USE OF THE GRAFT VERSUS HOST REACTION FOR SPECIFIC SUPPRESSION OF HOMOGRAFT IMMUNITY IN ADULT MICE

V. F. Semenkov

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The graft versus host reaction was evoked by injecting CBA mouse spleen cells into sublethally irradiated or intact (CBA × C57BL/6)F_1 mice. Subsequent injection of spleen cells of line A mice into the hybrids led to the appearance of partial specific areactivity to a skin graft from line A mice in the animals.

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Immunologic tolerance is produced with great difficulty in adult animals [5, 7], especially if a strong antigenic difference exists between donor and recipient, and this is an obstacle to the use of this method for overcoming tissue incompatibility under clinical conditions. Methods exist for facilitating the production of tolerance in adult organisms. They include procedures causing injury to the immune system or depressing its functions and facilitating the nonspecific suppression of immunologic reactivity (irradiation, antimetabolites, thymectomy, etc.). Some workers have suggested that the induction of tolerance may be facilitated by stimulating proliferation of donors' cells in the recipient's body by foreign antigens [6], or by producing parabiosis between donor and recipient [4].

In previous investigations [2, 3] the writer demonstrated nonspecific depression of homograft immunity during the graft versus host reaction (GVHR). In the present investigation, the possibility of using the GVHR as a method of specific depression of homograft immunity in adult mice was studied.

EXPERIMENTAL METHOD

Mice of inbred lines obtained from the nursery of pure-line animals, Academy of Medical Sciences of the USSR (Stolbovaya) were used in the experiments. In the first experiment the GVHR was induced in sublethally irradiated (400 R) 3-month hybrids (CBA × C57Bl/6)F_1 weighing 18-19 g by intraperitoneal injection of $3 \cdot 10^7$ CBA mouse spleen cells. The conditions of irradiation and the method of preparation of the cell suspensions were the same as those described previously [2, 3]. CBA mouse spleen cells were injected 1-2 h after irradiation of the hybrids. Line A mouse spleen cells were injected intravenously into the recipients in a dose of $5 \cdot 10^7$ cells 2-3 h after injection of the CBA mouse spleen cells. On the day after irradiation and injection of the CBA and A mouse spleen cells into the hybrids, they were given a second intravenous injection of $5 \cdot 10^7$ line A mouse spleen cells and skin homografts were transplanted to them on the same day. In the second experiment, unirradiated 3-month hybrids (CBA × C57BL/6)F_1 received an intravenous injection of $1 \cdot 10^8$ CBA mouse spleen cells to induce the GVHR. On the next 3 days the experimental hybrids were injected intravenously with $1 \cdot 10^8$ line A mouse spleen cells daily (total dose $3 \cdot 10^8$ cells). On the day after the last injection of line A mouse spleen cells, skin homografts were transplanted to the hybrids. Each recipient of the experimental and control groups was grafted in two experiments not only with a skin graft from a line A mouse to which areactivity had been produced, but also with a skin graft from a BALB/c mouse in order to test the specificity of the induced areactivity. The (CBA × C57BL/6)F_1 hybrids inherit from CBA mice the H-2^k-locus of tissue compatibility, and from C57BL/6 mice they inherit the H-2^b-locus. Line A mice have a H-2^a-locus, and BALB/c mice possess the H-2^d-locus of tissue compatibility.

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TABLE 1. Induction of Specific Areactivity by Means of the Graft Versus Host Reaction in Sublethally Irradiated and Unirradiated Hybrids (CBA × C57BL/6)F_1

Expt.	Group No.	No. of mice	Dose of irradiation (in R)	No. of spleen cells ($\times 10^6$)			Period of survival of skin homografts (in days)			
							A		BALB/c	
				isologous	CBA	A	$M \pm m$	P	$M \pm m$	P
First	1	23	400	—	30	100	12:2±0,41	<0,01	9,3±0,4	>0,05
	2	11	400	—	—	100	8,6±0,3	>0,05	8,7±0,25	>0,05
	3	10	400	—	30	—	9,9±0,58	>0,05	9,0±0,45	>0,05
	4	10	400	30	—	100	9,0±0,44	>0,05	9,1±0,2	>0,05
	5	5	400	—	—	—	8,7±0,32	—	8,1±0,4	—
	6	5	—	—	—	—	8,9±0,22	—	8,0±0,43	—
Second	7	10	—	—	100	300	12,9±0,3	<0,01	9,3±0,45	>0,05
	8	10	—	—	—	300	10,3±0,6	>0,05'	9,2±0,43	>0,05
	9	10	—	—	100	—	10,±0,8	>0,05	10,1±0,4	>0,05
	10	10	—	100	—	300	9,5±0,5	>0,05	9,2±0,23	>0,05
	11	10	—	—	—	—	8,9±0,44	—	8,7±0,5	—

EXPERIMENTAL RESULTS

In the first experiment a statistically significant increase in the survival period of skin grafts from line A mice was observed in the recipients of the experimental group, while immunologic reactivity to skin of BALB/c mice remained unchanged (Table 1, group 1). Evidence of homologous disease was observed in all the experimental mice, 13 hybrids recovered, while the other 10 hybrids died from the diseases. In 2 hybrids which recovered, skin homografts from line A mice showed no signs of rejection after 27 and 17 days, but signs of rejection of BALB/c mouse skin appeared on the 9th and 7th days. In most experimental recipients the survival period of skin grafts from line A mice varied from 12-15 days, and the longest period of survival of grafts from BALB/c mice in a few mice was 11 days.

Two intravenous injections of line A mouse spleen cells ($1 \cdot 10^8$) did not prolong the survival of skin grafts from line A and BALB/c mice or cause the development of homologous disease in sublethally irradiated hybrids (Table 1, group 2). If sublethally irradiated hybrids were injected intraperitoneally with spleen cells from CBA mice only ($3 \cdot 10^7$), no evidence of homologous disease or injury to reactivity of the recipient against skin homografts likewise was observed.

An intraperitoneal injection of $3 \cdot 10^7$ isologous spleen cells was given to 10 sublethally irradiated hybrids, after which two intravenous injections, each of $5 \cdot 10^7$ line A mouse spleen cells were given, followed by transplantation of two skin grafts from line A and BALB/c mice. All the hybrids gained in weight normally, and the period of survival of the skin grafts was the same as in the normal and sublethally irradiated hybrids (Table 1, group 4).

In the second experiment, on experimental unirradiated hybrids receiving an intravenous injection of $1 \cdot 10^8$ CBA mouse spleen cells followed by an injection of line A mouse spleen cells ($3 \cdot 10^8$), skin grafts from line A mice survived longer than grafts from BALB/c mice. Complete disintegration of skin grafts from BALB/c mice (loss of hair, decrease in thickness, necrosis) was observed in the experimental mice, whereas the skin grafts from line A mice still remained in a good condition. The survival period of skin homografts from line A mice in the experimental hybrids was 4-days longer than in mice of the control groups. Definite homologous disease developed in only one of the 10 experimental hybrids; this hybrid died on the 21st day from the beginning of the experiment. Manifestations of homologous disease in the remaining recipients appeared for only a short time, over a period of 5-9 days, and were followed by recovery.

Intravenous injection of line A mouse spleen cells over a period of three days into recipients not receiving CBA mouse spleen cells did not produce a statistically significant increase in the survival period

of skin grafts from line A and BALB/c mice (Table 1, group 8). The recipients of this group showed no definite signs of the disease. If unirradiated hybrids were injected intravenously with $1 \cdot 10^8$ isologous spleen cells followed by three daily intravenous injections of line A mouse spleen cells ($3 \cdot 10^8$), no increase in the survival period of the grafts or manifestations of homologous disease were observed (Table 1, group 10). Intravenous injection of $1 \cdot 10^8$ CBA mouse spleen cells was not accompanied by the development of homologous disease in the hybrids or by injury to immunologic reactivity against the grafts (Table 1, group 9).

In the experiments described above the recipients were injected with two types of immunologically active cells: spleen cells of CBA mice to evoke the GVHR and spleen cells of line A mice to induce specific areactivity against the background of the GVHR. The combined use of two types of cells produced homologous disease in the recipients and partial specific areactivity against skin grafts from line A mice (experimental groups), whereas the separate injection of cells from CBA and A mice did not lead to the development of homologous disease and did not significantly prolong the survival period of skin grafts from line A and BALB/c mice (control groups). The probable mechanism of specific inhibition of graft immunity is connected with the fact that the reaction of CBA mouse cells against the recipient led to longer persistence of the cells of the A genotype. Spleen cells from CBA mice, reacting against foreign antigens of the recipient, themselves were probably unable for some time to reject the line A mouse cells because of immunologic paralysis. It must be remembered that interaction between two types of grafted cells (A and CBA) in the hybrid, especially if irradiated, is dominant. During interaction the CBA cells may cause inactivation of stem cells of the A genotype, or the A cells may inactivate the CBA stem cells. The possibility of inactivation of stem cells during contact between genetically incompatible cell suspensions from l lymphoid tissues has been demonstrated experimentally [1]. It follows from the results of the present experiments that cells of line A mice, together with cells of CBA mice, participated in the production of homologous disease or stimulated the reaction of CBA mouse cells against the recipient. If the experimental hybrids were injected, not with spleen cells of CBA mice, but with isologous spleen cells, incapable of evoking the GVHR, no induction of specific areactivity against the skin grafts was observed.

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