ULTRASTRUCTURAL LOCALIZATION OF B10 ANTIGEN OF THE HEPATOCYTE PLASMA MEMBRANE IN MOUSE HEPATOMAS

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Polyclonal antibodies (AB) to a glycoprotein of the mouse hepatocyte plasma membrane, located mainly in the region of the biliary capillary, have been obtained in the Laboratory of Immunochemistry, Research Institute of Carcinogenesis, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR. This antigen (AG) was called AG 1, and it has been shown by immunocytochemical light-microscopic methods that location of AG 1 in spontaneous hepatomas of CBA mice is similar in part to its localization in the normal liver of the adult animal. AG 1 has been identified in hepatomas on the boundary between two tumor cells in the region of the biliary capillary. Unlike the adult mouse liver, acinar structures have been found in hepatomas in which AG 1 was located in the center of the acinus [6]. In some hepatocytes AG 1 was absent altogether [4]. Later monoclonal AB were obtained to AG B10, immunochemically identical with AG 1 [5], and possessing a similar localization. An immune electron-microscopic study of the distribution of AG B10 in the adult mouse liver has been undertaken with the aid of these AB [2]. The aim of the present investigation was to discover the particular features of the ultrastructural localization of AG B10 in mouse hepatomas and to give the characteristics of the biliary capillaries in which the reaction to AG B10 is weak.

EXPERIMENTAL METHOD

Material was studied from spontaneous hepatomas of five male CBH mice and from hepatomas of three mice of the same line after treatment with 1,2-dimethylhydrazine. Fixation was carried out for 10 min by perfusion through the spleen [3]. The composition of the fixing mixture was: 6% formaldehyde (from paraform), 0.05% glutaraldehyde, 0.05% saponin in 0.15M cacodylate buffer, pH 7.35. After perfusion the tumor fragments were kept for 3 h at 4°C in fixative of the same composition but without glutaraldehyde. After fixation the pieces were washed twice with 0.15 M cacodylate buffer, pH 7.3, with the addition of 3.5% sucrose, and they were allowed to stand in a fresh portion overnight at 4°C. Sections 15 μ thick, cut on a cryostat, were kept for 20 min in buffered physiological saline (BPS) with the addition of 0.1 M L-lysine, and then incubated in a solution of rat monoclonal AB to AG B10, containing 0.05% saponin, for 3 h at room temperature. They were then washed with BPS (3 times, 20 min each time) and incubated in a solution of a conjugate of goat AB to rat IgG with horseradish peroxidase for 12 h at 4°C. After washing, peroxidase activity was demonstrated with the aid of 3,3′-diaminobenzidine. The frozen sections were postfixed in 1.33% OsO_4 solution, dehydrated in acetone, and embedded in a mixture of Epon 812 and Araldite on the surface of specially prepared from the same embedding medium. Semithin and ultrathin sections were cut on an LKB III ultrotome from the specimens thus obtained. The sections were examined without preliminary staining in the JEM 100 CX electron microscope.

EXPERIMENTAL RESULTS

The study of the semithin sections showed that the hepatomas studied usually had a mainly mixed type of structure. Alternation of zones with solid and trabecular structure often was observed. In other parts either of these structures predominated.

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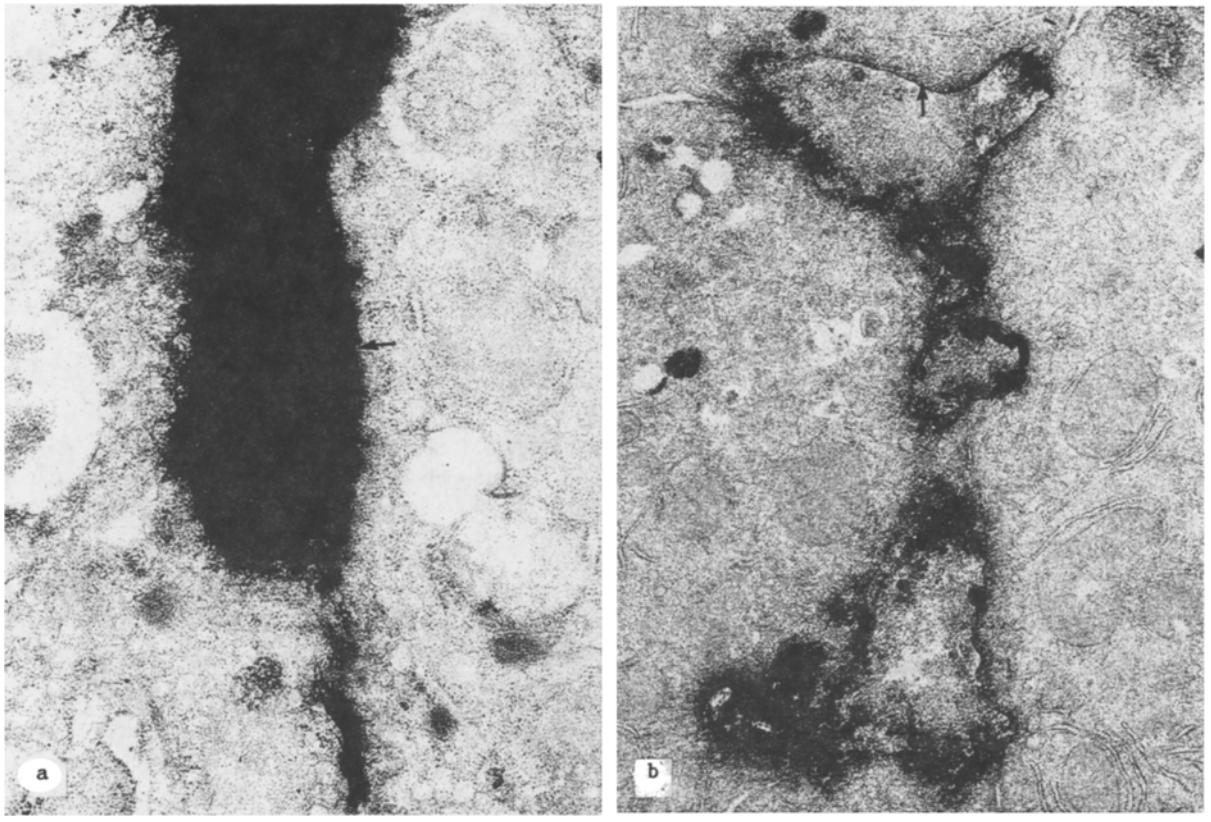

Fig. 1. Immunocytochemical localization of B10 antigen in mouse hepatomas. a) Intense reaction to AG B10 in region of biliary capillary, reaction product completely fills its lumen (arrow); b) weak reaction for AG B10 in lumen of two biliary capillaries in areas where plasmalemma is without microvilli (arrow). Ultrathin sections not stained. Magnification: a) 25,000, b) 12,000.

The product of the reaction for AG B10 could be seen in semithin sections on the boundary of contacting tumor cells in the form of small round formations, or of darkly stained strips, evidently consisting of biliary capillaries divided longitudinally. Zones of different sizes in which no reaction could be observed to AG B10 were often found in the hepatomas. Groups of cells forming acinar structures were found. In this case the reaction product deposited along radii from the lumen to the periphery of the acinus.

Electron microscopy showed that AG B10 was located on the membrane of the microvilli of the biliary capillaries and in the form of fine grains between the microvilli. The product of the reaction for AG B10 in some cases was discovered on the plasmalemma of the lateral domain of the hepatoma cells. Under normal conditions localization of the lateral zones along the plasmalemma in the liver of adult mice has been observed in a narrow layer of hepatocytes around the central veins [2]. In hepatomas, dependence of the reaction on the location of the cell in the lobule cannot be traced because of the absence of a lobular structure in the lobule. The reaction was weak on the plasmalemma of hepatoma cells adjacent to the perivascular space, in agreement with the character of deposition of the reaction product in the normal liver.

The intensity of the reaction for AG B10 in different parts of the hepatomas and in different hepatomas varied. Biliary capillaries with a very intense positive reaction for AG B10 were found in the hepatomas (Fig. 1a), but their structure was difficult to examine because of the abundant electron-dense reaction product filling their lumen.

Some biliary capillaries with a moderate or weak reaction for AG B10 were characterized by the absence of microvilli on definite segments of their surface. The granules in the lumen of these biliary capillaries either did not react for AB to AG B10 or gave a weak reaction (Fig. 1b).

Some biliary capillaries were found which contained unusual sickle-shaped structures with elongakd ends in their lumen (Fig. 2a). Narrow cytoplasmic projections also intruded into the lumen of these capillaries. All these formations in the biliary capillaries of hepatomas may perhaps be modified microvilli. They gave a weak reaction for AG B10.

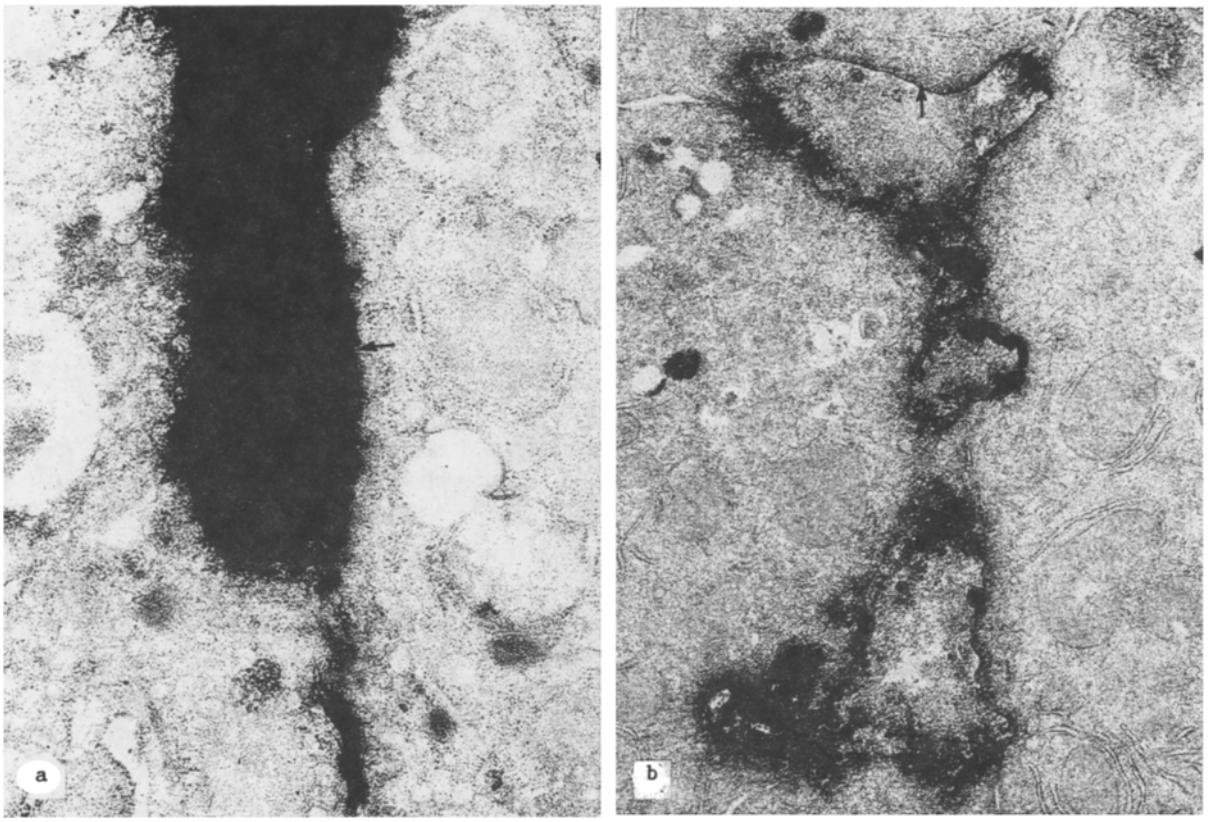

Fig. 2. Immunochemical localization of B10 antigen in mouse hepatomas: a) sickle-shaped structures in lumen of biliary capillary (arrow), weak reaction for AG B10; b) biliary capillary, longitudinal section through microvillus (arrow), containing granules reacting for AG B10; c) biliary capillary, reaction product for AG B10 on plasmalemma of capillary (arrow) in granules located in lumen. Unstained ultrathin sections. Magnification: a) 25,000, b) 95,000, c) 70,000.

In certain parts of hepatomas the intercellular spaces were widened, including in areas in direct contact with biliary capillaries. In this case a reaction for AG B10 was absent both in the biliary capillaries and on the plasmalemma of the lateral zones of the tumor cells. Sometimes biliary capillaries not reacting for AB to AG B10 or reacting for them only weakly, despite the integrity of their structure, were seen in the hepatomas. These capillaries had a small lumen, in which microvilli were located close together, and there were few granules in the lumen.

Definite relationship was observed between the intensity of the reaction for AG B10 and the ultrastructural features of the tumor cells. The reaction was strongest in the biliary capillaries located between tumor cells, and containing many peroxisomes in their cytoplasm. An equally strong reaction was found in regions of hepatomas consisting of cells whose cytoplasm contained extensive zones without organelles, and which were evidently regions from which the glycogen had been eluted. A moderately strong reaction was observed in areas of hepatomas in whose cytoplasm numerous mitochondria were present, in addition to a well-developed rough and smooth cytoplasmic reticulum.

In biliary capillaries of hepatomas where the intensity of deposition of the reaction product was not too great, the sites of its localization could be studied in more detail. Usually the plasmalemma of the biliary capillary and the membrane of the microvilli stained intensely. The core of the microvilli also stained [2]. In our material, in longitudinal sections through the microvilli of the biliary capillaries, small granules stained with reaction product, and with a tendency in some places to form a line running parallel to the course of the microvillus (Fig. 2b) they could be seen inside the microvilli. We know that actin filaments pass through the microvilli of the biliary capillaries [8]. Oriented transport of protein molecules can take place along actin filaments [1]. Deposition of granules of the product of the reaction for AG B10 in a line is perhaps the ultrastructural reflection of transport of AG B10 molecules through the microvilli of the biliary capillaries. The fine granules distributed in the lumen of the biliary capillaries, including in those regions where no microvilli are present, often reacted for AG B10 (Fig. 2c). The significance of this phenomenon is not clear.

These investigations revealed a somewhat varied picture of the intensity of the reaction for AG B10 in the biliary capillaries of spontaneous and induced mouse hepatomas. The tumors, as we know, are heterogeneous and consist of a large number of clones. It has been shown, in particular, that heterogeneity of tumor cells with respect to expression of α-fetoprotein is observed in rat hepatomas [7]. The diversity of tumor cells of mouse hepatomas which we found, in relation to the intensity of the reaction for AG B10 and the ultrastructural features of their cytoplasm and biliary capillaries, also probably may reflect the cellular heterogeneity of hepatomas. Correlation was found between disturbance of the ultrastructure of the biliary capillaries of hepatomas and the reduced expression of AG B10.

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USE OF MAGNETICALLY CONTROLLED MICROCAPSULES IN COMBINATION (CHEMOTHERMOMAGNETO-) THERAPY OF EXPERIMENTAL TUMORS

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The main obstacle to the use of antitumor preparations (ATP) in clinical oncology is the nonselectivity of their action, which gives rise to serious levels of poisoning and ultimately lowers the natural resistance of the organism to the development of the tumor process. Often the depression of immunity as a result of chemotherapy leads (after a short remission) to recurrences and to the development of latent metastases. One way of obtaining selective accumulation of ATP in the zone of tumor growth is to use magnetically controlled microcapsules (MC), covered with a biocompatible membrane, and which may contain various ATP [1-4, 5]. In this way the concentration of an ATP in the zone of tumor growth can be greatly increased, the duration of action of the ATP lengthened, and the total therapeutic dose of the ATP substantially reduced. We know that chemotherapy of tumors is most effective when combined with hyperthermia. Meanwhile the use of hyperthermia is limited by the impossibility of obtaining a strictly local rise of temperature in the zone of tumor growth. The use of magnetically controlled MC in conjunction with a high-frequency field enables strictly local hyperthermia to be attained in the tumor tissue, as a result of the hysteresis effect in the ferromagnetic material of MC, and this sensitizes the tumor cells to the action of ATP incorporated in MC.

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