Depression of the intensity of the immune response after injection of the anti-DBA/2 and antimacrophagal sera on the first 2 days after transplantation of the antigen-processing cells of the peritoneal exudate evidently indicated that MPH induced antibody formation during the first 2 days after their transplantation into the recipient, and that it was at that time that the antisera prevented interaction between the donor's MPH and the recipient's immunocytes. However, the number of AFC never fell to the background level as was observed after injection of antierythrocytic serum. If anti-DBA/2 serum was injected on the first 2 days after transplantation of the cells the intensity of the immune response corresponded to values obtained when free antigen was used [2]. It can be postulated that specific destruction of the immune MPH by anti-DBA/2 antibodies injected on the 3rd-4th day after transplantation of the antigen-processing peritoneal exudate cells did not effect the final intensity of the immune response, because the induction process was already complete. Consequently, it is extremely likely that the immune rejection was unable to exert its eliminating action for the induction period ended before maturation of the rejection factors could be completed.

Other workers using adherent and nonadherent spleen cells of two inbred strains and allogeneic antiserum against adherent cells also reached similar conclusions [3]. Analysis of the accumulation of AFC in a nonsyngeneic combination between the donor of immune MPH and the recipient suggests that the effectiveness of induction depends not only on the immunogen of the MPH cell surface and the antigen-recognizing receptors of the immunocytes, but also on the genetic correspondence (structural similarity) of the interacting cellular units. The most effective induction by immune MPH takes place under conditions of complete genotypical similarity of the interacting immunocompetent cells.

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PARTICIPATION OF PHYTOHEMAGGLUTININ-TRANSFORMED MOUSE LYMPHOCYTES IN THE GRAFT VERSUS HOST REACTION

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Transformed lymphocytes obtained by stimulating lymph node cells of CBA mice with phytohemagglutinin (PHA) do not give the graft versus host reaction (GVHR) if injected into sublethally irradiated (CBA x C57BL/6)F_1 hybrids. In a population of PHA-stimulated cells the GVHR was induced by small lymphocytes having the same concentration of antigens, detectable by antilymphocytic serum, as intact lymphocytes.

KEY WORDS: phytohemagglutinin; blast transformation; graft versus reaction.

It was shown previously that cultivation of lymph node cells with phytohemagglutinin (PHA) for 44 h led to a considerable reduction in their activity in the graft versus host reaction (GVHR). It was suggested that

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the decrease in the ability of PHA-stimulated cells to induce this reaction is connected with their transformation into blast cells [2].

In the investigation described below this hypothesis was tested by studying the participation of PHA-transformed lymphocytes in the GVHR.

EXPERIMENTAL METHOD

Lymph node cells from adult CBA mice weighing 20-22 g were cultivated in vitro with PHA for 2 or 44 h. The methods of obtaining the cells, of cultivation and of assessing the blast-transformation effect were described previously [2]. Cell suspensions were treated with specific antisera and the lymphocytes against which they were obtained were selectively eliminated. The following antisera were used to treat the intact and PHA-stimulated cells: antilymphocytic serum (ALS) with a broad spectrum of action and antiserum against PHA-transformed lymphocytes (ATLS). ALSwas obtained from the Department of Immunology of the Moscow Institute of Epidemiology and Microbiology, Ministry of Health of the RSFSR. Rabbits were immunized with intact mouse lymphocytes and the resulting serum was absorbed with mouse erythrocytes, liver cells, and serum [1]. The ATLS was obtained by immunizing rabbits with mouse lymphocytes stimulated for 68 h with PHA, after which the serum was absorbed with mouse erythrocytes, liver cells, serum, and intact lymphocytes. In the cytological tests the ATLS was nontoxic against mouse lymphocytes, both intact and cultivated for 2 h with PHA, and it killed 60-70% of the cells in the population of lymphocytes stimulated with PHA for 68 h. The toxicity of the ATLS was not connected with increased sensitivity of the transformed lymphocytes to treatment with antisera, for intact lymphocytes were more sensitive to the ALS [3]. A sample of 1 ml of a suspension of intact of PHA-stimulated lymph node cells ($2 \cdot 10^7$ cells in 1 ml medium) was incubated for 40 min at 37°C with 0.1 ml of a known dilution of the corresponding antiserum and 0.05 ml of blood serum of an intact rabbit (the source of complement). After incubation the cells were washed by centrifugation in medium No. 199 at 1000 rpm for 10 min and used in a model of the GVHR based on depression of spontaneous endogenous colony formation by parental lymphocytes in sublethally irradiated F_1 hybrids [5]. An intravenous injection of $3 \cdot 10^6$ living lymph node cells from CBA mice, treated with antisera, was given to the (CBA x C57BL/6) F_1 recipient mice 24 h after their irradiation with ^{137}Ce γ-rays on the "Stebel'-3A" apparatus in a dose of 750 R. The number of colonies in the recipients' spleen was counted on the 9th day and the percentage inhibition of the colony-forming units (CFU) was calculated by the usual formula $(a - b)/a \cdot 100\%$, in which a is the number of CFU in the spleen of mice of the control group (irradiation only), and b the number of CFU in the spleen of mice of the experimental group. Statistical analysis of the results was carried out by Lord's method and confidence intervals were calculated with a probability of 0.95 [4].

EXPERIMENTAL RESULTS

The cell composition of the intact lymph node cells and of the suspension of cells cultivated with PHA is given in Table 1. The number of transformed cells in the culture of lymphocytes with PHA after 44 h was more than 40% (above 3% in the culture without PHA). Among intact lymph node cells and cells stimulated with PHA for 2 h there were 1-2% of transformed lymphocytes and 93-97% of small lymphocytes.

Data showing the ability of the antisera to abolish the GVHR are given in Table 2. ALS equally depressed the ability of the intact of PHA-stimulated lymphocytes to induce the GVHR. In a dilution of 1:10 ALS abolished endogenous colony formation by lymphocytes of all three groups completely, but in a dilution of 1:50 it cause virtually no change. Lymphocytes responsible for the GVHR in the population of intact or PHA-stimulated cells evidently carry equal concentrations of antigens detectable by ALS on their surface.

According to an earlier hypothesis, a new antigen appears on the surface of lymphocytes transformed by PHA [6-8]. An antiserum (ATLS), selectively toxic for PHA-transformed lymphocytes [3], has been obtained against an antigen present on proliferating lymphocytes. In a population of cells stimulated for 44 h by PHA lymphocytes accounted for over 40% (Table 1) and the ATLS in the cytotoxic tests killed 45-50% of the cells of this group. Meanwhile, if a suspension of cells cultivated for 44 h was treated with ATLS, their ability to depress endogenous colony formation was unchanged (Table 2). Transformed lymphocytes evidently do not participate in the induction of the GVHR. ATLS had a similar action on lymph node cells whether intact or cultivated for 2 h with PHA: It did not inhibit their activity in GVHR (Table 2).

The following conclusions can be drawn from these results. Transformed lymphocytes obtained as a result of stimulation by PHA cannot give the GVHR in the experimental model used. Lymphocytes transformed into blast cells through the action of a nonspecific polyclonal stimulator (PHA) perhaps differentiate

TABLE 1. Cell Composition of CBA Mouse Lymph Node Cells before and during Cultivation with PHA

Time of cultivation (in h)	Transformed lymphocytes (in%)	Small lymphocytes (in %)
—	1,3±0,2	97,7±0,9
2	1,7±0,1	93±1,1
2*	2,1±0,1	92,9±0,8
44	3±1,6	87±2,6
44*	45±2,4	40±4,3

* Cultivation with PHA

TABLE 2. Ability of Antisera to Abolish GVHR Induced by Different Cell Groups

Antiserum	Dilution of antiserum	% inhibition of CFU in recipient's spleen after injection of lymph node cells		
		Normal	Cultivated for 2h with PHA	Cultivated for 44 h with PHA
—	—	100	100	100
ALS	1:10	13±10	15±12	0
	1:50	86±12	100	87±18
	Whole	100	100	96±3
ATLS	1:2	100	100	100
	1:10	100	100	100

into cells that are inactive from the immunological point of view, cells that after contact with the specific stimulator (transplantation antigens) are unable to give an immunological response (the GVHR). In a population of PHA-stimulated cells the GVHR is induced by small lymphocytes which have the same concentration of antigens detectable by ALS as intact lymphocytes.

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