Carcinoembryonic Antigen in Normal and Diseased Liver Tissue

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Many reports have demonstrated an elevation of circulating carcinoembryonic antigen (CEA) in the majority of patients with alcoholic liver disease and, less frequently, in patients with nonalcoholic liver disease. Several explanations for this finding have been proposed, eg, increased production or release of CEA by the damaged liver, decreased hepatic metabolism, or diminished excretion of CEA of extrahepatic origin. In an attempt to clarify the mechanism of CEA elevation in liver disease, we have compared the CEA plasma level as measured by radioimmunoassay with CEA demonstrable in liver tissue by the indirect fluorescent antibody technique in 7 patients without significant changes in the liver biopsy specimen, 23 patients with alcoholic liver disease, and 16 patients with miscellaneous liver diseases such as acute or chronic nonalcoholic hepatitis or extrahepatic biliary obstruction. The mean CEA plasma level in patients with alcoholic liver disease was significantly higher than in patients with nonalcoholic liver disease (8.8 ± 9.5 vs 2.7 ± 2.5 ng/ml; $P < 0.02$). In normal liver tissue, CEA was observed in the apical cytoplasm and along the luminal surface of bile duct epithelial cells, suggesting that under normal conditions CEA accumulates in and is excreted by bile ducts. In patients with alcoholic hepatitis and/or cirrhosis there was marked bile ductular proliferation and prominent cytoplasmic CEA-specific staining and both were associated with elevated CEA plasma levels in more than 80% of cases. In the group of miscellaneous liver diseases, bile ductule counts and CEA-specific staining did not correlate with CEA plasma levels. These observations suggest that proliferating bile ductules contribute to elevated plasma CEA in alcoholic patients. (Am J Pathol 92:671–680, 1978)

ELEVATED LEVELS of circulating carcinoembryonic antigen (CEA) have been reported in many nonneoplastic disorders,[1] including a variety of liver diseases.[1-6] Patients with alcoholic liver disease had a high incidence of elevated CEA levels, ranging from 45 to 88%. The association of CEA elevation with chronic active hepatitis was 22%; with primary biliary cirrhosis, 46%; with cryptogenic cirrhosis, 78%; with extrahepatic biliary tract obstruction and inflammation, 52%; and with hepatitis (not further specified), 30%. Bullen et al found raised CEA levels in 50% of patients with acute liver damage, 84% of patients with chronic hepatitis, 71% of patients with cryptogenic cirrhosis, and 89% of patients with alcoholic hepatitis.[7] Several explanations for increased CEA activity in

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patients with liver disease have been given [2,4-6]: a) The damaged liver is less efficient in the excretion or metabolism of CEA produced outside the liver. This hypothesis was supported by a study of the metabolism of human CEA in animals which showed a rapid disappearance of radiolabeled CEA from the serum with its coincident accumulation in the liver.[8,9] b) The damaged or regenerating liver cells release or produce increased amounts of CEA or CEA-like glycoproteins. This possibility remained unproved since the extraction of CEA from normal or cirrhotic liver tissue [10,11] does not resolve the question whether CEA is produced or excreted by the liver. Therefore, we embarked on a study of CEA in patients with different liver diseases, comparing the CEA plasma level with CEA demonstrable in liver tissue by the fluorescent antibody technique. For brevity, "CEA" will be used in this report instead of "CEA-like activity," recognizing that CEA represents a family of glycoproteins.

Materials and Methods

Patients

Plasma and needle biopsy specimens of liver obtained at the same time from 50 patients were studied. The diagnoses, based on clinical and laboratory data and histologic findings, were as follows: nonspecific changes (7 patients), alcoholic liver disease (19 patients had alcoholic hepatitis; in 11 of these with alcoholic cirrhosis, 2 patients had central hyaline sclerosis and 2 had only steatosis), acute viral hepatitis (3 patients), chronic persistent hepatitis (3 patients), chronic hepatitis with cirrhosis (8 patients), and extrahepatic biliary obstruction (2 patients). None of these patients had evidence of malignancy. Four patients with colonic adenocarcinoma metastatic to the liver were included as positive controls. In addition, surgical (wedge) liver biopsy specimens from 5 patients without liver diseases were studied to examine large bile ducts, which are usually not present in needle biopsy specimens. Finally, bile ducts and proliferating bile ductules were examined in one surgical and five needle biopsy specimens from 5 patients with extrahepatic biliary obstruction. Plasma for CEA determination was not available in the last two groups of patients.

Bile ductule proliferation in the liver biopsy specimens was quantitated by counting the number of bile ductules per portal area and graded as follows: 1+ = 1 to 2 bile ductules per portal area; 2+ = 3 to 5 bile ductules per portal area; 3+ = more than 5 bile ductules per portal area.

Immunohistochemical Staining

The indirect fluorescent antibody technique was employed to demonstrate CEA in routine histologic liver specimens which had been fixed in 4% buffered formalin and embedded in paraffin. Sections were deparaffinized in xylene and acetone, washed in phosphate-buffered saline pH 7.2 (PBS), and digested in 0.1% trypsin for 30 minutes to reduce nonspecific background staining.[12] After washing for 30 minutes in PBS, the sections were incubated with antiserum to CEA (anti-CEA). The antiserum had been prepared in rabbits by injection of purified CEA (kindly supplied by Dr. T. Edgington, Scripps Clinic and Research Foundation, La Jolla, Calif.) and had been purified by repeated absorptions with washed, formalinized, human AB and O red blood cells and

normal human plasma linked to cyanogen-bromide–activated sepharose.[13] The antiserum did not react with fresh human red blood cells type A, B, and O by hemagglutination. It stained the surface of colonic carcinoma cells and of normal colonic epithelium by the indirect fluorescent antibody technique. After three washes in PBS, the sections were treated with fluoresceinated sheep antirabbit γ-globulin, washed again, cover-slipped, and examined under an Ortholux fluorescence microscope (E. Leitz, GMBH, Wetzlar, Germany). Rabbit antihuman γ-globulin and anti-CEA absorbed with purified CEA were used as controls for the specificity of the staining by anti-CEA. The absorption was performed at a concentration of 0.1 mg CEA per ml anti-CEA for 30 minutes at 37 C followed by an overnight incubation at 4 C. An equal volume of PBS was used as control. The intensity and the extent of specific fluorescence were graded as negative to 3+ by two independent observers without knowledge of the histologic diagnosis or CEA plasma levels.

CEA Determinations in Plasma

All tests were done in duplicate by radioimmunoassay using the CEA-Roche Test Kit (Hoffman-LaRoche Inc., Nutley, N.J.). Values greater than 2.5 ng/ml plasma were considered elevated.

Results

The only structures showing specific staining with anti-CEA in 61 liver specimens studied were bile ducts, bile ductules, and metastatic colonic carcinoma, whereas hepatocytes were uniformly negative. This staining was abolished by absorption of anti-CEA with purified CEA, proving the specificity of the reaction. The antiserum to γ-globulin reacted with plasma cells and lymphoid cells, which were particularly prominent in chronic hepatitis, but not with the CEA-containing structures.

CEA in Normal Liver

The bile ducts in surgical wedge and needle biopsy specimens of liver from 12 patients without significant changes showed linear staining along the luminal aspect of the columnar epithelial cells after reaction with anti-CEA (Figure 1). The apical cytoplasm of the bile duct cells contained CEA-positive granules. Bile ductules were negative or weakly positive.

CEA in Alcoholic Liver Disease

Of 19 patients with alcoholic hepatitis or cirrhosis, 9 showed 3+ and 7 showed 2+ CEA-specific staining of many proliferating bile ductules (Figures 2 and 3). One case of alcoholic cirrhosis and 1 case of mild alcoholic hepatitis with only a few proliferating bile ductules were graded as 1+ and negative for CEA, respectively. Only 1 case of severe alcoholic hepatitis with transition to cirrhosis was CEA-negative despite the presence of many proliferating bile ductules. One case with central hyaline sclerosis contained many strongly positive bile ductules. The second case

with central hyaline sclerosis and the 2 cases of hepatic steatosis exhibited only rare bile ductules which did not contain CEA. There was a significant correlation between the CEA-specific staining and count of bile ductules (Kendall's Tau = 0.4709, $P < 0.01$).

In contrast to the linear luminal staining of bile duct epithelium, the entire cytoplasm of low cuboidal ductular cells was CEA-positive. The bile ductules usually did not arrange around a visible lumen but proliferated in double file in the expanded portal tracts and sclerotic areas.

CEA in Nonalcoholic Hepatitis

CEA-specific staining was graded as 3+ in 1 case each of acute viral hepatitis B and chronic active hepatitis, 2+ in 2 cases of chronic active hepatitis and 1 case of acute viral hepatitis, and 1+ in 2 cases of chronic persistent hepatitis and 1 case of acute viral hepatitis. Five cases of chronic active hepatitis and 1 case of chronic persistent hepatitis were negative. There was no significant correlation between the CEA-specific staining and count of bile ductules (Kendall's Tau = 0.2449, $P < 0.18$).

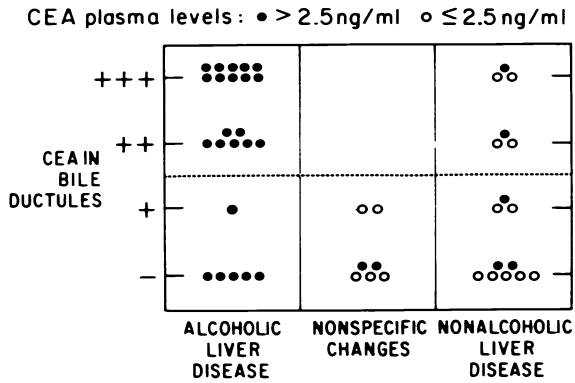
CEA in Extrahepatic Biliary Obstruction

To demonstrate CEA in a condition with prominent bile ductule proliferation other than alcoholic liver disease, the liver specimens from 7 patients with extrahepatic biliary obstruction were examined. Despite the presence of many proliferating bile ductules, reaction with anti-CEA was 3+ only in 2 cases, 1+ in 2 cases, and negative in 3 cases. Bile ducts, observed in 5 cases, showed moderate to strong staining along the luminal surface of the epithelial cells.

Relationship Between CEA in Bile Ductules and Plasma (Text-figure 1)

The plasma level of CEA was elevated in all 23 patients with alcoholic liver disease, with a mean of 8.8 ± 9.5 ng/ml. This was significantly higher than the mean CEA plasma level of 2.7 ± 2.5 ng/ml in patients with nonalcoholic liver disease ($P < 0.02$ by Student t test). In 16 of 19 patients (84%) with alcoholic hepatitis or cirrhosis, the elevated CEA level was associated with 2 to 3+ CEA-specific staining of proliferating bile ductules.

In the group of nonalcoholic liver diseases, the CEA level was elevated in 2 patients with acute viral hepatitis (5.1 and 10.1 ng/ml), in 2 patients with chronic active hepatitis and cirrhosis (3.7 and 5.9 ng/ml), and in 1 patient with chronic persistent hepatitis (2.8 ng/ml). The elevated CEA plasma level was associated in only 2 of these 5 patients with 2+ to 3+ CEA-specific staining of proliferating bile ductules. In 11 patients, the



TEXT-FIGURE 1—Chart demonstrating the relation between CEA in bile ductules as determined by immunofluorescence and the CEA plasma level measured by radioimmunoassay.

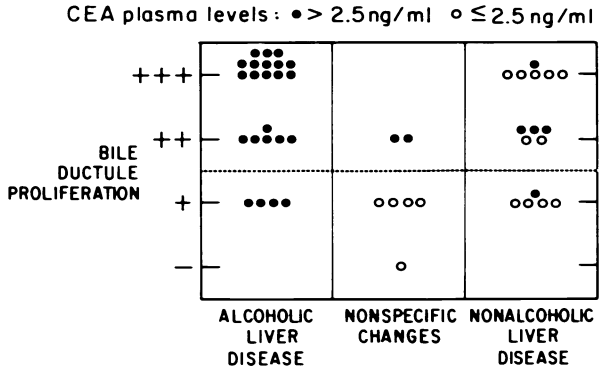
CEA level was below 2.5 ng/ml; however, in 4 of these, 2 to 3+ CEA was found in proliferating bile ductules. Thus, there was no relationship between plasma and ductular CEA among patients with nonalcoholic liver diseases.

Relationship Between Bile Ductule Count and CEA Plasma Level (Text-figure 2)

In 19 of 23 patients (83%) with alcoholic liver disease, the elevated plasma level of CEA was associated with marked bile ductule proliferation (three or more bile ductules per portal tract). In the group of patients with nonalcoholic liver diseases, there was no relationship between elevated plasma CEA and bile ductular proliferation.

Discussion

Current knowledge suggests that CEA represents a family of glycoproteins which can be found in a variety of normal and diseased tissues and neoplasms. The CEA-like molecules differ slightly in their physico-



TEXT-FIGURE 2—Chart demonstrating the relation between bile ductule proliferation as determined by counting bile ductules per portal area and the CEA plasma level measured by radioimmunoassay.

chemical and immunochemical characteristics according to their tissue of origin, but they share group-specific antigenic determinants.[14] Recently, progress has been made in the isolation and purification of certain species of CEA, and antiserums with specificity for these species have been prepared.[14-16] However, most antiserums, including our own, react with "conventional" CEA, defined as a group of CEA-like molecules.

Our studies demonstrate CEA in the cytoplasm and glycocalyx at the luminal surface of normal bile duct epithelial cells. It is not known whether these findings indicate absorption, concentration, synthesis, or secretion of CEA. Svendberg has demonstrated CEA-like glycoproteins in normal bile,[17] consistent with the hypothesis that, under normal conditions, glycocalyx antigens are not secreted into the blood but into the lumen of the biliary tree.[6] Bile ductules proliferate markedly in alcoholic liver disease, often without formation of a visible lumen, and penetrate portal tracts, fibrous septums, central sclerotic areas, and sometimes the adjacent hepatic parenchyma.[18] Quantitation of bile ductules documented a marked increase of their number in 83% of patients with alcoholic liver disease. CEA-specific staining was prominent in the cytoplasm of proliferating bile ductules in 84% of patients with alcoholic hepatitis or cirrhosis. Plasma levels of CEA were elevated in all patients with alcoholic liver disease. Therefore, the association of proliferation of CEA-containing bile ductules with elevated CEA plasma levels suggests that the bile ductules contribute to circulating CEA in alcoholic patients. This may be due to increased synthesis of CEA by proliferating bile ductules and/or secretion of CEA (or ductular or extrahepatic origin) into the blood rather than into the biliary tree by damaged bile ductules without a lumen. In 4 cases, bile ductule proliferation and CEA-specific staining were insignificant; however, the CEA plasma levels were augmented, suggesting additional conditions leading to CEA elevations in alcoholic patients. No association between proliferation of CEA-containing bile ductules and CEA plasma levels was demonstrable in a group of patients with acute viral hepatitis, chronic active hepatitis, chronic persistent hepatitis, or extrahepatic biliary obstruction, although the bile ductule count in the majority of these diseases was as high as in alcoholic liver disease. Alcohol might have a specific effect on bile ductules, resulting in quantitative as well as qualitative changes as suggested by the significant correlation between CEA-specific staining and count of bile ductules in alcoholic patients but not in nonalcoholic patients with liver disease. This possibility is supported by the higher incidence of increased levels of circulating CEA in alcoholic liver disease compared with nonalcoholic liver disease as observed in this and previous studies.[1,2,4]

The findings of this report do not exclude the possibility that impaired metabolism of CEA of extrahepatic origin by the damaged liver contributes to elevated plasma levels of CEA in alcoholic and nonalcoholic liver diseases. Future investigations directed at isolation and characterization of a CEA species with specificity for bile ductules could identify the source of increased CEA in patients with liver diseases and might lead to the development of a serologic test for bile ductule proliferation.

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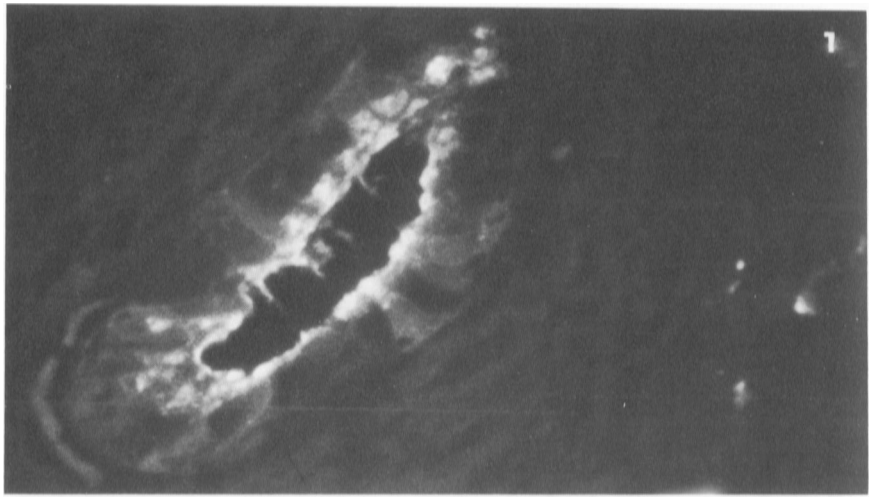
Figure Legends

Figure 1—Bile duct in a liver without significant changes shows CEA-specific staining along the luminal aspect and in the apical cytoplasm of epithelial cells. (Indirect immunofluorescence for CEA, × 400). **Figure 2**—Proliferating bile ductules in alcoholic cirrhosis contain CEA in their entire cytoplasm. Note the absence of a visible lumen in many bile ductules. (Indirect immunofluorescence for CEA, × 250). **Figure 3**—Consecutive sections of proliferating bile ductules in alcoholic cirrhosis. **A**—Indirect immunofluorescence with anti-CEA. **B**—Indirect immunofluorescence with anti-CEA absorbed with purified CEA. Note that the CEA-specific staining of bile ductules seen in 3A is abolished in 3B. (× 250)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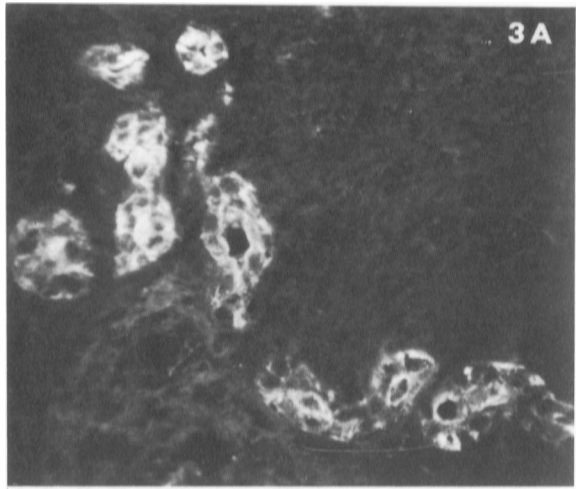
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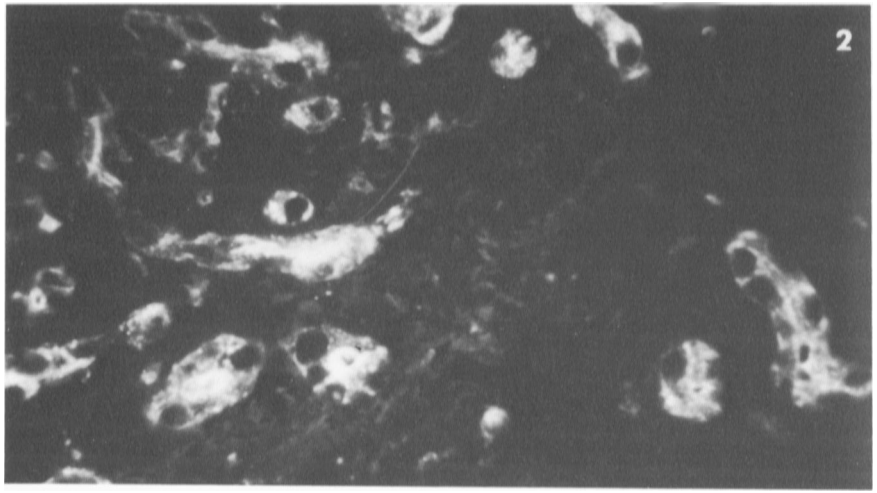
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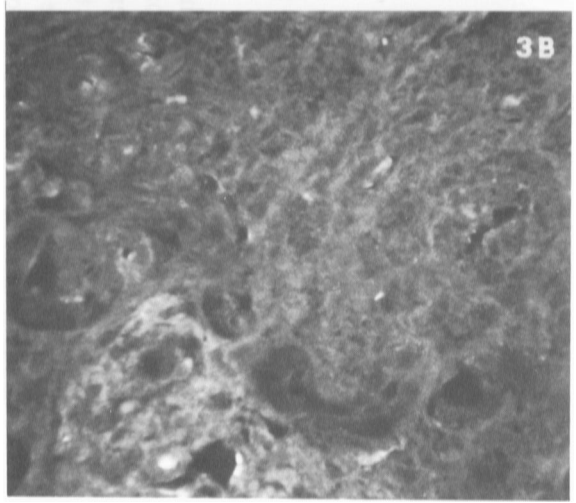
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