ANTIBODY-FORMATION IN THE REGENERATING SPLEEN OF INTACT AND THYMECTOMIZED MICE

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The ability of the regenerating spleen in intact and thymectomized mice to form antibodies was studied. The titer of Vi-antibodies in the serum and the number of antibody-forming cells in the regenerating spleen of thymectomized adult mice were much lower than the same indices for normal mice with a regenerating spleen.

A previous investigation [3] showed that antibody synthesis in the mouse spleen regenerating after resection of two-thirds of its tissue is sharply inhibited by comparison with the same process in the intact spleen of control animals.

In the investigation described below the role of the thymus in the antibody-forming function of the regenerating spleen was studied. Data in the literature on this problem are contradictory [2, 4, 6].

EXPERIMENTAL METHOD

The thymus was removed from adult (18-20 g) noninbred mice. Five days later, through an incision in the left lumbar region, 70-75% of the spleen tissue was removed from the thymectomized and intact mice. Sixteen days after this operation all the animals received an intravenous injection of antigen: either Vi-antigen of Salmonella typhi in a dose of 1 μg per mouse, or sheep's erythrocytes ($3 \cdot 10^7$ cells per mouse). The animals were sacrificed 4 days after the injection of antigen.

Four groups of animals were used in all the experiments: 1) thymectomized mice with regenerating spleen; 2) thymectomized mice with intact spleen; 3) mice with regenerating spleen (not thymectomized); 4) intact mice (control).

The criteria of splenic function were the accumulation of Vi-antibodies in the mouse serum (by the method described previously [1]) and the number of cells producing antibodies against sheep's erythrocytes (determined by the method of Jerne and Nordin [7]). In addition, in every series the spleen was weighed and cytological specimens of the tissue of the regenerating spleens of thymectomized and intact mice were studied. Statistical analysis of the results was carried out by the Fisher–Student method.

EXPERIMENTAL RESULTS

The weight of the intact spleen 5 days after thymectomy was reduced by 10-12%. The decrease in weight was accompanied by a marked decrease in the number of small lymphocytes. Regeneration of the spleen took place both in the control (group 3) and experimental (group 1) series: its weight increased until it was almost twice that of the fragment remaining after operation (Table 1).

The cell composition of the regenerating spleen of the thymectomized mice differed in the relatively small number of lymphocytes and larger number of cells of the myeloid and erythroid series.

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TABLE 1. Weight (in mg) of Regenerating Spleens of Thymectomized and Intact Mice

Group	Weight of intact spleen	Weight of residual spleen after operation	Weight of regenerating spleen
1	79.5 (72.7-86.4)	27	51.5 (42.2-60.7)
3	90.2 (92.4-88.0)	35	66.3 (53.0-79.5)

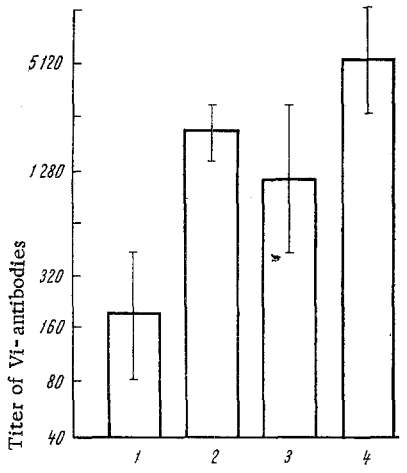

Fig. 1. Production of Vi-antibodies by mice: 1) thymectomy and removal of $^2/_3$ of the spleen; 2) thymectomy; 3) removal of $^2/_3$ of the spleen; 4) control.

Results showing the ability of the mice of the various experimental groups to produce Vi-antibodies are shown in Fig. 1. The titer of Vi-antibodies in the serum of the thymectomized mice with a regenerating spleen (group 1) was only one-quarter that in the serum of mice with a regenerating spleen and intact thymus (group 3) and was 20 times less than that in the serum of the control animals (group 4).

Results showing the changes in weight and in the antibody-forming function of the regenerating spleen in intact and thymectomized mice after receiving an injection of antigen (sheep's erythrocytes) are shown in Table 2. The weight of the spleen in all groups of animals increased after the injection of antigen. The number of cells forming antibodies against sheep's erythrocytes in the regenerating spleen was four times less than in the intact spleen. These results confirm those obtained previously [3].

Thymectomy led to a marked decrease in the number of antibody-forming cells in the intact spleen; the mean number of antibody-forming cells found in the spleen of the mice of group 4 was 68,710, compared with only 20,990 in the spleen of the group 2 mice. There was a particularly marked decrease in the number of antibody-forming cells in the regenerating spleen of the thymectomized mice (group 1). In these animals it was five times smaller than in the intact spleen of the thymectomized mice and 17 times smaller than in the intact spleen of the control mice.

These results indicate that regeneration of the spleen is possible in thymectomized animals: the weight of the spleen increases. However, the two criteria (titer of antibodies in the serum after immuniz-

TABLE 2. Effect of Thymectomy on Antibody Synthesis in the Spleen

Group	Opertn.		Number of mice	Weight of spleen		Number of antibody-forming cells			
	thymectomy	removal of $^2/_3$ of spleen		absolute (in mg)	in % of control	absolute		in % of control	
						per spleen	per 10^6 cells	per spleen	per 10^6 cells
1	+	+	20	84,6 (69,6—99,6)	26	4 198 (2 138—8 433)	69,1 (39,9—129,4)	6	27
2	+	—	17	153,2 (126,2—180,2)	68	20 990 (11 670—37 610)	111,9 (92,0—136,1)	31	44
3	—	+	23	122,5 (62,1—174,1)	35	16 370 (10 140—26 420)	178,3 (99,3—284,5)	24	68
4	—	—	22	217,2 (180,4—242,0)	100	68 710 (33 500—140 900)	253,2 (111,2—566,2)	100	100

ation with Vi-antigen and the number of antibody-forming cells in the spleen after immunization with sheep's erythrocytes) showed that the functional activity of the regenerating spleen is sharply reduced in thymectomized animals. The thymus evidently exerts a substantial effect on the formation of immunological reactivity during regeneration of the spleen. At the same time, it must be emphasized that thymectomy significantly depressed the ability of mice with an intact spleen to give an immune response, for it reduced the number of antibody-forming cells by 67%.

The results described above clearly demonstrate that the immunological function of the lymphoid system is dependent on the presence of the thymus. The results described above are in full agreement with those obtained by Miller [6] in mice, who found inhibition of antibody-formation in thymectomized and irradiated animals.

Immunological reactivity of the thymectomized animals is probably depressed through a disturbance of differentiation of stem cells into immunocompetent cells [6]. However, this hypothesis requires experimental verification.

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