Significance of AFP in hepatitis

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During the initial stage of study on α-fetoprotein (AFP), the specificity of this protein for primary hepatoma and embryonal carcinoma, as well as for the fetuses, has been emphasized[1]. Later, however, the appearance of this protein in some cases of gastric or pancreatic carcinoma having metastasis to the liver has been reported[2], thus the problem of the specificity seems to require re-evaluation. The recent development of radioimmunoassay of AFP enabled detection of even a minute amount of this protein in serum, and it was found that a considerable number of cases with hepatitis or cirrhosis, in addition to hepatoma and other malignant tumor cases, had detectable amounts of AFP in their sera. We have therefore attempted to study the mechanism by which AFP is produced in the liver of patients suffering from hepatitis, and to study the clinical and pathological significance of the appearance of this protein in hepatitis.

MATERIALS AND METHODS

For the radioimmunoassay of AFP in sera, the double antibody method-(α-FETO · RIAKIT, Dainabott) was used.

Liver biopsy specimens were divided into three portions, and each portion was fixed in 95% ethanol, 4% formaldehyde or 2% osmium tetroxide. Both the formaldehyde-fixed tissue and the ethanol-fixed tissue were embedded in paraffin, and the former was subjected to conventional histological stains, while the latter was subjected to the indirect method of fluorescent antibody technique using anti-AFP rabbit serum (Behringwerke) and FITC-labelled anti-rabbit γ-globulin goat γ-globulin. After fluorescence microscopic observation, the latter was further subjected to counterstaining with hematoxyline and eosin. The osmium-fixed tissue was embedded in Epon 812, and subjected to electron microscopy.

RESULTS

Results of radioimmunoassay of serum AFP in healthy control subjects and in patients with various diseased conditions are shown in Fig. 1-a.

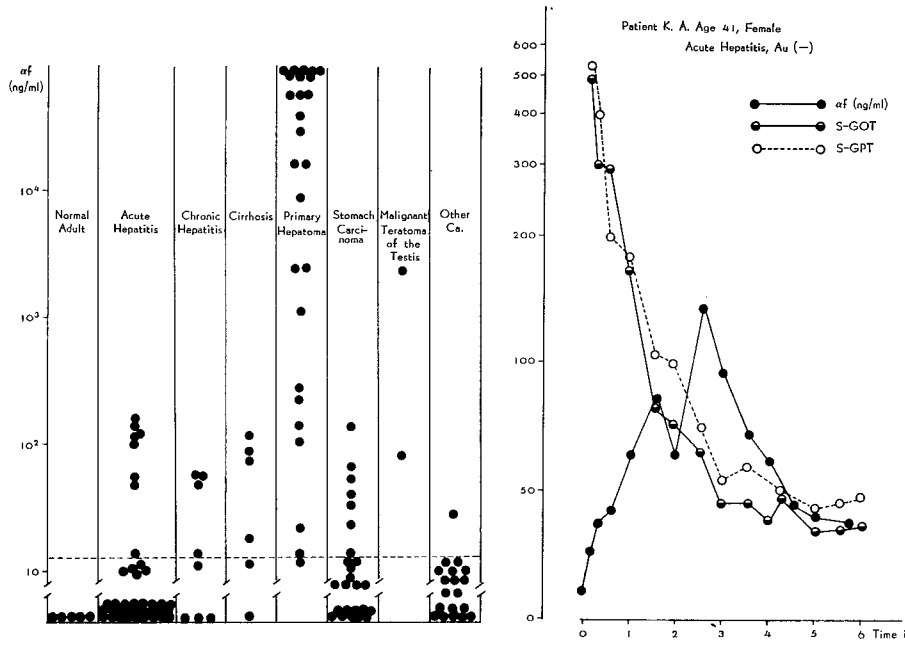

Fig. 1-a. **Fig. 1-b.**

Fig. 1-a: Serum AFP levels in healthy control subjects and in patients with liver diseases and malignancies.

Fig. 1-b: An example of the course of changes of AFP levels in acute hepatitis.

Out of 26 patients with hepatocellular carcinoma, all but one case had AFP in their sera in amounts exceeding the upper normal limit.

Increased amounts of serum AFP levels were also found in 4 out of the 6 cases with cirrhosis of the liver, and in 4 out of 8 cases with chronic hepatitis. Out of 39 cases with acute hepatitis, 8 cases, or 20.5%, had AFP in excess of the upper normal limit in their sera.

Fig. 1-b shows an example of the course of changes of AFP levels in acute hepatitis. Serum transaminase activities, which initially showed markedly elevated levels, decreased rapidly and reached normal values within 5 to 6 weeks. On the other hand, serum AFP level in this case was within a normal limit in the earliest stage, and showed a rapid increase within 3 weeks until the highest level of approximately 150 ng/ml was reached, followed by a gradual decrease thereafter. Most of the cases with acute hepatitis showed a pattern similar to that in this case, and this pattern was considered typical of acute viral hepatitis with a benign course.

However, in a small number of cases, AFP level was already high before the decrease of the elevated transaminase levels were attained, and

in still other cases, no apparent increase of AFP level could be observed despite increased transaminase levels. In chronic hepatitis and in cirrhosis, serum AFP levels were not necessarily in parallel with the transaminase levels. In some episodes of aggravation, serum AFP level increases, and decreases in coincidence with the remission, but in other episodes of aggravation, serum AFP remained at low levels in spite of the elevated transaminase levels.

The immunofluorescent visualization of AFP-producing cells in the liver tissue obtained by needle biopsy resulted in a clear demonstration of specific immunofluorescence in the cytoplasms of some of the small parenchymal cells in the peripheral zone of the lobule. None of the mature hepatocytes inside the lobule was found to have specific immunofluorescence. The hematoxyline and eosin stain of the same microscopic field clearly showed that the cells which had specific immunofluorescence were small cells composing the so-called pseudo-bile duct-like structures lying in the peripheral zone of the lobule immediately adjacent to the portal area (Fig. 2).

Electron microscopic observation of the pseudo-bile duct-like structure showed that this structure was composed of at least three types of cells: (1) cells having organelles similar to those of biliary epithelial cells, while at the same time having a few glycogen particles of rosette-like type; (2) cells having large ellipsoidal nuclei, with a large ratio of nucleus/cytoplasm, and scanty organelles, assuming an overall characteristic of the so-called "oval

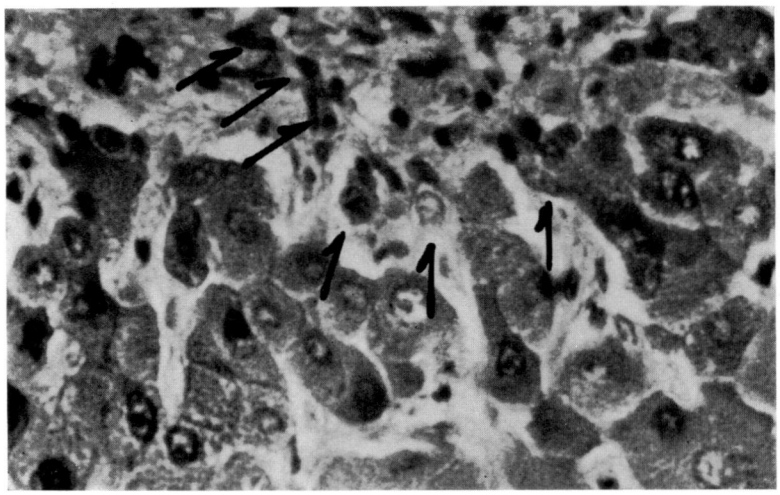

Fig. 2. A liver specimen from an acute hepatitis patient, stained with hematoxylin and eosin after immunofluorescent technique. Arrows show the cells which showed AFP specific fluorescence. × 600.

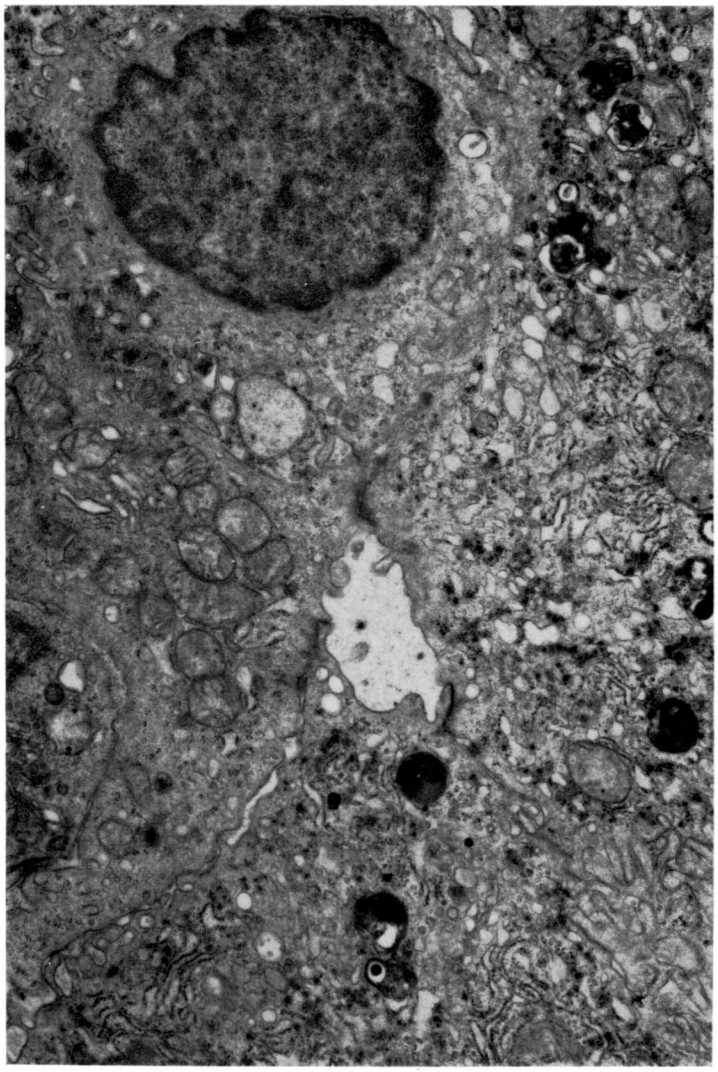

Fig. 3. An electron micrograph of the pseudo-bile duct-like structure of the liver of acute hepatitis patient. × 7,000.

cells"; and (3) mature hepatocytes. These three types of cells were connected with each other by junctional complexes to form a ductular lumen, and were surrounded by a basement membrane along their basal surfaces (Fig. 3).

DISCUSSION

The electron microscopic findings of the so-called pseudo-bile duct-like structures indicates, as Sakamoto[3] previously reported, that this structure corresponds to the canal of Hering composed of transitional cells having dual characteristics of hepatocytes and biliary epithelial cells, and oval cells, as well as cells undergoing differentiation from oval cells towards mature hepatocytes. The fact that specific immunofluorescence of AFP was found in some of the constituent cells of this structure suggests that the transient AFP production in hepatitis is achieved by cells undergoing differentiation from oval cells towards mature hepatocytes, and that in some, if not all cases with acute viral hepatitis, the regeneration of parenchymal cells are achieved through such a differentiation process, although the division of mature hepatocytes may no doubt be the main and most important process of the parenchymal regeneration.

Onoè *et al.*[4] reported that, during the process of experimental hepatocarcinogenesis, small hepatocytes having characteristics intermediate between oval cells and mature hepatocytes were responsible for the transient AFP production known as the "primary reaction"[5,6]. It is of interest to find that similar cells are also responsible for the transient appearance of AFP in sera in human viral hepatitis, inasmuch as this may reflect an intermediate stage of cell differentiation in the process of parenchymal regeneration.

It is already known that, even in primary hepatoma, not all hepatoma cells necessarily participate in AFP production, and that the difference in the rate of de-differentiation may be related to these functional discrepansies between different hepatoma cells[7,8]. It is suggested that a similar relation between AFP production and the stage of differentiation may be applied to the benign regenerating parenchymal cells undergoing differentiation from oval cells towards mature hepatocytes.

Whether this transient AFP production has any connection with a covert potency over future carcinogenesis or not may require further study.

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