MORPHOLOGICAL CHANGES IN THE THYMUS AND PERIPHERAL LYMPHOID ORGANS DURING THE SYSTEMIC GRAFT VERSUS HOST REACTION

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If parental lymphoid cells are injected into (CBA × C57BL/6)F_1 hybrid mice 2-12 days after resection of two-thirds of the spleen, the resistance of these animals to the systemic graft versus host reaction (GVHR) is increased [1]. This resistance develops irrespective of the time and completeness of recovery of the hematopoietic component of the resected spleen [2].

Since the brunt of the immunologic conflict in the GVHR falls on the spleen [11], it was interesting to discover to what extent its regeneration modifies the morphological manifestations of the GVHR in it, and to what degree this is reflected in the structure of the other lymphoid organs. The present investigation is devoted to the study of these problems.

EXPERIMENTAL METHOD

Experiments were carried out on 90 inbred female (CBA × C57BL/6)F_1 mice and 50 C57BL/6 mice weighing 19-23 g, obtained from the Stolbovaya nursery, Academy of Medical Sciences of the USSR. The F_1 hybrids were divided into four groups. Two-thirds of the spleen tissue was removed from the group 1 recipients (experiment) under ether anesthesia by the usual method [4], and 2 days later they were injected with parental spleen cells prepared by the method described previously [1, 2, 5], in a dose of 75×10^6 cells in 0.4 ml of medium No. 199, into the retro-orbital venous sinus. The control animals of group 2 did not undergo any operation, but a systemic GVHR was induced in them (nonsplenectomized recipients). In the control animals of group 3 with a regenerating spleen, no GVHR was induced; control group 4 consisted of intact animals.

On the 5th, 10th, and 25th days after induction of the GVHR, five animals from each group were sacrificed. The thymus and spleens were weighed and fixed simultaneously with the mesenteric lymph nodes, in Carnoy's mixture. Paraffin sections 5 μ thick were stained with methyl green and pyronine, hematoxylin and eosin, and hematoxylin and picric acid. Mitotic activity of the lymphoid cells was determined by counting the number of dividing cells among a total of 2000-3000 cells. The mitotic index was expressed in promille. Megakaryocytes were counted in histological preparations of the spleen in the whole area of the preparation, after which their number per square millimeter was calculated. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Mitotic activity in cortical lymphocytes of the thymus in hybrids with a regenerating spleen (Table 1) was indistinguishable at all times of investigation (up to the 25th day) after injection of parental lymphoid cells from that in animals with a regenerating spleen in which the GVHR was not induced, and in intact hybrids (groups 1, 3, and 4).

Meanwhile the injected parental lymphoid cells significantly affected the number of dividing lymphocytes in the cortex of the thymus of the nonsplenectomized recipients, and led to a significant decrease in their number compared with that in intact animals (groups 2 and 4). By the 25th day of the GVHR the proliferative zone beneath the capsule was almost absent in the thymus of the nonsplenectomized recipients and there were practically no Hassall's corpuscules, whereas in the experimental animals of group 1 proliferation in the thymus was undisturbed, a zone of large thymocytes was present beneath the capsule, and the Hassall's corpuscles were preserved.

The sharpest changes in the experimental and control animals were observed in the white and red pulp of the spleen. For instance, 5 days after induction of the GVHR hemorrhages were found in the spleen of the nonsplenectomized control recipients, with proliferation in the stroma of the organ. Myeloid hematopoiesis was intensified in the red pulp, as reflected

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TABLE 1. Mitotic Index (in %) in Cortical Lymphocytes of Thymus and Germinative Centers of Malpighian Bodies and Secondary Follicles of Lymph Nodes in F_1 Hybrids with Regenerating and Intact Spleen (M ± m)

Time after operation, days	Time after induction of GVHR, days	Test organ	Group of animals				P_{1-2}	P_{2-4}	P_{1-3}	P_{3-4}
			1	2	3	4				
7	5	Thymus	37,06±2,03	25,8±5,13	30,45±6,13	29,16±1,24	>0,05	>0,05	>0,05	>0,05
		Spleen	82,20±10,6	26,4±12,1	30,8±8,4	58,0±8,1	<0,001	<0,01	>0,05	>0,05
		Lymph nodes	97,6±7,3	31,2±11,0	79,8±11,7	95,7±9,8	0,01	0,01	>0,05	>0,05
12	10	Thymus	29,96±7,99	20,79±1,78	34,06±4,34	29,16±1,24	>0,05	<0,05	>0,05	>0,05
		Spleen	62,2±12,0	0	70,71±9,0	58,0±8,1	—	—	>0,05	>0,05
		Lymph nodes	107,5±22,5	33,7±9,9	66,2±7,3	95,7±9,8	<0,01	<0,01	<0,02	<0,05
27	25	Thymus	33,49±4,29	18,29±2,16	28,40±10,04	29,16±1,24	<0,05	<0,05	>0,05	>0,05
		Spleen	123,3±17,5	0	75,1±22,1	58,0±8,1	—	—	>0,05	>0,05
		Lymph nodes	118,6±13,5	21,0±10,0	94,4±19,7	95,7±9,8	<0,001	<0,01	>0,05	>0,05

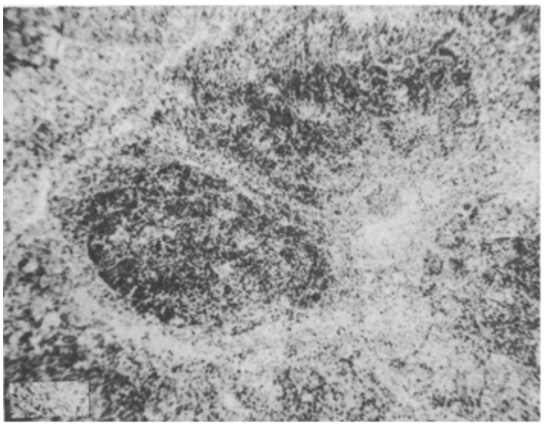

Fig. 1

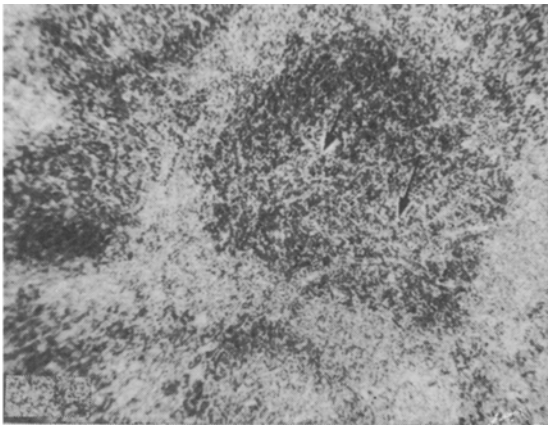

Fig. 2

Fig. 1. Malpighian body in mouse spleen during GVHR induced in mice with intact spleen. Germinative center absent, 400 X.

Fig. 2. Malpighian body with germinative center, with mitoses in it, in mouse with GVHR induced in mice with regenerating spleen, 400 X.

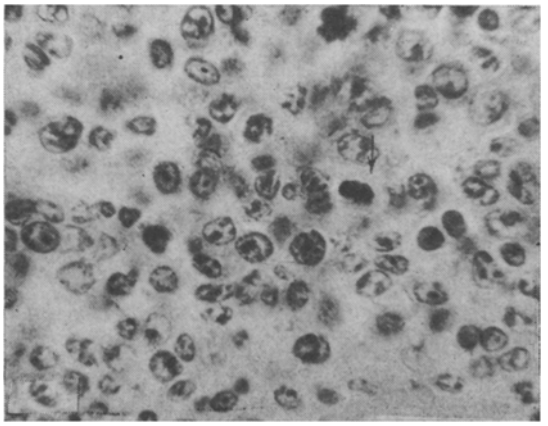

Fig. 3. Germinative center of the same mouse. 900 X.

in accumulation of immature and mature polymorphonuclear neutrophils and other cells of this series.

However, in recipients with a regenerating spleen the hemorrhages and myeloid hemtaopoiesis were much less marked, and there were only half as many megakaryocytes (8.56 ± 0.25 compared with 16.6 ± 1.60 in the nonsplenectomized recipients).

The number and size of the malpighian bodies in the animals of the experimental group were considerably greater than in the controls. In the clearly outlined germinative centers antibody formation continued to take place, as shown by proliferation of blast cells (82.2 ± 10.6% in the experiment, 58.0 ± 8.1% in the "pure" control). The periarterial zones were filled

with lymphocytes. The process of antibody formation in the control recipients was disturbed, as shown by a decrease in proliferative activity in the germinative centers of the malpighian bodies (26.4 ± 12.1% compared with 58.0 ± 8.1%).

All the differences in the regenerating and intact spleens mentioned above were increased 10 days after induction of the GVHR. In the solitary residual malpighian bodies in the spleen of the nonsplenectomized recipients no mitoses were observed, and cell mortality was increased. No new germinative centers were formed. The T-zones around the central arteries were less dense. Hemorrhages in the small and large blood vessels persisted at this time of observation. However, the number of megakaryocytes was down to normal. Hardly any malpighian bodies were present in the spleens of the non-splenectomized mice 25 days after induction of the GVHR, and no dividing blast cells were seen (Fig. 1).

Meanwhile, in the regenerating spleens of the experimental animals the malpighian bodies were clearly defined and their number was the same as in the intact mice, but the germinative centers were very active and they contained twice as many dividing blast cells as in intact animals – 123.3 ± 17.5 and 58.0 ± 8.1% respectively; (Figs. 2 and 3). The peri-arterial T zones were filled with lymphocytes and clearly outlined.

On the 5th day of the GVHR large cells of blast type, some of them dividing, could be seen in the paracortical zone of the lymph nodes of the nonsplenectomized recipients. Rare lymphoid follicles were presented in the cortical region, containing many dying cells and significantly fewer dividing cells than in animals of the experimental groups and the intact animals (Table 1). Many plasma cells, some of them dying, were found in the medulla of the lymph nodes. Well marked migration of lymphocytes, monocytes, and plasma cells in the vessels and sinuses of the lymph nodes also was found. The cortical zone of these lymph nodes in the experimental animals contained many secondary follicles with clearly defined germinative centers; migration of lymphocytes was observed in sinuses and blood vessels, but on a smaller scale.

By the 10th day of the GVHR the number of dividing cells in the secondary follicles of the experimental recipients was significantly greater than that in the nonsplenectomized recipients (Table 1). By the 25th day of the GVHR the differences were even more marked: In the lymph nodes of the nonsplenectomized recipients the number of secondary follicles fell progressively, as also did the number of dividing cells in them, whereas secondary follicles with dividing cells still remained in the recipients with a regenerating spleen.

The data on morphological changes found in the lymphoid organs of the nonsplenectomized recipients during the systemic GVHR thus agree with information in the literature on this equation [3, 5-12]. The most interesting fact is that the characteristic morphological changes of systemic GVHR were not found in the organs of the immune system of the recipients with a regenerating spleen: The histological picture in these organs corresponded to that observed in animals with a regenerating spleen in the absence of the GVHR. The results suggest that under the influence of certain factors (cellular or humoral) appearing during regeneration of the spleen, injected parental lymphoid cells remain inactive and do not induce the pathological changes characteristic of the systemic GVHR in the recipients' lymphoid organs; this probably explains the resistance of animals with a regenerating spleen to the systemic GVHR.

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