5. B. Bloom and P. Glade (editors), In Vitro Methods in Cell-Mediated Immunity, Academic Press, New York (1971).
6. H. George and J. Vaughan, Proc. Soc. Exp. Biol. Med, 111, 514 (1962).
7. J. Krejci, I. Kazakoz, J. Pekazekk, et al., Immunology, 27, 133 (1974).
8. J. Munos, J. Immunol., 99, 31 (1967).
9. R. Petrov, L. Koval'chuk, M. Stenina, et al., Ann. Immunol., 125C, 171 (1974).
10. W. Pincus, J. Flick, and T. Ingalle, J. Immunol., 91, 58 (1963).
11. M. Tyan, J. Immunol., 108, 65 (1972).
12. R. Williams, J. Exp. Med., 135, 1279 (1972).

GRAFT VERSUS HOST REACTION IN RECIPROCAL COMBINATIONS OF MOUSE STRAINS DIFFERING IN THEIR H-2 HISTOCOMPATIBILITY COMPLEX

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Spleen cells from C57BL mice injected intraperitoneally into newborn CBA recipient mice in doses of $0.5 \cdot 10^7$, $1 \cdot 10^7$, $2 \cdot 10^7$ induced runt disease in an acute form, from which 43, 86, and 95% of the recipients respectively died in the course of two or three weeks. Preliminary immunization of the C57BL donors with CBA isoantigens led to a marked increase, whereas immunization with "foreign" antigens (sheep's red cells) led to weakening of the reactions. With the reciprocal combination of strains runt disease followed a course 4-5 times less active and there was no "preimmunization effect." In the combination C57BL→CBA the reaction was accompanied by proliferation of pyroninophilic monocytes and by destruction of the splenic follicles, whereas in the combination CBA→C57BL their formation was delayed and no appreciable accumulation of blast cells took place in the zone of the follicle.

KEY WORDS: *Graft versus host reaction; runt disease; transplantation*

The graft versus host reaction (GVHR) resulting from transplantation of nonsyngeneic immunocompetent cells or their precursors into an immunologically inert recipient in the embryonic or early postnatal period of development, has been called "runt disease" [4]. It has been widely used in recent years as a model with which to estimate the functional activity of various categories of lymphocytes [3] and their interaction in reactions of transplantation immunity [5].

The course of runt disease as one form of the GVHR was studied in the investigation described below in relation not only to the degree of genetic differences between donor and recipient, but also to their reciprocal position in the combination of strains.

EXPERIMENTAL METHOD

Inbred mice of strains CBA ($H\text{-}2^k$) and C57BL/6 ($H\text{-}2^b$) were obtained from the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR. To induce the GVHR, donors' spleen cells, washed three times and suspended in 0.05 ml of medium No. 199, were injected intraperitoneally into the recipients during the first 24 h after birth. In series I, spleen cells of the C57BL genotype were injected into CBA recipients. In the experiments of series II the reciprocal combination of strains was used. For alloimmunization the donors were given an

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TABLE 1. Reproducibility and Activity of GVHR after Transplantation of Allogeneic Spleen Cells into Mice

Series of experiments	Experimental conditions	Dose of cells ($\times 10^7$)	No. of litters	No. of mice with SR > 1/ total number of mice	SI ($M \pm m$)
I	C57BL→CBA	0,5	8	10/23	1,47±0,055
		1	9	24/28	1,94±0,108
		2	16	52/55	2,15±0,115
	C57BL anti-CBA→CBA				
	A	0,5	10	21/26	3,16±0,320
	B	0,5	8	10/21	1,56±0,091
	C57BL anti-SRBC→CBA	2	8	4/21	1,23±0,057
II	CBA→C57BL	1	7	0/15	1,18±0,077
		2	7	4/22	1,24±0,113
	CBA anti-C57BL→C57BL	1	8	2/18	1,32±0,151
		2	5	1/11	1,15±0,207

Legend. After transplantation of disintegrated allogeneic cells SR was always below 1 (not included in Table 1). SI was calculated 10-12 days after cell transplantation.

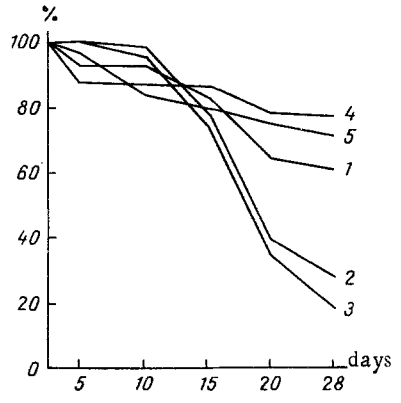

Fig. 1

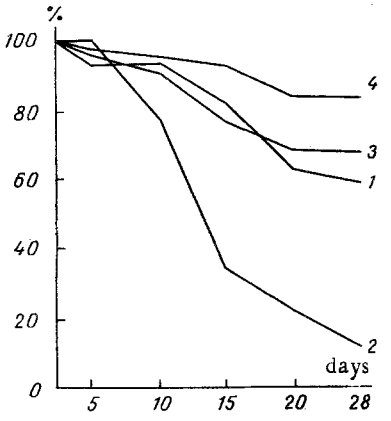

Fig. 2

Fig. 1. Curves of mortality among mice after transplantation of allogeneic spleen cells. Combination C57BL→CBA: 1) $0.5 \cdot 10^7$ cells, 2) $1 \cdot 10^7$ cells, 3) $2 \cdot 10^7$ cells; combination CBA→C57BL: 4) $1 \cdot 10^7$ cells, 5) $2 \cdot 10^7$ cells. Here and in Fig. 2: Abscissa, days of experiment; ordinate, %.

Fig. 2. Mortality curves after transplantation of normal and sensitized C57BL cells into CBA mice: 1) $0.5 \cdot 10^7$ C57BL spleen cells; 2) $0.5 \cdot 10^7$ C57BL cells obtained 7-14 days after immunization of donors with CBA isoantigens (C57BL anti-CBA cells); 3) $0.5 \cdot 10^7$ C57BL anti-CBA cells obtained 2-3 months after immunization of donors; 4) $2 \cdot 10^7$ C57BL cells obtained 7-14 days after immunization with sheep's red cells.

intraperitoneal injection of 0.2 ml of 50% whole liver homogenate + 10^8 living spleen cells of the recipient's strain. In the control, two or three mice from the same litter were injected with syngeneic spleen cells and one or two cells from allogeneic donors, disintegrated by freezing and thawing 4 or 5 times. Activity of the GVHR was estimated from the mortality and the value of the splenic index (SI), the ratio between the weight of the spleen and the body weight in % (the splenic ratio, SR) in the experimental series, divided by the same index in the "syngeneic" control.

EXPERIMENTAL RESULTS

In the experiments of series I, after transplantation of $0.5 \cdot 10^7$, $1 \cdot 10^7$, or $2 \cdot 10^7$ spleen cells of the C57BL genotype into CBA mice, a GVHR developed in 43, 86, and 95% of cases respectively (Table 1). Most of the recipients died 2-3 weeks after the transfer of cells (Fig. 1). Dose-effect relationship was seen most clearly with cell doses of between $0.5 \cdot 10^7$ and $1 \cdot 10^7$ ($P<0.05$) and less clearly with doses of $1 \cdot 10^7$ to $2 \cdot 10^7$ ($P>0.05$). Starting from the 10th-12th day growth of the recipients was completely inhibited and splenomegaly was accompanied by atrophy of the thymus. After injection of C57BL cells obtained 7-14 days after alloimmunization of the donors with CBA ("A") antigens, the GVHR ran a course several times less active than after transplantation of the same dose of "normal" cells ($P<0.05$). Meanwhile, if the cells were obtained from C57BL mice sensitized against CBA H-alloantigens 2-3 months before transplantation ("B"), no appreciable increase in GVHR was observed ($P>0.05$). After transplantation of $2 \cdot 10^7$ C57BL spleen cells preimmunized with "foreign" antigens (sheep's red cells), the reproducibility of the GVHR was reduced more than fourfold (Table 1, Fig. 2), and SI was significantly lower than after transplantation of the same dose of cells of intact mice ($P<0.05$).

On the 5th-7th day after transplantation pyroninophilic monocytes accumulated in the zone of reduced primary splenic follicles, but on the 10th-12th day destructive processes developed in the territory of the follicles (Fig. 3).

In the experiments of series II and in the combination CBA→C57BL the GVHR followed a less active course (Table 1, Fig. 1). On the 10th-12th day after transplantation of $1 \cdot 10^7$

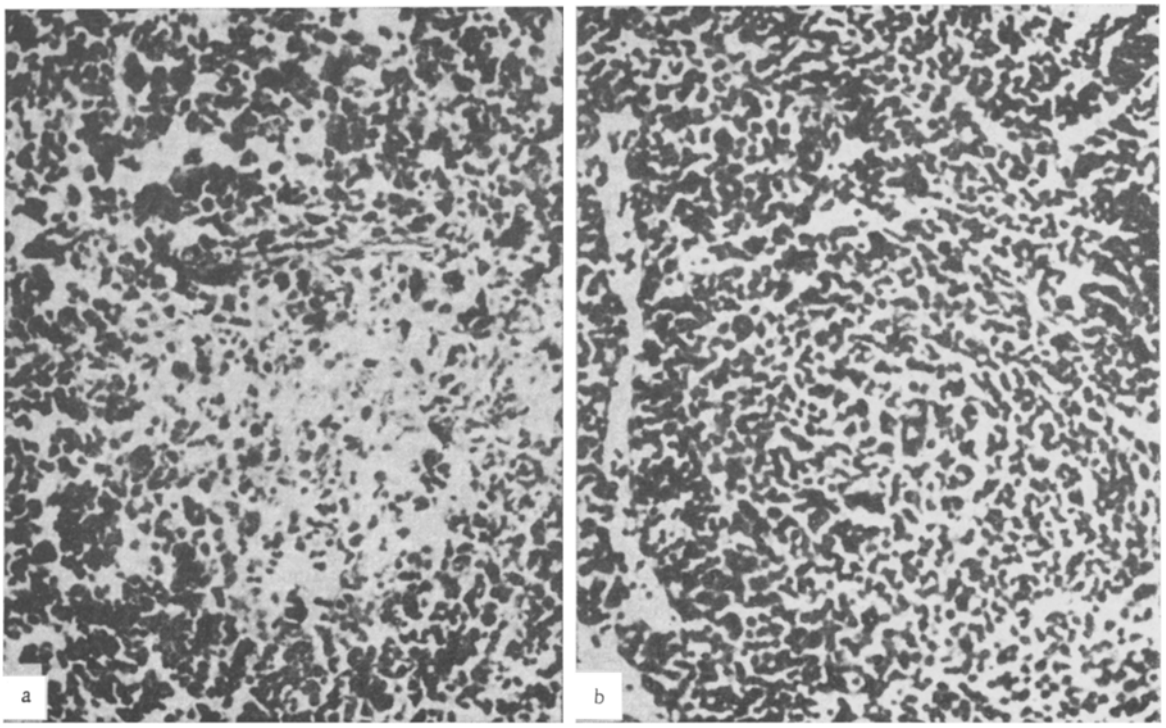

Fig. 3. Sections through spleen of CBA mice: a) Control, 12th day after transplantation of $2 \cdot 10^7$ syngeneic cells; primary follicle; b) experiment, 14th day after transplantation of $2 \cdot 10^7$ cells of C57BL genotype: necrosis of white pulp. Stained with methyl green—pyronine by Brachet's method, 140×.

cells no visible signs of the GVHR could be observed in the recipient, whereas after transplantation of $2 \cdot 10^7$ cells they were observed in only 18% of the animals. SI was significantly lower than during reciprocal combinations of strains ($P<0.05$, $P<0.02$). To judge from the value of SI, after immunization of the donors with the recipient's H-alloantigens no appreciable increase in the intensity of the GVHR took place ($P>0.05$). The formation of primary follicles in the spleen was inhibited without any appreciable destruction of the white pulp.

Differences in the course of the GVHR in the combinations C57BL→CBA and CBA→C57BL in these experiments were due, it can be suggested, to the greater sensitivity of lymphocytes of the C57BL genotype to CBA H-alloantigens. Another fact to be remembered is that the greater part of the pool of the donor's stem cells in the C57BL→CBA combination could be inactivated or "used up" in the direction of immunopoiesis, whereas in the reciprocal combination it is preserved and can participate in the "finishing" of hematopoietic cells [1, 2].

LITERATURE CITED

1. R. V. Petrov, Usp. Sovrem. Biol., 69, No. 2, 261 (1970).
2. V. N. Shvets, L. S. Seslavina, and V. V. Shikhodyrov, Byull. Éksp. Biol. Med., No. 6, 89 (1972).
3. H. Cantor and R. Asofsky, J. Exp. Med., 135, 764 (1972).
4. M. Simonsen, Prog. Allergy, 6, 349 (1962).
5. R. E. Tigelaar and R. Asofsky, J. Exp. Med., 135, 1059 (1972).