HISTOCHEMISTRY OF THE SPLEEN AND THYMUS IN RUNT DISEASE

D. N. Mayanskii and M. E. Viksman

UDC 616-056.3-02:612.6.02]-07:[616.411+616.438]-008.9-092.18-07

During the first 24 h after birth, CBA mice received an intraperitoneal injection of 1.92-2.38 × 10^7 spleen cells of adult male mice of lines C57BL. All these recipients developed an acute form of runt disease, from which they died 18-22 days after transplantation. On the 7th-8th day of the experiment we noted a decrease in the activity of lactate, succinate, glucose-6-phosphate, α-glycerophosphate, malate, and glutamate dehydrogenases, NAD- and NADP-diaphorases, and cytochrome oxidase in the center of the newly formed primary follicles. Clusters of highly active reticulum cells were found in the red pulp of the spleen along the course of the blood vessels and sinuses, where they alternated with areas of atrophy of the lymphoid elements. In the thymus of the mice with runt disease, activity of the enzymes in the cortical cells was sharply increased. Starting from the 7th day after transplantation, areas of cell degeneration were found in the medulla, in which diformazan was deposited as large, polymorphic granules or as clusters resembling bunches of grapes.

The "graft versus host" reaction is used now days to investigate many of the problems in transplantation immunity.

This paper describes an investigation of the distribution of activity of oxido-reductases in the spleen and thymus of mice at different times after transplantation of allogeneic lymphoid cells.

EXPERIMENTAL METHOD

During the first 24 h after birth, 32 CBA mice ($H\text{-}2^k$ histocompatibility locus) received an intraperitoneal injection of 1.92-2.38 × 10^7 spleen cells from male C57BL mice ($H\text{-}2^b$ histocompatibility locus) aged 12-14 weeks. The technique of preparing and assessing the viability of the cell suspension was described previously [2]. In the control series the same doses of syngeneic spleen cells (14 mice) and allogeneic spleen cells, disintegrated by rapid freezing and thawing five times, together with homogenization at 2000 rpm for 3 min in the cold (12 mice) were injected. The recipients were sacrificed on the 3rd-4th, 7th-8th, 12th-15th, and 18th-22nd days after transplantation. The development of runt disease was judged from an increase in the splenic index over 1.0 [7], aplasia of the thymus, hepatomegaly, and peripheral necrosis of the liver [5]. The histochemical tests were carried out on frozen sections of the spleen and thymus 10 μ in thickness. Activity of the following enzymes was determined simultaneously: succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), α-glycerophosphate dehydrogenase (α-GDH), NAD- and NADP-diaphorases, glucose-6-phosphate dehydrogenase (G6PDH), glutamate dehydrogenase (GLDH), and cytochrome oxidase (CCO) [3]. Some sections in parallel tests were stained with hematoxylin and eosin.

EXPERIMENTAL RESULTS

High activity of the oxido-reductases was detected in the cells of the primitive follicles in the spleens of the intact mice and also of the recipients of syngeneic and disintegrated allogeneic lymphoid cells on the

Department of Pathological Physiology and Central Research Laboratory, Kazan' Medical Institute.
(Presented by Academician of the Academy of Medical Sciences of the USSR A. I. Strukov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 74, No. 9, pp. 108-111, September, 1972. Original article submitted December 29, 1971.

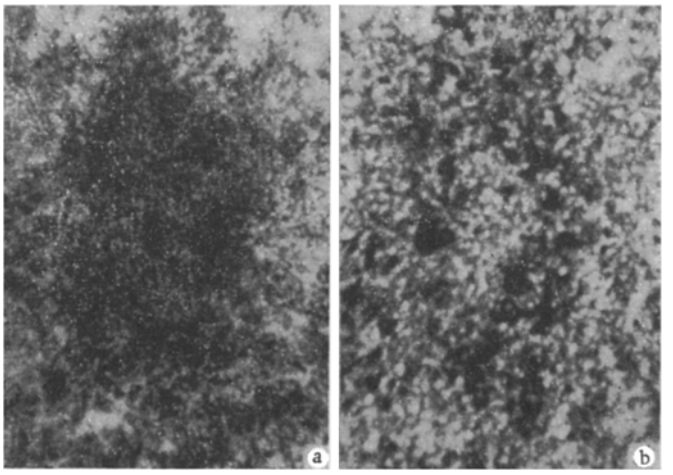

Fig. 1. Sections through spleen of 7-day CBA mice (160×): a) control; high LDH activity in lymphocytes of primitive follicle; 2.38×10^7 disintegrated spleen cells of a C57BL mouse were injected; b) experiment; sharp decrease in G6PDH activity in lymphocytes of follicle, collection of highly active reticulum cells at periphery of follicle, 2.38×10^7 spleen cells of a C57BL mouse injected.

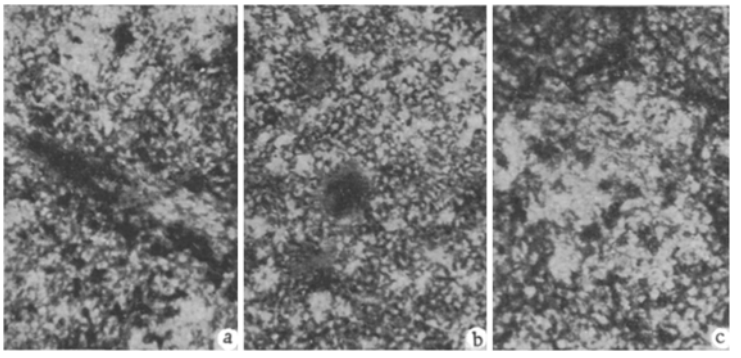

Fig. 2. Sections through spleen of CBA mice during graft versus host reaction; 2.2×10^7 spleen cells of a C57BL mouse were injected: a) 14 days; accumulation of reticulum cells along the course of blood vessels and sinuses of the red pulp; reaction for G6PDH (160 ×); b) 12 days; high CCO activity in megakaryocytes and in cells of hematopoietic foci of red pulp (350 ×); c) 18 days; zone of sharp decrease in NADP-diaphorase activity (350 ×).

3rd-5th day after birth. The tests for LDH and NAD-diaphorase showed higher activity at the periphery of the follicle (Fig. 1a), while those for NADP-diaphorase, SDH, α-GDH, GLDH, and CCO characteristically showed a uniform distribution of diformazan throughout the section through the follicle. In the walls of the central arteries and in the endothelium of the sinuses, high LDH, NAD- and NADP-diaphorase, and CCO activity was detected. As the animals grew older the number of follicles in the spleen increased.

On the 3rd-5th day after transplantation of the spleen cells of the C57BL mice, activity of the above-mentioned enzyme decreased at the sights of formation of the primitive follicles in the spleen of the CBA recipients. On the 7th-8th day of the experiment this tendency was more marked still, especially in the center of the follicle, where a zone of complete disappearance of enzyme activity had appeared, or cells containing very few granules of average size were detected. Some such follicles were surrounded by a barrier of active stellate reticulum cells. This process was seen most clearly in the test for G6PDH (Fig. 1b). On the 12th-15th day of the experiment and later, reticulum cells giving off processes and

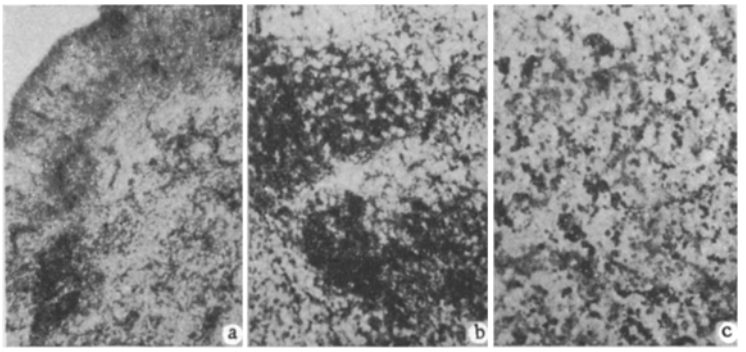

Fig. 3. Sections through thymus of CBA mice: a) control, 7 days; 2.38×10^7 disintegrated spleen cells of a C57BL mouse were injected; reaction for NAD-diaphorase; three zones are clearly visible: a narrow subcapsular, cortical, and medullary zone (explanation in text, 63 ×); b) experiment, 7 days; 2.38×10^7 spleen cells of a C57BL mouse injected; sharp increase in G6PDH activity in cortical cells (140 ×); c) experiment, 12 days; accumulation of "pathological" granules in medulla of thymus; reaction for α-GDH (140 ×).

containing extremely high finely granular and diffuse activity were clustered along the sinuses (Fig. 2a). Intermingled with these cells with processes there were large round cells containing deep blue granules at the periphery of the cytoplasm. In many of the sections, activation of hematopoiesis could be seen (Fig. 2b). In all the animals at the height of the graft versus host reaction (18th-22nd day of the experiment) zones of atrophy, from which all enzyme activity had completely disappeared, were found in the spleen (Fig. 2c). Near the zones of atrophy of the lymphocytes there were highly active reticulum cells, and also zones in which large, deep blue granules of diformazan were irregularly clustered.

In the thymus of the intact and control mice, three zones could be distinguished: a narrow subcapsular zone containing cells with numerous large diformazan granules; a less active cortical zone, consisting mainly of thymocytes, in which activity of the dehydrogenases was distributed as small, dark granules, forming a narrow rim around the nucleus; finally, a medullary zone in which the total enzyme activity was higher than in the cortex (Fig. 3a). Together with the thymocytes, the medullary zone also contained a high proportion of reticulum cells. In the character of their diformazan deposits they showed considerable polymorphism. Of the enzymes studied in the reticulum cells, those with highest activity were LDH and NAD- and NADP-diaphorases.

In the graft versus host reaction the histochemical picture differed sharply from the control. In the cortical zones there were large areas which consisted of cells in which diformazan was deposited as numerous dark blue medium-sized granules (Fig. 3b). Solitary cells and groups of cells, packed with dark blue granules, showing a tendency toward agglomeration, appeared in the medulla of the thymus. In the test for G6PDH and for α-GDH, areas of cells in which diformazan was deposited as polymorphic "pathological" granules, which can be regarded as an indication of the early stages of cellular degeneration, were clearly visible both in the cortex and in the medulla of the thymus (Fig. 3c). The zone of migration of the active reticulum cells was widened, so that the boundaries between the cortex and medulla were obliterated. The highest enzyme activity was observed in the reticulum cells along the line of transition from cortex to medulla. Meanwhile the number of active reticulum cells in the central regions of the medulla was reduced.

After transplantation of allogeneic spleen cells in the system C57BL–CBA, a characteristic redistribution of activity of the oxido-reductases was thus found in the spleen and thymus. The sharp decrease in LDH and NAD- and NADP-diaphorase activity as well as that of other enzymes in the center of the newly formed follicles and the formation of areas with total disappearance of enzyme activity can be regarded as functional and morphological evidence of different stages of lymphoid aplasia in the animals with runt disease [1]. Two opposite processes took place simultaneously in the thymus. On the one hand, there was functional activation of the cells, as shown by the accumulation of uniformly enlarged diformazan granules

in their cytoplasm. These changes in the intracellular enzyme pattern may arise as the result of an increase in the permeability of the mitochondrial membranes to substrates or an increase in the number of functioning mitochondria [4]. On the other hand, death of the cells and replacement of lymphoid structures by reticulum cells were observed in the thymus. The changes in the cell composition of the thymus evidently do not depend on the direct cytopathogenic action of the donor's lymphocytes on the host's thymocytes, but are stressor in character. This hypothesis is confirmed by the fact that allogeneic spleen and lymph gland cells do not penetrate into the thymus [8], unlike bone marrow cells which "filter" through the thymus before spreading into the lymphoid organs [6].

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