341

Liver Allograft

Its Use in Chronic Active Hepatitis With Macronodular Cirrhosis, Hepatitis B Surface Antigen

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• A patient suffering from chronic active hepatitis with macronodular cirrhosis, positive for hepatitis B surface antigen (HB,Ag), was treated with an orthotopic liver allograft. The HB, antigenemia, as measured with several precipitation tests and by complement fixation, became negative after transplantation and remained so for about 2½ months. During the interval, very low titers of the antigen were detectable by radioimmunoassay. At about three months after transplantation, she had an attack of acute hepatitis, at which time HB,Ag became detectable by all tests. She recovered, but progressive liver disease developed during the remaining 1½ years of her life. She died of disseminated nocardiosis and candidiasis with deteriorating hepatic function. The homograft at autopsy showed no evidence of rejection, but was the site of chronic active liver disease, although of a different pathologic pattern than that affecting her native liver. The differences in histology may reflect the influence of chronic immunosuppression on the features of chronic active hepatitis.

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In August 1970, a patient with end-stage chronic aggressive hepatitis and cirrhosis, hepatitis B surface antigen (HB,Ag)[1] positive, was treated at the University of Colorado Medical Center, Denver, by total replacement of the liver. Her clinical course is reported and discussed in this report, with particular reference to the infection of the new liver by the hepatitis B virus.

REPORT OF A CASE

In 1964, a 22-year-old woman, a chronic intravenous drug user, was admitted to a Los Angeles hospital with acute hepatitis. She appeared to recover fully, but five years later, jaundice and ascites developed and she was admitted to the University of Southern California Liver Unit, John Wesley Hospital, Los Angeles. The HB,Ag was positive by counterelectrophoresis (CEP).

A liver biopsy specimen was taken in 1969. At that time, the lobular architecture was completely distorted by large discrete regenerative nodules. There were scattered foci of hepatocytolysis surrounded by lymphocytes. Many of the hepatocytes were ballooned. The biopsy specimen was considered to represent a coarsely nodular cirrhotic liver in a relatively quiescent stage of chronic active hepatitis.

Over the next year, there was progressive deterioration of liver function, necessitating several admissions to the hospital for the treatment of ascites, hepatic encephalopathy, and gastrointestinal bleeding. In early August 1970, the patient was transferred to the University of Colorado Medical Center, Denver, for liver transplantation. She had massive ascites, bilateral pleural effusions, and jaundice. The laboratory findings (Fig 1) were typical of advanced cirrhosis, showing the following levels: serum total bilirubin, 6.4 mg/dl (direct, 4.6 mg/dl); SGOT, 240 IU; and alkaline phosphatase, 330 IU. The prothrombin activity was less than 20%. The serum albumin level was 1.9 g/dl, and the serum globulin level was 4 g/dl.

On Aug 9, 1970, she underwent total hepatectomy, orthotopic liver transplantation,[2] and splenectomy. The procedure was made difficult by the portal hypertension and numerous venous collaterals. During the operation, she required 18 units of blood, all of which were negative for HB,Ag antibody by CEP. Aliquots of the transfusion blood were not kept for subsequent radioimmunoassay.

The excised liver weighed 688 g (Fig 2). Its lobular pattern was entirely distorted by bulging, well-defined, 0.5 to 0.6 cm-regenerative nodules separated by loose connective tissue septa. Microscopically, the discrete nodules were made up of markedly hydropic hepatocytes with vesicular nuclei. There were only occasional foci of hepatocytolysis, and most of the lymphocytic and plasmacytic infiltrates were confined to the loosely arranged fibrous septa. Kupffer cells were only moderately prominent.

Postoperatively, she was treated with a triple drug immunosuppressive program consisting of azathioprine or cyclophosphamide, prednisone, and horse antilymphocyte globulin[3,4] (Fig 1). Despite her poor condition prior to transplantation, the early convalescence was uneventful and the patient was discharged from the hospital 32 days after the operation. Postoperatively, HB,Ag was

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not detectable by CEP or agarose gel immunodiffusion (AG).

Eighty days after transplantation, she was readmitted to the hospital with malaise, fever, arthralgia, and anorexia. There was a marked increase in the levels of the serum albumin, SGOT, and alkaline phosphatase (Fig 1); prothrombin activity was 50% of normal. Because of the reappearance of AG- and CEP-detectable HB,Ag, this illness was thought to be acute viral hepatitis, rather than a rejection episode, and her program of immunosuppression was therefore not altered. During the ensuing few weeks, there was further deterioration of hepatic function, and ascites was transiently present. Her condition then gradually improved, with return of the liver function test values to essentially normal levels (Fig 1).

A needle biopsy of the liver was taken in September 1971, approximately 13 months after transplantation and ten months after the episode of acute hepatitis. Microscopically, this specimen showed a decidedly atypical pattern, with regularly arranged but more closely approximated portal structures from which insidious arachnoid collagen fibers extended varying distances into the lobules. The cord pattern was completely effaced by the hydropic parenchymal cells that formed irregularly distributed clusters of cells that bulged against adjacent areas of less swollen hepatocytes. The central veins were difficult to find, but were normal when found. There was only a modest increase of cells in the portal areas, consisting mostly of duct epithelial cells, fibroblasts, endothelial cells, and a few lymphocytes and plasma cells (Fig 3).

The bloated hepatocytes had vesicular nuclei with prominent nucleoli. Inflammatory infiltration into the lobule was meager, but there were occasional relatively inconspicuous clusters of Kupffer cells and a rare eosinophilic body, but no other frank necrosis (Fig 4). Vascular structures in portal areas were not sclerotic. It was thought that the pattern probably represented an early stage of chronic active hepatitis, modified by immunosuppressive therapy.

Soon after this, the patient was readmitted to the hospital with fever and headaches. A complete neurological evaluation was normal. A month later, *Nocardia asteroides* was isolated from a skin nodule, and she was treated with sulfonamides.' She was subsequently readmitted to the hospital on several more occasions with headaches and fever. By January 1972, seventeen months after transplantation, her liver function had deteriorated (Fig 1), with recurrence of jaundice and ascites. In late March 1972, focal neurologic signs developed. Craniotomy was performed, revealing multiple intracerebral *Nocardia* abscesses. Liver function continued to worsen, and she died of hepatic failure and neurologic dysfunction on April 21, 1972, twenty months after transplantation. At postmortem examination, multiple *Nocardia asteroides* and *Candida albicans* abscesses were found in the brain, heart, lungs, liver, and kidneys.

The allografted liver at autopsy weighed 1,120 g. Since the liver was obtained from a male donor who weighed more than 70 kg, its weight at the time of transplantation was estimated to have been 1,800 g, based on a liver weight/total body weight ratio of 2.5%. The surface of the allograft had a fine, sandpaper appearance (Fig

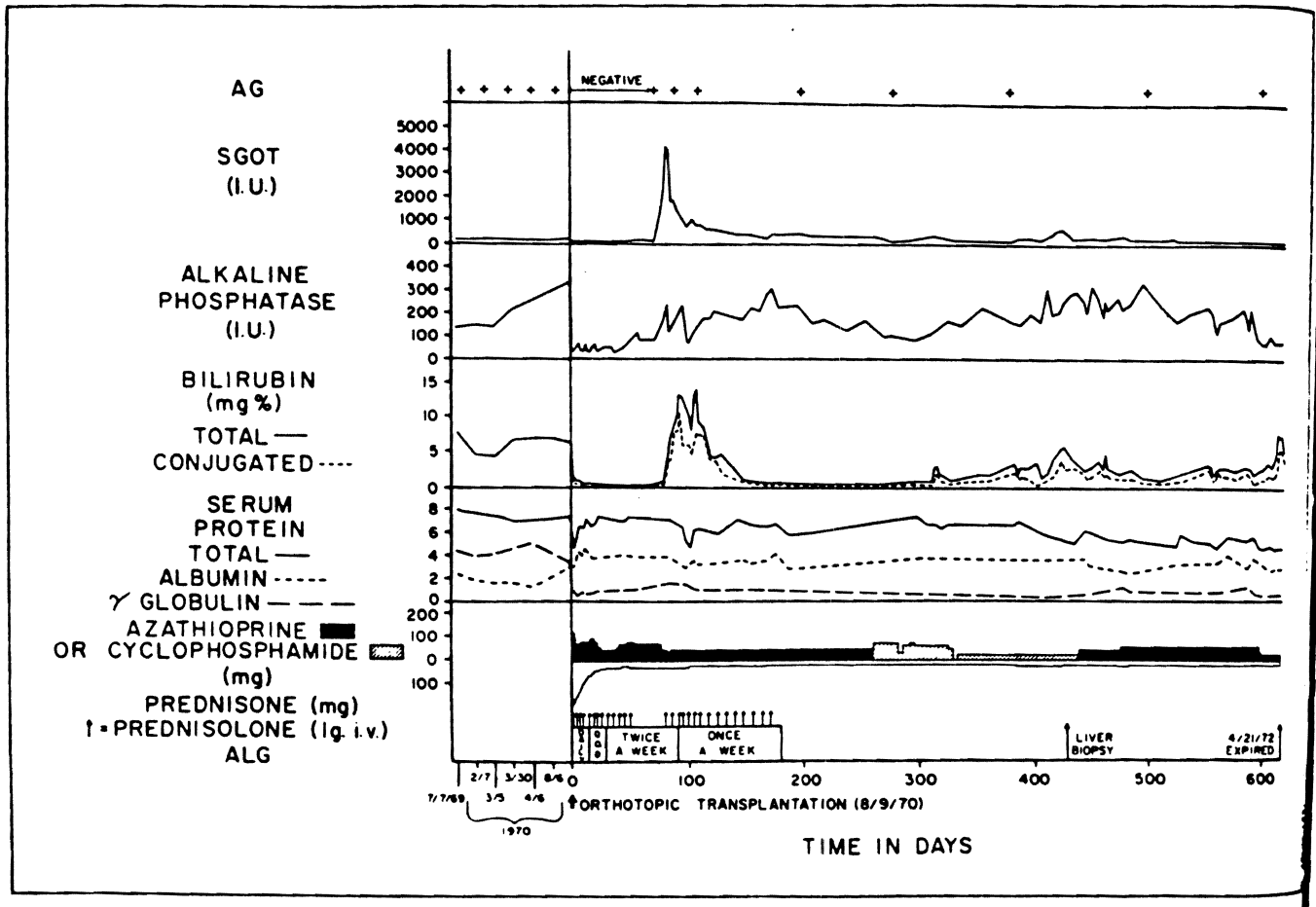

Fig 1.—Clinical course of patient before and after liver replacement. AG indicates agarose gel immunodiffusion test for hepatitis B surface antigen; ALG, antilymphocyte globulin.

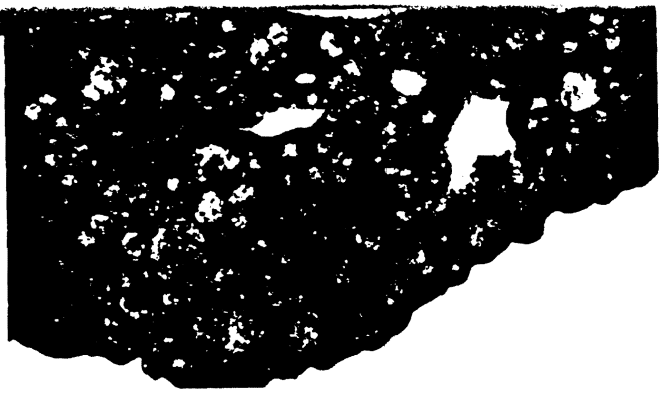

Fig 2.—Cut surface of shrunken nodular liver removed at time of transplantation on Aug 9, 1970 (×2.4).

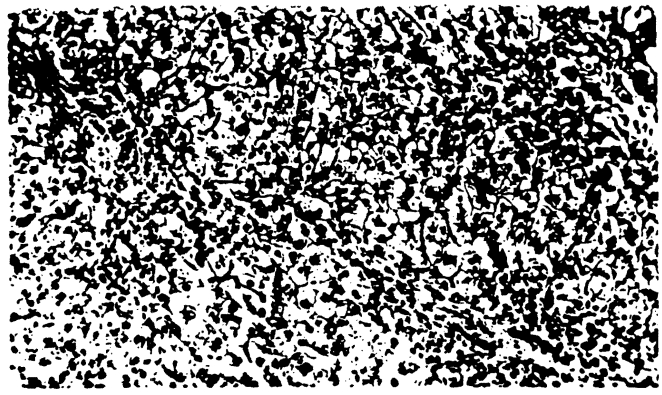

Fig 3.—Liver biopsy specimen taken of allografted liver ten months after appearance of elevated transaminase activity and hepatitis in B surface antigen serum. Note widened portal tracts and bloated hepatocytes with very little lobular inflammatory infiltrate (hematoxylin-eosin, ×120).

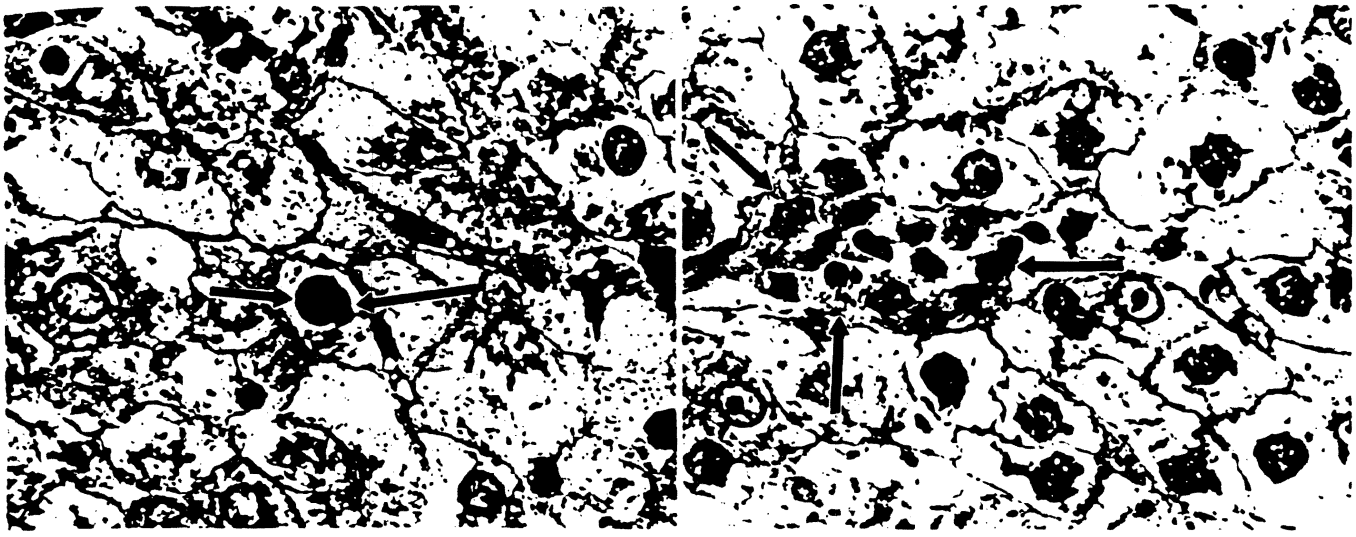

Fig 4.—High-power photomicrographs of liver biopsy specimen shown in Fig 3. Hepatocytes are hydropic and nuclei are enlarged, with finely divided chromatin. Left, Eosinophilic body (arrows) is shown. Right, Focus of hepatocytolysis (arrows) filled with swollen Kupffer cells, macrophages, and a few lymphocytes (hematoxylin-eosin, ×600).

Fig 5.—Cut surface of allografted liver removed at autopsy. Note that liver, although toughened, is not cirrhotic, and that portal structures are regularly distributed (×2.4).

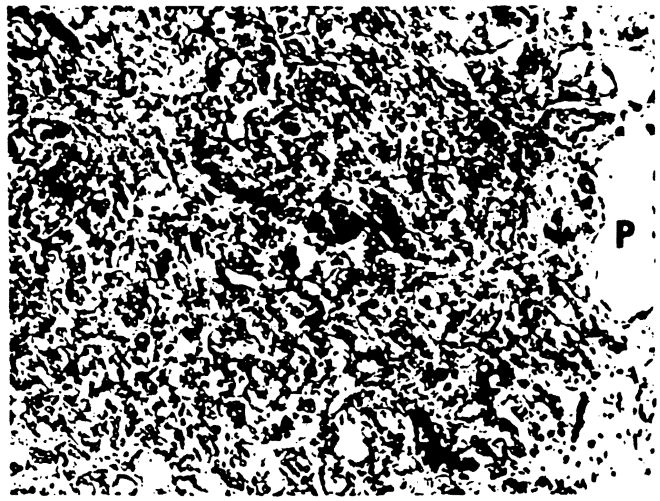

Fig 6.—Photomicrograph of allografted liver at autopsy. There are no fibrous septa incorporating portal areas (P). Centrilobular areas (C) are obliterated by fibrosis and there is diffuse interstitial fibrosis with collagen enveloping hepatocytes. (hematoxylin-eosin, ×240).

5). The parenchyma was toughened, but the lobular architecture was intact (Fig 5) and the liver was not truly cirrhotic. Microscopically, there were no regenerative nodules. The portal and central areas had a proper relationship to one another. However, there were fine collagen fibers that extended throughout the sinusoids, enveloping segments of hepatic cords that were arranged in restricted, acinar-like structures (Fig 6). The short hepatic cord structures were not arranged in a radial fashion, but were in disarray. The centrilobular areas had many finely foamy hepatocytes, with the vesicles ranging from about 1 to 3 μ. Canaliculi were dilated with bile plugs. Kupffer cells were numerous and had unusually abundant cytoplasm. There was no exudate into the lobules, nor was there inflammatory hyperplasia in portal regions.

Serologic Studies

During the patient's hospital course, multiple serum specimens were tested for the hepatitis B antigen in laboratories at the Universities of Southern California and Colorado by a variety of techniques, including micro-Ouchterlony agarose gel immunodiffusion, counterelectrophoresis, and complement fixation (CF) and radioimmunoassay (RIA).[3]

Serum specimens tested in California at intervals during the year prior to transplantation were all positive (Fig 1) for HB_sAg by AG and CEP. These findings were confirmed in the same samples at the Colorado laboratories using all the methods but RIA.

Serum samples were obtained for analysis in Colorado immediately before transplantation, after hepatectomy but before placement of the homograft, and at 30-minute intervals after revascularization of the graft. The preoperative sample was positive in all test systems, while the specimen taken during the anhepatic phase was positive only with the more sensitive complement fixation test.[4] The five subsequent intraoperative serum samples were all negative by the AG, CEP, and CF tests.

The patient remained HB_sAg negative by the above techniques for 43 days after transplantation. At that time, although hepatic function tests were essentially normal, HB_sAg was again detectable, first by CF and later by CEP. Henceforth, until her death 18 months later, all serum specimens tested were positive by all techniques.

After the patient's death, serum samples that had been obtained during the early postoperative period (days 5, 9, 17, and 31) were retested by radioimmunoassay. Although these four samples had initially been found to be HB_sAg negative by AG, CEP, and CF, all were positive by RIA (positively confirmed by blocking with anti-HB_sAG antibody).[5] Hepatitis B surface antigen subtype determinations were done in the California laboratory. Both the pretransplant antigen and the antigen detected during the posttransplant acute hepatitis were subtype *ay*.

COMMENT

The outcome after orthotopic hepatic transplantation in this patient was not readily predictable. It could be theorized that the new and genetically different liver might be affected differently by the hepatitis virus than the native organ. Furthermore, the response of the new liver, if infected, would be conditioned by an iatrogenically altered host immune response.

Removal of the host liver resulted in an immediate, marked reduction in HB_sAg titer, suggesting that the liver was the major source of circulating HB_sAg. Apparently, the amount of infectious material retained extrahepatically, as reflected by the positive RIA test, was sufficient to infect the new organ. The only alternative, and an unlikely one since the HB_sAg screening tests of the blood baths with CEP were negative, was that the infection came from one of the blood transfusions. Whatever its origin, viral replication then occurred, presumably mainly in the freshly infected hepatic homograft, leading eventually to the release of large amounts of HB_sAg into the blood circulation. The situation was thus somewhat comparable to post transfusion type B hepatitis. The development of clinical symptoms and biochemical abnormalities about 80 days after transplantation terminated the "incubation period," after which the less sensitive serologic tests readily detected HB_sAg in the serum. The bout of acute hepatitis at that time was not obviously different than would have been expected in a patient with a normal immune system.

Although it was hoped that provision of new liver tissue of a different genetic constitution than the native organ or the use of an immunosuppressive program would protect against redevelopment of chronic active hepatitis, such was not the case in our patient. A chronic and progressive liver disorder developed, but one that was histologically dissimilar to chronic active viral hepatitis in that collagen developed in fine intrasinusoidal strands not forming septa, hepatocyte regeneration was minimal, and inflammatory cell infiltration was meager. The histological features that developed in the liver homograft were also unlike the changes that can be induced by either rejection or immunosuppression. It seems most likely that the posttransplantation hepatic disorder in our patient was chronic active viral hepatitis modified by immunosuppression.

In future cases, treatment should be considered at the time of operation to neutralize the residual virus with human anti-HB_sAg antibody. Successful therapy of this kind would make similar patients attractive candidates for liver replacement. One patient recently treated by us with hyperimmune serum has had complete clearing of HB_sAg for the entire 4½ months of postoperative follow-up. It is not known to what extent this approach has been used at other centers.

Nonproprietary Name and Trademark of Drug

Azathioprine–*Imuran*.

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