EFFECT OF TRANSPLANTATION OF THYMUS, BONE MARROW, AND SPLEEN CELLS ON REPAIR PROCESSES IN THE PATHOGENETICALLY CHANGED LIVER

I. F. Kolpashchikova

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Experiments on CBA × C57BL mice with experimental hepatitis induced by carbon tetrachloride showed that transplantation of cells from lymphoid organs of healthy donors stimulates repair processes in the recipients' pathologically changed liver. Normalization was most marked in the liver of animals receiving thymus cells only, which suggests that a deficiency of these cells is present in experimental toxic hepatitis and that the thymus plays a definite role in repair processes in the damaged liver.

KEY WORDS: lymphocytes; transplantation; hepatitis.

In the study of the mechanisms of chronic liver damage considerable attention is paid to autoimmune disturbances [3, 4, 9]. According to the most recent theories put forward to explain the mechanism of autoimmune disorders, they are based on an immunodeficiency [8] and, in particular, an immunodeficiency in the T-system [6]. Considering that these observations and also the role of immunological mechanism in the regulation of repair processes in the liver [1, 2], it was decided to study the effect of transplantation of syngeneic thymus, bone marrow, and spleen cells on repair processes in the pathologically changed liver.

EXPERIMENTAL METHOD

Experiments were carried out on 52 CBA × C57BL mice weighing 25-27 g. Experimental liver damage was produced by subcutaneous injection of carbon tetrachloride (0.2 ml of a 60% solution on alternate days) for 2 weeks. Of the 48 animals with experimental toxic hepatitis, three were killed in order to study the initial morphological picture in the liver, 15 were kept as controls, and cells from lymphoid organs taken from four intact animals by the usual method [7], using medium No. 199, were transplanted into 30 animals. The recipients were divided into two groups with 15 animals in each group. Thymus cells only, in a dose of 2×10^6 viable cells, were transplanted into the first group, and 2×10^6 cells from the thymus, bone marrow, and spleen, contained in equal numbers in the suspension, were injected into the second group. The animals were killed 5 and 14 days and 1 month after transplantation. Control animals with experimental hepatitis were sacrificed at the same times.

Pieces of liver were fixed in 10% neutral formalin. Paraffin sections, 5 μm thick, were stained with hematoxylin–eison, by Shabadash's method and with cresyl violet, and the tetrazonium coupling reaction was carried out by the method of Danielli and Pearse.

By means of an ocular grid for stereometric measurements, with an area of 0.25 mm^2, the total number of liver cells was counted in 50 fields of vision, and at the same time, the number of normal hepatocytes and of hepatocytes with signs of fatty degeneration and cloudy swelling, and the total number of hepatic nuclei and the number of normal and degenerating nuclei, were counted.

EXPERIMENTAL RESULTS

In animals with experimental hepatitis the liver was pale brown in color with a well-marked pattern of the lobules. Microscopic examination revealed marked fatty, granular, hydropic, and balloon degeneration of the hepatocytes (Fig. 1A), polymorphism of the liver cells and their nuclei, vacuolation of the nuclei, and pycnosis. Along the course of the portal tracts and near the central veins, infiltration with lymphocytes was observed.

Departments of Pathological Physiology and General Biology, S. M. Kirov Gor'kii Medical Institute.
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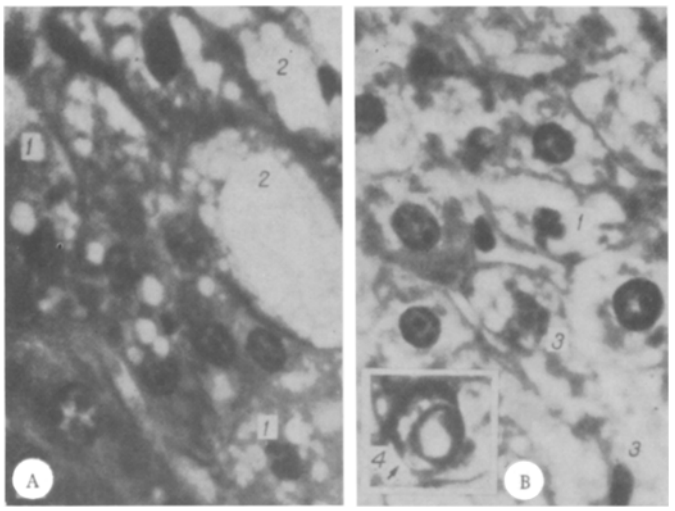

Fig. 1. Liver of CBA × C57BL mice with experimental toxic hepatitis immediately (A) and 1 month after (B) end of injections of carbon tetrachloride. 1 and 2) Hydropic and balloon degeneration of hepatocytes respectively; 3) pycnosis of nuclei; 4) vacuolation of nucleus. Hematoxylin-eosin, 600×.

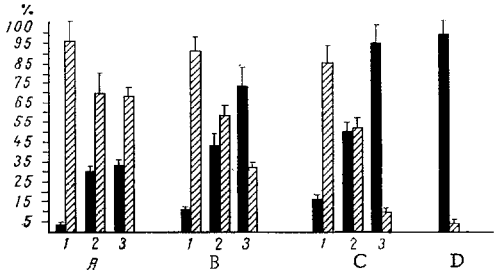

Fig. 2. Number of normal and damaged hepatocytes in mice with hepatitis in early stages after transplantation of lymphocytes from intact animals of the same line. A, B, and C) Five and 14 days and 1 month respectively after transplantation; D) intact animals. 1) Hepatitis (control); 2) hepatitis + mixture of cells from lymphoid organs; 3) hepatitis + thymus cells. Black columns represent normal hepatocytes; obliquely shaded columns damaged hepatocytes.

Five days after transplantation of cells from the lymphoid organs, the liver of all the recipients was darker in color than that of the control animals with toxic hepatitis. Hepatocytes with cloudly swelling and fatty degeneration in the control group accounted for 96.2 × 13.2% of cells, but in the experimental groups they were considerably fewer (Fig. 2: 1). The percentage of degenerating nuclei in the recipients' liver was reduced: from 27.9 ± 2.6 in the control animals with hepatitis to 15.4 ± 2.3 in animals with hepatitis receiving the suspension of thymus cells, and to 18 ± 2.1 in animals receiving a mixture of cells from lymphoid organs. Well marked cloudy swelling and fatty degeneration was seen microscopically in the liver of both the control and the experimental animals, the glycogen content of the organ was considerably reduced, and lymphocytes infiltrated along the course of its vessels.

The intensity of degenerative changes in the liver of the recipients was reduced 14 days after transplantation. The percentage of normal hepatocytes was considerably increased, especially in animals with transplanted thymus cells, whereas the percentage of cells with cloudy swelling and fatty degeneration was reduced (Fig. 2B). Compared with the control, the percentage of degenerating nuclei was reduced by half: 30.1 ± 4.2 in the control animals with hepatitis, 15.6 ± 2 in animals receiving the suspension of thymus cells and 17 ± 2 in animals receiv-

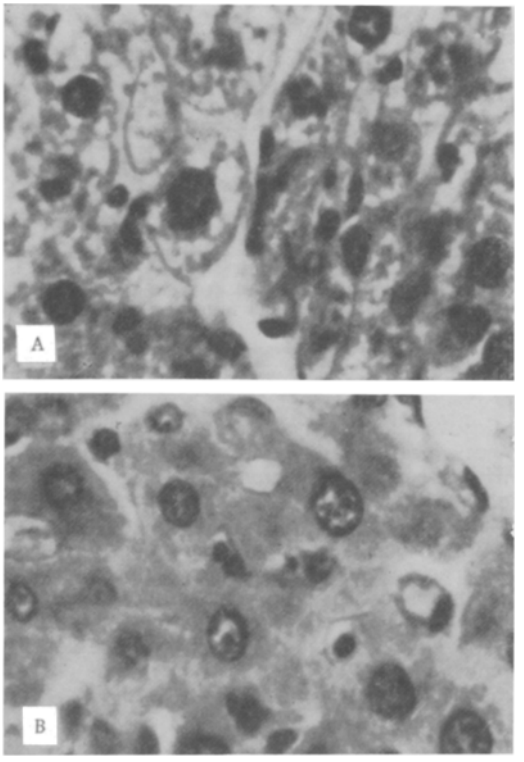

Fig. 3. Liver of CBA × C57BL mice with experimental toxic hepatitis 1 month after transplantation of mixture of cells from lymphoid organs (A) and thymus cells only (B). Hematoxylin–eosin, 600×.

ing a mixture of cells from various lymphoid organs. The glycogen content in the Kupffer cells was increased, probably in connection with their increased functional activity.

Practically no pathological changes could be observed in the liver 1 month after transplantation in animals with experimental hepatitis receiving the suspension of thymus cells from healthy donors (Fig. 3B). The number of normal hepatocytes was 92.1 ± 11.6% and the number of hepatocytes with cloudy swelling and fatty degeneration 8 ± 1%. The number of degenerating nuclei was 4.7 ± 0.8%, i.e., almost the same as in intact animals (Fig. 2: C3, and D). The liver was dark brown in color.

The liver of recipients receiving thymus, bone marrow, and spleen cells simultaneously was, however, paler. Microscopically, cloudy swelling and fatty degeneration of the hepatocytes was observed (Fig. 3A). Compared with the previous time of investigation, at this period the number of normal hepatocytes showed a tendency to increase (Fig. 2C), and the percentage of degenerating nuclei was 11.1 ± 1.2.

In control animals with toxic hepatitis marked hydropic degeneration of the hepatocytes with vacuolation of the nuclei were observed (Fig. 1B) and there were many hepatocytes with features of cloudy swelling, fatty degeneration, and degenerating nuclei (83.2 ± 9.7 and 26.8 ± 2.7% respectively).

Transplantation of cells from lymphoid organs of healthy donors into animals with experimental toxic hepatitis thus considerably stimulated repair processes in the damaged liver. The most marked normalizing effect was observed in recipients receiving thymus cells only. This suggests that in experimental hepatitis there is a deficiency of thymocytes, and that the thymus plays a similar role in repair processes in the liver. The less marked normalizing effect observed in animals receiving a mixture of thymus, bone marrow, and spleen cells is probably attributable to the smaller number of thymus cells in the transplanted suspension.

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MITOTIC ACTIVITY OF LYMPHOCYTES IN THE THYMUS CORTEX DURING HYPOKINESIA AND READAPTATION

G. V. Kharlova and S. E. Li

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Changes in the weight and mitotic index in the thymus cortex of Wistar rats were studied during hypokinesia for 10 days followed by recovery for the same period. The mitotic index was reduced by half, 24 h after immobilization of the animals. During readaptation a stage of secondary stress (when the mitotic index was reduced by 71%) was followed by a stage of true readaptation after 10 days.

KEY WORDS: hypokinesia; mitotic activity; thymocytes; readaptation.

Exposure to stressors leads to involution of lymphoid organs [3, 4, 7]. Atropic changes have been observed in the lymphoid organs of mice [6] and rats [5] during immobilization. The object of the present investigation was to study stress changes during hypokinesia and recovery of the thymus after immobilization of the animals ended.

Attention was concentrated on the cellular mechanisms of stress, as reflected in the mitotic activity of cortical lymphocytes.

EXPERIMENTAL METHOD

Wistar rats weighing 130-160 g were used. Hypokinesia was induced by placing the animal in a closely fitting transparent plastic mold, the lid and one wall of which were movable. The mold was so designed that all movements of the animal except of its head were prevented. Immobilization continued for 12 h and 2, 8, and 10 days. After immobilization for 10 days the animals were allowed out into a general cage. The animals were tested 6, 12, and 18 h and 2, 8, and 10 days after the beginning of recovery. They were killed in the mornings, 5 to 8 rats at each time. In histological section cells and mitoses were counted in the cortex of the thymus (the zone located 2-3 fields of vision away from the capsule). The mitotic index (MI) was expressed in promille.

EXPERIMENTAL RESULTS

After the animals had been secured in the molds, the weight of the thymus fell very quickly (Fig. 1), by 60% of its initial value on the 10th day.

A statistically significant increase in MI of the cortical lymphocytes (19.1‰), control (13.1‰) was observed. However, comparison of the phases of mitosis in the experimental and control groups showed that the increase

Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. Institute of Marine Biology, Vladivostok. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 10, pp. 480-482, October, 1979. Original article submitted March 5, 1979.

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