EFFECT OF CORTISONE-RESISTANT THYMOCYTES ON ENDOGENOUS COLONY FORMATION IN INBRED MICE

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Endogenous colonies were counted in the spleen of sublethally irradiated (CBA×C57BL/6)F_1 hybrid mice after injection of thymocytes and lymph node cells treated with hydrocortisone and in intact CBA mice. Cortisone-resistance thymoctyes did not inhibit endogenous colony formation, whereas lymph node cells had a marked suppressive effect on endogenous colonies. In individual recipients the number of colonies in the spleen after injection of cortisone-resistant thymocytes was twice the number found in control irradiated hybrids.

KEY WORDS: *cortisone-resistant thymocytes; endogenous colony formation; graft versus host reaction.*

Cortisone-sensitive thymocytes are known to occur in the cortex of the thymus. During maturation they migrate into the medulla of the thymus and become cortisone resistant [11]. Cortisone-resistant thymocytes account for $^1/_{25}$ of the total number of thymus cells and, according to data given by several workers, they are immunologically competent, as revealed by the graft versus host reaction (GVHR) which can be induced in newborn and irradiated mice [10, 12]. Since the GVHR can be tested in relation to depression of endogenous colony formation [5], it is interesting to study the effect of cortisone-resistant thymocytes on endogenous colony formation in mice.

The object of this investigation was to study the possible effect of cortisone-resistant thymocytes of the parental CBA strain on endogenous colony formation in sublethally irradiated (CBA×C57BL)/6)F_1 hybrids.

EXPERIMENTAL METHOD

Experiments were carried out on CBA and (CBA×C57BL/6)F_1 mice aged 3-4 months (the mice were obtained from the Inbred Animals Nursery of the Academy of Medical Sciences of the USSR, Stolbovaya). Cortisone-resistant thymocytes were obtained by the method described by Blomgren and Anderson [8]. CBA mice (weighing 20 g) were given an intraperitoneal injection of hydrocortisone in a dose of 125 mg/kg body weight. The thymus and lymph nodes were removed from these mice 48 h after the injection of hydrocortisone to prepare cell suspensions. The F_1 hybrids were irradiated in a dose of 520 R and, during the 2 h after irradiation, they received an intravenous injection of thymus cells and lymph node cells of CBA mice. The method of preparation of the cell suspensions and the conditions of irradiation were indistinguishable from those in the previous investigation [6]. The spleen was taken from the F_1 hybrids on the ninth day after irradiation and injection of the cells from CBA mice, fixed in Bouin's fluid, and the number of colonies in it was then counted macroscopically. In the first experiment hybrids weighing 23 g were used and they were injected with thymocytes and lymph node cells in a dose of $1 \cdot 10^6$ nucleated cells. In the second experiment smaller hybrids (18 g) were injected with $3 \cdot 10^6$ nucleated cells. Altogether 62 CBA mice and 120 (CBA×C57BL/6)F_1 hybrids were used. The results of these experiments were subjected to statistical analysis [1].

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TABLE 1. Effect of Cortisone-Resistant Thymocytes of CBA Mice on Endogenous Colony Formation in Sublethally Irradiated (CBA×C57BL/6)F_1 Hybrids

Experiment	No. of group of mice	Number of cells of CBA mice injected ($\times 10^6$)				Number of mice	Number of endogenous colonies (M ± m)	Index of inactivation, %
		treated with hydrocortisone		intact				
		T	LNC	T	LNC			
1	1	1	—	—	—	20	15,3±1,65	0
	2	—	1	—	—	10	0,3±0,23	97,6
	3	—	—	1	—	20	16,5±0,5	0
	4	—	—	—	1	20	2,3±0,6	83,8
	5	—	—	—	—	10	11,8±1,6	—
2	6	3	—	—	—	10	7,4±1,1	0
	7	—	3	—	—	10	0	100
	8	—	—	—	3	10	0	100
	9	—	—	—	—	10	7,8±1,2	—

Legend. T) Thymocytes, LNC) lymph node cells.

EXPERIMENTAL RESULTS

Treatment of CBA mice with hydrocortisone in the above dose led to a 75% decrease in the size of the thymus compared with that of intact CBA mice. As Table 1 shows, thymocytes obtained from donors treated with hydrocortisone did not depress endogenous colony formation in doses of $1 \cdot 10^6$ and $3 \cdot 10^6$ (groups Nos. 1 and 6). Cells from the lymph nodes of these donors had a definite suppressive action on endogenous colony formation (groups Nos. 2 and 7). In the first experiment the mean number of endogenous colonies in the hybrids after injection of thymocytes treated with hydrocortisone and in the intact CBA mice was greater than the number of colonies in the control hybrids which were irradiated only (groups Nos. 1, 3, and 5); in individual recipients of groups Nos. 1 and 3, moreover, the number of colonies in the spleen was twice or three times greater than the number of colonies in the hybrids of group No. 5.

The results of these experiments show that cortisone-resistant thymocytes of CBA mice do not depress endogenous colony formation in (CBA×C57BL/6)F_1 hybrids, whereas lymph node cells treated with hydrocortisone from CBA donors had a marked suppressive action on endogenous colony formation in the hybrids. Thymocytes of intact CBA mice likewise did not suppress endogenous colony formation. According to results obtained by other workers, injection of thymocytes during the first 12 h after sublethal irradiation accelerated the restoration of the cellular composition of the bone marrow and spleen [7], thymus cells induced a much weaker local GVHR [4], and cooperation between T and B lymphocytes is essential for the GVHR to occur in mice [2]. T lymphocytes entering the bone marrow after treatment of mice with hydrocortisone [9] may potentiate the proliferative activity of polypotent stem cells [3]. Consequently, it can be concluded from the results of these experiments and those obtained by other workers that the effect of cortisone-resistant thymocytes on endogenous colony formation is similar to a certain extent to the action of cortisone-resistant T lymphocytes on stem cells in the bone marrow.

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TRANSFORMATION OF *Bacillus subtilis* IN MICE AFTER IMMUNOLOGICAL SUPPRESSION OF EXOGENOUS DEOXYRIBONUCLEASE 1 ACTIVITY

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Transformation of *Bacillus subtilis* was carried out intraperitoneally in mice. The frequency of transformation was considerably reduced by intraperitoneal injection of bovine deoxyribonuclease 1 (DNase 1) into the animals in a dose of 3-5 μg. Immune rabbit γ-globulins, containing antibodies against bovine DNase 1, inhibit the activity of DNase 1 in vivo, thus protecting the transforming DNA against the hydrolytic action of that enzyme. The model suggested can be used to search for ways of preserving a nucleic acid introduced into an animal for the purposes of genetic engineering.

KEY WORDS: *transformation in vivo; γ-globulins; DNase 1; inhibition in vivo.*

One of the main problems facing genetic engineering, a new field of investigation in molecular biology and genetics, is correction of defects of the genetic material of man and animals at the DNA level. However, whereas for prokaryotes and, in some cases, for eukaryotes also (in cell culture) the question of introducing foreign genetic information into a recipient cell is to some extent solved already [13, 14], with regard to the whole organism this problem still remains open. The reason is that simulation of the transmission of genetic material in higher animals by the nonsexual route is difficult because they possess a powerful system of protection, including the immune system, the system of interferon synthesis, and the nuclease barrier [3, 4]. The last of these merits the closest attention when experiments are carried out in genetic engineering, for nucleases and, in particular, deoxyribonucleases (DNases), enzymes specifically hydrolyzing DNA, are constantly present in all animal tissues [6] and are capable of destroying exogenous DNA introduced into the organism [4, 13]. Difficulties arise when this problem is studied in experiments involving transformation of eukaryote cells. Meanwhile, in bacterial models, where transformation can easily be achieved, factors inhibiting transformation in vivo are absent. The writers accordingly suggested a new model combining the technical simplicity and advantages of bacterial transformation with the introduction of conditions preventing transformation in vivo into the experimental system. For this purpose, experiments on bacterial transformation were carried out in animals. The use of such a model provides an approach to the development of methods of overcoming the obstacles existing in the intact organism in the way of introducing foreign DNA in the course of genetic engineering. Anti-DNase γ-globulins, specifically inhibiting the activity of these enzymes in experiments in vitro [2], were used as one of the factors protecting DNA against the destructive action of DNases.

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