ($P < 0.01$). The mice developed signs of homologous disease on the 10th day after injection of the spleen cells. Most mice lost considerable weight and developed untidiness of the hair and diarrhea; only one or two mice showed less marked manifestations of the disease and merely ceased to gain weight. The grafts survived 13 days on two mice and their rejection was of the chronic type. The skin grafts on these mice gradually became thinner and peeled at the edges, and the hair fell out, but they were soft to the touch. The graft on one mouse showed no signs of rejection for 35 days, and its subsequent rejection was of the chronic type. The grafts on the control mice survived 8-9 days: they became hard to the touch at once, the hair fell out, the grafts were lifted above the surface of the recipient's skin, and they fell off to leave scarring of the graft bed. Sublethal irradiation alone or injection of small doses of spleen cells alone did not increase the survival period of the grafts in the hybrids.

Hybrids of the other two experimental groups were grafted with skin from line A mice 7 and 15 days after sublethal irradiation and intraperitoneal injection of spleen cells from CBA mice (Table 1). All the mice of the experimental groups developed signs of homologous disease. Longest survival of the grafts was noted in animals skin grafted on the 7th day of the experiment ($P < 0.01$). If the grafts were transplanted onto hybrids on the 15th day of the experiment, signs of rejection appeared sooner ($P < 0.05$).

Intraperitoneal injection of isologous spleen cells (50 million) into sublethally irradiated F_1 (C57Bl/6 × CBA) hybrids caused no signs of homologous disease and did not prolong survival of skin grafts from line A mice transplanted 1, 7, and 15 days after injection of the cells.

In the experiments of series II rejection of skin grafts from line A mice by unirradiated F_1 (C57Bl/6 × CBA) hybrids 1, 7, and 15 days after injection of spleen cells of the parent strains CBA and C57Bl/6 was

studied. Spleen cells of CBA mice were injected intraperitoneally into the hybrids in doses of 200 million each. Ten hybrids were grafted with skin from line A mice next day (group 1), 10 on the 7th day (group 2), and 10 on the 15th day after injection of the spleen cells (group 3; Table 2). A second graft was transplanted onto the mice of all three groups 19 days after the first graft. Lengthening of the survival period of the first graft was observed in the mice of group 1. Survival of the first graft on mice of groups 2 and 3 was indistinguishable from the control. The anamnestic response to the second graft was impaired in the mice of group 3 ($P < 0.05$). All the experimental mice remained well and gained normally in weight.

Spleen cells of C57Bl/6 mice were injected intravenously into 30 F_1 (C57Bl/6 × CBA) hybrids in a dose of 75 million cells per mouse. An increase in the survival time was observed in the case of mice on which the skin was grafted on the day after injection of spleen cells ($P < 0.05$). The graft on one stunted mouse showed no signs of rejection for 17 days, and its eventual rejection was of the chronic type (see figure). In these experiments 180 primary skin grafts and 40 secondary grafts were applied.

In the experiments described above the reaction of the graft against the host was induced in the genetic system of parent strain–first generation hybrid, in which only a unidirectional reaction of the lymphoid cells of the parent strain is possible against the foreign components of the other parent in the hybrid [17]. Spleen cells of CBA mice in a small dose induced homologous disease in sublethally irradiated hybrids. Transplantation immunity was disturbed to a greater degree in recipients on which the graft was applied on the first and seventh days of the experiment. Larger doses of spleen cells from CBA mice did not induce signs of homologous disease in unirradiated hybrids, but the survival of the first graft was prolonged in the mice grafted on the day after injection of spleen cells. Impairment of the reactivity of the recipients to a second graft was observed 34 days after injection of large doses of spleen cells. Howard and Woodruff [11] grafted the skin of line A mice on F_1 (C57Bl × CBA) hybrids 19 days after intravenous injection of CBA spleen cells in doses of 60-70 million and found no increase in the survival period of the graft. In our experiments intravenous injection of spleen cells of the parent strain C57Bl/6 (75 million) induced the disease in only one mouse. In a stunted mouse the graft survived 17 days and its rejection was of the chronic type. Immunological reactivity against the first graft was disturbed in two series of experiments in recipients skin grafted during the first days after injection of parent spleen cells. Probably the taking of the graft in the mice of these groups coincided with the early phase of reaction of the graft against the host, accompanied by proliferation of plasma cells and lymphoid cells [10, 18], facilitating the induction of areactivity against foreign antigens [6]. The possibility is not ruled out that rejection of the skin grafts from line A mice by F_1 (C57Bl/6 × CBA) hybrids is brought about by the injected immunologically competent cells of CBA and C57Bl/6 mice.

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