Primary Hepatocellular Carcinoma Following Non-A, Non-B Posttransfusion Hepatitis

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Knowledge of the etiologic factors resulting in primary hepatocellular carcinoma (PHC) remains incomplete. The worldwide association of this disorder with persistent hepatitis B virus (HBV) infection has been recently stressed (1). This relationship has been strengthened by epidemiologic studies (2–6), morphologic findings (7), and explorations in molecular biology. The latter observations have shown the integration of sequences of hepatitis B viral DNA in the tumor host genome (8). The virus–cancer linkage is also supported in nature; PHC has evolved after chronic viral hepatitis in woodchucks, Chinese ducks, and California ground squirrels (9). In the United States approximately one-fourth of PHC patients in one study had no evidence of HBV markers (6), suggesting a possible role of other viruses in pathogenesis (10). In this regard we recently observed PHC in a patient whose initial hepatic injury in 1964 appeared to be acute non-A, non-B (NANB) posttransfusion hepatitis.

CASE REPORT

E.K., a 59-year-old housewife, was admitted to Beth Israel Hospital in September 1981 with complaints of nausea and vomiting, fever, and increasing jaundice of four weeks' duration.

In 1964 E.K. was admitted to Beth Israel because of excessive vaginal bleeding. Adenocarcinoma of the endometrium was diagnosed on dilation and curettage; the patient was subsequently returned to the operating room for an abdominal hysterectomy and bilateral salpingo-oophorectomy with apparent total ablation of the tumor. Six units of whole blood were administered during these procedures. Of note is the presence of normal liver function one year earlier: total bilirubin, 0.2 mg/100 ml (3.4 μmol/liter); cephalin flocculation, 1+: thymol turbidity, 2.5; aspartate aminotransferase (AST),* 17 U/ml (0.12 μmol/sec/liter); and alkaline phosphatase, 3.1 Bodansky units/liter (0.52 μmol/sec/liter). The postoperative course was complicated by bleeding from the vaginal suture line on day 13. All liver function tests were normal at that time with the exception of the alkaline phosphatase of 10.7/liter (1.78 μmol/sec/liter); protein electrophoresis showed albumin, 3.5 g/100 ml (35 g/liter); α_1-globulin, 0.31 g/100 ml (3.1 g/liter); α_2-globulin, 0.78 g/100 ml (7.8 g/liter); beta globulin, 0.60 g/100 ml (6.0 g/liter); and gamma globulin, 0.82 g/100 ml (8.2 g/liter).

Thirty-four days after receiving the first two of six whole-blood transfusions, the patient experienced malaise, anorexia, and dark urine. Two weeks later because of progressive jaundice, hospitalization was required. There was no exposure to shellfish, contact with icteric patients, or use of hepatotoxic substances. Hepatic function tests as reported on admission and subsequently are found in Table 1. A serum iron determination was normal. The diagnosis was considered to be posttransfusion hepatitis. Rapid clinical improvement allowed discharge after two weeks, but in three weeks recurrent symptoms and signs of liver damage required readmission. Liver edge was now 6 cm below the costal margin, and the splenic tip was palpable. Again rapid clinical and biochemical remission permitted discharge at three weeks. The following year, in May 1965, a bleeding duodenal ulcer required hospitalization. Physical examination and hepatic function tests were normal except for an admission ALT of 72 units/ml (0.5 μmol/sec/liter). Between 1965 and 1980, E.K. was considered to have a chronic hepatic disorder, but major complications of liver injury or need for hospitalizations did not occur. Liver function tests performed annually between 1970 and 1978 showed mild elevations of AST on eight occasions (maximally, 112 units/ml) 0.8 μmol/sec/liter); alkaline phosphatase was raised slightly in four of nine assays.

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*SGOT, normal: 40 units/ml; SGPT is recorded as alanine aminotransferase (ALT), normal: 40 units/ml.

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TABLE 1. COURSE OF HEPATIC FUNCTION TESTS*

Date	*Total bilirubin (mg/100 ml)*	*ALT (units/ml)*	*AST (units/ml)*	*Alkaline Phosphatase (units/liter)†*
8/19/64	13.2 (224 μmol/liter)	1480 (11.0 μmol/sec/liter)	3050 (22.6 μmol/sec/liter)	47.5 (7.7 μmol/sec/liter)
9/3/64	2.0 (34 μmol/liter)	40 (0.3 μmol/sec/liter)	20 (0.2 μmol/sec/liter)	11.2 (1.8 μmol/sec/liter)
9/23/64	7.8 (133 μmol/liter)	300 (2.3 μmol/sec/liter)	400 (3.1 μmol/sec/liter)	20.0 (3.3 μmol/sec/liter)
July 1980	1.8 (30.8 μmol/liter)	46 (0.3 μmol/sec/liter)	45 (0.3 μmol/sec/liter)	115 IU/liter‡
Sept 1981	7.5 (128 μmol/liter)	73 (0.5 μ mol/sec/liter)	244 (1.6 μ mol/sec/liter)	249 IU/liter

* See text for data observed in years 1964–1980.
† Bodansky units $N \leq 4$.
‡ $N \leq 130$ IU/liter.

In July 1980, pneumococcal pneumonia required readmission to hospital. Significant laboratory tests revealed total bilirubin, 1.8 mg/100 ml (30.8 μmol/liter); AST, 45 units/ml (0.3 μmol/sec/liter); ALT, 46 units/ml (0.3 μmol/sec/liter); and alkaline phosphatase, 115 IU/liter* (0.58 μmol/sec/liter). Three months later at the time of a routine office visit the abdominal examination was unremarkable without organomegaly. Liver function tests remained mildly disordered as follows: total bilirubin, 0.5 mg/100 ml (8.5 μmol/liter); AST, 57 units/ml (0.4 μmol/sec/liter); alkaline phosphatase, 154 IU/liter (0.77 μmol/sec/liter), albumin, 3.7 g/100 ml (37 g/liter); and globulin, 3.6 g/100 ml (36 g/liter). The serum was negative for HB_sAg by radioimmunoassay (RIA).

In September 1981, progressive jaundice, constitutional symptoms, and a hard, nodular enlarged liver were recorded at entry to Beth Israel Hospital. The patient was moderately icteric, appeared chronically ill and had stigmata of chronic liver disease; the liver edge was observed 5 cm below the right costal margin. Laboratory values included total bilirubin, 7.5 mg/100 ml (128 μmol/liter); ALT, 73 units/ml (0.5 μmol/sec/liter); AST, 244 units/ml (1.6 μmol/sec/liter); and alkaline phosphatase, 249 IU/liter (1.24 μmol/sec/liter). Serologic tests by RIA were negative for HB_sAg,† anti-HB_s, anti-HB_c, and hepatitis A antibody. Serum alphafetoprotein was greater than 516,000 ng/ml. Alpha-1-antitrypsin concentration was 380 mg/100 ml (normal: 200–400 mg/100 ml). A ^{99}Tc liver scan demonstrated hepatomegaly with the right hepatic lobe largely replaced by a tumor mass; a substantial shift of colloid from liver to spleen and bone marrow was observed, consistent with cirrhosis. A percutaneous liver biopsy specimen was interpreted as primary hepatocellular carcinoma, clear cell type.

Description of Surgical Specimen. On histologic examination the entire specimen was formed of neoplastic epithelial cells in most areas organized into trabeculae, several cell layers thick, separated by endothelial-lined sinusoids. Occasional pseudoglandular areas were also identified. There were no portal areas or bile ducts. Most of the malignant cells were uniform in size and shape, with hyperchromatic, relatively small nuclei with an appearance resembling those of hepatocytes. The cytoplasm varied from finely granular and eosinophilic in a few areas to clear in most of the cells. The clear cytoplasm stained slightly for glycogen. The cytoplasm contained neither hyaline or evidence of bile production. A small population of cells was very atypical, having pleomorphic bizarre nuclei and increased size; some of these cells were multinucleated (Figure 1).

A relentless downhill course followed in spite of intravenous Adriamycin therapy. The patient succumbed to liver failure after a three-week hospitalization; postmortem permission was denied.

DISCUSSION

The histologic features of the neoplasm were most typical of hepatocellular carcinoma, particularly the trabecular and pseudoglandular growth patterns and the overall resemblance of the cells to hepatocytes. Hepatocellular carcinomas often have areas of marked pleomorphism, as noted in this case, and the "clear cell" appearance is a well-documented variant (11).

The course of this patient may be explained by the hypothesis that infection by NANB hepatitis initiated a sequence of pathophysiologic events ultimately leading to PHC. This conclusion, however, must be presumptive rather than definitive; a barrier to acceptance is the obvious lack of identifiable serologic markers for the NANB pathogens. The epidemiologic characteristics of the case described, nevertheless, are consistent with the current understanding of NANB-induced posttransfusion hepatitis. The comparatively short incubation

*Normal <130 IU/l.

†Absence of serologic markers for HBV was confirmed independently by Dr. Edward Tabor, Hepatitis Branch, Bureau of Biologics, Bethesda, Maryland.

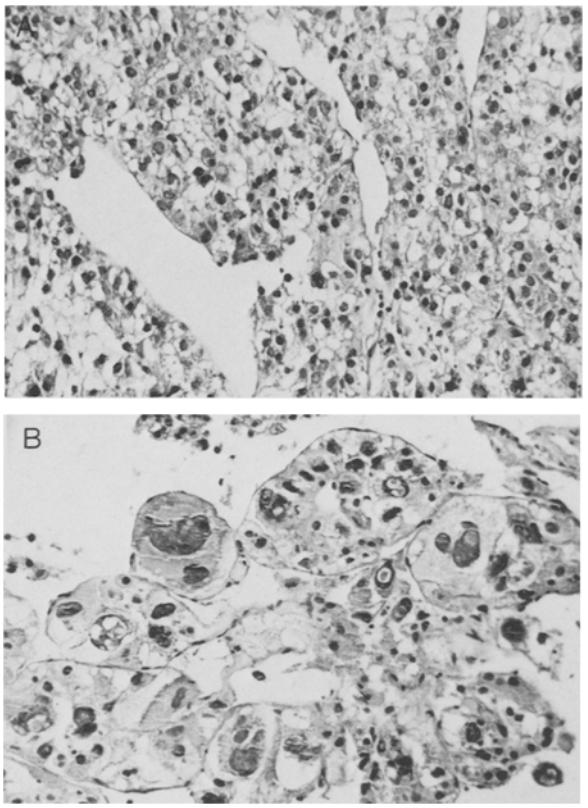

Fig 1. (A) Neoplastic hepatocytes form trabeculae several layers thick separated by sinusoids. In this area the cells are relatively uniform in size and have hyperchromatic nuclei (×50). (B) In some foci, the malignant cells are large with pleomorphic nuclei (×50).

period (12) and substantial fluctuations in transaminase values during the course of the illness are features of acute NANB viral hepatitis. HBV has been excluded by highly sensitive serologic testing (negative for HB_sAg, anti-HB_s, and anti-HB_c by RIA). HBV markers other than HB_sAg may occur in serum of some PHC patients who demonstrate integrated HBV–DNA in the host genome (13). [Seronegativity for HBV coexisting with integrated HBV–DNA in hepatocytes has surprisingly been observed in PHC associated with alcoholic cirrhosis (14)]. PHC has also been associated with HB_sAg-negative chronic active hepatitis (15), but no relationship to NANB virus has been established. Hepatitis A virus has been excluded in our patient by lack of association with chronic hepatitis (16) and PHC (17). A chronic carrier state of NANB virus may have existed in our case as shown by retrospective and prospective (18) observations. Studies are currently in progress to determine the infectivity of our patient's serum in the chimpanzee model (19, 20).

As previously noted, a substantial body of information implicates HBV as an initiating cause of PHC (21). The concept is reinforced by prospective studies of cirrhotic patients with HBV antigenemia who subsequently developed PHC (22, 23). Common epidemiologic patterns of HBV and NANB viral infection could suggest a shared potential for inducing malignant transformation.

An alternative concept to direct viral-induced oncogenesis is the enhanced capacity for neoplasia associated with cirrhosis. PHC may complicate cirrhosis, but varying rates of occurrence appear to be based on etiology (eg, hemochromatosis vs Wilson's disease) and morphology (macronodular vs micronodular). Although we lack histologic confirmation in our patient, cirrhosis was believed to be present because of a characteristic ^{99}Tc liver scan, chronicity, and stigmata of chronic hepatic disease. Other potential explanations for PHC in our subject, such as alcoholism, anabolic and sex steroids, hemochromatosis, and α_1-antitrypsin deficiency have been excluded. Conceivably cocarcinogens such as mycotoxins could be important in pathogenesis by suppressing cellular immunity and thereby enhancing viral cytotoxicity (24).

In conclusion we report a case of acute NANB posttransfusion hepatitis followed by anicteric chronic liver disease and ultimate development of PHC. Although this sequence has not been previously observed, caution appears to be warranted because of an anticipated prolonged latent period. Awareness of possible malignant transformation in populations at high risk for chronic NANB viral hepatitis may be indicated. Periodic alphafetoprotein determinations may assist in the earlier detection of hepatocarcinogenesis, but the implications regarding therapeutic efficacy are uncertain.

SUMMARY

In 1964 a 42-year-old woman was hospitalized with clinical and laboratory signs of posttransfusion hepatitis five weeks after administration of six whole blood transfusions. During the following 17 years anicteric chronic liver disease was repeatedly documented by elevations of serum aspartate aminotransferase (SGOT) and alkaline phosphatase enzymes. In 1981 hepatomegaly, progressive jaundice, and a serum alphafetoprotein level of 516,000 ng/ml were observed. Percutaneous liver biopsy

showed a primary hepatocellular carcinoma (PHC). Serologic examinations failed to reveal markers for hepatitis B virus including HB_sAg, anti-HB_s, and anti-HB_c by radioimmunoassay; antibody to hepatitis A virus was also absent. This sequence of events demonstrates a presumptive association of PHC and the agent(s) of non-A, non-B viral hepatitis.

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