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EXPERIMENTAL BIOLOGY

ATYPICAL CELLS SYNTHESIZING α-FETOPROTEIN IN THE REGENERATING MOUSE LIVER

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The tumor and embryonic protein α-fetoprotein (AFP) is synthesized during normal development by yolk sac cells and by fetal hepatocytes. AFP synthesis is temporarily resumed in the regenerating liver of adult mice. The results of immunomorphologic studies at the light-optical level have led to the view that, depending on the character of the repair process, AFP can be synthesized in the adult liver by two types of cells: differentiated hepatocytes during regeneration of the liver after partial hepatectomy or a single poisoning with hepatotoxins, and by hepatite precursor cells (transitional cells and small hepatocytes) in the acute phase of chemical hepatocarcinogenesis [1]. However, an immunoelectron-microscopic study of the AFP localization in the regenerating liver of SWR mice [4] revealed synthesis of this protein not only in typical hepatocytes, but also in smaller cells similar in their ultrastructure to oval and transitional cells of the rat liver during chemical carcinogensis [11].

This paper describes a comparative electron-microscopic study of AFP containing cells in the regenerating liver of different lines of mice, using monospecific and monoclonal antibodies (AB) against AFP.

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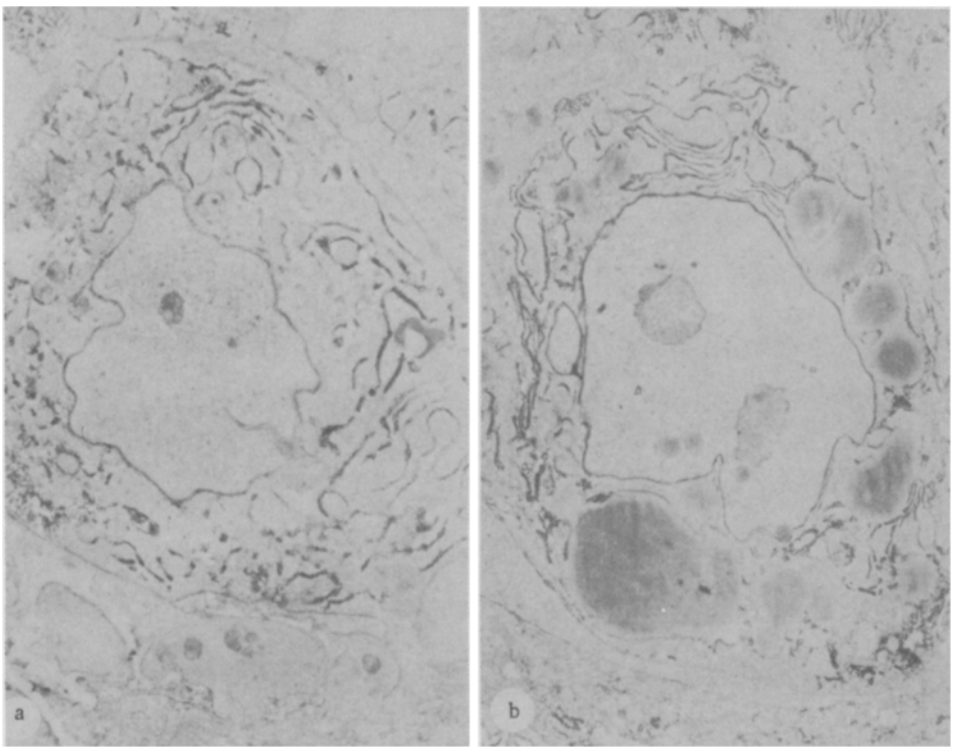

Fig. 1. Detection of AFP synthesis in regenerating mouse liver: a) AFP synthesis by atypical cell from liver of SWR mouse. Material treated with monospecific antibodies against AFP, 6500 ×; b) AFP synthesis by atypical cell from liver of BALB/c/J mouse. Material treated with monoclonal antibodies against AFP, 6200 ×.

EXPERIMENTAL METHOD

SWR, CC57BR, and BALB/c/J mice aged 2-3 months were obtained from the Nursery of the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR. The liver of normal mice and 72 h after poisoning with CCl_4 [3] was studied. Rabbit monospecific AB against mouse AFP were obtained, their specificity verified, and immunoelectron microscopy carried out by the methods described previously [4]. Unlike in the previous study [4], Fab'-fragments of donkey AB against rabbit IgG [13], labeled with horseradish peroxidase (PZ-3, type 6, from Sigma, USA) by the periodate method [14], were used.

Monoclonal rat AB against mouse AFP were isolated on Sepharose immunosorbent from the culture medium of a clone of rat-mouse hybridoma [7] and dialyzed against buffered physiological saline, which substantially increased the clarity and intensity of the subsequent immune reaction on sections. To detect AFP, frozen sections through the liver were incubated consecutively with rat monoclonal AB against mouse AFP, with rabbit AB against rat IgG, and with a conjugate of Fab'-fragments of donkey AB against rabbit IgG with horseradish peroxidase. It was discovered in the course of the work, however, that antigenic similarity between rat and rabbit IgG allows the second stage of incubation to be omitted without any appreciable loss of intensity of the immune reaction. Working dilutions of AB and Fab'-fragments of AB were chosen on serial paraffin sections of the liver under light microscope control [5].

EXPERIMENTAL RESULTS

Preliminary light-optical study of serial paraffin sections of the regenerating mouse liver, incubated with monospecific or monoclonal AB against AFP revealed the identical cellular localization of AFP [7]. On immune electron microscopy with these AB it was found that AFP is present in the same types of cells and has the same intracellular localization (Fig. 1). These observations showed that the rabbit AB against mouse AFP which were used possessed the same absolute specificity as monoclonal AB, thus ruling out any possibility of detection of foreign antigens.

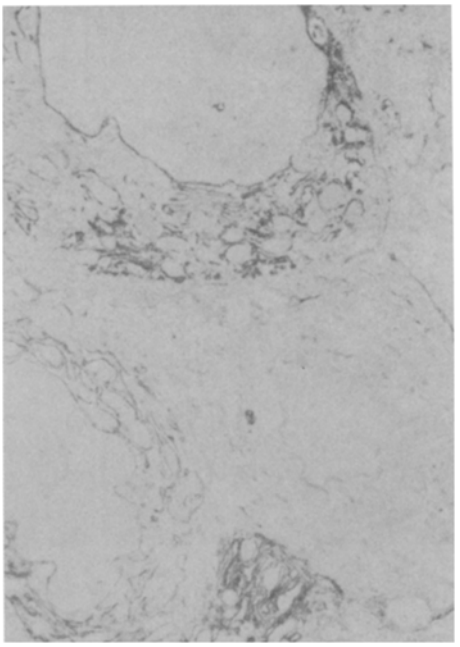

Fig. 2. Group of three atypical cells in liver of BALB/c/J mouse with different intensities of intracellular immune reaction. Material treated with monoclonal antibodies against AFP, 5200 ×.

The selective localization of the electron-dense reaction product during detection of antigens in the perinuclear space, on membrances and ribosomes, and in the lumen of cisterns of the rough endoplasmic reticulum and in elements of the Golgi complex by the immunoperoxidase method indicates synthesis and secretion of the test substance by the corresponding cells. This localization of AFP was found in the present investigation of regenerating liver cells of mice of all three lines. In SWR and CC57BR mice these cells were concentrated as a rule in three or four narrow layers of cells in perinecrotic zones. In their ultrastructure, they were mainly typical mature hepatocytes, with one or two nuclei. However, smaller cells with an intense intracellular immune reaction also were found, although not in all animals. They differed definitely from differentiated hepatocytes not only in size (10-15μ), but also in ultrastructural organization (Fig. 1). Characteristically they had a large nucleus, often with invaginations, one or more large nucleoli, and lipid inclusions. The cytoplasm of these small cells, relatively small in volume, contained single mitochondria, a well developed rough endoplasmic reticulum, and single inclusions resembling multivesicular bodies or lysosomes. These cells as a rule formed bile capillaries on contact with neighboring mature hepatocytes or similar small cells.

In BALB/c/J mice, characterized by a higher background blood AFP level [12] and by an active rise in AFP synthesis in response to liver damage [9], mature hepatocytes synthesizing AFP were found both in perinecrotic zones and at the periphery of the lobules, including in periportal regions. Small AFP-positve cells were more numerous than in SWR and CC57BR mice, and formed considerable groups resembling bands or trabeculae in type (Fig. 2), and they were found only in the zone of cells directly contiguous with the necrotic foci or in the zones of necrosis themselves. Sometimes, besides the extreme forms of cells just described, something resembling transitional cell forms could be seen, i.e., cells combining individual ultrastructural features of both mature hepatocytes and of small cells. The question of the existence of analogous small cells in the normal adult mouse liver arises. During ordinary and immune electron micoscopy of normal liver of the three lines of mice, no cells structurally or functionally similar to these small cells could be found. Only a certain purely external resemblance between these cells and Herring's epithelial cells could be noted. However, in the regenerating mouse liver, neither Herring's cells nor the epithelium of the bile ducts contained AFP (Fig. 3). In this respect the present results differ from those of Kuhlmann and Wurster [10], who described the localization of AFP in the epithelium of bile tubules and ducts in rats after administration of galactosamine.

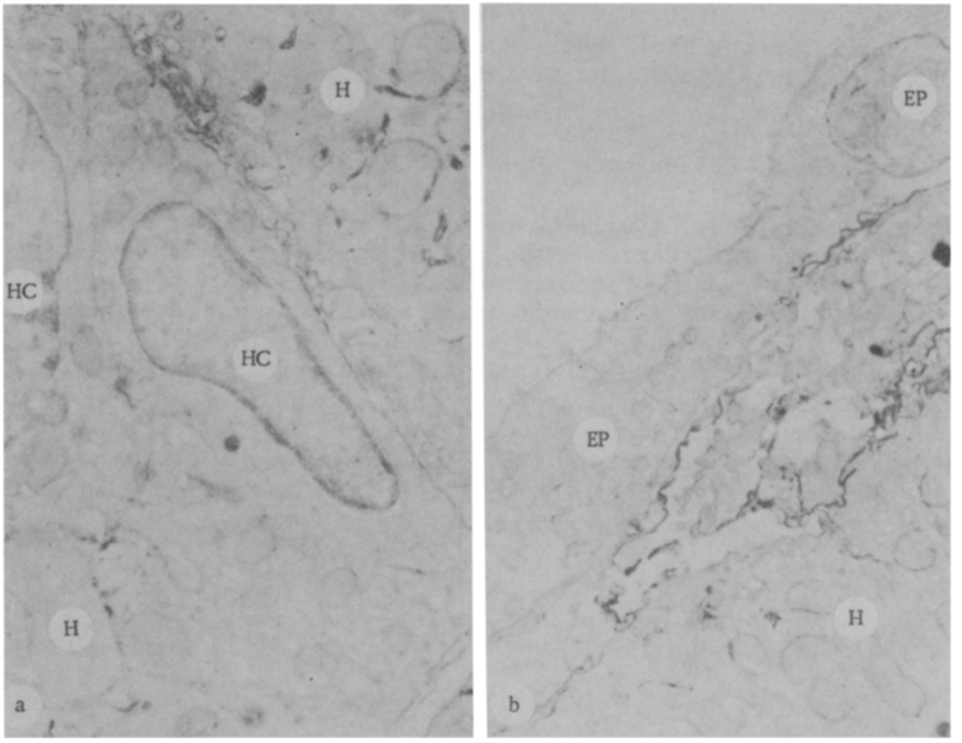

Fig. 3. Localization of AFP in regenerating liver of BALB/c/J mouse detected by treatment with monoclonal antibodies against AFP. Reaction product absent in Herring's cells (a) and epithelium of bile duct (b). AFP located in single cisterns of rough and endoplasmic reticulum of adjacent hepatocytes (H). HC) Herring's cell, EP) epithelium, H) hepatocytes. Magnification: a) 13,000 ×; b) 8000 ×.

Atypical small cells activiely snythesizing AFP were thus found in the liver of SWR, CC57BR, and BALB/c/J mice on the 3rd day after poisoning with CCl_4. The genesis of these cells is a very important problem. Foci of small AFP-containing cells have been described in the mouse liver after repeated poisoning with CCl_4 in the course of carcinogenesis [2] and also during spontaneous hepatocarcinogensis [8]. The appearance of small cells with AFP on the 3rd-4th day after a single poisoning of the mice with CCl_4 was observed previously at the light-optical level [5]. However, only an immunoelectron-microscopic study showed conclusively that these cells synthesize AFP, and revealed their definite ultrastructural similarity with hepatocyte precursor cells in rats [11].

Following exposure to CCl_4, besides proliferation of adult hepatocytes in the mouse liver it is possible that new hepatocytes are formed from precursors, as is observed during chemical hepatocarcinogenesis in rats and, consequently, the atypical small cells correspond to transitional and small hepatocytes in rats. To verify this hypothesis, a combination of methods of immunomorphology and autoradiography must be used. The elucidation of the physiological specialization of the atypical cells and, in particular, the problem of their ability to synthesize other serum proteins, like hepatocytes, is extremely interesting.

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