DYNAMICS OF FORMATION OF THE IMMUNOLOGICAL MEMORY TO TRANSPLANTATION ANTIGENS

A. P. Bogdanov

UDC 612.6.02.017.1

Second and subsequent skin grafts in mice undergo "second set" rejection regardless of the time of the second grafting operation (maximal interval between grafts 1 year). The rate of rejection of the third and subsequent grafts is the same as the rate of rejection of the second graft ($P > 0.05$), regardless of the length of the interval between grafting operations. During the adaptive transfer of viable cells of immune lymph glands (50 million cells) to a syngeneic irradiated (1000 R) recipient, protected with syngeneic bone marrow (10 million cells), accelerated rejection of both the primary and secondary (relative to the primary) grafts was observed if the interval between grafting operations or transfer of cells was 2 or 3 weeks or 1, 2, and 3 months.

A second graft in the same donor has been shown to be rejected much faster than the first. This rule has been confirmed in experiments on various animals at different levels of evolutionary development, ranging from cold-blooded animals to man [7, 9, 13, 14, 17]. The more rapid rejection of the second graft has been called the "second set" phenomenon. Rejection of second set type is also observed if another tissue from the same donor is used as the first graft [8, 10].

Immunity to allografts can be transferred to syngeneic recipients in most cases only through immune lymphoid cells ("adaptive immunity") [11-13, 15, 16, 18]. The state of immunity transmitted with the cells develops immediately after their transfer [5]. The state of sensitization of the lymph gland cells persists for a long time and can be transferred by them to a recipient as long as 1 year after immunization [5].

The object of the present investigation was to study the dynamics of formation of the immunological memory to strong (H-2) transplantation antigens at various times after transplantation of a primary skin graft, the effect of the number of skin grafts on the immunological memory, and the ability of lymph gland cells to produce adaptive transfer of immunological memory at different times after immunization.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA and C57BL/6 mice weighing 19-21 g. The CBA mice received skin grafts from C57BL/6 donors by the method of Billingham and Medawar [6]. The postoperative wound was covered with a dressing [2]. Signs of rejection of the graft were taken to be edema, induration, loss of hair, and drying of the edges of the graft. The adaptive transfer of immunological memory was carried out by means of lymph gland cells from immune CBA mice (a single immunization by a skin graft from C57BL/6 donors). Lethally irradiated (1000 R) CBA mice were given an intravenous injection of 50 million lymph gland cells from immune donors 1-2 h after irradiation. All the irradiated mice received an intravenous injection of 10 million syngeneic bone marrow cells at the same time from intact CBA mice. In the control groups, in one case irradiated mice were injected only with syngeneic bone marrow, and in the other case with bone marrow mixed with intact syngeneic lymph gland cells (in the same doses). The conditions of irradiation and the technique of preparing the cell suspensions were described previously [1].

Department of Microbiology, Smolensk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 74, No. 9, pp. 70-74, September, 1972. Original article submitted November 25, 1971.

TABLE 1. Effect of Number of Transplantations of Skin of C57BL/6 Mice on Intensity of Immunological Memory to Transplantation Antigens in CBA Mice.

Number of mice	Interval between grafts	Order of grafts	Time of rejection of graft (in days)		
			$M \pm m$	P_1	P_2
70	—	First	12,3±0,54	—	—
10	2 Weeks	Second	7,1±0,43	<0,01	—
		Third	7,0±0,43	<0,01	>0,05
		Fourth	6,9±0,22	<0,01	>0,05
		Fifth	6,8±0,43	<0,01	>0,05
		Sixth	5,9±0,65	<0,01	>0,05
10	3 »	Second	7,2±0,22	<0,01	—
		Third	6,1±0,33	<0,01	>0,05
		Fourth	6,8±0,22	<0,01	>0,05
		Fifth	6,0±0,43	<0,01	>0,05
		Sixth	5,9±0,22	<0,01	>0,05
10	1 Month	Second	6,0±0,22	<0,01	—
		Third	6,1±0,32	<0,01	>0,05
		Fourth	6,0±0,43	<0,01	>0,05
		Fifth	6,1±0,32	<0,01	>0,05
		Sixth	5,9±0,22	<0,01	>0,05
10	2 Months	Second	7,0±0,43	<0,01	—
		Third	6,8±0,22	<0,01	>0,05
		Fourth	6,9±0,22	<0,01	>0,05
		Fifth	5,9±0,65	<0,01	>0,05
		Sixth	6,7±0,57	<0,01	>0,05
10	3 »	Second	6,8±0,43	<0,01	—
		Third	7,0±0,32	<0,01	>0,05
		Fourth	6,3±0,87	<0,01	>0,05
10	6 Months	Second	8,3±0,32	<0,01	—
		Third	7,1±0,52	<0,01	>0,05
10	12 »	Second	8,0±0,97	<0,01	—

Note: P_1 represents degree of significance relative to time of rejection of first graft; P_2, degree of significance relative to time of rejection of second graft.

In the different groups of animals the skin grafting and adaptive transfer with subsequent skin grafts were carried out at different time intervals: 2 and 3 weeks, 1, 2, 3, 6, and 12 months.

EXPERIMENTAL RESULTS

In the experiments of series I the effect of the number of grafts on the expression of the immunological memory to transplantation antigens differing in their H-2 locus, and the duration of this effect were studied. The results are given in Table 1. They show that the second and subsequent grafts were rejected much more rapidly than the first ($P < 0.01$) regardless of the time of the second grafting operation. The immunological memory persisted for more than 1 year. The rate of rejection of the third and subsequent grafts was the same as that of the second graft ($P > 0.05$) regardless of the interval between grafting operations.

Simultaneously with this series of experiments the effect of the number of skin grafts transplanted on C57BL/6 mice on the intensity of their immunological memory of CBA mice was studied during the adaptive transfer of immune lymph gland cells from CBA mice to them. As Table 2 shows, the transfer of lymph gland cells from a sensitized donor led to the more rapid rejection of the graft than in the control group ($P < 0.1$), but the second graft of these same animals was still rejected faster than the first, except when the adaptive transfer was carried out after 6 months.

In can be concluded from the results of both series of experiments that the cells of immune lymph glands can transfer immunological memory to transplantation antigens and can repopulate in a syngeneic bone marrow. Comparison of the results of the two experiments shows that during adaptive transfer of immune lymph gland cells the rejection of the first graft was of the second set type, but by contrast with

TABLE 2. Effect of Number of Skin Grafts from C57BL/6 Mice on Intensity of Immunological Memory to Transplantation Antigens during Adaptive Transfer of Immune Lymph Gland Cells of CBA Mice to Lethally Irradiated Syngeneic Recipients

Number of mice	Interval between sensitization and adaptive transfer	Order of grafts	Number of cells injected ($\times 10_6$)			Time of rejection of graft (in days)		
			intact BMC	intact LGC	immune LGC	$M \pm m$	P_1	P_2
10	—	First	10	—	—	18,7±0,97	—	—
10	—	First	10	50	—	15,2±0,43	—	—
		Second				8,3±0,22	<0,01	—
		First				9,9±0,22	<0,01	—
		Second				7,9±0,34	<0,01	<0,01
10	2 Weeks	Third	10	—	50	7,0±0,11	<0,01	<0,01
		Fourth				6,4±0,43	<0,01	<0,01
		Fifth				6,2±0,22	<0,01	<0,01
		First				9,4±0,43	<0,01	—
		Second				7,5±0,32	<0,01	<0,01
10	3 »	Third	10	—	50	6,0±0,22	<0,01	<0,01
		Fourth				5,9±0,11	<0,01	<0,01
		Fifth				5,6±0,32	<0,01	<0,01
		First				9,0±0,43	<0,01	—
		Second				5,7±0,22	<0,01	<0,01
10	1 Month	Third	10	—	50	5,5±0,22	<0,01	<0,01
		Fourth				6,0±0,22	<0,01	<0,01
		Fifth				5,6±0,11	<0,01	<0,01
		First				8,5±0,32	<0,01	—
		Second				5,6±0,22	<0,01	<0,01
10	2 Weeks	Third	10	—	50	5,4±0,22	<0,01	<0,01
		Fourth				6,0±0,11	<0,01	<0,01
		Fifth				5,7±0,01	<0,01	<0,01
		First				8,1±0,32	<0,01	—
10	3 »	Second	10	—	50	5,5±0,22	<0,01	<0,01
		Third				6,0±0,32	<0,01	<0,01
		First				6,8±0,43	<0,01	—
10	6 Months	Second	10	—	50	6,6±0,11	<0,01	>0,05

Legend: LGC) lymph gland cells; BMC) bone marrow cells; P_1) degree of significance relative to first graft transplanted to lethally irradiated CBA mice receiving intact LGC; P_2) degree of significance relative to first graft transplanted to CBA mice receiving adaptive transfer of 50 million LGC from immune animals.

the experiments of series I the rejection of the second graft in these same animals was also by the second set type compared with the first, except in the case of adaptive transfer after 6 months.

The "double second set" phenomenon observed in these experiments was evidently due to the fact that at the time of the second grafting operation the irradiated syngeneic recipients, protected with syngeneic bone marrow against the transplanted cells, were able to form a fresh pool of lymphocytes which, after their production, were sensitized against the antigens of the primary graft. In these experiments the more rapid rejection of the primary grafts was due to a reaction of the transferred cells of the sensitized donor, whereas the more rapid rejection of the secondary grafts was due to an immunological reaction of the memory cells of the new pool of sensitized cells, which could be the progenies of both donor and recipient precursor cells. If the interval between the grafts was 6 months, cells arising from precursor cells of the sensitized donor, from cells arising from the transferred bone marrow, and from cells of the hematopoietic tissue of the recipient itself, which had regenerated after irradiation, could participate in the reaction to the first graft in the irradiated recipient. During the 6 months which elapsed in these experiments after adaptive transfer of the immune lymph gland cells, the transferred memory cells were evidently able to transmit the corresponding immunological information to the newly arising cells. In this experiment the phenomenon of accelerated rejection of the first graft was therefore expressed to the maximal degree, while the "double second set" phenomenon was absent.

LITERATURE CITED

1. A. P. Bogdanov, in: Problems in Noninfectious Immunology [in Russian], Smolensk (1971), p. 17.
2. V. F. Semenkov, Éksper. Khir., No. 1, 43 (1969).
3. J. Berrian and C. McKhann, Ann. New York Acad. Sci., 87, 106 (1960).

4. R. E. Billingham et al., Proc. Roy. Soc. B., 143, 43 (1954).
5. R. E. Billingham et al., J. Exp. Med., 118, 397 (1963).
6. R. E. Billingham and P. B. Medawar, J. Exp. Biol., 28, 385 (1951).
7. R. Erikson, Plast. Reconst. Surg. Bull., 30, 137 (1962).
8. E. Friedman et al., J. Clin. Invest., 40, 2162 (1961).
9. W. Hieldemann and E. Cooper, Fed. Proc., 22, 1145 (1963).
10. L. Mannick et al., Am. J. Surg., 105, 167 (1963).
11. J. P. Merrill et al., Ann. New York Acad. Sci., 87, 266 (1960).
12. N. A. Mitchison, J. Exp. Med., 102, 155 (1955).
13. E. Peacock et al., Ann. New York Acad. Sci., 87, 175 (1960).
14. F. Rapaport et al. Ann. New York Acad. Sci., 87, 217 (1960).
15. G. D. Snell et al., J. Exp. Med., 112, 293 (1960).
16. G. D. Snell et al., J. Immunol., 87, 1 (1961).
17. M. Tyan and L. Cole, Transplantation, 1, 546 (1963).
18. H. J. Winn, J. Immunol., 86, 228 (1961).