INDUCTION OF α-FETOPROTEIN SYNTHESIS IN HIGHLY DIFFERENTIATED SPONTANEOUS MOUSE HEPATOMAS

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Although production of the embryonic serum protein α-fetoprotein (AFP) by hepatomas is now firmly established in experimental and clinical situations, the causes of this phenomenon are not yet known. In particular, it has not been explained why liver tumors similar in morphology and degree of differentiation can differ sharply in their level of AFP synthesis. Furthermore, heterogeneity of the cells for AFP production always exists within a tumor, and no morphological differences have been found between cells producing and not producing AFP [1].

AFP synthesis is reversibly repressed in the liver of adult animals. It can be restored, if the liver structure is damaged, in the region of injury and accompanying information [3, 8, 5]. Abelev has suggested that the mechanism of regulation of AFP repression present in normal liver is preserved in highly differentiated hepatomas, which usually produce only very small quantities of AFP or none whatever. If this hypothesis is correct it might be expected that surgical injury to tumor nodules of highly differentiated hepatomas will lead to free-expression of AFP in regions adjacent to the zone of injury.

The present investigation was devoted to testing of this hypothesis on spontaneous hepatomas. The writers showed previously that spontaneous hepatomas, consisting in most cases of highly differentiated tumors, very similar in microscopic structure to normal liver tissue, are found in the liver of old CBA mice in 100% of cases. AFP-producing cells are found in these nodules very rarely [6]. It is evident that such tumors are most suitable for the study of the possibility of AFP induction in tumor tissue.

EXPERIMENTAL METHOD

Sixteen male CBA mice aged 13-15 months, with spontaneous hepatomas in the initial stage of development, were used. Tumor nodules in all animals measured 1-3 mm in diameter.

After laparotomy, shallow parallel incisions were made on the tumor with a razor. On the 3rd day after the operation the mice were decapitated and the tumor nodules fixed in acetone—formalin—phosphate buffer mixture [2] and embedded in Histoplast. In five cases the mice were given colchicine in physiological saline (50 μg/g body weight) by intraperitoneal injection 30 min before sacrifice [9]. Usually tumor nodules with no incisions from the same animals were used as the control. In some cases during the operation part of a tumor nodule was removed during the operation and fixed as a control, and incisions were made on the remaining part of the same nodule.

The serum AFP level of animals undergoing the operations was determined by immunodiffusion in agar with a standard test system [4]. AFP was investigated in sections by the indirect immunoperoxidase method [10] with monospecific rabbit antibodies (AB) against mouse AFP and Fab-fragments of donkey antibodies against rabbit immunoglobulins, conjugated with horseradish peroxidase. As the control for nonspecific uptake of serum proteins by the tumor cells, parallel serial sections were treated with antiserum against mouse γ-globulin [7].

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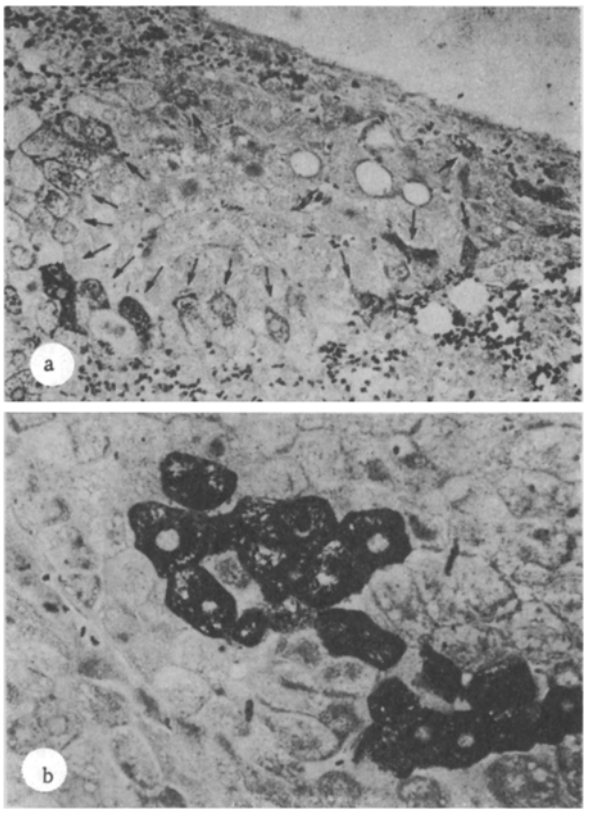

Fig. 1. Sections through spontaneous hepatoma of CBA mouse treated with AB against mouse AFP: a) AFP-positive tumor cells (dark) on border of inflammatory barrier (arrows), 80 ×; b) AFP-positive cells located in zone of a large blood vessel, 150 ×.

EXPERIMENTAL RESULTS

No AFP was found in the serum of mice with highly differentiated tumors by the immunodiffusion method in any of the 16 cases.

An immunomorphologic study of control tumor nodules and also of fragments of nodules removed before the incisions were made showed that AFP-positive cells were absent in every case. Morphological analysis showed that all control and experimental tumor nodules consisted of highly differentiated hepatomas.

AFP-positive cells were found only in sections of some nodules subjected to surgical injury. Two types of distribution of AFP-positive cells were found: 1) usually the AFP-positive cells were located in the zone immediately adjacent to the inflammatory barrier, caused by injury (Fig. 1a); 2) in some cases groups of AFP-positive cells were found near large blood vessels, evidently draining the injured part of the tumor (Fig. 1b). Usually staining of the cells with AB against AFP was very weak. Tumor cells containing AFP did not differ significantly in morphology from AFP-negative cells.

To detect AFP-positive cells more conclusively, in five cases secretion of serum proteins from the hepatocytes was blocked by injection of large doses of colchicine [9]. In these cases the distribution of AFP-positive cells on the boundary of the inflammatory barrier formed after the incision was much clearer (Fig. 1a). These cells stained with varied intensity and in some cases they were smaller than most cells of the tumor nodule.

In all cases staining sections of hepatomas with minimal injuries by AB against mouse γ-globulin showed no nonspecific uptake of blood serum proteins by the tumor cells.

The morphological picture of induction of AFP synthesis in highly differentiated spontaneous hepatomas after minimal mechanical injuries was thus very similar to that in normal mouse liver. Just as in Poltoranina's experiments [5], AFP-positive cells were located either in the inflammatory barrier or rather more distantly, in contact with large blood vessels.

These findings, in the writers' view, indicate that ATP synthesis in highly differentiated hepatomas is regulated in exactly the same way as in normal adult mouse liver tissue, and is closely connected with local disturbances of the trabecular structure.

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