PARTICIPATION OF MOUSE T AND B LYMPHOCYTES IN THE GRAFT VERSUS HOST REACTION

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Transplantation of spleen or lymph node cells from CBA mice into sublethally irradiated (CBA × C57BL/6)F_1 mice induced the development of a graft versus host reaction (GVHR). The lymphocytes lost their ability to give this reaction after treatment *in vitro* with specific sera against both mouse T lymphocytes and B lymphocytes. The development of the GVHR in mice is evidently connected with cooperative interaction between T and B lymphocytes.

KEY WORDS: T and B lymphocytes; graft versus host reaction; cellular interaction.

It has now been shown that the graft versus host reaction (GVHR) is effected by lymphocytes. However, data showing whether these cells belong to the T or B lymphocyte populations and on whether cooperative interaction between T and B cells is necessary for this reaction are contradictory. It has been clearly shown that cells of lymphoid organs of mice which contain both T and B lymphocytes (spleen, lymph nodes, and so on) are more able to give the GVHR than cells of the thymus or bone marrow [8, 11, 14, 17]. Some workers consider that during development of the GVHR synergism takes place between thymus and bone marrow cells [7, 10, 12], but this is denied by others [9, 15].

Various workers [5, 6, 13, 16] and the present writer [1, 2] showed previously that antilymphocytic serum (ALS) abolishes the development of the acute GVHR in mice injected with spleen cells taken from donors receiving ALS or with spleen cells treated *in vitro* with ALS.

The object of this investigation was to study the participation of mouse T and B lymphocytes in the GVHR. The basic experimental method was elimination of one of the lymphocyte populations from the cell suspension with the aid of antisera against T or B cells, followed by observation of the ability of the "defective" suspension to give the GVHR.

EXPERIMENTAL METHOD

Male CBA and (CBA × C57BL/6)F_1 mice were used. The ability of the T and B lymphocytes of the mice to carry out the GVHR was tested in two experimental models.

The first model was the development of an acute GVHR with lethal outcome in (CBA × C57BL/6)F_1 mice irradiated in a dose of 700 rad and then injected with spleen cells from mice of the parental genotype. The F_1 mice, 24 h after irradiation with ^{137}Ce γ rays on the Stebel" 3A apparatus (dose rate 900 rad/min), received an intravenous injection of $3 \cdot 10^7$ nucleated spleen cells from CBA mice. The GVHR was assessed from the survival rate of the recipients during the 25 days after transplantation.

The second experimental model of the GVHR was based on the fact that lymph node cells of CBA mice, if injected intravenously into sublethally irradiated (CBA × C57BL/6)F_1 mice, inhibit the formation of endogenous hematopoietic colonies in the recipients' spleens [3]. The (CBA × C57BL/6)F_1 mice were irradiated in a dose of 750 rad and, 24 h after irradiation, were injected intravenously with $4 \cdot 10^6$ nucleated lymph node cells of CBA mice. The mice were killed 9 days later, the spleens fixed in a mixture of 1 part glacial acetic acid and 3 parts

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TABLE 1. Inhibition of Endogenous Colony Formation in Sublethally Irradiated (CBA × C57BL/6)F_1 Mice by T and B Lymphocytes from CBA Mice

Cells injected into recipients	Number of recipients	Number of colonies per spleen M ± m	Percentage of inhibition
LN	30	2,4± 1,2	73
LN + C	22	2,0± 0,9	77
LN + C + ATS	33	10± 2,4	0
LN + C + ABS	25	7,9± 1,1	9
(LN + C + ATS) + (LN + C + ABS)	9	2,3± 0,7	74
	26	8,8± 2,3	0

Legend. LN) lymph node cells; C) complement.

absolute ethanol, and the number of hematopoietic colonies on their surface was counted macroscopically. The intensity of the GVHR was assessed from the reduction in the number of colonies.

The transplanted lymphocytes (spleen cells in the first case, lymph node cells in the second) were either intact or previously treated *in vitro* with antisera against T (ATS) or B (ABS) mouse lymphocytes. As a result of treatment with ATS a suspension of B lymphocytes was obtained; treatment with ABS yielded a suspension of T lymphocytes. The suspension was treated as follows: To 1 ml ($2 \cdot 10^7$ nucleated cells) of the suspension 0.1 ml antiserum and 0.05 ml freshly frozen rabbit serum (as complement) were added. The mixture was incubated for 45 min at 37°C, after which the cells were washed by centrifugation in cold medium No. 199 for 10 min at 1500 rpm. The washed cells were resuspended in medium No. 199 and injected into the recipients in the doses given above.

Monospecific antisera against mouse T and B lymphocytes were prepared as described previously [2]. ATS in the presence of complement killed 98% of the thymus cells but did not act on the bone marrow cells; ABS had no effect on thymus cells but killed 30-40% of the bone marrow cells. ABS completely abolished the ability of the antibody-forming cells to form zones of hemolysis in Jerne's method; ATS had no effect on antibody-forming cells.

The results were subjected to statistical analysis by Lord's method [4].

EXPERIMENTAL RESULTS

Experiments with the first model showed that after transplantation of intact spleen cells an acute GVHR developed in the recipients, causing death of all of them by the 15th day after transplantation and irradiation (Fig. 1). Treatment of intact spleen cells with rabbit complement did not alter their ability to induce the GVHR. Treatment of the spleen cell suspension with both ATS and ABS rendered it incapable of causing death of the recipients from acute GVHR. In these groups, by the 25th day after transplantation, the survival rate of the mice was indistinguishable from that in the "irradiation control" group.

The results of the three experiments obtained with the second model of GVHR are summarized in Table 1. This group of experiments confirmed the patterns observed in the experiments described above. Intact lymphocytes completely inactivated colony formation in the recipients' spleens, whereas both antisera (ATS and ABS) rendered the allogeneic lymphocytes incapable of inhibiting endogenous colony formation. The number of hematopoietic colonies in the spleens of recipients of the lymph node cells treated with antisera did not differ statistically from the number in the spleens of irradiated mice not receiving lymph node cells. Experiments with the same model of GVHR showed that ATS and ABS act on different lymphocyte populations. The suspension of lymph node cells treated with ATS and the suspension of lymph node cells treated with ABS were mixed in equal proportions and injected into the recipients. This mixture inhibited endogenous colony formation just as did the cells of intact lymph nodes.

Experiments with two different models thus showed that treatment of a suspension of lymphocytes *in vitro* with both ATS and ABS makes the cells unable to carry out the GVHR. Under the experimental conditions used, neither the population of T cells nor the population

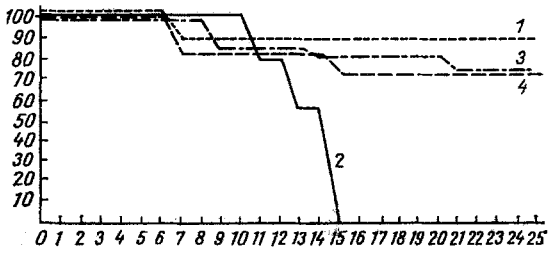

Fig. 1. Participation of mouse T and B lymphocytes in graft versus host reaction: 1) irradiation control; 2) intact spleen cells; 3) spleen cells treated with ATS; 4) spleen cells treated with ABS. Abscissa, days after transplantation; ordinate, percentage of surviving mice.

of B cells by itself could give rise to a normal GVHR. It can be postulated that an essential role in the development of the GVHR is played by cooperative interaction between T and B lymphocytes.

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