A STUDY OF THE LYMPH-NODE TYPE OF GRAFT VERSUS HOST REACTION IN MICE

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A local form of the graph versus host reaction (GVHR) was induced in adult (CBA × C57BL)F_1 hybrid mice by subcutaneous injection of semiallogeneic spleen, thymus, or bone marrow cells from CBA mice into the right hind footpad. The criteria of activity of the GVHR were an increase in the number of blast forms in the region of popliteal lymph node and in its weight 7 days after transplantation of cells. After transplantation of 5×10^6 and 20×10^6 spleen cells the absolute weight of the regional lymph node was increased by 3-5 times and was significantly higher than in the control (injection of living syngeneic or fragmented semiallogeneic cells from the same source). By contrast with the control, in the experimental animals the effect clearly depended directly on the dose of transplanted cells. Enlargement of the lymph nodes was accompanied by the regular appearance of blast forms in them. Thymus and bone marrow cells had a much weaker action than spleen cells.

KEY WORDS: *transplantation; graft versus host reaction; lymph nodes.*

The graft versus host reaction (GVHR) is widely used as a model for assessing the H-alloantigen-identifying function of lymphocytes with respect to H-alloantigens in different situations [3, 5]. In the writers' view, the most promising models of GVHR are those in which allogeneic test lymphocytes are injected subcutaneously into the footpad of rats [6] or F_1 hybrid mice [7] and the effect is recorded in the regional lymph node [4].

In this investigation the character of manifestation of the local "lymph-node" GVHR was studied after transplantation of spleen, thymus, and bone marrow cells from CBA mice into (CBA × C57BL)F_1 hybrids.

EXPERIMENTAL METHOD

CBA and (CBA × C57BL)F_1 hybrid mice obtained from the "Stolbovaya" nursery of laboratory animals, Academy of Medical Sciences of the USSR, were used. Spleen, thymus, and bone marrow cells were obtained from male CBA mice aged 4-5 months and injected into male (CBA × C57BL)F_1 mice aged 3-4 months and weighing 18-24 g. The method of preparing the suspension of spleen and thymus cells was described previously [1]. The bone marrow was flushed out of the femur and tibia, forced under pressure through a Kassirskii needle, and washed 3 times with medium No. 199. The number of viable cells in the suspension was 85-90%. The cells were injected subcutaneously into the hind footpad of the F_1 hybrids in doses of 5×10^6 and 20×10^6 in 0.05 ml medium No. 199. Control F_1 recipients received 5×10^6 and 20×10^6 syngeneic spleen cells or equivalent doses of fragmented CBA spleen cells. In the latter case, the cells were quickly frozen and thawed 3 times, and then homogenized in the RT-2 microgrinder at 300 rpm for 2 min, under the control of the trypan test. The recipient mice were killed 7 days after transplantation. The criteria of GVHR activity were an increase in

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TABLE 1. Weight of Regional Lymph Nodes and Number of Blast Forms in Them after Transplantation of CBA Thymus, Spleen, and Bone Marrow Cells into (CBA × C57BL)F_1 Recipients (M±m)

Source of cells	Transplantation of 5×10^6 cells			Transplantation of 20×10^6 cells		
	number of animals	weight of regional lymph node (in mg)	number of blast cells (in ‰)	number of animals	weight of regional lymph node (in mg)	number of blast cells (in ‰)
CBA spleen	45	4,8±0,27	3,0±0,33	40	8,1±0,37	5,9±0,40
CBA thymus	29	2,1±0,16	1,4±0,35	29	2,8±0,25	2,2±0,17
CBA bone marrow	26	1,8±0,37	1,3±0,31	26	3,2±0,20	2,6±0,52
Fragmented CBA spleen cells (control 1)	8	3,7±0,52	—	8	3,2±0,46	—
(CBA × C57BL)F_1 spleen cells (control 2)	14	2,1±0,40	1,3±0,20	10	2,5±0,17	1,3±0,21

weight of the regional lymph node, the ratio between the weight of the regional lymph node and the contralateral equivalent, and the percentage of immunoblasts in the regional popliteal lymph node. The lymph nodes were weighed with an accuracy of 0.1 mg. Immunoblasts were counted in squash preparations of lymph nodes stained with azure II-eosin in 10,000 cells, using the criteria of Pokrovskaya et al. [2]. Statistical analysis of the numerical results was carried out by Student's t-test.

EXPERIMENTAL RESULTS

The weight of the popliteal lymph node in intact (CBA × C57BL)F_1 mice was 1.6±0.06 mg. As Table 1 shows, after transplantation of syngeneic cells an insignificant (by a factor of 1.3 to 1.5) enlargement of the regional lymph node occurred which was practically independent of the dosage of injected cells. The deviation from the weight of the lymph nodes of intact mice was unreliable for a dosage of 5×10^6 ($P > 0.05$) and statistically reliable for a dosage of 20×10^6 ($P < 0.01$). Following injection of equivalent doses of fragmented CBA spleen cells, the regional lymph node was enlarged by 2-2.5 times irrespective of the dose of material injected ($P < 0.01$). After transplantation of viable CBA spleen cells, enlargement of the lymph node depended directly on dose. It must be emphasized that the weight of the regional lymph node was significantly greater following transplantation of living CBA spleen cells than after injection of fragmented ($P = 0.05$ for a dose of 5×10^6 and $P < 0.001$ for a dose of 20×10^6) or living syngeneic spleen cells ($P < 0.01$ and 0.01 respectively). After transplantation of 5×10^6 and 20×10^6 CBA thymus cells the absolute weight of the regional lymph gland was increased by 1.3 ($P < 0.01$) and 1.8 ($P < 0.001$) times respectively compared with the contralateral node. After transplantation of 5×10^6 CBA bone marrow cells into F_1 hybrids practically no increase was found in the size of the regional lymph node, but after transplantation of 20×10^6 bone marrow cells it was almost doubled in size ($P < 0.001$). Meanwhile the effect of injection of thymus and bone marrow cells was much weaker than that of transplantation of the same doses of semiallogeneic spleen cells ($P < 0.001$). The ability of thymus and bone marrow cells to induce of GVHR was about equal.

After transplantation of 5×10^6 and 20×10^6 syngeneic spleen cells into the F_1 hybrids, equal numbers of blast forms were found in the regional lymph node (Table 1). Meanwhile transplantation of CBA spleen cells into F_1 hybrids led to a considerable accumulation of immunoblasts. The level of blast transformation in the regional lymph node was directly proportional to the dose of cells transplanted and it differed significantly from the corresponding values after syngeneic transplantation ($P < 0.001$). About equal numbers of immunoblasts were found in the regional lymph nodes of F_1 hybrids receiving 5×10^6 thymus or bone marrow cells, almost the same as the number of blast forms found in recipients of syngeneic spleen cells ($P > 0.5$). The same pattern was found when the effect of injection of 20×10^6 thymus and bone marrow cells was compared, but in this situation the percentage of immunoblasts was significantly higher than in the "syngeneic control" ($P < 0.001$). Dependence of effect on dose was observed for both thymus and bone marrow cells. After transplantation of thymus and bone marrow cells, only half the number of blast forms were found in the regional lymph node compared with the number after transplantation of semiallogeneic spleen cells ($P < 0.001$). On the whole, the degree of enlargment of the regional lymph node was directly proportional to the number of immunoblasts which accumulated in it.

After injection of semiallogeneic spleen, thymus, and, to a lesser degree, bone narrow cells of parental origin subcutaneously into the footpad of F_1 hybrids, under conditions in which donor and recipient differed with respect to the powerful H-2 histocompatibility complex, a characteristic immunospecific process developed in the regional lymph node and reflected the response of a particular pool of donor's lymphocytes with the recipient's isoantigens. This conclusion is supported by the absence of any analogous effect when living syngeneic or fragmented semiallogeneic spleen cells were transplanted.

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