THE MECHANISM OF LIVER DAMAGE IN THE GRAFT VERSUS HOST REACTION

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A graft versus host reaction was induced in CBA mice by intraperitoneal injection of spleen or lymph gland cells from a rat immunized with mouse liver antigens during the first 24 h after birth. A sharp increase in acid phosphatase activity was found in the endothelium of the blood vessels and sinusoids and in the Kupffer cells of the liver. Three days after transplantation numerous phosphatase-positive foci were found in the parenchyma. They consisted of mononuclear cells with a high diffuse and granular acid phosphatase activity and of hepatocytes in which a spontaneous increase in activity of the lysosomal enzymes was observed. Some of the mononuclear cells in the foci were proliferating donor's cells. After 6 days or more, the separate foci in the liver merged with each other to form zones of coagulation necrosis. At least two types of cells may participate in the destruction of the hepatocytes during the graft versus host reaction: donor's immune lymphocytes coming into close contact with the recipient's target cells, and the recipient's macrophages which are "attracted" toward the site of the immune reaction by special mediators of cellular immune reactions.

The writers have shown previously that after transplantation of lymphoid cells of rats stimulated primarily or secondarily by mouse liver antigens into immunologically immature mice a typical graft versus host reaction (GVHR), resembling runt disease in its form [3], develops. One of the most characteristic features of the GVHR is the formation of foci of necrosis in the liver, initially along its free border but later extending throughout the hepatic parenchyma [6]. The causes and mechanism of this process have not been studied.

In the investigation described below the role of acid hydrolases of the lysosomes in the development of necrotic lesions in the liver in the GVHR was examined. According to some reports these enzymes are mediators of the reactions of transplantation immunity and they are liberated from destroyed immune mononuclear cells in the immediate neighborhood of target cells [1, 13].

EXPERIMENTAL METHOD

During the first 24 h of postnatal life CBA mice were injected intraperitoneally with 1.78×10^7–2.34×10^7 spleen cells or 1.90×10^7–2.12×10^7 lymph gland cells of a Wistar rat which had first been immunized with a whole homogenate of mouse liver by the following scheme: the first injection consisted of 15 mg lyophilized material subcutaneously into all the limbs plus 30 mg intraperitoneally, followed by an interval of 30 days; the second injection was of 40 mg subcutaneously into the hind limbs. The cells were obtained 10–14 days after the final immunization. The technique of preparation and evaluation of the viability of the cell suspension was described previously [2]. The cytopathogenicity of the cells was judged by the development of runt disease in the mice: the animals were sacrificed 1, 3, 5–7, and 10–12 days after transplantation and the thymic, splenic, and hepatic indices were determined [3]. Acid phosphatase (AP) activity was

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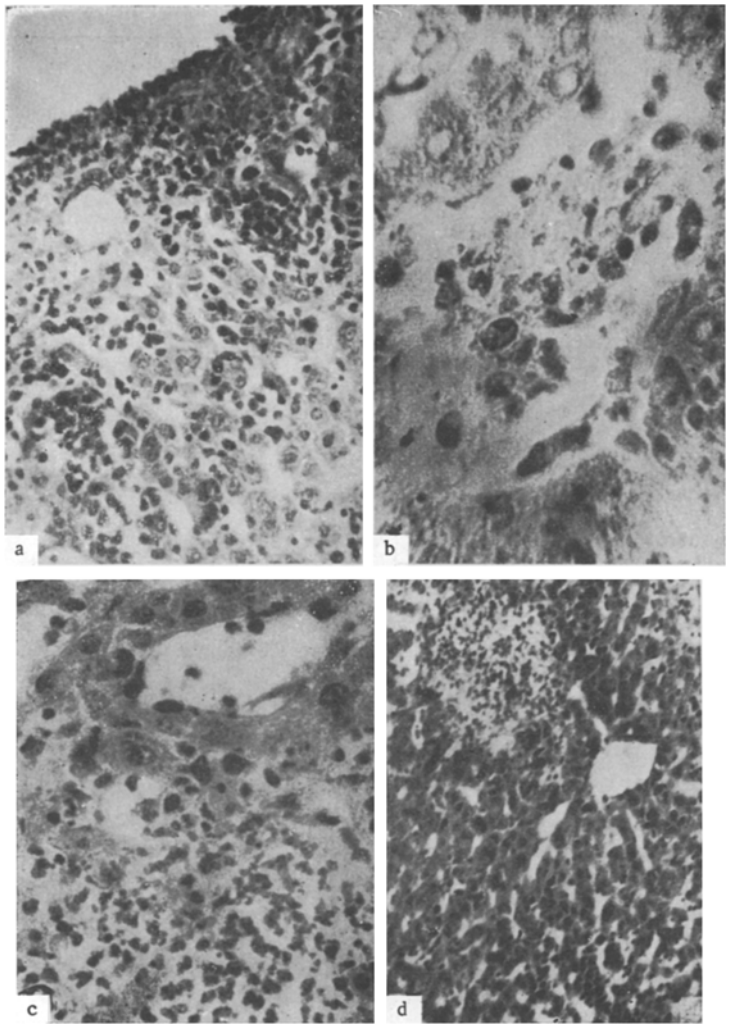

Fig. 1. Mouse liver after transplantation of sensitized rat lymphoid cells: a) 6th day of experiment; foci of proliferation of mononuclear cells in liver parenchyma (methyl green–pyronine, 200×); b) same time; accumulation of pyroninophilic blast cells in dilated hepatic sinusoid (methyl green–pyronine, 400×); c) 10th day of experiment; necrosis in zone of intralobular proliferation of mononuclear cells (hematoxylin-eosin; 140×); d) same section; edge of necrosis; remmants of nuclei of dying cells (300×).

determined by Gomori's method in frozen sections of the liver fixed in calcium formol and in unfixed freshly frozen sections [5]. In the latter case true activity of the lysosomal enzymes and the actual permeability of the lysosome membranes were obtained [5]. The substrate mixture consisted of equal volumes of α- and β-glycerophosphates. The incubation time for the fixed sections was 2 h and for the unfixed sections 40–50 min. Parallel series of paraffin sections were stained with hematoxylin-eosin and methyl green–pyronine and treated by the indirect Coon's method. Rabbit immune serum against rat globulins, previously exhausted with a mixture of mouse serum proteins and tested by immunophoresis and immunofluorescence, was used to detect species-specific rat globulins. A luminescent ass serum against rabbit globulins (prepared at the N. F. Gamaleya Institute of Epidemiology and Microbiology, Moscow) was used as the second reagent. The same doses of cells, but previously disintegrated by freezing and thawing five times, were used in the control tests.

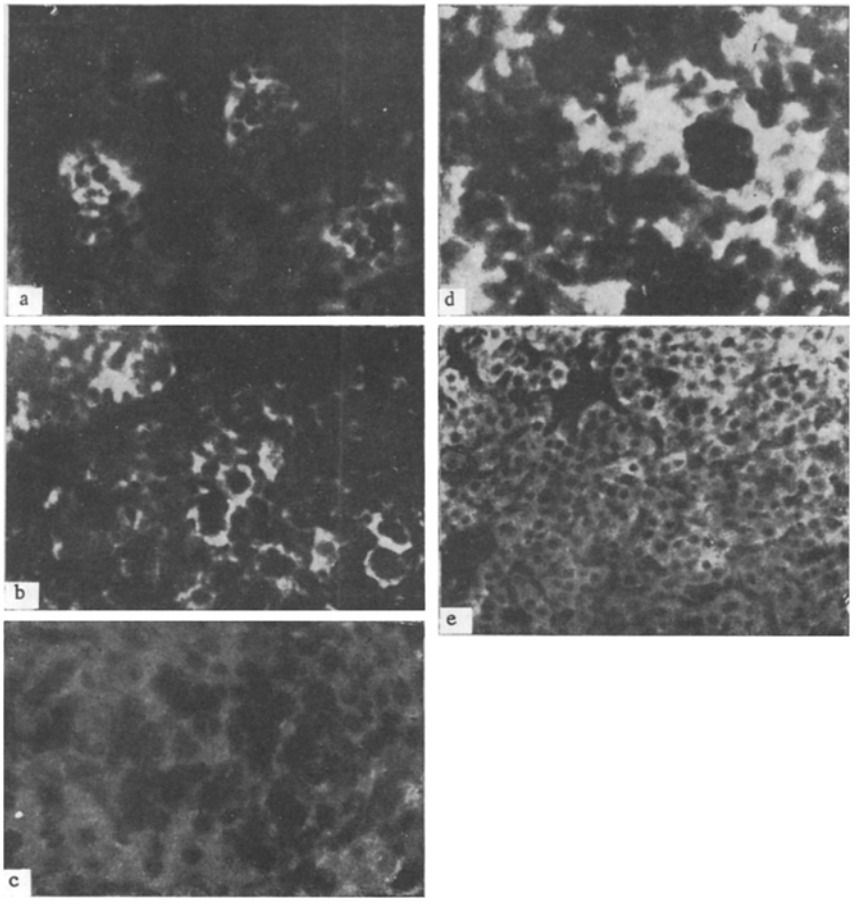

Fig. 2. Sections of mouse liver treated by indirect Coons' method after transplantation of rat spleen cells: a) 6th day of experiment; rat mononuclear cells in sinusoids (320×); b) same time; donor's cells among foci of perivascular infiltration (400×); c) 10th day of experiment; fluorescence of hepatocytes along the course of the central vein (320×); d) specific neutralization of fluorescence of cells in focus of infiltration (serum against rat globulins exhausted with rat serum proteins; (400×); e) control section; 2.3×10^7 disintegrated spleen cells injected at birth (320×).

EXPERIMENTAL RESULTS

After transplantation of the rat spleen cells about 90% of the mice died from an acute form of GVHR on the 8th-12th day of the experiment. After injection of a cell pool from the inguinal, axillary, retroperitoneal, and mesenteric lymph glands all the mice had died by the 5th-7th day after transplantation. At the height of the GVHR atrophy of the thymus and marked splenomegaly and hepatomegaly were observed.

The formation of primary follicles was disturbed in the spleen and lymph glands and proliferation of reticulum cells and macrophages was observed.

On the 3rd day after transplantation of the spleen cells, multiple foci of cell proliferation of two types appeared in the liver. The foci of the first type were found along the course of the portal vessels and consisted of dense collections of mononuclear cells with hyperchromic nuclei and basophilic cytoplasm, and also of large, pyroninophilic blast cells with high mitotic activity. The second type of foci consisted of intralobular foci of hematopoiesis and nodules of proliferating mononuclear cells of different degrees of maturity* (Fig. 1a, b). Later destruction and disintegration of the hepatocytes took place in these foci

*Not only cells of the lymphoid series, but also Kupffer cells, which are components of a single "mononuclear phagocytic system" [12], are included among the mononuclear cells.

with the formation of characteristic zones of coagulation necrosis with round indistinct outlines (Fig. 1c, d). Sometimes the layers of hepatocytes which entered the zone of perivascular proliferation of mononuclear cells underwent necrosis.

The injuries to the hepatocytes in the recipients of the lymph gland cells were more widespread in character.

In fixed sections of the liver from intact mice AP was detected in a few Kupffer cells in the form of dark brown polymorphic granules against the background of weak diffuse staining of the cytoplasm, and also as uniform black granules of lysosomes in the hepatocytes. In the unfixed sections AP was discovered along the course of the biliary capillaries and in individual Kupffer cells. After transplantation of disintegrated rat lymphocytes, the number of AP-positive Kupffer cells was increased and AP activity was increased in the endothelium of the portal and hepatic veins.

Three days after transplantation of the rat spleen and lymph gland cells numerous AP-positive foci were found within the lobules and along the course of the portal tracts of the liver. These foci consisted of round, diffusely stained mononuclear cells and also of large, polymorphic Kupffer monophages with high diffuse and granular enzyme activity. In the adjacent hepatocytes there was appreciable activation of AP, which was concentrated in the enlarged granules and distributed diffusely in the cytoplasm of the cell. In individual cases the boundaries between the mononuclear cells and hepatocytes within the focus were partly or completely obliterated. The diffusely stained component was predominant because of damage to the cells, and the entire focus consisted of primary necrosis of hepatocytes with very high AP activity.

On the 6th day and later, besides the changes described above, fusion of the necrotic foci had occurred: layer after layer in the hepatocytes showed spontaneous activation of lysosomal enzymes, leading to an increase, initially of the granular, and later of the diffuse, activity in the cytoplasm. The clearly positive Gomori's reaction in the unfixed liver sections was evidence of spontaneous activation of AP in the zones of damage to the hepatocytes. High AP activity, in the form of granules of different sizes, shapes, and intensity of staining, was detected in fixed sections in the newly formed foci of secondary necrosis. In many areas an intense, diffuse staining by Gomori's method was observed. At these times of the experiment proliferation of the Kupffer cells, the endothelium of the sinusoids and of the portal veins was well marked, and they showed high AP activity. At the sites of established necrosis of the hepatocytes AP activity was sharply reduced.

The times of accumulation of mononuclear cells in the liver coincided with the times of appearance of fluorescent cells of donor's type. On addition of the antiserum against rat globulins, exhausted with mouse serum proteins, to the liver sections, in 18 of 23 cases specific fluorescence of the cells of the intrahepatic and perivascular foci of infiltration was observed (Fig. 2). At the height of the GVHR the number of fluorescent cells was reduced, but by way of compensation the diffuse fluorescence of the hepatocytes along the course of the veins and sinusoids was increased on account of fixation of rat globulins (Fig. 2c). Rat globulins were regularly found in the blood of these mice [3]. In mice without the GVHR after inoculation of normal or sensitized spleen cells, no fluorescent donor's cells could be seen in the liver.

It can thus be concluded that sensitized lymphoid cells of a donor, when introduced into the recipient, on the one hand died from contact with an excess of specific antigens [9] and, on the other hand, they were converted through a stage of pyroninophilic blast cells into immune lymphocytes, which can be regarded as inducers of the GVHR [4, 8]. At the same time, through close contact between the immunologically active lymphocytes and specific antigens, a group of "molecular mediators" of hypersensitivity reactions of delayed type and of transplantation immunity [10, 11] was liberated into the medium. These substances, together with many other effects, can exert positive chemotaxis on the blood monocytes, from which all classes of tissue macrophages are formed [12]. From the moment that sufficient macrophages have accumulated in the zones of primary immunological damage to the hepatocytes, the nonspecific phase of the GVHR begins and the macrophages themselves can be regarded as effectors of this phase, just as is accepted for other cellular reactions of immunity [11].

In the course of immunological transformation hypertrophy of the lysosomal apparatus takes place in the lyphoid cells [4, 5]. In the experiments described above a sharp increase in AP activity also was observed in the mononuclear cells at the sites of necrosis of the hepatocytes. Spontaneous activation of the lysosomal enzymes may have occurred on account of definite disturbances of intracellular metabolism arising through contact between the immune lymphocyte and the corresponding target cell and leading to the

development of acidosis [7]. As the result of autolysis of the cell membrane the lysosomal enzymes were able to penetrate into the surrounding tissues and cause damage to the hepatocytes. An important role in the formation of the extensive zones of secondary necrosis was played by the acid hydrolases of the macrophages, which accumulated in the sites of the primary reaction between the donor's immune lymphocytes and the recipient's hepatocytes.

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