EFFECT OF ADRENALECTOMY ON DONORS AND RECIPIENTS ON REPOPULATION OF PARENTAL LYMPHOCYTES IN FIRST GENERATION HYBRIDS

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Lymph gland cells from sensitized and normal CBA/HT6T6 mice were injected into F_1(CBA/HT6T6 × C57BL/6) mice subjected to bilateral adrenalectomy 7 days before injection of the donor cells. In one of the experiments both adrenals were removed from the donors four weeks before the lymph gland cells were taken. Adrenalectomy on the recipients led to an increase in the percentage of donor mitoses in the spleen at all times of transfer, provided that the lymphocytes were taken from sensitized donors, whereas the number of donor mitoses in the lymph glands was indistinguishable from that in the control groups. An increase in the percentage of donor mitoses in the spleen of the adrenalectomized mice was observed only on the 5th day after transfer if lymphocytes from unsensitized donors were used; on the first day after injection of the cells an increase in mitotic activity of the donor cells in the lymph glands was observed. If the lymphocytes were taken from adrenalectomized and sensitized donors, the injected cells retained their power of increased mitotic activity in the spleen of the irradiated recipient on the first day after injection.

KEY WORDS: adrenalectomy; repopulation of lymphocytes; sensitization.

Glucocorticoids produced by the adrenal cortex are widely used in clinical and experimental medicine to suppress tissue incompatibility reactions [4]. Glucocorticoids probably participate in the regulation of tissue incompatibility reactions and in the proliferation of lymphocytes in vivo, for adrenalectomy in animals induces marked thymico-lymphoid hyperplasia [6, 8] and increases the intensity of reactions of hypersensitivity of delayed type [2]. Adrenalectomy increases the severity of transplantation sickness in F_1 hybrids receiving injections of parental lymphocytes [3, 6].

It was therefore decided to study the effect of adrenalectomy on donors and recipients on the repopulation of parental lymphocytes in F_1 hybrids, i.e., under the conditions of the graft versus host reaction.

EXPERIMENTAL METHOD

In experiments Nos. 1 and 2 the donors were CBA/ HT6T6 mice weighing 20-22 g and the recipients were F_1(CBA/HT6T6× C57BL/6 mice weighing 20 g. Bilateral adrenalectomy was performed on the recipients 7 days before the injection of lymph gland cells from CBA/HT6T6 mice. Recipients undergoing a mock operation acted as the control. In experiment No. 1 lymph gland cells were taken from donors which were given an intravenous injection of 30 million lymph gland cells from the recipient three weeks before the experiment. In experiment No. 2, lymph gland cells from unsensitized donors were used. The recipients were killed on the first, third, and fifth days after the transfer of the donor cells and the percentage of donor mitoses was calculated in the spleen and the lymph gland. In experiment No. 2 the donors were CBA/H mice weighing 19 g and age 3 months, whereas the recipients were F_1(CBA/HT6T6 × C57BL/6) mice weighing 21 g and aged 4 months. The CBA/H donor mice underwent bilateral adrenalectomy and a mock operation was performed on the control CBA/H mice. On the 7th day after the operation the donors

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TABLE 1. Effect of Adrenalectomy on F_1(CBA/HT6T6 × C57BL/6) Recipients on Repopulation of Sensitized and Unsensitized Lymph Gland Cells from CBA/HT6T6 Mice in Spleen and Lymph Glands

Expt. No.	Group	Day after injection of lymphocytes	Organ studied	Adrenalectomy		Mock operation	
				mitoses			
				total	% donor	total	% donor
1	1st	1st	Lymph glands	123	34,96±4,3	168	26,19±3,4
	2nd	1st	Spleen	397	17,0±1,88	400	11,5±1,68
	3rd	3rd	Lymph glands	201	28,86±3,19	193	22,8±3,0
	4th	3rd	Spleen	401	25,94±2,19	407	15,23±1,78
	5th	5th	Lymph glands	178	20,79±3,0	300	14,33±2,0
	6th	5th	Spleen	297	16,84±2,2	246	8,13±1,75
2	7th	1st	Lymph glands	260	30,38±2,85	257	22,57±2,6
	8th	1st	Spleen	259	8,1±1,7	423	5,9±1,14
	9th	3rd	Lymph glands	220	24,1±2,88	155	18,7±3,14
	10th	3rd	Spleen	239	10,88±2,0	412	7,28±1,28
	11th	5th	Lymph glands	241	16,0±2,4	167	10,78±2,4
	12th	5th	Spleen	215	8,27±1,8	243	4,94±1,4

Note. An intravenous injection of 50 million lymphocytes was given, from sensitized donors in experiment No. 1 and from unsensitized donors in experiment No. 2.

TABLE 2. Repopulation of Lymphocytes from Immunized CBA/H Mice in Spleen and Lymph Glands of Sublethally (600R) Irradiated F_1(CBA/HT6T6 × C57BL/6) Mice

Organ studied	Day after injection of lymphocytes	Adrenalectomy		Mock operation	
		mitoses			
		total	% donor	total	% donor
Lymph glands	1st	349	34,38±2,5	298	33,56±2,74
Spleen	1st	362	32,60±2,47	389	23,14±2,14
Lymph glands	2nd	329	44,98±2,74	203	38,42±3,42
Spleen	2nd	423	40,90±2,39	307	38,76±2,78
Lymph glands	5th	159	90,57±2,32	—	—
Spleen	5th	—	—	132	87,88±2,85

of the lymphoid cells were immunized by a single graft of skin from the recipient mice. The donors were killed three weeks after the skin grafting, a suspension of their lymph gland cells was made, and it was injected intravenously in a dose of 50 million cells into recipients irradiated sublethally (600R) 1.5-2 h before the injection of the cells. Two of these mice at a time were killed on the 1st, 2nd, and 5th day after injection of the donor cells for chromosomal analysis. In all three experiments, preparations for counting the donor mitoses were made by Ford's method.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that adrenalectomy on the recipient in experiment No. 1 stimulated repopulation of lymphocytes taken from sensitized donors in the recipient's spleen on the 1st, 3rd, and 5th days after transfer (groups 2, 4, 6; $P < 0.01$) compared with animals undergoing the mock operation. The number of donor mitoses in the lymph glands of the adrenalectomized recipients was not statistically significantly different from the number in the control groups of mice undergoing the mock operation (groups 1, 3, and 5). In experiment No. 2 adrenalectomy led to an increase in the number of donor mitoses in the recipient's lymph glands on the first day after transfer (group 7; $P < 0.05$), and by the 5th day the percentage of donor mitoses was increased in the spleen (group 12; $P < 0.01$) compared with the recipients undergoing the mock operation. In experiments Nos. 1 and 2 a higher percentage of donor mitoses was observed in the lymph glands of the recipients on the 1st, 3rd, and 5th day after injection of the lymphocytes than in the spleen ($P < 0.01$). By the 5th day after injection of the cells the percentage of donor mitoses in the spleen and lymph glands was reduced, and this effect was more marked in the animals undergoing the mock operation.

Following injection of lymphocytes from sensitized donors, the number of donor mitoses observed on the first and third day after transplantation was greater in the spleen of the adrenalectomized recipients and recipients undergoing the mock operation (experiment No. 1, groups 2 and 4) than in the corresponding recipients receiving cells from unsensitized donors (experiment No. 2, groups 8 and 10). In experiment No. 3 (Table 2) injection of lymph gland cells from the adrenalectomized and sensitized CBA/H mice into sublethally irradiated F_1(CBA/HT6T6 × C57BL/6) mice was accompanied by an increase in mitotic activity of the donor cells in the lymph glands of the second day after transplantation. Higher mitotic activity of cells from adrenalectomized donors in the recipient's spleen was observed on the first day after transplantation, whereas on the second day the percentage of donor mitoses in the spleen was about equal to that in the animals undergoing the mock operation. In this experiment a tendency was observed for the number of donor mitoses to increase in the spleen and lymph glands by the 5th day in both the experimental and the control mice.

The experimental results indicate that adrenalectomy on the recipients stimulated repopulation of the injected donor cells. Removal of the adrenals presumably led to a decrease in the glucocorticoid concentration in the recipient and stimulated proliferation of the donor cells in response to the recipient's foreign antigens. In the adrenalectomized mice the glucocorticoid concentration in the blood plasma was reduced by 33-50% compared with its value in the intact recipients and in animals after the mock operation. Adrenalectomy on the recipients led to an increase in the percentage of donor mitoses in the spleen at all periods after transplantation if the lymphocytes were taken from sensitized donors, whereas the number of donor mitoses in the lymph glands was the same as in the control groups. An increase in the percentage of donor mitoses in the spleen of the adrenalectomized mice was observed only on the 5th day after transplantation if lymphocytes from unsensitized donors were used; in that case on the first day after injection of the cells an increase in the mitotic activity of the donor cells was observed in the lymph glands. These results can be explained on the grounds that a higher proportion of the sensitized lymphocytes enter the spleen and proliferate in it than in the lymph glands. When unsensitized cells were used the percentage of donor mitoses in the spleen was increased on the 5th day, possibly on account of an increase in the number of sensitized cells in the adrenalectomized recipients by this time and their migration from the lymph gland into the spleen. It must be remembered that adrenalectomy can increase sensitization to transplantation antigen [1]. There is evidence that lymphocytes spread from the lymph gland into other lymphoid organs [7] and that spleen cells from sensitized donors are more able to induce the graft versus host reaction than lymphocytes from the thoracic duct of the same donor [5]. When lymphocytes were taken from adrenalectomized sensitized donors, the injected cells remained capable of increased mitotic activity in the spleen of the irradiated recipient on the first day after injection.

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