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INCREASED RESISTANCE OF (CBA × C57BL/6)F_1 HYBRIDS TO THE SYSTEMIC GRAFT versus HOST REACTION DURING REGENERATION OF THE SPLEEN

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Partial splenectomy was performed on (CBA × C57BL/6)F_1 hybrid mice 2, 12, 15, and 20 days before induction of the graft versus host reaction (GVHR) and 2 and 10 days after injection of parental immunocompetent cells. Recipients with an intact spleen, and those undergoing total splenectomy or a mock operation served as the control. Hybrids with a regenerating spleen up to 12 days of regeneration were shown to have increased resistance to GVHR, whereas splenectomy increased the resistance of the hybrids to GVHR only if carried out 2 days before induction of GVHR or 2 days after it.

KEY WORDS: graft versus host reaction; partial splenectomy; regeneration.

In the early stage of the systemic graft versus host reaction (GVHR) splenomegaly is known to develop, and this may indicate a role of the spleen in the pathogenesis of the GVHR. Removal of the spleen 2 days after injection of donors' immunocompetent cells has been shown experimentally to cause weakening of this reaction [5, 6]. However, this hypothesis is contradicted by the fact that splenectomy, if performed 1, 2, or 7 days before induction of the GVHR, had no significant effect on the intensity of the reaction. Admittedly the same operation, if performed 12 days before induction of GVHR, aggravated its course [5-7].

No reference to the study of the effect of partial splenectomy (PS) on the course of the GVHR could be found in the accessible literature. However, such an investigation appeared interesting because during regeneration of the spleen immunological changes are observed in the animal, affecting both its cellular and its humoral immunity [1-4].

The object of this investigation was to study the effect of PS, performed at different times before and after induction of the GVHR, on the course of this reaction.

METHODS

Experiments were carried out on 524 female (CBA × C57BL/6)F_1 hybrids weighing 19-22 g, obtained from the "Stolbovaya" Nursery of Inbred Animals, Academy of Medical Sciences of the USSR. Under superficial ether anesthesia, two-thirds of the tissue of the spleen was removed from the recipients of the experimental group by the usual method [2, 4] or total splenectomy (TS) was performed 2, 12, 15, and 20 days before induction of GVHR and 2 and 10 days after injection of parental immunocompetent cells. The control recipients underwent either a mock operation or no operation at all.

C57BL/6 female mice served as donors for induction of GVHR. A suspension of spleen cells was prepared by homogenization in medium 199 and filtered twice through a fine Kapron filter. Viable cells were counted with the aid of trypan blue. A systemic GVHR was induced by injection of $75 \cdot 10^6$ living parental spleen cells in a volume of 0.4 ml into the retro-orbital venous sinus. Development of the GVHR was assessed clinically from the reduction in weight of the recipients, falling out of the hair, the stooping posture, diarrhea, and death of the animals.

Animals of each group were killed five at a time on the 10th and 25th day after induction of the GVHR and the weight of the spleen and body weight were determined.

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TABLE 1. Effect of Partial (PS) or Total (TS) Splenectomy on Mean Length of Survival of Animals with Induced Systemic GVHR (M ± m)

Series of experiments	Time of operation relative to time of induction of GVHR, days	Group of recipients			
		1 (PS)	2 (TS)	3 mock operation	4 no operation
		mean length of survival of animals, days			
I	Before 2	171,1±4,9 †	180,0±0†	85,0±17,6	80,7±18,2
	» 12	127,8±14,94*	66,0±17,0	—	80,7±18,2
	» 20	68,1±7,82	67,0±10,32	—	73,9±16,12
II	After 2	57,5±2,47	60,0±0	—	57,0±1,67
	» 10	56,8±1,91	56,8±1,91	—	54,7±2,26

*P < 0.05.
†P < 0.001.

The longest period of observation on the animals was 180 days in the experiments of series I and 60 days in series II.

The experimental results were subjected to statistical analysis by the Fisher—Student method and by the use of a nonparametric criterion (Fisher's method for fourfold tables).

RESULTS

As Table 1 shows, PS performed 2 and 12 days before induction of GVHR significantly prolonged the mean life span of the animals compared with the control group of mice not undergoing any operation (series I, groups 1 and 4). A further increase in the interval between the operation and injection of the parental immunocompetent cells did not give this effect.

Total splenectomy 2 days before induction of GVHR also significantly increased the mean period of survival of the animals compared with the group of mice not undergoing any operation, but did not affect the length of survival of recipients if the operation was performed 12, 15, or 20 days before induction of GVHR (series I, group 2).

However, if PS was performed 2 or 10 days after induction of GVHR, no increase in the resistance of the hybrids to this reaction was observed.

In hybrids undergoing PS and TS 2 days before induction of GVHR the body weight rose steadily and, on the 25th day of GVHR, it significantly exceeded the body weight of recipients with an intact spleen. For instance, at this period of observation the weight of the intact recipients and recipients undergoing the mock operation was 18.5 ± 0.5 and 19.5 ± 1.2 g respectively, whereas the weight of partially and totally splenectomized mice with a GVHR reached 23.4 ± 0.2 and 23.1 ± 0.5 g. In other words, operations on the spleen protected the animals and the GVHR followed a milder course in them. During 100 days of observation, none of the recipients undergoing PS 2 days before injection of the GVHR died, and the mortality observed later (between 100 and 180 days) was significantly lower than in mice not undergoing the operation (30 and 80% respectively; $P < 0.025$).

If PS was performed 12 days before the induction of GVHR, none of the recipients died during the first 30 days of observation, and the mortality observed over a period of 60 days was significantly lower than that found in intact recipients (13.3 and 60% respectively; $P < 0.025$), whereas TS did not alleviate the course of the GVHR in this period.

PS performed 15 days before induction of the GVHR did not significantly reduce the mortality among the recipients, but the clinical course of their reaction was milder, and the absolute mortality among these animals compared with that among mice with an intact spleen was as follows: Four of 15 mice with PS died, compared with eight of 15 intact recipients (period of observation 60 days); this suggests a tendency for the resistance of hybrids with PS to GVHR to increase.

The course of GVHR in recipients with PS performed 20 days before induction of GVHR did not differ significantly from that in control mice with an intact spleen. If the spleen was removed completely 2 days after induction of GVHR there were no clinical signs of disease and none of the recipients died during 60 days of observation, whereas removal of the spleen at the height of the proliferative phase of GVHR (on the 10th day), while not causing a signif-

icant decrease in mortality among the recipients, nevertheless did not cause a sharp decrease in the body weight of the animals, and on the 25th day of GVHR it was significantly greater than the body weight of recipients not undergoing an operation ($P < 0.01$).

Investigation of the weight of the regenerating and intact spleen in hybrids on the 10th day after induction of GVHR showed a significant increase in the weight both of the residual part of the spleen and in the weight of the spleen as a whole in both series of experiments compared with the corresponding figures for control mice not exposed to the GVHR.

In mice on which PS was performed 2, 12, and 15 days before induction of GVHR no significant differences were found in the weight of the residual part of the spleen on the 10th and 25th days of GVHR ($P > 0.05$). In recipients undergoing PS 2 and 10 days after induction of GVHR a significant increase in the weight of the residual part of the spleen was observed on the 25th day of observation ($P < 0.01$). The GVHR did not facilitate the development of regeneration of the spleen, for the weight of the residual part of the spleen accounted for approximately the same percentage of its initial value as in animals without GVHR, and sometimes it was lower than in the case of the spleen of the control animals.

Processes of regeneration of lymphoid tissue in the spleen or in the system of immunogenesis as a whole evidently interfere with the development of the GVHR, possibly in connection with the action of cellular or humoral factors of immunity.

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