EFFECT OF THYMECTOMY ON FORMATION OF ENDOGENOUS COLONIES OF HEMATOPOIETIC CELLS IN THE SPLEEN OF MICE OF VARIOUS LINES

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Thymectomy was performed on CBA and C57B1 mice and also on (CBA × C57B1)F_1 hybrids at the age of 6-7 weeks. One month after thymectomy or after a mock operation the animals were irradiated in a dose of 650 R, and on the 9th day after irradiation the number of endogenous colonies of hemotopoietic cells in the spleen was counted. Thymectomy led to an approximately twofold increase in the number of endogenous splenic colonies compared with the mock operation in CBA mice and (CBA × C57B1)F_1 hybrids but did not affect their number in C57B1 mice. In the thymectomized animals of both lines and the hybrids the relative percentage of erythroid colonies was considerably reduced and the percentage of undifferentiated colonies correspondingly increased.

There is every reason to suppose that hematopoietic stem cells which, when transplanted into lethally irradiated recipients, form exogenous colonies of homogeneous hematopoietic cells in their spleens, and which spontaneously form endogenous splenic colonies after sublethal irradiation of intact animals, are precursors for cells of the erythroid and myeloid series [3, 5, 6].

Thymectomy in mice during the first few days after birth has been shown to cause an increase in the number of hematopoietic stem cells in their spleen [1] and bone marrow [4]. In the investigations cited, methods of exogenous [4, 5] and endogenous [4] splenic colonies of hematopoietic cells were used to record the number of stem cells.

In the investigation described below the effect of thymectomy in adult animals was studied on the number of hematopoietic stem cells recorded by the method of endogenous splenic colonies [2].

EXPERIMENTAL METHOD

Mice of lines CBA and C57B1 and (CBA × C57B1)F_1 hybrids were used. Thymectomy was performed on the experimental animals at the age of 6-7 weeks and control mice of the same age and sex underwent a mock thymectomy at the same time. The number of polypotent hematopoietic stem cells was estimated by the endogenous splenic colony method [2, 3], for which purpose the experimental and control animals were irradiated 1 month after the operation in a dose of 650 R. On the 9th day after irradiation the mice were sacrificed, their spleens were fixed in Bouin's fluid, and the number of endogenous colonies was then counted. Irradiation was carried out on the RUD-200-20-3 apparatus (dose rate 50 R/min, voltage 180 kV, filter Al-3). The morphology of the endogenous colonies of hematopoietic cells was studied after staining serial longitudinal sections (5 μ) through the spleens of the control and experimental animals with hematoxylin

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TABLE 1. Number of Endogenous Colonies Appearing after Irradiation in a Dose of 650 R in the Spleen of Thymectomized Mice and Mice Undergoing the Mock Operation (M ± m)

Group of animals	No. of endogenous colonies in mice of different genotypes		
	C57Bl	CBA	F_1 (CBA×C57Bl
Mock operation	6,6±2,3 ($^2/_{20}$)	1,8±0,42 ($^2/_{12}$)	2,1±0,3 ($^2/_{15}$)
Thymectomy	6,6±5,2 ($^2/_{20}$)	3,5±0,66 ($^2/_{16}$)	4,1±1,85 ($^2/_{19}$)

Note. In parentheses: numerator – number of experiments, denominator – number of animals investigated.

TABLE 2. Composition of Endogenous Colonies Formed after Irradiation in a Dose of 650 R in Spleen of Thymectomized Mice and Mice Undergoing the Mock Operation

Genotype of mice	No. of mice	Composition of colonies (% in their total number)				
		erythroid	myeloid	megakaryocytic	undifferentiated	mixed
C57Bl:						
Mock operation	9	60	10	19	4	7
Thymectomy	8	38	12	10	38	2
CBA:						
Mock operation	7	50	22	5	20	3
Thymectomy	7	19	4	8	69	0
F_1 (CBA×C57Bl):						
Mock operation	4	53	7	13	27	0
Thymectomy	8	15	7	4	71	3

and eosin. The following types of hematopoietic colonies were counted in the sections: erythroid, myeloid, megakaryocytic, undifferentiated, and mixed. The significance of the results was estimated by Student's method.

EXPERIMENTAL RESULTS

As Table 1 shows, 1 month after the operation the number of endogenous splenic colonies in thymectomized CBA and hybrid mice after a single irradiation in a dose of 650 R was approximately twice as high as their number in animals of the same genotype undergoing the mock operation. In the thymectomized C57Bl mice the number of endogenous colonies was indistinguishable from their number in animals of this line undergoing the mock operation.

Morphological study of the endogenous colonies (Table 2) in the thymectomized CBA and C57Bl mice and in the hybrids revealed a considerable (by 1.5-3 times) decrease in the relative percentage of erythroid colonies compared with animals of the same genotype undergoing the mock operation. The decrease in the relative percentage of erythroid colonies in the thymectomized mice was compensated by an increase in the percentage of undifferentiated colonies.

Thymectomy performed on CBA and hybrid mice at the age of 6-7 weeks thus led to an increase in the number of endogenous colonies formed in their spleens after irradiation when tested 1 month after thymectomy. In the absence of the thymus migration of stem cells into the spleen is presumably stimulated, with the result that the number of endogenous colonies observed in the thymectomized CBA and hybrid mice was increased. However, thymectomy may also delay proliferation and differentiation of the stem cells forming endogenous splenic colonies. This hypothesis is confirmed by the results of the present investigations which showed that the relative percentage of undifferentiated endogenous splenic colonies was considerably higher in the thymectomized animals than in those undergoing the mock operation. Nevertheless, the decrease in the relative percentage of erythroid colonies observed in the thymectomized mice of both lines and the hybrids investigated by comparison with the animals undergoing the mock operation does not rule out the possibility of abolition of the inducing effect of the thymus on differentiation of the hematopoietic stem cells toward erythropoiesis.

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