SUCCESSFUL PREVENTION OF HOMOLOGOUS DISEASE IN MICE BY MEANS OF SYNGENETIC HEMATOPOIETIC CELLS

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UDC 617-089.843-097.5-092.9-085.361.41/42

Homologous disease in the "graft versus host" reaction did not develop if 24 h after transplantation of the parents' lymphocytes into F_1 hybrid mice, syngenetic lymphocytes were injected. Injection of syngenetic bone marrow cells under the same conditions was less successful but it did produce an effect after preliminary irradiation of the recipient. The disease, once it had started, could be completely suppressed only if a mixture of syngenetic bone marrow cells and lymphocytes was injected and if the injection was preceded by irradiation of the animals.

The transplantation of allogeneic hematopoietic tissue is followed in some cases by the development of transplantation sickness, in the development of which an important role is played by the "graft versus host" reaction [3, 6, 8, 14]. The immediate cause of the recipient's death under these conditions is considered to be lymphoid atrophy [2, 7, 13] and the associated increased sensitivity to infectious complications [4]. For this reason the study of the mechanism of the "graft versus host" reaction and of transplantation sickness and the search for methods of preventing or minimizing these conditions are of urgent importance. One such method could be to give the recipient an additional graft of hematopoietic tissue in order to prevent lymphoid atrophy.

The object of the investigation described below was to study the possibility of preventing or reducing the severity of homologous disease produced in F_1 hybrids by injection of parents' lymphocytes by means of an additional transplantation of various syngenetic hematopoietic cells at a time when the first signs of the disease are appearing and also during the first few days after injection of the allogeneic cells.

EXPERIMENTAL METHOD

Mice of inbred lines obtained from the Stolbovaya Nursery of Inbred Animals, Academy of Medical Sciences of the USSR, were used in the experiment. Homologous disease was induced in unirradiated (CBA × C57BL/6) F_1 hybrid mice weighing 16-18 g and aged 6-8 weeks, by injecting parent spleen and lymph gland cells from adult C57BL/6 mice into the retro-orbital venous sinus in a dose of 100-300 million cells.

Syngenetic cells of normal mice (cells from the thymus, lymph glands, and bone marrow) were additionally injected intravenously 1 and 10 days after transplantation of the parent cells into the F_1 hybrids. Some of the experimental hybrids were irradiated in a dose of 800 R 1-2 h after the injection of syngenetic cells. The conditions of irradiation were; RUM-11 apparatus, voltage 190 kV, current 15 mA, filters 1 mm Al and 0.5 mm Cu, field 20 × 20 cm, focal distance 40 cm, dose rate 44-48 R/min. The suspension of spleen, thymus, and lymph gland cells was prepared by a method described previously [2, 5]. The suspension of bone marrow cells was obtained by flushing out the femur and tibia with Hanks's solution.

The control to the those experiments consisted of irradiated or unirradiated hybrids injected the day before with parent's lymphoid cells. The effectiveness of the additional injection of hematopoietic cells

Department of Microbiology, Smolensk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR, N. N. Zhukov-Verezhnikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny. Vol. 73, No. 3, pp. 71–74, March, 1972. Original article submitted June 4, 1971.

TABLE 1. Effect of Transplantation of Syngenetic Hematopoietic Cells Combined with Preliminary Irradiation on Development outcome of Homologous Disease Developing in the Misc (CBA × C57BL/6) F_1 Hybrids after Injection of Parental Lymphocytes of C57BL/6 Mice

Experiment	Group	Number of mice*	Injection of syngenetic cells and irradiation				Number of mice surviving for 200 days			Life span of dying mice (in days), M ± m
			time of irradiation and (or) injection of syngenetic cells after injection of cells from C57BL/6 mice (in days)	dose of irradiation (in R)	type of cells	number of cells (in millions)	abs.	% ($M \pm m$)	P	
1	1	10	1	—	LGC	60	9	90±10	<0,05	36
	2	10	1	—	BMC	60	6	60±16,3	>0,05	39,2±4,5
	3	10	—	—	—	—	0	0±28,5	—	28,3±4,2
	4	10	1	800	LGC BMC	30 30	10	100±28,5	<0,05	—
2	5	10	10	—	LGC	60	1	10±10	>0,05	28,1±7,6
	6	10	10	—	TC	60	1	10±10	>0,05	27,0±5,7
	7	10	10	—	LGC BMC	30 30	2	20±13,3	>0,05	31,7±4,9
	8	10	10	—	TC BMC	30 30	2	20±13,3	>0,05	29,7±3,0
	9	10	10	—	BMC	60	1	10±10	>0,05	28,3±4,5
	10	10	—	—	—	—	0	0±28,5	—	32,0±5,2
3	11	10	10	800	BMC	5	0	0±28,5	>0,05	19,3±3,7
	12	10	10	800	BMC	60	3	30±15,2	>0,05	34,0±4,0
	13	10	10	800	—	—	0	0±28,5	—	15,7±1,2
	14	10	10	800	BMC LGC	30 30	7	70±15,2	<0,05	13,0±1,3
	15	15	10	800	BMC LGC	30 100	13	86,6±11,3	<0,05	24 ±5
	16	20	—	—	—	—	0	0±16,6	—	27,2±4,9

*All mice received intravenous injections of 100-130 million living lymphocytes of C57BL/6 mice.

Legend: BMC – bone marrow cells; LGC – lymph gland cells; TC – thymus cells.

was judged from the number of surviving mice or from the increase in life span of the experiment mice compared with the control.

EXPERIMENTAL RESULTS

The experimental results are given in Table 1. The control mice developed signs of homologous disease (loss of body weight, reduced mobility, untidiness of the hair) 10-12 days after receiving the injection of parental lymphocytes, and in most cases the animals died 20-40 days after transplantation. Death of most mice from homologous disease could be prevented (experiments of series I) if 24 h after the transplantation of parental lymphocytes, a further injection of 60 million syngenetic lymphocytes was given (group 1). Injection of syngenetic bone marrow at this period led to an increase in the survival period of the mice and prevented the development of the disease and death in 60% of cases (group 2), but these differences were not statistically significant ($P > 0.05$) compared with the control (group 3). If the hybrids were irradiated 24 h after receiving the injection of parental cells, subsequent transplantation of syngenetic bone marrow completely prevented death of the animals (group 4).

In the next experiment (series II) the possibility of preventing death of the mice 10 days after transplantation of the parental cells, i.e., when the first signs of homologous disease were appearing, was studied. The results showed that additional transplantation of hematopoietic tissue cells (lymph gland, thymus, and bone marrow cells), obtained from normal syngenetic hybrids, and injected in various combinations, did not prolong the life span or reduce the severity of the disease in any of the mice (group 5-9) compared with the control (group 10). Furthermore, after injection of thymus or lymph gland cells (group 5 and 6) the life span of the mice was slightly reduced by comparison with the control (group 10), but these differences were not statistically significant.

Death of most of the mice from transplantation sickness could be prevented only (experiments of series III) if the hybrids were irradiated 10 days after transplantation of the parental cells and if they received an injection of a mixture of lymphocytes and bone marrow cells from syngenetic mice (group 14; $P < 0.05$). With an increase in the number of lymphocytes in the mixture injected, the therapeutic effect was increased (group 15). Transplantation of a mixture of cells into the animal of these groups caused disappearance of the manifestations of homologous disease, the mice began to gain in weight, and no further deaths were observed (period of observation 200 days). Bone marrow cells alone, if injected after irradiation even in large doses, did not prevent the development of homologous disease in most animals and only slightly increased the life span of the mice (group 12) by comparison with unirradiated control hybrids (group 16). After transplantation of the parental cells the hybrids showed increased sensitivity to irradiation and died during the first 5-8 days after irradiation (group 13), whereas control mice did not die within 1 month after receiving this dose.

The results largely confirm the evidence in the literature that attempts to prevent deaths of animals from transplantation sickness by injection of syngenetic hematopoietic cells later than the 5th-6th day after transplantation of allogeneic cells are unsuccessful (1, 9, 15-18], even after preliminary irradiation of the recipient in which a "graft versus host" reaction had previously been induced [10]. Accordingly many authorities consider that the immunocompetent cells of the allogeneic graft are capable of inducing irreversible changes in the recipient's tissues, and these changes contribute to the further development of the transplantation sickness even in the absence of donors' cells. As evidence it is pointed out that in the study of chimerism in unirradiated hybrids a few days after transplantation of parental cells a very small number of donor mitoses can be detected [11, 12]. By contrast with those authors cited above, who in all their experiments, used only one type of hematopoietic cells (bone marrow or lymphocytes) for treatment and did not obtain positive results, in the present experiments the further development of homologous disease was successfully prevented by the use of irradiation of the experimental mice followed by injection of a mixture of syngenetic bone marrow cells and lymphocytes.

It can be concluded from the analysis of the present investigations and results obtained by other workers that donors' cells remain for a long time in the recipient's body and facilitate the development of homologous disease without exhibiting a high level of mitotic activity. The possibility cannot be ruled out that after injection of donors' cells their number decreases rapidly, but before this they modify the host's immune system to such an extent that its cells become capable of reacting against its own antigens, and also against injected syngenetic cells, abolishing their therapeutic action. Further experimental investigations are necessary to confirm these hypotheses.

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