1190

Reprinted from HEPATOLOGY, St. Louis
Vol. 13, No. 4, pp. 619-626, April, 1991, (Printed in the U.S.A.)

Orthotopic Liver Transplantation for Patients with Hepatitis B Virus–related Liver Disease

SATORU TODO,[1] ANTHONY J. DEMETRIS,[2] DAVID VAN THIEL,[1] LEWIS TEPERMAN,[1] JOHN J. FUNG[1] AND THOMAS E. STARZL[1]

Departments of [1]Surgery and [2]Pathology, University Health Center of Pittsburgh, University of Pittsburgh, Pittsburgh, Pennsylvania 15213

Fifty-nine patients with prior hepatitis B virus infection underwent orthotopic liver transplantation. During the first 2 mo, mortality was not significantly different in the hepatitis B virus–infected group (25.5%) vs. a hepatitis B virus–immune control group (21%). Beyond 2 mo, the mortality, rate of graft loss, need for retransplantation and incidence of abnormal liver function were significantly higher in the hepatitis B virus–infected group. Treatment of the hepatitis B virus infection was attempted with passive immunization, combined active and passive immunization, α-interferon or nothing. The clinical outcome was not significantly influenced by any of these therapies. However, of the patients who lived more than 60 days, 6 of 22 treated with active plus passive immunization were cleared of HBsAg, something achieved once in 16 patients treated with α-interferon, never in 3 patients with passive immunization only and once in 4 patients with no therapy. In patients with recurrent hepatitis B virus infection, the pace of hepatitis development in the graft appeared to be accelerated, and this was particularly striking in patients who underwent multiple retransplantations at progressively shorter intervals. None of the patients who became HBsAg-negative had HBeAg preoperatively. (HEPATOLOGY 1991; 13:619-626.)

How to manage patients with HBV who undergo transplantation after developing end-stage chronic disease or fulminant hepatic failure (FHF) has been one of the unanswered questions in hepatology ever since it was realized that HBV can cause early or later graft loss (1, 2). Liver replacement transiently lowers the viral titer in the blood, as determined by serial HBsAg testing (1-5). However, the virus is difficult to eradicate, almost ensuring that the viral infection will persist and that recurrent hepatitis will occur and impair the recovery and subsequent health of most recipients (3, 4, 6). Hepatitis B hyperimmune globulin (HBIG) (5-7), hepatitis B vaccine (HBVx) (5) and α-interferon (α-IFN) (8) have been administered alone or in some combination to such patients to prevent recurrent HBV infection in the new liver. The effectiveness of such therapies at clearing either the infection or the antigenemia is not established.

This study reports observations on 59 adult patients who had chronic or fulminant HBV infection at the time of liver transplantation. Follow-ups are at least 19 mo. We have compared the courses of those 59 recipients with those of 38 other patients with postnecrotic cirrhosis (PNC) who were HBsAg negative but whose serum had anti-HBs, reflecting a previous HBV infection and presumed subsequent immunity.

MATERIALS AND METHODS

Case Material. From March 1, 1981, to February 28, 1988, 924 consecutive patients who were at least 16 yr old were treated with orthotopic liver transplantation at the Presbyterian-University Hospital of the University of Pittsburgh. Throughout this time, preoperative screening for HBV infection was routine for all potential recipients.

At the time of their transplantation, 38 patients were found to have anti-HBs but not HBsAg in their serum. These were designated as group 1 (HBV immune control) (Table 1). All 38 of these patients had PNC, and 6 (15.8%) of these 38 had coexisting primary liver cancer (PLC).

Fifty-nine patients who were positive for HBsAg (HBV infected) were the subjects of this investigation (Table 1). Fifty-one of the 59 had PNC as a result of the HBV infection (group 2); 11 (22%) of the 51 cirrhotic livers had PLC. The remaining eight patients had no evidence of prior liver disease but FHF developed from the HBV infection (group 3). Five of these eight were HBV-DNA positive as determined by Abbott Laboratories' HBV-DNA genostics kit (Abbott Laboratories, North Chicago, IL); all were IgM anti-core positive at the time of transplantation.

The severity of illness of each patient was defined by a six-tier classification system (9) that defined the distribution of donor livers by the United Network for Organ Sharing (UNOS) by urgency of need: (a) working or in school, (b) confined to home with self-care, (c) confined to home and requiring professional care, (d) hospital bound but not in an intensive care unit (ICU), (e) ICU bound without ventilator support and (f) in an ICU on a ventilator and usually unconscious.

Differences in the general condition, performance and sex of

Received March 21, 1990; accepted November 14, 1990.
Aided by research grants from the Department of Veterans Affairs and Project Grant No. DK 29961 from the National Institutes of Health, Bethesda, Maryland.
Address reprint requests to: Thomas E. Starzl, M.D., Ph.D., Department of Surgery, 3601 Fifth Avenue, Falk Clinic, Pittsburgh, PA 15213.
31/1/27819

TABLE 1. Liver transplant recipients with past or present HBV infection

Characteristics	Group 1 (HBV immune)	Group 2 (HBV infected)	Group 3 (HBV fulminant)
N	38	51	8
Age (range)	45.6 (23.2 to 56.8)	46.6 (16 to 66.5)	30.6 (21 to 51.1)
Men/Women	25/13	46/5[a]	4/4
Patients with tumor (%)	6/38 (15.8%)	11/51 (21.6%)	0/8 (0)
Duration of disease (range)	7.6 yr (0.3 to 26)	6.9 yr (0.5 to 22)	3.3 wk (1 to 8)
Urgency status (old UNOS)[b]	3.1 ± 1.3	3.4 ± 1.1	5.1 ± 1.0
Gastrointestinal bleeding (%)	12/38 (37%)	12/51 (23%)	0/8 (0)
Encephalopathy (%) (Stage ≥ 3)	11/38 (29%)	19/51 (37%)	7/8 (87.5%)
Hepatorenal (%) (Cr > 3 mg/dl)	3/38 (8%)	4/51 (8%)	3/8 (37.5%)
Total bilirubin (mg/dl)	6.8 ± 9.1	7.9 ± 12.1	21.6 ± 9.1
Serum albumin (gm/dl)	2.8 ± 0.4	2.7 ± 0.5	2.9 ± 0.5
Prothrombin time (sec)	15.5 ± 2.1	16.0 ± 2.9	24.3 ± 6.6
Creatinine (mg/dl)	1.1 ± 0.5	1.2 ± 0.8	1.2 ± 0.8

Values are mean ± S.D. unless otherwise stipulated.
[a] $p < 0.05$ compared with the other two groups.
[b] UNOS = United Network for Organ Sharing.

TABLE 2. Serological profile of the 59 HBV-infected patients in groups 2 and 3

Serological marker	Number of patients tested	Positive results (%)	Positive results in 45 patients who eventually survived more than 60 days (%)
HBsAg	59	59 (100)	45 (100)
Anti-HBs	58	9 (15.5)	5 (11.1)
HBeAG	56	26 (46.4)	21 (46.7)
Anti-HBe	57	25 (43.8)	20 (44.4)
Anti-HBc	59	59 (100)	45 (100)
Anti-delta	55	15 (27.3)	12 (26.7)

Not all tests were performed on every patient.

the patients in the three groups are shown in Table 1. Significantly fewer women were seen in group 2 than in either group 1 ($\chi^2 = 6.59, p < 0.05$) or group 3 ($\chi^2 = 5.81, p < 0.05$). Most group 1 and group 2 patients were extremely ill before transplantation. More than 30% had a history of variceal bleeding, advanced encephalopathy or both. All patients in group 3 were in the ICU and seven of the eight had stage 3 or 4 coma (Table 1).

Serological Analysis of HBV Infection. The preoperative serological markers of HBV and hepatitis delta virus (HDV) infection are summarized in Table 2 for all 59 patients of groups 2 and 3. Also included is a culled tabulation of the 45 patients from these two groups who survived at least 60 days after their operations (Table 2). The tests included HBsAg and anti-HBs, HBeAg and anti-HBe, anti-HBc and anti-hepatitis delta antibody (anti-HD). Commercially available RIA kits (Abbott Laboratories) were used for each of these determinations. The same tests were performed at irregular intervals after transplantation.

Operative Procedure and Immunosuppressive Regimens. The method of orthotopic liver transplantation has been standardized at the University of Pittsburgh including the use of a venovenous bypass (10). Immunosuppression after the operation consisted of cyclosporine and low maintenance doses of prednisone. Injections of methylprednisolone (1 gm) or a 5-day burst of methylprednisolone or prednisone was added when a rejection episode occurred. If the response to such "rejection" therapy was not satisfactory, the monoclonal antithymocyte antibody (OKT3), azathioprine or both were added to the immunosuppressive regimen.

Adjuvant Immunotherapy Used to Treat HBV Infection. Three different treatments were used in an attempt to eliminate the HBV and to avoid recurrent HBV disease. When HBIG (Abbott Laboratories) was used as either the sole therapy or in combination with Hepatavax (HBVx, Merck, Sharp & Dohme, West Point, PA), 100 ml of the HBIG was given intravenously during the anhepatic phase of the liver replacement and again after concluding the operation. A third and final dose was given 1 mo later. For the first three cases so treated, 10 ml of HBIG rather than 100 ml was used at each time point.

Active immunization with HBVx was attempted preoperatively in patients who were well enough to allow sufficient time for three intramuscular injections spread at monthly intervals before transplantation. If the patients were too ill to wait the necessary 2 mo, the vaccine was started after their operation. In either event, the vaccine was administered as a 1 ml (20 μg) dose in the deltoid muscle.

Recombinant α-INF (Intron A; Shering Corporation, Kenilworth, NJ) was administered subcutaneously at a dose of 2.5×10^6 units/day for a minimum of 2 wk before and a total of 6 wk after transplantation. Patients with FHF were given HBVx or α-INF only after their operation. α-IFN therapy was difficult to administer, particularly before transplantation, and the development of hematological abnormalities often necessitated a reduction in the dose used or a shortening of the course.

Four patients, two in group 2 and two in group 3, were not treated by any of these adjuvant therapies.

Postoperative Follow-up and Pathological Studies. When recipients were found to have liver function abnormalities after the operation, a complete workup was initiated that in each case included an ultrasound examination of the liver and its vessels, a percutaneous transhepatic cholangiogram and/or a needle biopsy of the liver to establish the correct diagnosis

TABLE 3. Patients who died or who had retransplantation within 60 days after liver transplantation

Characteristic	Group 1	Group 2	Group 3
N	38	51	8
Patients who died within 60 days after transplantation (%)	8 (21)	13 (25.5)	1 (12.5)
Causes			
Sepsis	5	11	0
Pulmonary complication	0	1	0
Myocardial infarction	0	1	0
Death during surgery	2	0	1
Lymphoproliferative disease	1	0	0
Patients who had retransplantations within 60 days (%)	4 (10.5)	6 (11.8)	3 (37.5)
Causes			
Primary graft nonfunction	3	4	2
Hepatic artery thrombosis	1	2	1

TABLE 4. Patients who died or who had retransplantations 60 days or later after liver transplantations

Characteristic	Group 1	Group 2	Group 3
N	38	51	8
Patients who continued to survive more than 60 days (%)	30 (79)	38 (74.5)	7 (87.5)
Patients who died after 60 days (%)	6	15	0
Causes			
Multiple organ failure related to recurrent HBV infection	0	11	0
Multiple organ failure unrelated to recurrent HBV infection	3	0	0
Cancer recurrence	3	3[a]	0
Intracerebral bleeding	0	1[b]	0
Patients who had retransplantations after 60 days (%)	3	7	1
Causes			
Recurrent HBV infection	0	7	0
Chronic rejection	2	0	1[c]
Hepatic artery thrombosis	1	0	0

[a]All patients except one (OT #767) had CAH from recurrent HBV infection.

[b]Patient (OT #501) who had hemophilia, had AIDS and CAH from recurrent HBV infection.

[c]Patient (OT #788) had CAH by recurrent HBV infection but chronic rejection was predominant.

and treatment. All patients were observed until their death or to August 1989. The follow-up through August 1989 allowed for a minimum potential follow-up of at least 19 mo for patients with the most recent transplants.

Liver biopsy specimens were obtained only for specific indications rather than by a set schedule. Thus the availability of a tissue specimen for examination implied either an indication for liver biopsy, retransplantation or an autopsy. All liver specimens were fixed in buffered formalin, embedded in paraffin and routinely stained with hematoxylin and eosin, trichrome and reticulin. In addition, expression of HBsAg and HBcAg by liver cells was determined routinely in these tissues using immunoperoxidase techniques (11, 12).

The tissue changes of the liver in patients who experienced a recurrence of their viral liver disease were classified as follows: *0* = chronic carrier state with little or no evidence of hepatic damage; *1* = acute hepatitis, including examples of fulminant progression; *2* = the transition phase from acute hepatitis to CAH, defined by bridging necrosis and a transition from panlobular to periportal hepatocyte destruction; *3* = CAH and *4* = cirrhosis. All histopathological samples were reviewed by one pathologist who did not know the clinical course or outcome.

Statistical Analysis. All data are presented as mean ± S.D. Student's *t* tests and χ^2 tests were used for the statistical analyses. Patient survival was calculated using the method of Kaplan-Meier. Comparisons between groups were made by the methods of Breslow and Mantel-Cox using the BMDP statistical software (BMDP Statistical Software, Inc., Los Angeles, CA).

RESULTS

Patient Survival for 60 Days. Consistent with the degree of patient illness, heavy mortality occurred in group 1 and group 2 patients within 60 days after liver transplantation. Eight patients (21.1%) died in group 1, and 13 patients (25.5%) died in group 2, after a mean survival time of 23.7 ± 24.2 (S.D.) days and 29.8 ± 15.1 (S.D.) days, respectively. The causes of these early deaths are shown in Table 3. No statistical differences existed between group 1 and group 2 in the incidence and causes of death within 60 days. One of the eight patients in group 3 (Table 3) died perioperatively. No patient in groups 2 or 3 died within 60 days because of recurrence of HBV. Thirteen retransplantations were performed during that period either because of primary graft nonfunction or hepatic artery thrombosis (Table 3).

Survival after 60 Days. Twenty-four (80%) of the 30 recipients in group 1 who survived for at least 60 days are still alive, with follow-ups ranging from 586 days to 1,962 days; this is 63.2% of the original 38. Twenty-three (60.5%) of the 38 recipients in group 2 who lived for at least 60 days are still alive, with follow-ups ranging from 518 days to 2,036 days; this is only 45.1% of the original 51. One of the eight patients in group 3 died during the operation, but the other seven are still alive 558 days to 1,694 days after transplantation. When the survival rates between groups 1 and 2 were compared, patients with HBV infection (group 2) had a lower survival rate than those who were immune to the HBV (group 1) ($p < 0.05$).

Causes of Delayed Mortality and Retransplantation. Reinfection by HBV of the new liver (Tables 4 and 5) was the leading cause of mortality that occurred after 60 days. Recurrent HBV infection was an important factor in 11 (73%) of the 15 deaths of group 2 patients after 60 days. Moreover, HBV infection was the exclusive reason for seven retransplantations in this group (Table 4). Four of the seven recipients who underwent retransplantation died during the perioperative period of the second grafting, and two more died within 100 days from recurrence of HBV in the second transplant. Only one of these seven patients is alive, and he has already required

TABLE 5. Influence of adjuvant therapy on HBsAG incidence of retransplantation and eventual survival of the 45 HBV-infected patients who lived for at least 60 days after liver transplantation

Adjuvant therapy	HBIG	HBIG plus HBVx	IFN	None
Patients who lived more than 60 days	3	22	16	4
HBsAg evolution				
Continuous HBsAG clearance				
N (%)	0 (0%)	6 (27.3%)	1 (6.25%)	1 (25%)
Mean follow-up days (range)	–	1,185 (815-1,714)	555	530
Transient HBsAG clearance				
N (%)	2 (66.7%)	7 (31.8%)	2 (12.5%)	1 (25%)
Mean disappearance days (range)	21 (10-32)	43 (13-216)	151 (137-166)	89
Mean reappearance days (range)	140 (26-253)	202 (86-335)	257 (162-351)	260
Continuous HBsAG positivity				
N (%)	1 (33.3%)	9 (40.9%)	13 (81.3%)	2 (50%)
Mean follow-up days (range)	149	554 (141-1,259)	591 (138-994)	609 (315-918)
Patients who died, survived or had retransplantations				
Patients who had retransplantations				
N (9%)	1 (33.3%)	5 (22.7%)	1 (6.3%)	0 (0%)
Mean days of retransplantation (range)	2,073	480 (203-1,293)	131	–
Patients who died				
N (%)	3 (100%)	8 (36.4%)	3 (18.7%)	1 (25%)
Mean days of death (range)	1,033 (149-2,073)	502 (141-1,356)	292 (150-489)	305
Patients who survived[a]				
N (%)	0 (0%)	14 (63.6%)	13 (81.3%)	3 (75%)
Mean days follow-up (range)	–	1,218 (999-2,056)	698 (599-920)	726 (530-918)

An accounting of mortality before 60 days is in Table 3.

[a]Bilirubin normal in all survivors. ALT and AST normal in 12 of the 30 survivors and variably elevated in the others (more than 50 to 281 IUs).

a third transplantation because of HBV reinfection of the second liver.

Although all seven of the 60-day survivors in group 3 are still alive, one patient has required late retransplantation after 595 days because of chronic rejection plus HBV reinfection of her graft.

Changes in HBV Markers in 60-day Survivors. No patients in group 1 became HBsAg positive at any time after transplantation. In the 45 patients of group 2 and group 3 who survived for at least 60 days, the first of the five HBV markers to increase after the operation was anti-HBs antibody. This antibody was detectable preoperatively in the sera of only 5 of the 45 patients studied (Table 2). An additional 18 patients became anti-HBs positive at an average postoperative time of 23.1 days (range = 2 to 66 days) (Fig. 1), presumably because of the administration of HBIG alone or HBIG plus HBVx. This artifact of therapy was not evident in the anti-HBe antibodies that did not increase (Fig. 1). During the time of HBIG therapy and increased anti-HBs, a decline occurred in HBeAg and HBsAg (Fig. 1). By about 5 mo, the number of patients with HBeAg had rebounded to the pretransplant level, the HBsAg that had cleared in several patients had now recurred in most and the anti-HBs antibodies were declining. Each of these changes, and all of them together, correlated well with the clinical diagnosis of hepatitis B recurrence (Fig. 1) that was made at an average of 178 ± 81 days. By 351 days, only eight patients remained HBsAg negative. In the ensuing follow-up, which has been 19 mo to 57 mo (1,090 ± 465 [S.D.] days), none of these eight patients has become HBsAg positive (Table 5).

One of the eight patients whose seroconversion to negative was permanent was among the 7 who survived more than 60 days after FHF (group 3); this was a surgical resident who had been infected while on duty. The other 7 patients who remained HBsAg negative were among the 38 who survived more than 60 days in the chronic disease group 2.

Therapy Vs. HBV Markers. Examples of long-term antigen clearing occurred with two of the three treatment protocols and in one of four patients who had no treatment at all; this last patient was one of seven with FHF. Using the three treatment regimens, only HBIG plus HBVx resulted in permanent clearing of HBsAg in more than one patient (Table 5); 27.3% of patients treated with HBIG plus HBVx (6 of 22) have become HBsAg free, and 6% (1 of 16) treated with α-INF have become HBsAg free (Table 5). The features of all eight patients with permanent antigen clearing are summarized in Table 6. All eight were HBeAg negative at the time they were first seen, but six of the eight were HBV-DNA positive. All except one of the eight were and have remained anti-HBs negative.

Among the group 2 patients with prior chronic HBV infection, women had a 75% (3 of 4) permanent conversion to HBsAg vs. 11.8% (4 of 34) for men ($\chi^2 = 5.75$, $p < 0.05$). The three women in group 2 who became permanently negative were premenopausal (age 35.4, 38.8 and 47.9 yr old). The single patient with

TABLE 6. Features of the eight (of 45) patients with permanent clearing of HGsAG

Feature	Number
Group	
Group 2 (chronic HBV infection)	7/38
Women	3/4
Men	4/34
Group 3 (acute HBV infection)	1/7
Women	0/3
Men	1/4
Treatment	
None	1/4
HBIG	0/3
HBIG plus HBVx	6/22
IFN	1/16
Preoperative markers	
HBsAG	8/8
HBeAG	0/8
anti-HBs	1/8
anti-HBe	6/8
anti-HD	4/8
anti-HBc	8/8
Postoperative markers	
HBsAG	0/8
HBeAG	0/8
anti-HBs	3/8
anti-HBc	4/8
anti-HBc	8/8

All patients lived more than 60 days.

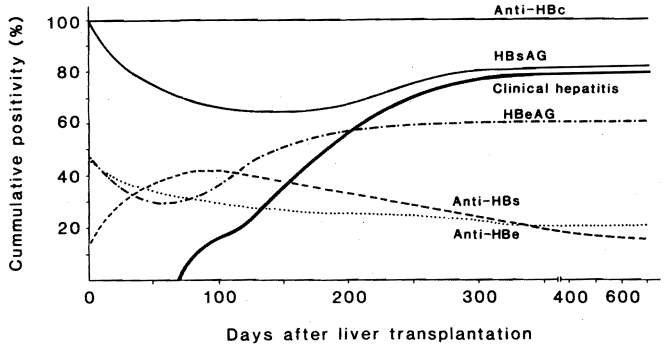

FIG. 1. Percentage of patients who survived more than 60 days who became positive as time passed for the serological or clinical parameters shown. Once a change to positive occurred, this was permanent and contributory to subsequent cumulative scores with the exception of the anti-HBs antibodies. The rise in anti-HBs antibodies in the first 100 days and subsequent decline reflected HBIG administration and later discontinuance.

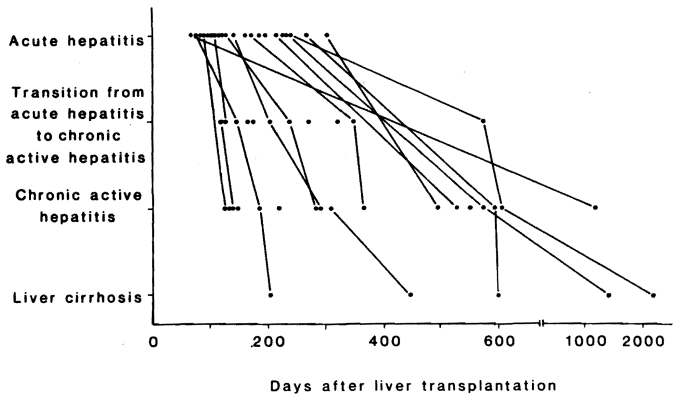

FIG. 2. Summary of histopathological structure in allografts of 35 patients who developed recurrent HBV infection after liver transplantation. Note the changes in histopathological classification that occurred in many of the recipients who had serial biopsies.

FHF (group 3) who became HBsAg negative was a man (Table 6).

Clinicopathological Correlations. Twenty-one of the 30 patients in group 1 who survived more than 60 days had biopsies done later in the course of treatment. None of the patients had histological or immunohistochemical evidence of HBV in the allograft. The biopsy findings in this group of patients differed markedly from those found in the HBV-infected groups 2 and 3. Non-B hepatitis was seen in seven group 1 patients. Unresolved lobular hepatitis was seen in two patients, and CAH developed in another patient. None of these patients had evidence of known viral liver pathogens, leading to a diagnosis by exclusion of non-A, non-B hepatitis. In the four other patients, cytomegalovirus was responsible for the hepatitis. None of the biopsy specimens or failed grafts from this group of patients was cirrhotic.

Liver tissue was studied from 43 of the 45 patients of groups 2 and 3 who lived for at least 60 days. Eight (17.8%) of these patients (seven from group 2 and one from group 3) were those whose HBV markers cleared. Two of these recipients (OT #920 and #630) had elevated liver enzymes at 797 days and 829 days, respectively. Both patients were diagnosed as having non-A, non-B hepatitis because serological and histopathological studies failed to reveal another cause.

Of the 37 patients with serological evidence of HBV reinfection 60 days or longer after transplantation, 35 had their diagnosis confirmed by examination of the liver. Two patients who were serologically HBsAg positive refused biopsy. One (OT #955) had normal liver function, whereas the other (OT #914) had abnormal function.

In those four patients of groups 2 and 3 who had retransplantation because of recurrence of HBV and who survived retransplantation, recurrence was diagnosed in the second grafts at 68, 87, 89 and 178 days. Three of these four patients always had HBeAg-positive sera, and all four had unrelieved HBsAg positivity. Recurrence of HBV infection occurred faster with the second transplantation than after primary liver replacement. One patient (OT #528) died after 104 days with massive hepatic necrosis, a second (OT #869) died after 99 days with recurrent acute hepatitis and multiple organ failure and the remaining two (OT #539 and #788) are alive but with CAH after 128 days and 395 days, respectively.

By reviewing specimens from the foregoing 35 patients with HBV reinfection, it was possible to reconstruct a progression of pathological changes of recurrent

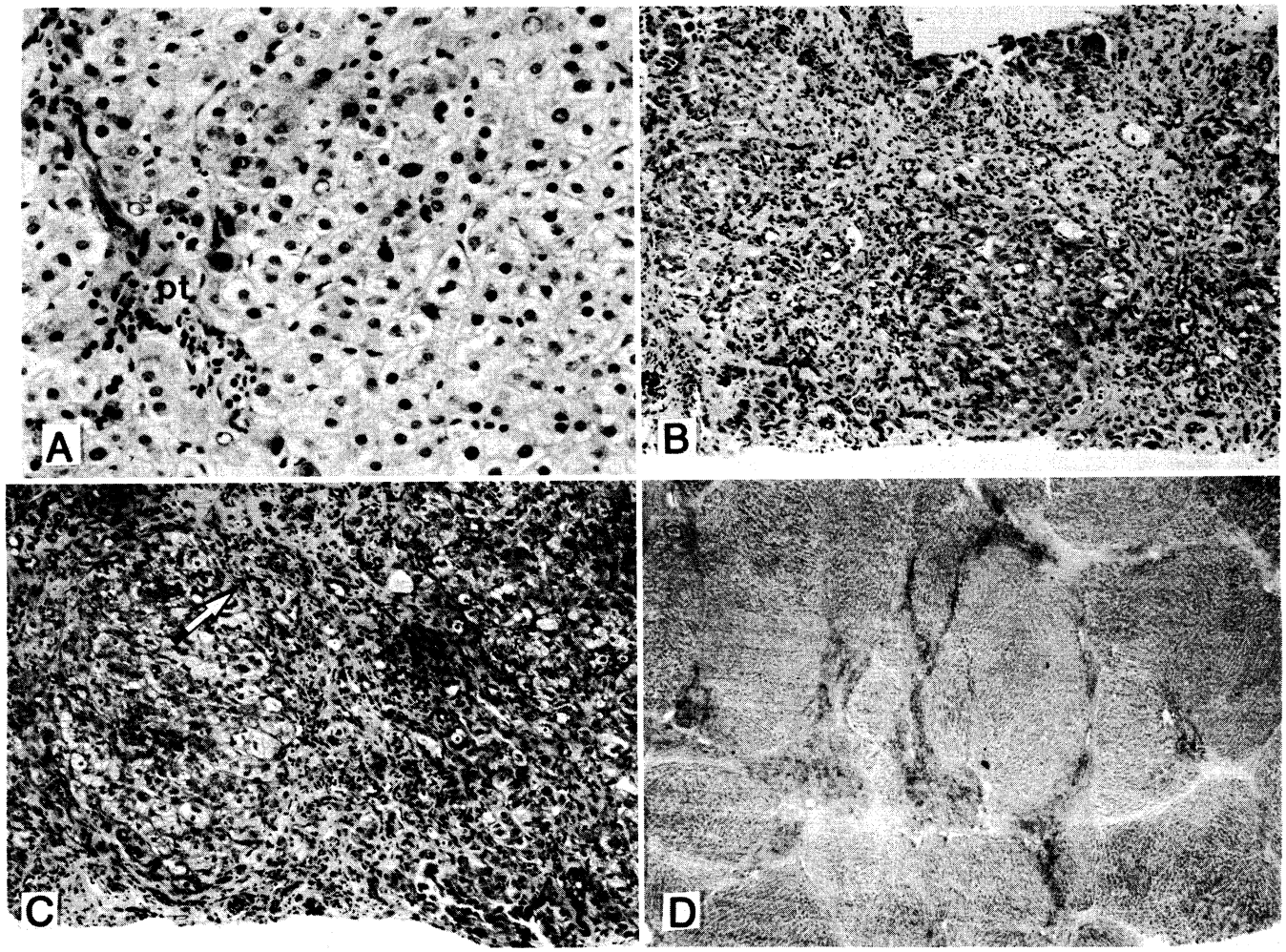

FIG. 3. Evolution of histopathological changes in serial liver biopsies of patient OT #810 with recurrent HBV-induced cirrhosis. (a) At 71 days, mild spotty hepatocyte necrosis was seen. *PT* = portal tract. (H & E, original magnification ×300). (b) At 147 days, features of acute hepatitis were evident, superimposed on bridging and piecemeal necrosis *(arrow),* that were suggestive of a transition to chronic hepatitis with progression (H & E, original magnification ×300). (c) At 187 days, early regenerative nodule formation was already present (H & E, original magnification ×300). (d) At 203 days, failed graft removed at retransplantation that showed well-developed cirrhosis (H & E, original magnification ×100).

HBV (Fig. 2). The first histological sign of recurrent HBV infection, as early as 2 wk to 5 wk after transplantation, was the presence of HBcAg in the cytoplasm of the homograft hepatocytes. Usually, little other evidence of pathological changes in the liver was seen at this time. After 70 days to 298 days (average = 151) days, mild lobular disarray, hepatocellular swelling, mild spotty acidophilic necrosis and regenerative changes signaled the onset of hepatitis activity in 21 patients. The changes in most of these specimens were similar to those seen in nonimmunosuppressed patients with HBV, but lobular or portal inflammation was less. The usual pathological diagnosis was mild acute hepatitis.

At an average of 242.2 days (range = 117 days to 567 days), 11 patients showed transitional change from acute hepatitis to CAH. The transition was characterized by the appearance of bridging necrosis and predominantly periportal hepatocyte destruction. This was in contrast to the panlobular activity observed during the acute phase. By 128 days to 1,140 days, 17 patients had established CAH. Finally, five patients had cirrhotic grafts at 203 days to 2,054 days (average = 912.8 days).

When biopsy specimens were obtained frequently, it was possible to see these stages evolve in a single graft (Fig. 2). Patient OT #810 was found to have acute hepatitis at 71 days, a transitional form of hepatitis at 147 days, CAH at 187 days and cirrhosis at 203 days (Fig. 3). This patient, who was treated with HBIG plus HBVx, also had PLC that recurred. He died shortly after the retransplantation procedure.

A clinical syndrome of FHF developed in three patients from 5 to 12 mo after transplantation. Only one of the three (OT #528) had a liver with the typical histological picture of massive hepatic necrosis and marked inflammation. The grafts in the other two

showed marked hepatocellular ballooning with focal bridging necrosis in the absence of significant hepatic inflammation. However, a striking expression of HBcAg by the hepatocytes was seen (Fig. 4).

Five other patients had grafts with a histological pattern resembling a chronic carrier state with the typical ground glass appearance of the hepatocytes but little evidence of inflammation. These patients were exclusively in the subgroup given α-IFN therapy (Table 5). The presence of anti-delta agent antibodies did not predictably alter the histopathological findings.

Current Hepatic Function. All but 1 (OT #372) of the 24 patients who are alive in group 1 have no significant abnormality of hepatic function. In contrast, only 12 of the 30 patients who are alive in groups 2 and 3 have perfect graft function (Table 5). Patients who were treated by α-IFN tended to have worse liver function than those given HBIG plus HBVx ($\chi^2 = 3.41$), although five patients treated with α-IFN had a reassuring histopathological figure resembling that seen with the chronic carrier state.

DISCUSSION

The observations in these patients confirm previous reports (2-7) that the prognosis after transplantation is poor for recipients with persistent HBV infection. It is not known whether therapy with active immunization, HBIG or α-IFN can improve the outlook. The most optimistic reports of therapy have come from Hanover, Germany, (5), where active and passive immunization have been combined, as we did in one of our therapeutic subgroups.

The development and progression of recurrent HBV disease was slow enough so that many of our patients experienced prolonged rehabilitation before some variant of the disease that had destroyed the native liver was repeated. Even so, the pace was considerably faster than that occurring with the naturally acquired infection, which was determined by the pretransplant medical history of these same individuals. In patients who underwent a second retransplantation, the second graft was even more vulnerable than the first transplant, which was determined by the time required to manifest graft failure or cirrhosis. These findings in patients under effective immunosuppression are not congruent with hypotheses that the principal damage in HBV hepatitis is by way of an autoimmune mechanism or that the HBV is not cytopathic.

The most encouraging observation from this study is that the HBV could be cleared, as determined by HBsAg positivity, in about one of every six patients. It should be noted that all eight patients who converted to HBsAg negativity were HBeAg negative before transplantation, suggesting a low rate of viral replication even though six of these eight patients were HBV-DNA positive.

Six of the eight patients who cleared their HBsAg had been treated with HBIG plus active immunization with HBVx, one was given α-IFN and one (with FHF) converted to negative HBsAg with no treatment at all. α-IFN was not efficacious, contrary to our hopes, which were based on α-IFN's effectiveness in nontransplant settings (13-15). The fact that continuous immunosuppression was required to prevent graft rejection in these cases may have accounted for the poor outcome with IFN therapy.

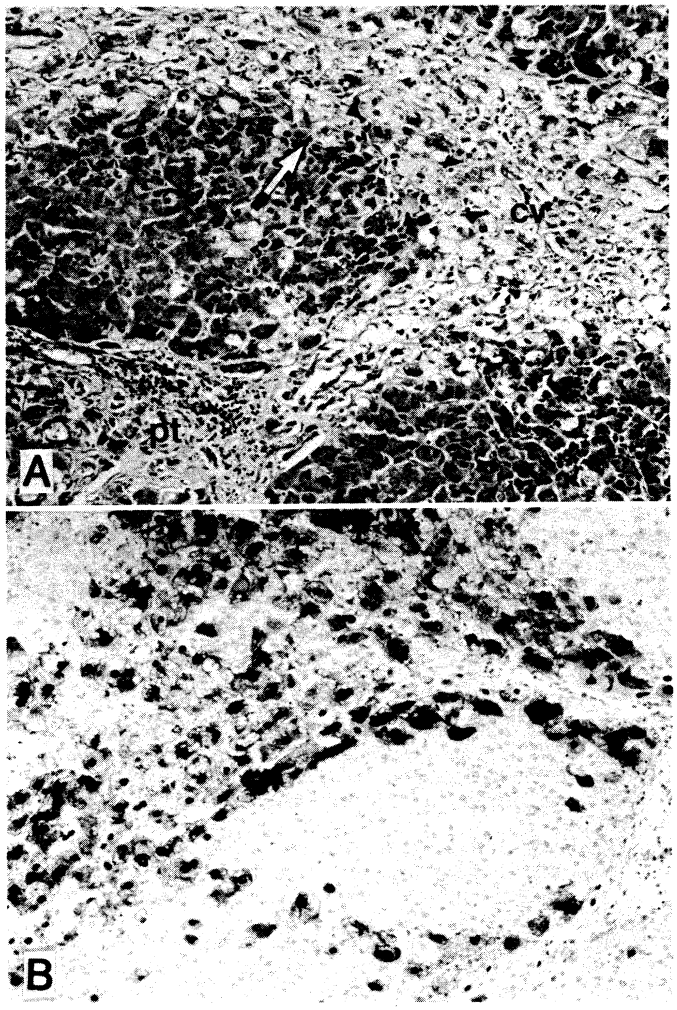

FIG. 4. Failed allograft removed at 378 days in a patient who had subacute hepatic failure. (a) Very little inflammation but marked hepatocellular swelling and degeneration in the centrilobular regions. Bridging *(arrow)* between central veins and between portal and central veins was seen. *PT* = portal tract, *CV* = central vein. (H & E, original magnification ×300). (b) Tissue expression of HBcAg. This was most intense and largely restricted to the degenerating hepatocytes, where both nuclear and cytoplasmic core antigen were detected (immunoperoxidase stain for HBcAg, original magnification ×120).

No conclusions are warranted from this study about the efficacy of the therapies that were tried. Instead, the results emphasize the difficulties of the management problems that were frequently encountered long after transplantation. However, the possibility cannot be excluded that combined active plus passive immunization or better designed α-IFN therapy might allow a significant number of long-term serological conversions. This possibility should stimulate additional trials with even more intensive immunization or therapeutic

schedules. In addition, passive immunization may become more effective by using the anti-HBs monoclonal antibodies that have been produced by Ostberg and Pursch (16) from a complex hybridoma that produces human IgG. These hybridoma immunoglobulins are nearly 50,000 times more potent than the best currently available immunoglobulin preparations. Preliminary encouraging but inconclusive trials with such reagents have been reported (17) and are still in progress; these cases were not part of this report.

Another unknown factor in future trials could be improved immunosuppression. The new drug FK 506, which is having its first clinical trials (18), can be used with very low or no steroid doses. When used alone, FK 506 does not reduce the number or function of natural killer cells that are important in viral immunity (19). In addition to this potential advantage, FK 506 has been shown in experimental models to stimulate or promote hepatic regeneration and repair (20). Such considerations and the possibility of better immunoprophylaxis justify continued efforts to use transplantation in treating these difficult cases.

REFERENCES

1. Torisu M, Yokoyama T, Amemiya H, Kohler PF, Schroter G, Martineau G, Penn I, et al. Immunosuppression, liver injury, and hepatitis in renal, hepatic, and cardiac homograft recipients: with particular reference to the Australia antigen. Ann Surg 1971;174: 620-639.
2. Corman JL, Putnam CW, Iwatsuki S, Redeker AG, Porter KA, Peters RL, Schroter G, et al. Liver allograft: its use in chronic active hepatitis with macronodular cirrhosis, hepatitis B surface antigen. Arch Surg 1979;114:75-78.
3. Demetris AJ, Jaffe R, Sheahan DG, Burnham J, Spero J, Iwatsuki S, Van Thiel DH, et al. Recurrent hepatitis B in liver allograft recipients: differentiation between viral hepatitis B and rejection. Am J Pathol 1986;125:161-172.
4. Rizzetto M, Macagno S, Chiaberge E, Verme G, Negro F, Marinucci G, DiGiacomo C, et al. Liver transplantation in hepatitis delta virus disease. Lancet 1987;2:469-471.
5. Muller R, Lauchart W, Farle M, Klein H, Niehoff G, Pichlmayr R. Simultaneous passive-active immunization for preventing hepatitis B virus reinfection in hepatitis B surface antigen-positive liver transplantation recipients. In: Zuckerman AJ, ed. Viral hepatitis and liver disease. New York: Alan R. Liss, Inc., 1988:810-812.
6. Portmann B, O'Grady J, Williams R. Disease recurrence following orthotopic liver transplantation. Transplant Proc 1986;18 (suppl 4):136-141.
7. Johnson PJ, Wansborough-Jones MH, Portmann B, Eddleston AL, Williams R, Maycock WD, Calne RY. Familial HBsAg-positive hepatoma: treatment with orthotopic liver transplantation and specific immunoglobulin. BMJ 1978;1:216.
8. Mancini C, Gaeta A, Lorino G, Filadoro F, Marinucci G, Bachetoni A, Pretagostini R, et al. Alpha interferon therapy in patients with hepatitis infection undergoing organ transplantation. Transplant Proc 1989;21:2429-2430.
9. Starzl TE, Gordon RD, Tzakis A, Staschak S, Fiorvanti V, Broznick B, Makowka L, et al. Equitable allocation of extrarenal organs: with special references to the liver. Transplant Proc 1988;20:131-138.
10. Starzl TE, Iwatsuki S, Esquivel CO, Todo S, Kam I, Lynch S, Gordon RD, et al. Refinements in the surgical technique of liver transplantation. Semin Liver Dis 1985;5:349-356.
11. Hsu SM, Raine L, Fanger H. The use of avidin-biotinperoxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 1981;29:577-580.
12. Sternberger LA, Hardy PH, Cuculis JJ, Meyter HG. The unlabeled antibody-enzyme method of immunohistochemistry: preparation and properties of soluble antigen-antibody complex horseradish peroxidase-antiperoxidase and its use in identification of spirochetes. J Histochem Cytochem 1970;18:315-333.
13. Lai CL, Lin HF, Yeoh E, Lok A, Wu P, Yeung C. Placebo-controlled trial of recombinant alpha-2 interferon in Chinese patients with chronic hepatitis B infection. Lancet 1987;2:877-880.
14. Greenberg HG, Pollard RP, Lutwick LI, Gregory PB, Robinson WS, Merigan PC. Effect of human leukocyte interferon on hepatitis B virus infection in patients with chronic active hepatitis. N Engl J Med 1976;295:517-522.
15. Hoofnagle JH, Peters M, Mullen KD, Jones DE, Rustgi V, Bisceglie A, Hallahan C, et al. Randomized controlled trials of recombinant human alpha-interferon in patients with chronic hepatitis B. Gastroenterology 1988;95:1318-1325.
16. Ostberg L, Pursch E. Human X (mouse X human) hybridomas stably producing human antibodies. Hybridoma 1983;2:361-367.
17. Starzl TE, Todo S, Tzakis A, Gordon R, Makowka L, Stieber A, Podesta L, et al. Liver transplantation: an unfinished product. Transplant Proc 1989;21:2197-2200.
18. Starzl TE, Todo S, Fung J, Demetris AJ, Venkataramanan R, Jain A. FK 506 for human liver, kidney, and pancreas transplantation. Lancet 1989;2:1000-1004.
19. Markus PM, Van der Brink MRM, Luchs BA, Fung JJ, Starzl TE, Hiserodt JC. Effects of in vivo treatment with FK 506 on NK and T-cell function in rats [in press].
20. Starzl TE, Porter KA, Mazzaferro V, Todo S, Fung J, Francavilla A. Hepatotrophic effects of FK 506 in dogs. Transplantation 1991;51:67-70.