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Evolution of Hepatitis B Virus Liver Disease After Hepatic Replacement

Practical and Theoretical Considerations

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The morphologic evolution of hepatitis B virus (HBV) liver disease in 45 hepatic allograft recipients who were HBV surface-antigen positive (HBsAg+) at the time of liver replacement and who survived for more than 60 days was studied by routine histologic and immunocytochemical analysis of serial pathology specimens. The findings in these patients were compared to a control group of 30 individuals who were immune to the HBV (anti-HBs antibody positive), but required hepatic replacement for other reasons. Eight of the forty-five (18%) HBsAg-positive patients have no serologic evidence of HBV reinfection after transplantation. All 37 remaining patients are reinfected; 21 (47%) developed chronic active hepatitis and/or cirrhosis, 3 (7%) developed submassive necrosis, and 6 (14%) developed chronic lobular hepatitis. One patient lost her graft to chronic rejection, despite reinfection with the B virus. Four other patients (9%) developed a chronic carrier state. No long-term follow-up biopsies were available in the remaining two patients. The histologic features associated with dysfunction related to recurrent HBV infection evolved from an acute to chronic phase and were similar to hepatitis B seen in nonallografted livers. Furthermore HBV-related lesions could be separated from rejection using routine histology alone. The only exception to this conclusion was the occurrence of a peculiar HBV-related lesion in two recipients, described herein. Immunohistochemical analysis demonstrated the presence of viral antigens in almost all cases. Hepatic inflammation also was commonly present during HBV disease and consisted mostly of accessory cells and T lymphocytes. Analysis of the effect of major histocompatibility complex matching revealed no clear association between the number of class I or II matches or mismatches and the development, or pattern, of active hepatitis in the allograft. Peculiar pathologic alterations in several of the biopsies and failed allografts after HBV reinfection suggests that, under special circumstances, the B virus may be cytopathic. (Am J Pathol 1990, 137:667–676)

The rationale of using liver replacement as therapy for a disease such as hepatitis B virus (HBV)-induced cirrhosis, which predictably recurs in a high percentage of patients after transplantation, is debatable.[1–3] Nevertheless some of these recipients will clear the virus and others will evolve into chronic carriers, with little or no disease activity, and thus benefit from liver transplantation.[3–9] One cannot question, however, the value of the information obtained from studying these patients to gain insight into the pathobiology of hepatitis B.

Most known immune effector mechanisms have, at some time, been implicated in HBV-induced liver damage because the virus itself is thought to be unable to directly injure hepatocytes.[10–15] Presently the consensus opinion suggests that cellular immunity is primarily responsible for HBV-associated liver damage and humoral immunity protects the liver from infection or reinfection when the virus enters the blood stream.[10–15] An unsubstantiated contention suggests that class I major histocompatibility complex (MHC)-restricted cytotoxic T lymphocytes (CTL) directed against the core antigen on the hepatocyte membrane are thought to lyse the virally infected cells.[12] However MHC class II (DR) restricted generation of T-helper cell function (*via* accessory cells) also appears to be important because the development of the final effector pathways (CTL and antibodies) is dependent on this rate-limiting step.[15] One must remember that the newly

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Table 1. *Pathologic Manifestations of Hepatitis B Viral Liver Disease and Distribution of Native Diseases in the HBsAb-positive Group*

Disease	HBsAg+	HBsAb+
CAH with cirrhosis	34	24*
Submassive necrosis	7	3
CAH/cirrhosis and hepatoma	4	2
Secondary biliary cirrhosis	0	1
Total	45	30

* Most of these patients were presumed to have non-A, non-B CAH or cryptogenic cirrhosis.

placed liver may be only partially MHC matched or even be totally MHC incompatible.

The following study had three goals. The first was to document the pathologic evolution of HBV disease after liver replacement and identify histopathologic features useful for the diagnosis and management of these recipients. The second was to analyze the immunopathologic findings associated with recurrent HBV disease in the allograft. The third goal was to study the effect of MHC matching or mismatching on the evolution of the disease after liver replacement.

Patients and Methods

Two groups of patients were selected for study. The first consisted of hepatic allograft recipients who were serologically HBsAg positive at the time of liver transplantation and survived for more than 60 days after the operation (n = 45). The second, or control group, consisted of 30 patients who were given livers because of non-B acute or chronic liver disease, but were immune to the HBV (anti-HBs positive, HBsAb+). These patients also survived for at least 60 days after transplantation. The distribution of the native liver diseases in both groups is shown in Table 1. All the patients received their primary allografts between January 1981 and July 1988; follow-up ranged from 18 months to more than 8 years.

All of the HBsAg-positive patients were treated with various modalities in an attempt to clear the HBV after liver replacement. Hepatitis B Immune Globulin (HBIG, Abbott Laboratories, North Chicago, IL), HBIG plus Hepatovax (HBVx, Merck, Sharp & Dohme, West Point, PA), or recombinant alpha interferon (Intron A, Schering Corp, Kenilworth, NJ) were the treatments used. Three doses of HBIG (100 ml/dose) were given intravenously, intraoperatively during the anhepatic phase, after completion of the operation, and 1 month later. When possible the three monthly spaced doses of HBVx were given before transplantation or completed after the operation in those who required more immediate liver replacement. The alpha interferon was administered for 2 weeks before and for 6 weeks after transplantation. The HBV serologic profile for both groups of patients is shown in Table 2. Twelve of the patients were serologically positive for anti-delta agent antibodies.

All pathology specimens (Table 3) were obtained at the discretion of the clinical physicians and were fixed in neutral buffered formalin, embedded in paraffin, sectioned at 4 μ, and stained routinely with hematoxylin and eosin and trichrome. In addition, all the specimens from the HBsAg-positive group and those from the control group with a 'hepatitic' histology were stained for the presence of HBcAg and HBsAg with commercially available reagents (DAKO, Carpinteria, CA) using standard avidin-biotin-complex methods (Vector, Burlingame, CA). The failed allograft specimens from the HBsAg-positive group were also stained for accessory cells (Mac 387, DAKO), T cells (L60, Becton-Dickinson, Mountainview, CA), B cells (L26, DAKO), interdigitating reticulum cells (S100 protein, DAKO), beta-2-microglobulin (DAKO), and IgM and IgG (DAKO). Appropriate formalin-fixed, paraffin-embedded positive control tissue for the immunohistochemical studies were included. For T, B, interdigitating, and accessory cells, tonsillar tissue was used; for the hepatitis antigens, known positive liver tissue was stained.

Results

Serology and Clinical Course After Transplantation

Briefly, in the HBsAg-positive group, eight of the patients had no serologic or tissue evidence of the HBV surface antigen

Table 2. *Pretransplantation HBV Serologic Profile of Patient Groups*

	Before transplantation		After transplantation*		
Serologic marker	HBsAg+†	HBsAb+	HBsAg+/HBeAg+	HBsAg+/HBeAg−	HBsAg−
HBsAg+, HBeAg+, HBeAb−	18	0	16	2	0
HBsAg+, HBeAg+, HBeAb+	3	0	3	0	0
HBsAg+, HBeAg−, HBeAb+	17	0	5	6	6‡
HBsAg+, HBeAg−, HBeAb−	7	0	4	1	2‡
HBsAg−, HBsAb+	0	30	—	—	30
Total	45	30	28	9	38

* At least 18 months of follow up of survivors.
† All of the HBsAg+ patients were anti-HBc positive as well.
‡ Despite serologic clearance of the HBsAg, only three patients became anti-HBs positive.

Table 3. *Number of Patients and Number of Types of Pathology Specimens Obtained After Transplantation*

Patients and Specimens	HBsAg+	HBsAb+
Total patients	45	30
Liver biopsies	177	113
Failed allografts	14	8
Autopsies	4	1
Specimens obtained after 60 days	102	57
Total pathology specimens	195	122

and remained negative and disease free with a minimum follow-up of at least 1.5 years. Presumably these patients have cleared the virus. All of them were HBeAg negative before transplantation and six of the eight were positive for anti-HBe.[17] Six of the eight were treated with a combination of HBIG and HBVx, one was given IFN alone, and the remaining patient was not treated at all. Despite apparent 'clearing' of the virus by serum antigen assays, only three converted to anti-HBs seropositivity.[17]

Four other patients, although still serologically HBsAg positive after liver replacement, have been stable and appeared to have evolved as chronic carriers with minimal or no evidence of liver enzyme elevations. In the remaining patients (n = 33), recurrent infection with serologic evidence of disease activity of varying severity was seen. Other than the absence of the e antigen before transplantation, no clinical or serologic parameter could predict which patient would clear the virus. In general, the effect of treatment modalities were difficult to evaluate, although six of the eight patients, who apparently have cleared the virus after transplantation, were given this regimen. At the time of writing, 15 of 45 (33%) of the HBsAg-positive patients who survived for more than 60 days have died. Eleven died because of multiple-organ failure associated with recurrent HBV infection, three to recurrent carcinoma, all of whom had recurrent CAH and one from an intracerebral bleed.[17] Greater detail into the clinical course, serology, and effects of the various treatment modalities will be presented elsewhere.[17]

In the HBsAb-positive group, only six patients (20%) developed any serologic evidence of hepatic injury, which histologically was diagnostic or compatible with a viral etiology. None of the HBsAb-positive patients became HBsAg positive after transplantation. Cytomegalovirus (CMV) was identified as the cause of dysfunction in four and the two others were presumed to have non-A,non-B hepatitis.

At the time of writing, 6 of the 30 (20%) HBsAb-positive patients died. Sepsis with multiple-organ failure (n = 3) and recurrent carcinoma (n = 3) were the major causes of late death.[17]

Histopathologic Evolution of Disease

HBsAg-positive Patients

For convenience of comparison, the postoperative course was separated into three time periods (0 to 60, 60 to 300, and more than 300 days) based on our previous experience with HBsAg-positive patients.[3] Acute cellular rejection and liver damage associated with preservation of the organ[18] were the most frequent diagnoses in the first 60 days, with most episodes of acute cellular rejection occurring in the first 30 days. Acute hepatitis or acute with transition to chronicity was the most common diagnosis between 60 and 300 days. Thereafter a chronic carrier state or CAH and/or cirrhosis represented the disease manifestations associated with persistent infection of the allograft with HBV. Each of these time periods is discussed in more detail below.

Episodes of acute cellular rejection (ACR) were histologically documented in 25 of 45 (56%) patients and all except two occurred within the first 30 days and were responsive to steroids and/or OKT3 therapy. The histologic appearance was similar to that previously reported.[3,12] In these specimens, only minimal lobular activity was seen and tissue stains for HBV antigens were either negative or showed focal cytoplasmic and/or nuclear positivity for core antigen, although surface antigen could be detected in the sera of some of these patients. Two of the patients developed CMV hepatitis after successful treatment of rejection, both of whom responded to lowered immunosuppression.

Only one patient developed a late onset of ACR (at 549 days), associated with a reduction of immunosuppressive therapy because of pregnancy; the diagnosis of ACR was made pathologically despite the presence of core antigen in almost every hepatocyte nucleus, as demonstrated by immunoperoxidase staining (Figure 1). Although this patient transiently responded to increased immunosuppressive therapy with a decrease of portal inflammation on biopsy and improvement of serologic parameters of liver injury, she lost her graft 46 days later to chronic rejection. Examination of the failed graft revealed findings typical of chronic rejection with severe bile duct loss and obliterative arteriopathy (Figure 1). Fewer hepatocytes contained nuclear core antigen, and those that were infected were found predominantly in the centrilobular regions.

During the 60- to 300-day period and thereafter, the biopsies represented the morphologic consequences and evolution of persistent HBV infection of the allografts. Early in the 60- to 300-day time slot, the histologic findings were typical of those reported for acute viral hepatitis in nongrafted livers. Initially there was Kupffer cell hypertrophy and spotty acidophilic necrosis of hepatocytes accompanied by a mild lobular mononuclear infiltrate. Varying degrees of lobular disarray, hepatocyte ballooning, and a mild portal and/or lobular mononuclear infiltrate (Figure 2) soon followed. Neither bile duct damage nor subendothelial infiltration of portal or central veins, such as that seen in acute cellular rejection, were prominent features. The only apparent difference between these

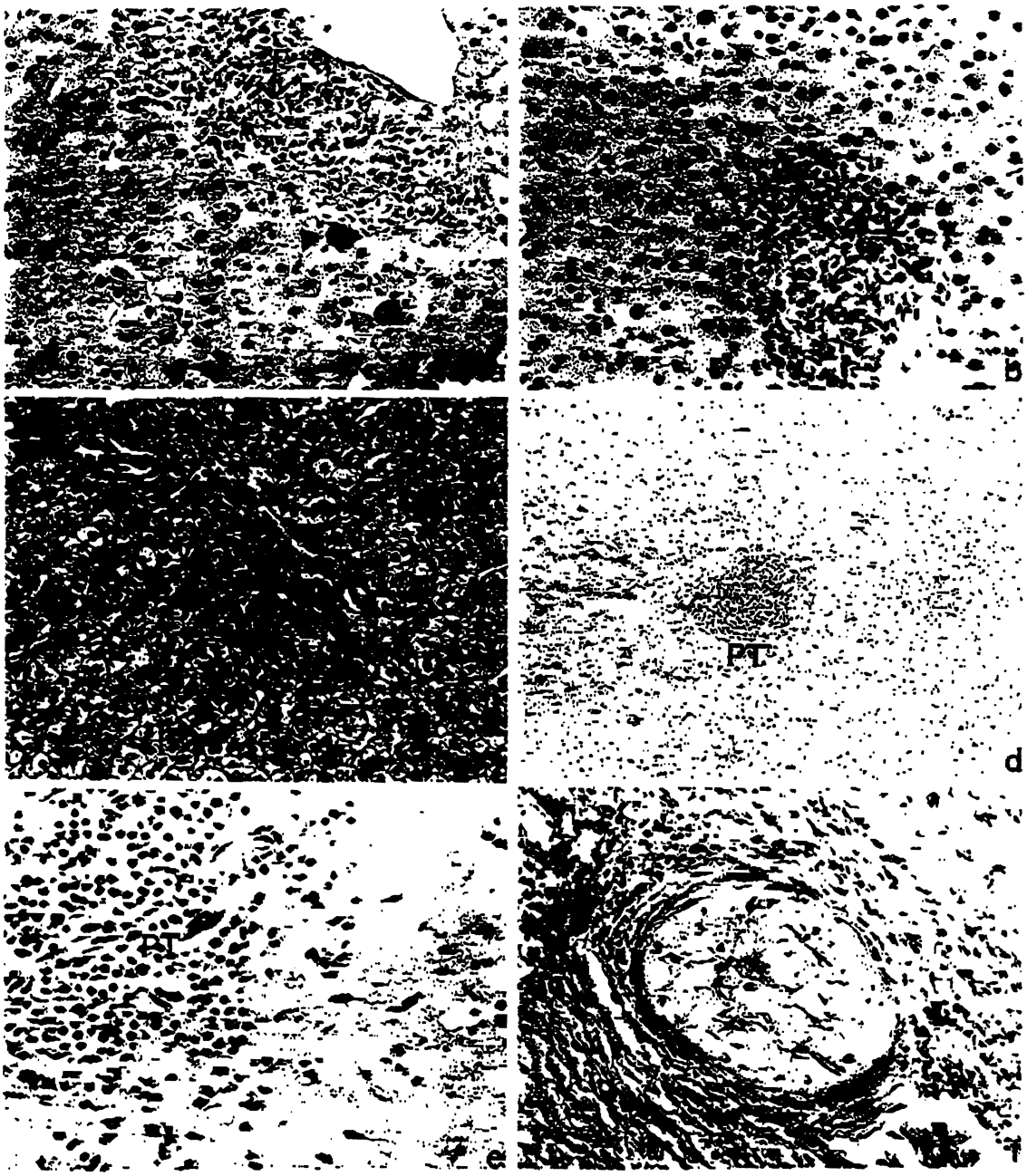

Figure 1. *Rejection of an allograft can be diagnosed histologically, despite infection with the B virus.* A: *Needle biopsy of allograft 549 after transplantation with acute cellular rejection and infection with the B virus (arrowhead) (immunoperoxidase [IPEX] for HBsAg, original magnification ×300).* B: *Same biopsy as in A shows panlobular expression of HBcAg in hepatocyte nuclei (IPEX for HBcAg, original magnification ×300). Note the confinement of the infiltrate to the portal triads, bile duct damage (arrows), and lack of piecemeal necrosis.* C: *Several weeks after steroid therapy for rejection, the portal cellularity diminished (H&E, original magnification ×300).* D: *Unfortunately, this patient lost her graft 46 days later to chronic rejection (IPEX for Mac 387, original magnification ×48).* E: *Higher-power view of D demonstrates the loss of bile duct with residual portal inflammation (IPEX for Mac 387, original magnification ×480). Note the lack of bile ducts, cirrhosis, ongoing piecemeal necrosis, and Mac 387+ accessory cells in D and E as opposed to the prominence of these findings in failed grafts with CAH/cirrhosis from HBV (PT, portal tract; see Figure 6).* F: *Prominent obliterative arteriopathy was seen in this graft as well.*

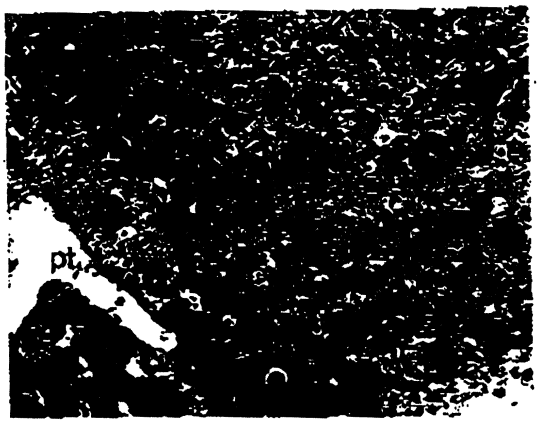

Figure 2. *The typical appearance of acute HBV hepatitis was similar to that seen in nonallograft livers and was characterized by a mononuclear portal and/or lobular infiltrate, lobular disarray, and hepatocyte necrosis. Note the lack of bile duct damage (arrow: pt, portal tract; H&E, original magnification ×300)*

findings and those seen in nonallograft livers with acute hepatitis was a greater degree of Kupffer cell hypertrophy and less inflammation. During the latter stages of this time period, changes of acute hepatitis overlapped with those portending chronic disease, which included a greater degree of portal inflammation, portal–portal and portal–central bridging, and piecemeal necrosis.

No change of immunosuppressive therapy was instituted in most of these patients after the diagnosis of acute hepatitis, except for the first few patients, who were reported on previously.[3] In others, in whom no change in therapy was instituted,[3] a typical clinical course followed. The following is one example. Patient 1 presented 195 days after transplantation with ALT of 1627 (nl < 50 IU), AST 1667 (nl < 40 IU), and malaise. A biopsy diagnosis of acute HBV was made. No particular therapeutic intervention was taken and 4 weeks later the levels of ALT and AST normalized. He again presented at 644 days with mildly elevated transaminases, at which time a biopsy diagnosis of chronic active hepatitis B was made. By 1293 days the patient developed jaundice, encephalopathy, and severe portal hypertension. He was given a new liver. The failed allograft was cirrhotic. No obliterative arteriopathy or significant duct loss were seen. Several other patients, however, experienced progressive deterioration after the initial onset of hepatic dysfunction and either died from hepatic failure or had to be given new livers (see below).

Those patients who survived for longer than 300 days with the same allograft demonstrated changes in later liver biopsies of unresolved or 'chronic' lobular hepatitis, carrier state, or ongoing CAH with progression toward cirrhosis. The major features of those with active disease were portal lymphohistiocytic and plasmacytic inflammation with piecemeal necrosis (Figure 3). Again the changes were similar to those reported for nonallografted livers and no significant bile duct damage nor subendothelial infiltration was seen.

There were two cases, however, that were similar to each other and different from the rest and from nonallografted livers that merit special description because of their unique clinic course and histopathologic findings. Clinically the first patient developed mild acute hepatitis at 271 days that spontaneously resolved. He remained infected. Because of this, he was treated with IFN, but again presented at 349 days with severe acute hepatitis, which rapidly progressed to liver failure and hepatic coma, requiring retransplantation on the 378th day. The second patient was well after liver replacement until he presented at 115 days with nausea, vomiting, and abdominal pain. A liver biopsy diagnosis of acute hepatitis with severe centrilobular ballooning was made. He rapidly

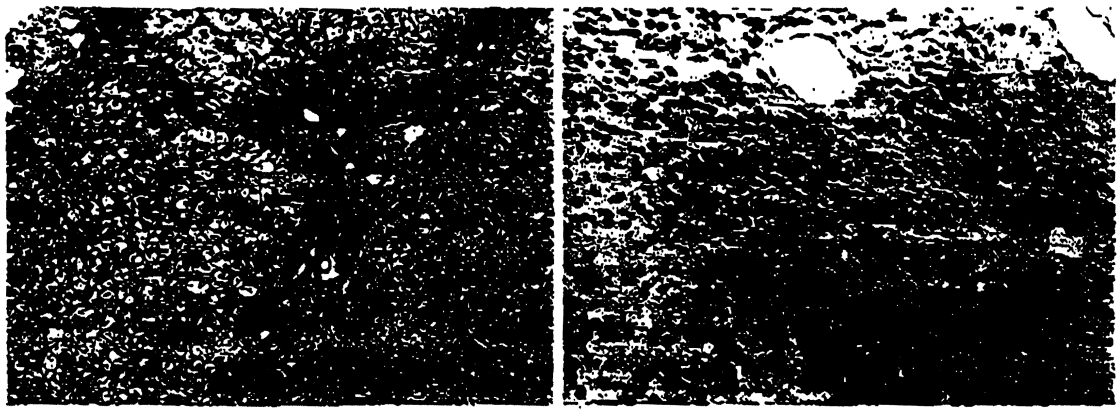

Figure 3. *The histologic appearance of CAH also was similar to that seen in nonallograft livers. A: Needle biopsy 1.5 years after transplantation. Note the activity at the limiting plate, the bridging portal fibrosis, and the lack of bile duct damage (arrow; H&E, original magnification ×120). B: Higher-power view of A illustrating the periportal activity, intact bile duct (arrow), and expression of HBcAg (arrowhead, IPEX for HBcAg, original magnification ×300). pt, portal tract.*

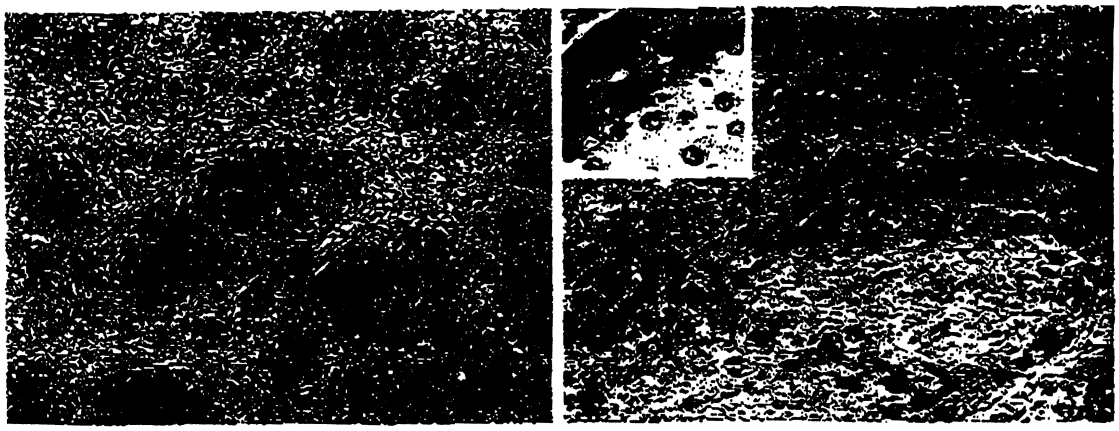

Figure 4. *Failed graft at 378 days in a patient who presented with subacute hepatic failure. A: Note the prominent central-central and portal-central distribution of swollen and degenerating hepatocytes, paucity of inflammation, and nodules of healthy appearing hepatocytes (H-E, original magnification ×48). B: Note that the viral antigen expression is largely restricted to the degenerating hepatocytes, where both nuclear and cytoplasmic core antigens were detected (IPEX for HBcAg, original magnification ×120). The inset (IPEX for HBcAg, original magnification ×480) shows the sharp demarcation between the infected degenerating cells and noninfected more healthy-appearing ones. p, portal; c, central.*

progressed to liver failure in the next 16 days, necessitating retransplantation. Unfortunately both patients died within 1 week of retransplantation from multiple-organ failure and sepsis. Examination of both failed primary allografts revealed marked centrilobular hepatocyte ballooning and cholestasis with central-central and central-portal architectural collapse (Figure 4). There was only a scant focal mononuclear portal infiltrate and few, if any, lymphocytes were present in the distribution of injured hepatocytes. It was as if the virus itself was responsible for the hepatocellular degeneration. Neither of these patients were positive for the delta agent. Interestingly both were completely mismatched at all A, B, and DR MHC loci (see Effect of MHC Matching).

In addition, there was one other patient who experienced fulminant hepatic failure in two consecutive allografts. After the first transplant, he presented at 223 days with severe acute hepatitis. Immunosuppressive therapy was lowered and the liver failed 19 days later, at which time it was replaced. The patient again did well initially but became ill only 89 days after placement of the second graft. Severe acute hepatitis, histologically indistinguishable from that seen in the first graft, was documented on biopsy. He died 15 days later because of hepatic and subsequent multiple-organ failure. The histology at initial presentation in both grafts was characterized by a brisk lobular inflammatory response, disarray, and hepatocyte necrosis typical of that seen with severe acute hepatitis. All three of these patients (two above and this one) had chronic active disease with cirrhosis in their native livers.

Finally there were eight patients who apparently had cleared the HBV after liver replacement. Minimal pathologic changes were seen in six, one had ongoing lymphocytic bile duct injury, and the last patient had low-grade chronic active hepatitis and was serologically positive for antibodies to the hepatitis C virus. A summary of the evolution of the HBV disease is shown in Figure 5.

Delta agent coinfection did not noticeably alter the course of HBV liver disease after transplantation. Of the 12 patients who were positive, 4 cleared the HBV, 7 developed CAH, 1 of whom has apparently cleared the virus after his third transplant, and 1 developed chronic lobular hepatitis.

Seven patients were given new livers after their first grafts succumbed to hepatitis B. Three died within 2 weeks of retransplantation because of multiple-organ failure. Interestingly the second grafts in the four others again developed severe HBV liver disease, confirmed by biopsy, but in almost exactly one half the time observed for the first graft. One of these latter patients developed chronic hepatitis, during which he apparently cleared the virus (serologically and immunohistochemically) and eventually lost the graft to chronic rejection. He remains free of the B virus 3 months after placement of his third liver.

HBsAb-positive Patients

The histologic findings in this group of patients was similar to those seen in the HBsAg-positive group during

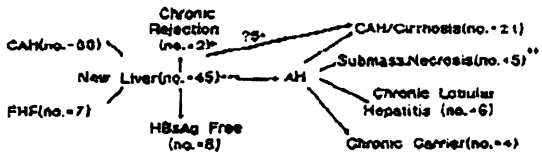

Figure 5. *Evolution of HBV disease after liver replacement based on graft survival of more than 60 days. §The first biopsy in some patients demonstrated CAH. It is uncertain whether they went through an acute hepatitis phase. **Two of these were secondary grafts and occurred in patients who lost their first grafts to HBV. ***No long-term biopsies were available in two patients. †One of these was a secondary graft in a patient who lost his first graft to HBV.*

Table 4. *Causes of Graft Failure Based on Clinicopathologic Analysis of Specimens Obtained at the Time of Retransplantation*

	HBsAg+		HBsAb+	
Cause of graft failure	N	Survival days	N	Survival days
Submassive/massive necrosis*	3	131–378	0	
Chronic active hepatitis/cirrhosis	4	203–2074	0	
Chronic rejection	2	258–632	4†	72–1021
Hepatic artery thrombosis	4	1–3	2	2–358
Primary nonfunctioning	1	18	2	1–28

* These three patients are from the group of five grafts that failed because of fulminant hepatic failure secondary to submassive necrosis.
† Three of these grafts were from the same patients.

the first 60 days. Acute cellular rejection was histologically verified in 20 of the 30 patients (67%), all of whom responded to antirejection therapy. Four of the patients developed CMV hepatitis after treatment of rejection, which resolved with temporary lowering of the immunosuppressive regimen.

Thereafter (after 60 days) the histologic findings and diagnoses differed dramatically from those of patients infected with the B virus. The predominant findings in follow-up biopsies in this group were nonspecific changes (n = 13) or chronic portal inflammation with or without ongoing bile duct damage (n = 10) and little or no evidence of piecemeal necrosis, consistent with an ongoing rejection reaction or chronic persistent hepatitis. Four of the grafts were eventually lost to chronic rejection. Two HBsAb-positive patients, however, had biopsies in this time period, which were similar to those seen in the HBsAg-positive group, but no HBV antigens were detected serologically or immunohistochemically. Both were diagnosed as hepatitis pathologically. One lost his graft at 150 days from chronic rejection and the other is still being followed. The remaining five patients have shown little or no evidence of allograft dysfunction and no long-term follow-up biopsies were available.

Failed Allografts

There was a total of 22 failed grafts removed at the time of retransplantation from the two groups: 14 from the HBsAg-positive and eight from the HBsAb+ patients. The causes of graft failure are shown in Table 4. Pathologically it was not difficult to differentiate between the various etiologies. Nonimmunologic causes of graft failure were similar between the two groups. However graft loss from hepatitis and/or cirrhosis was seen only in HBsAg-positive patients. None of the grafts removed from the HBsAb+ group, or because of chronic rejection from either group of patients, was cirrhotic.

Immunopathologic Studies

All but one of the 37 patients in whom the HBsAg reappeared in the serum after transplantation had tissue deposits as well. The first evidence of HBV antigens in the allografts was the presence of HBcAg in the cytoplasm and/or nucleus within 3 weeks after transplantation in one case. During episodes of acute hepatitis, HBV antigens were usually detected in the allografts.[19] There was a gradual increase in the number of positive cases and by 250 to 300 days all but one of the patients who were HBsAg-positive and who had a late biopsy after transplantation had tissue deposits of HBV antigens.[19]

After reinfection, there was a gradual increase in the percentage of hepatocytes containing HBcAg, both nuclear and cytoplasmic, particularly during the evolution of acute to chronic disease. Nearly all patients who developed CAH continued to express cytoplasmic HBcAg. Among the chronic carriers (n = 4; 9%), generalized cytoplasmic HBsAg was seen in one, while the three others showed generalized nuclear core antigen and fewer cells with cytoplasmic HBsAg.[19] In those patients with progressive disease, a gradual decrease in HBcAg+ cells and an increase in the HBsAg-positive ones was found.

One of the two peculiar cases mentioned previously demonstrated panlobular expression of HBcAg+ at 271 days and at the time of graft failure (378 days) nuclear and cytoplasmic core antigen staining was limited to the degenerating centrilobular hepatocytes (Figure 4). More healthy appearing clusters of hepatocytes were generally located in zone 1 of the acinus and only some cells contained nuclear HBcAg.

In the failed allografts with CAH/cirrhosis, inflammatory cells in the portal tracts and lobules contained a predominance of T cells (L60+), although appreciable numbers of IgG- and IgM-containing plasma cells were present in the portal spaces in some cases as well. A conspicuous finding was the large number of irregular- and dendritic-shaped accessory cells (Mac 387+) present in the triads, especially at the edge of the limiting plates in areas of piecemeal necrosis (Figure 6). These cells also were conspicuous in the lobules in areas of active hepatocyte necrosis. Despite the large number of Mac 387+ cells, there were only a few S100+ cells in the triads and these were located in the center of the triads.

Beta-2-microglobulin expression was difficult to interpret but appeared to be present on the hepatocyte sur-

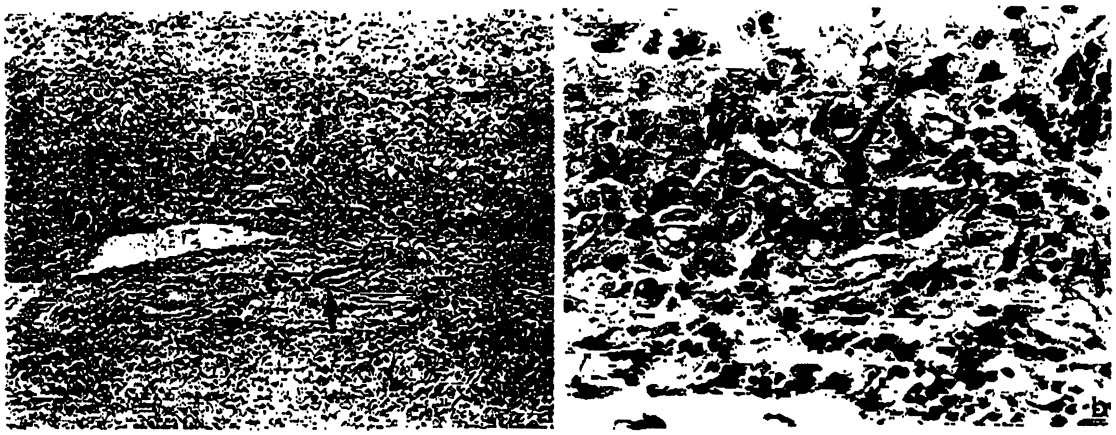

Figure 6. A: *The most impressive finding on immunohistochemical staining for inflammatory cells was the presence of many accessory cells in the portal tracts and at the limiting plate in allografts that failed because of CAH/cirrhosis (IPEX for Mac 387, original magnification ×48). Note the intact bile ducts (arrows).* B: *Higher-power of A demonstrating the activity at the limiting plate (IPEX for Mac 387, original magnification ×480). Compare these photomicrographs to those in Figure 1, which were stained for Mac 387 and in which rejection was the cause of graft failure, although the graft was infected.*

face membrane in a panlobular distribution in grafts that failed from acute hepatitis, whereas in those with CAH and/or cirrhosis, the staining was generally restricted to the edge of the lobules/nodules in areas of ongoing piecemeal necrosis. Bile ducts and endothelia generally were positive.

Effect of MHC Mismatching on HBV Disease

Complete donor and recipient MHC profiles were available for 35 grafts, and in three others the class I antigens were typed but the DR antigens could not be classified. The effect of mismatching at the various MHC loci on graft survival is shown in Table 5. Most of the patients were mismatched at one or more class I and II MHC loci because no attempt was made to prospectively match the livers and recipients. No particular effect was noted for class I matching or mismatching other than two peculiar cases, previously described, who were completely mismatched at all A, B, and DR loci. It should be noted, however, that there were four other patients who were mismatched at all six loci, three of whom have developed CAH and/or cirrhosis, and one has chronic lobular hepatitis on last biopsy. There were only two patients completely matched at the DR locus: one evolved into a chronic carrier and the other had low-grade chronic lobular hepatitis at last follow-up. By contrast, there was one patient who became a chronic carrier who was mismatched at both DR loci. Overall there was no statistically significant association between the degree of matching or mismatching at class I or II MHC antigens and the occurrence of what appeared to be acute or chronic hepatitis in the allograft.

Discussion

The first goal of this study was to document the evolution of HBV liver disease after hepatic replacement. The results are best summarized in Figure 5. As might be expected, recurrent infection of the allograft occurred in 37 of 45 patients (82%): In 24 patients (53%), reinfection resulted in serious disease in the form of chronic active hepatitis (n = 21; 47%) or submassive necrosis (n = 3; 7%). The remaining patients had a histology at the last follow-up that could be classified as chronic lobular hepatitis (n = 6; 14%) or chronic carrier state (n = 4; 9%); the long-term outlook for these patients has yet to be determined. No long-term pathologic follow-up was available in two patients, who are still alive 942 and 999 days after transplant.

Table 5. *Effect of MHC Mismatching on Allograft Survival*

		MHC mismatches		
		0	1	2
MHC Loci	A	2 (1221)/1 (239)	5 (241)/11 (819)	6 (600)/10 (770)
	B	0 (–)/0 (–)	4 (285)/7 (696)	9 (585)/15 (697)
	DR	0 (–)/2 (798)	5 (452)/7 (877)	7 (522)/11 (802)

Numbers refer to number of failed grafts/functioning grafts with numbers in parentheses identifying mean survival times in days. Only one graft that failed from non-HBV–related causes was included in the analysis.

The pathologic features of liver injury associated with recurrent HBV infection in the allograft were not significantly different from those seen with hepatitis B in nongrafted livers. Furthermore biopsies from infected patients demonstrated both acute and chronic phases. Continuing periportal hepatocyte necrosis in the chronic phase eventually progressed to cirrhosis in a number of patients during the study period.

For the most part, histologic changes associated with HBV-related dysfunction were distinguishable from acute and chronic rejection. The key feature used to make the separation was the focus of the inflammatory cell damage. In acute and chronic rejection, the bile ducts were the main targets of lymphocytic injury. Venular injury could be seen in acute rejection as well. In hepatitis, the hepatocytes were preferentially targeted for damage. During acute disease, the injury was panlobular in distribution, whereas in the chronic active phase it was directed primarily at periportal hepatocytes. The only caveat was the presence of low-grade lobular 'hepatitic' changes in several patients during the development of chronic rejection in both the HBsAg-positive and HBsAb-positive groups, which led to a mistaken diagnosis of hepatitis in one of the HBsAb-positive patients. One must be alert, therefore, to the presence and severity of bile duct damage in all cases. When present, a component of rejection is likely to be involved. Therefore rejection can be diagnosed even in the presence of HBV infection of the allograft. This point is exemplified in the patient who lost her graft from rejection, even though the graft was infected.

Immunopathologic studies demonstrated that HBV antigens are almost invariably detected in the liver during disease related to recurrent HBV infection. Expression of core antigen was common during the acute stage and persistent cytoplasmic core antigen expression in the chronic phase generally correlated with ongoing disease activity.[19] Hepatic inflammation also was frequently, but not invariably, seen in the allografts during episodes of dysfunction related to recurrent HBV infection. Immunophenotypic analysis has shown that most of the inflammatory cells were T lymphocytes and accessory cells in biopsies with HBV-associated dysfunction. Compared to the grafts that failed because of chronic rejection, periportal accessory cells were more common and the T cells were distributed differently (see above). The observations strongly suggest that immunologic responses in the two disorders are different.

Static morphologic studies, however, give little or no information about the functional specificity of the infiltrating cells. Whether they are directed at viral, altered hepatocellular, or foreign MHC antigens or are merely secondary to the disease is unknown. Despite the lack of lymphocyte functional studies, the accelerated hepatic damage associated with reinfection of second allografts suggests that there may be an immunologic memory response or enhanced HBV virulence in this circumstance.

We also noted that MHC class I antigens are upregulated on hepatocytes in areas of ongoing injury associated with HBV infection, as detected by the presence of beta-2 microglobulin. One must remember, however, that these MHC antigens are those of the donor, and not the recipient effector cells.

Except for the two peculiar cases previously described, we were unable to detect a significant correlation between the degree of donor/recipient MHC matching or mismatching and the pattern or occurrence of what histologically appeared to be hepatitis. Although the number of patients in this study was relatively small, the findings suggest that immune mechanisms other than, or in addition to, MHC-restricted CTL could be responsible for the hepatic injury associated with HBV infection of allografts. Striking hepatocyte cytologic degenerative changes associated with marked viral antigen expression and little, if any, inflammation was seen. Under these special circumstances, the virus itself may be cytopathic. Whether the observations reported herein can be incorporated into a hypothesis regarding liver damage associated with HBV infection in the general population is open to speculation.

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