Expression of Hepatic Estrogen Sulfotransferase in Hepatocytes Transplanted into the Spleen of Syngeneic Animals

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An immunohistochemical assay showed patchy expression of liver-specific androgen-dependent estrogen sulfotransferase in hepatocytes of male rats transplanted into the spleen of castrated syngeneic males during 4-5 months after transplantation. Hepatocyte islets not arranged in hepatic lobules were found in the spleen. No regular distribution of cells with the maximum and minimal expression of estrogen sulfotransferase was revealed.

Key Words: *hepatocytes; transplantation; estrogen sulfotransferase; cell differentiation*

Expression of many genes in the hepatic lobule depends on the localization of cells around the central vein or in the periportal region [4,7,9,12]. Functional heterogeneity of centrolobular and periportal hepatocytes is associated with a concentration gradient of substrates, oxygen, cofactors, hormones, paracrine factors, and other substances, in the blood from the portal to central vein, while retrograde liver perfusion changes the pattern of this heterogeneity [7]. However, glutamine synthetase, cytochromes P-450b and P-450e, and α_2-microglobulin are expressed only in hepatocytes around the central vein and this expression does not depend on the blood flow direction in sinusoids. This is probably due to imprinting at the level of genes associated with various degrees of hepatocyte differentiation or due to regulation by nonhepatocyte factors [6,7].

Estrogen sulfotransferase (EST) is a liver-specific estrogen-binding protein absent in other rat tissues [1]. EST is expressed only in males and is characterized by a considerable gradient within the hepatic lobule, being maximum in the pericentral region [10]. Our studies showed that all male hepatocytes, regardless of their localization, have a complete androgenic program of EST synthesis. However, the expression of this protein is negatively regulated by factors prevalent in the hepatic triads [10]. Here we examined the contents of this protein in irregularly arranged male hepatocytes transferred from hepatic lobules to other tissue environment to establish the type of tissue regulation of the EST expression. Hepatocytes were transplanted into the spleen of syngeneic male rats. Recipients were castrated to prevent EST induction by endogenous androgens. EST expression was studied 4-5 months after transplantation. At this period, structure of the hepatic lobule was not formed [5,8].

MATERIALS AND METHODS

Male August rats weighing 150-200 g and castrated 2 weeks before the experiment were used. The suspension of isolated hepatocytes was obtained by a modified classic two-step perfusion of the liver taken from an intact August male rat with collagenase [11]. The liver was perfused through the vena cava inferior with 300 ml oxygenated Versen's solution (Institute of Poliomyelitis and Viral Encephalitis of the Russian Academy of Medical Sciences) containing 0.0001 M EDTA at 37°C for 15-18 min and with medium 199 on Hank's solution (Institute of Poliomyelitis and Viral Encephalitis of the Russian Academy of Medical Sciences) for 5-6 min. The liver was then reperfused with 0.05% collagenase ("Boehringer Mannheim" or "Sigma") on medium 199 (pH 7.5) at 37°C for 25-30

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min and then placed into 10-20 ml cold medium 199. Cell suspension was washed 3 times with 30-40 ml medium 199 and subjected to differential centrifugation at 50*g* for 3 min to isolate hepatocytes. Hepatocyte viability determined by 0.2% trypan blue exclusion was 50-75%. Cell suspension (0.7-1 ml) containing 10-13.5 million cells was injected into the spleen of castrated rats. The recipients underwent 2/3 partial hepatectomy 4 weeks after hepatocyte transplantation to stimulate hepatocyte proliferation [3]. EST distribution in spleenic hepatocytes was assessed 4-5 months after transplantation by an indirect immunoperoxidase reaction [2] using 7.2 μg/ml rabbit polyclonal antibodies isolated by immunoaffinity chromatography. On parallel spleen slices, anti-EST antibodies were replaced by immunoglobulin fraction from rabbit nonimmune serum to control the reaction specificity. Parallel slices were stained with hematoxylin and eosin for a histological assay of the cell structure.

RESULTS

In 3 of 5 recipients, islets of large (compared with spleen cells) mono- and binuclear cells were found in the red pulp 4-5 months after transplantation. They were not assembled into hepatic lobules and formed no bile ducts and sinusoid structures, but combined into clusters of 25-50 randomly arranged cells in the spleen tissue (Fig. 1, *a*). Such cells were not found in the spleen of sham-transplanted animals. On parallel slices, immunoperoxidase staining with anti-EST antibodies revealed a specific staining of cell cytoplasm. The intensity of staining varied from a maximum (corresponded to male centrolobular hepatocytes) to a minimum (no specific staining) (Fig. 1, *b*). On the control spleen slices without primary antibodies, such staining was observed only in spleen erythrocytes (Fig. 1, *c*). In sham-transplanted animals, no EST-specific staining in the spleen was revealed. Our findings indicate that these cells are hepatocytes.

The absence of a regular distribution of cells with different EST levels and patchy immunoperoxidase staining of EST in hepatocytes not arranged in acinar structures are probably due to retention of EST expression pattern in the progeny of centrolobular and periportal hepatocytes and the effects of tissue extrahepatic factors. The first assumption is vulnerable because there are data showing that the progeny of pericentral and periportal hepatocytes transplanted into the spleen progressively loss their differences [5,6,8]. On the 11th week after transplantation of individual pericentral and periportal hepatocytes into the spleen, the progeny of these cells expresses considerable amounts of cytochrome P-450 II E1 and glutamine synthetase around blood vessels [5]. This is typical of

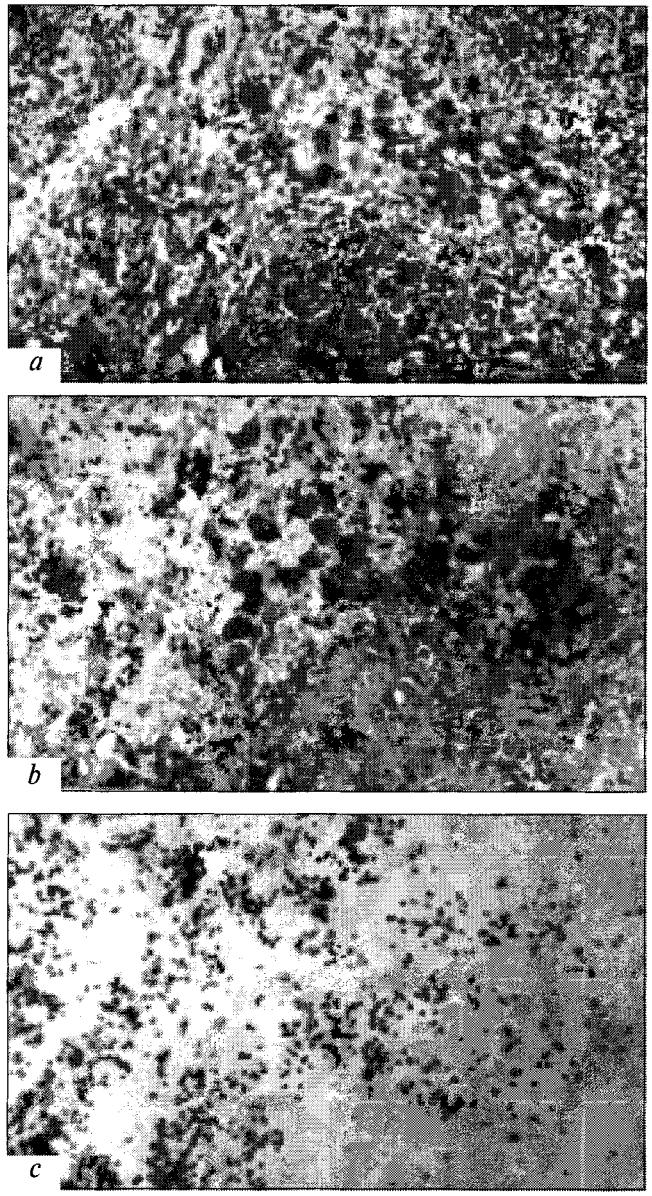

Fig. 1. Hepatocytes in the spleen of syngeneic rats 4 months after transplantation: staining with hematoxylin and eosin (*a*) and immunoperoxidase staining of a parallel slice with anti-estrogen sulfotransferase antibodies (*b*) and rabbit nonimmune serum immunoglobulin fraction (*c*), ×160.

pericentral hepatic cells. It can not be excluded that dedifferentiation rates of the hepatocyte progeny in relation to the expression of various proteins are different, and EST expression is a relatively stable index of differentiation. The assumption that tissue factors affect the expression of EST and other hepatocyte proteins seems to be more justified. Glutamine synthetase was shown to be expressed in both pericentral and periportal hepatocytes transplanted to the spleen and adjacent to vessels or fibrous tissues [5,8]. Depending on the site of transplantation, individual hepatocytes gain the capacity to express the gene of glutamine

synthetase (in the fat fascia) or carbamoylphosphate synthetase (in the spleen) over 2 weeks [8]. All hepatocytes become glutamine synthetase-positive after culturing with RL-ET14 epithelial cells [5]. A 355-bp sequence localized upstream of the phosphoenolpyruvate carboxykinase gene transcription start site is responsible for preferential expression of this gene in the hepatic periportal zone [5]. Intrahepatic factors of zonal expression of cholesterol 7α-hydroxylase and glutamine synthetase were identified [6]. It can be assumed that hepatic mesothelial and endothelial elements formed in the spleen to the 4-5th month after transplantation but not assembled in hepatic lobule inhibit EST synthesis only in adjacent hepatocytes (patchy EST expression).

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