

# Recurrence-Free Long-Term Survival After Liver Transplantation for Hepatitis B Using Interferon-Alpha Pretransplant and Hepatitis B Immune Globulin Posttransplant

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## Objective

The authors determined whether pretransplant reduction of hepatitis B virus (HBV) load using alpha-interferon-2b (IFN) and passive immunoprophylaxis using hepatitis B immunoglobulin (HBIG) posttransplantation can prevent HBV recurrence in patients undergoing liver transplantation (LT) for HBV cirrhosis.

## Summary Background Data

Liver transplantation in patients with HBV cirrhosis is associated with a high rate of recurrence and reduced survival. In patients with evidence of replicating virus (HBV-DNA or hepatitis B e antigen [HBeAg]-positive serum or both), recurrence is nearly universal. Passive immunoprophylaxis with HBIG alone is not effective in preventing HBV recurrence posttransplant, especially in patients with evidence of active viral replication pretransplant. Higher doses of HBIG posttransplant has reduced recurrence rates to 30% to 50%. Lamivudine, a nucleoside analogue that has shown early promise, also is associated with significant HBV recurrence. The authors report a reliable method of preventing viral recurrence in patients even with evidence for active HBV replication pretransplant.

## Methods

Pretransplant patients with evidence of replicating HBV were given IFN starting at 1 million IU 3 times per week subcutaneously. This dose was increased to 2 and then 3 million IU 3 times per week when patient's side effects permitted and was maintained until the patient underwent a LT. All patients were tested every 4 weeks for hepatitis B surface antigen (HBsAg), HBeAg, and HBV-DNA. When patients became negative for HBeAg and HBV-DNA, they were listed for LT. Patients that were only HBsAg positive were listed immediately and received a LT without prior IFN treatment. Post-LT, all patients began receiving HBIG 2000 IU (10 mL) daily from days 1 to 20 and then weekly for the first 2

years. After 2 years, all patients received 2000 IU (10 mL) monthly. Additional HBIg immunoprophylaxis was given during intense immunosuppression for rejection. Post-transplant serum was tested for HBsAg, HBeAg, and HBV-DNA in all patients 1 week, 1 month, and every 3 months thereafter. Liver biopsies were done at least yearly and when liver enzymes were abnormal and were always tested for HBsAg and HBcAg by immunoperoxidase.

## Results

Thirteen patients with decompensated HBV cirrhosis were transplanted. Pretransplant, eight patients had evidence of active viral replication at the initial assessment (HBeAg or HBV-DNA-positive serum or both). All eight were successfully treated with IFN (median duration, 24 weeks; range, 8–53) and converted to a negative status before transplantation. Side effects from IFN were minimal and well tolerated, except in one patient who required 6 million IU to convert to a nonreplicating status. The five patients that were only HBsAg positive were not treated with IFN pretransplant. After surgery, HBIg given as described achieved consistently serum levels greater than 1000 IU/L. Twelve of the 13 patients are alive with normal liver function and without serologic evidence of HBV recurrence at a median follow-up of 32 months (range, 9–56 months). None have evidence of HBV recurrence as measured by serum HBsAg/HBeAg/HBV-DNA at recent follow-up. The sera of the seven longest survivors has tested negative for HBV-DNA using the polymerase chain reaction method. In addition, a liver biopsy was obtained in six of these patients, the results of which also tested negative for HBV-DNA using polymerase chain reaction. Liver biopsy specimens have been negative for the presence of HBsAg and HBcAg by immunoperoxidase staining in all 12 patients.

## Conclusion

A reduction of viral load pretransplant with IFN and posttransplant HBIg prevents recurrence of hepatitis B and permits LT for HBV cirrhosis, even in patients with evidence of replicating virus. The IFN pretransplant was well tolerated, and the small frequent dosing of HBIg posttransplant did not cause side effects while achieving serum levels >1000 IU/L.

Liver transplantation (LT) in patients with hepatitis B virus (HBV)-induced liver cirrhosis is complicated by high recurrence rates and lower patient survival.<sup>1–14</sup> Recurrence of the grafted liver with HBV is almost universal and is associated with chronic active hepatitis with rapid progression to cirrhosis and, in some patients, fulminant hepatitis.<sup>4,5</sup> Occasionally, recurrent hepatitis B runs a subacute but rapidly progressive and deteriorating course characterized by a cholestatic clinical syndrome associated with a heavy viral load and histologically termed *fibrosing cholestatic hepatitis*.<sup>6</sup> As a result, at a recent consensus conference in Paris, France, on the indications for LT, a liver transplant in patients with hepatitis B-induced liver cirrhosis was deemed inappropriate unless it was under protocol to prevent recurrence.<sup>7</sup>

Numerous strategies to prevent HBV recurrence after

LT have been reported.<sup>7–17</sup> These studies showed that only passive immunoprophylaxis using anti-HBs polyclonal antibody (HBIg) currently has shown the ability to prevent recurrence after LT. However, even with passive immunoprophylaxis with HBIg, recurrence in patients with detectable HBV-DNA pretransplant occurred in 67% to 100% of transplanted patients. This occurred usually in the first 6 months after transplantation. Treatment with alpha-interferon (IFN) has been shown to be beneficial in the nontransplant setting in patients with chronic hepatitis B.<sup>18–21</sup> However, IFN alone given before or after transplantation does not reliably prevent HBV reinfection.<sup>13–15</sup>

Since August 1992, all patients with decompensated HBV cirrhosis requiring LT were given intensive post-transplant immunoprophylaxis with HBIg. In addition, patients who showed evidence of HBV replication (hepatitis B e antigen [HBeAg] or HBV-DNA positive) were treated pretransplant with IFN to reduce viral load before transplantation. We report results in eight patients with replicating virus treated pretransplant with IFN and in five additional hepatitis B surface antigen (HBsAg)-only positive recipients, all of whom received HBIg posttransplant without recurrence of hepatitis B after a median follow-up approaching 3 years after LT.

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**Table 1. DEMOGRAPHIC AND SEROLOGIC CHARACTERISTICS OF PATIENTS WITH HBV-CIRRHOSIS UNDERGOING LIVER TRANSPLANTATION TREATED WITH HBV PROTOCOL OF IFN AND HBIg**

Characteristic	Study Group
Number of patients	13
Sex (M/F)	12/1
Age (range, yr)	57 (37–64)
Evidence of HBV-replication at referral (%)	8/13 (61.5)
HBsAg/HBeAg/HBV-DNA positive (%)	3/13 (23.1)
HBsAg/HBV-DNA positive only (%)	4/13 (30.8)
HBsAg/HBeAg/positive only (%)	1/13 (7.7)
HBsAg positive only (%)	5/13 (38.5)
Patients receiving IFN treatment (%)	8/13 (61.5)
Response to IFN treatment (%)	8/8 (100)

HBV = hepatitis B virus; IFN = interferon.

## MATERIALS AND METHODS

Between August 1992 and August 1996, 119 LTs were done in 101 patients. Of these, 19 LTs were done in 15 patients with advanced HBV cirrhosis. Two patients were transplanted outside of this IFN and HBIg protocol and are not included in the study. Thirteen patients requiring LT were prospectively entered in this protocol designed to prevent HBV recurrence using interferon-alpha 2b (Schering—Plough Canada, Pointe Claire, Canada) pretransplant and HBIg (NABI, Boca Raton, FL) (Table 1). There were 12 men and 1 woman (median age, 57 years; range, 37–64). In 12 patients, the liver disease was caused by hepatitis B-induced cirrhosis. A single patient had a combination of hepatitis B and hepatitis C viral infection. There was no evidence of hepatitis D in any of the patients. Hepatitis B serologic status was determined before transplantation by use of standard, commercially available enzyme-linked immunosorbent assays (HBsAg, HBeAg, HBsAb, HBeAb, and HBcAb; Abbott Diagnostics, Chicago, IL). Serum also was tested for HDV-Ag and anti-HDV. Serum was tested for HBV-DNA by molecular hybridization spot test (Abbott Diagnostics, Chicago, IL) before IFN therapy was started. Of the 13 patients, 5 were in the nonreplicating state (HBsAg positive only) and 8 had evidence of HBV replication pretransplant (Table 1). Of the eight patients with evidence of active HBV replication pretransplant, three were HBsAg, HBeAg, and HBV-DNA positive, four were HBsAg and HBV-DNA positive, and one patient was HBsAg and HBeAg positive. After transplantation, all patients were screened 1 week, 1

month, and every 3 months thereafter for HBsAg, HBeAg, HBV-DNA, anti-HBs, anti-HBc, and anti-HBe. Serum levels of HBIg were measured quantitatively every 3 months posttransplant.

## Pretransplant Interferon Protocol

Patients were tested for HBsAg, HBeAg, and HBV-DNA at the initial visit and every 4 to 8 weeks after initiation of IFN treatment pretransplant (Table 2). The eight patients who were either HBeAg or HBV-DNA positive or both pretransplant, in addition to being HBsAg positive, received IFN initially at 1 million IU 3 times/week. The dose was increased by 1 million IU every week to a maximum of 3 million IU 3 times/week if the patient experienced no serious side effects or significant neutropenia or thrombocytopenia. The IFN was held or reduced for leukopenia  $<1.5 \times 10^6/\text{mL}$  and thrombocytopenia  $<30 \times 10^9/\text{L}$ . This dose of IFN (i.e., 3 million IU 3 times/week) was maintained for the next 8 weeks. At that time, if the patient was HBeAg and HBV-DNA negative, he or she was listed for LT. If the patient still was HBeAg or HBV-DNA positive or both, IFN was maintained at a dose of 3 million IU for an additional 8 weeks and reassessed. At that time, if the patient still was HBeAg or HBV-DNA positive or both, the dose of INF was increased to 6 million IU 3 times/week. In only one of the eight treated patients with IFN was the dose increased to 6 million IU after 16 weeks because he still was HBeAg positive. The IFN treatment was maintained in all patients until the day of transplantation.

## Posttransplant HBIg Protocol

After LT, all 13 patients received HBIg 2000 IU (10 mL) intramuscularly daily for the first 20 days starting in the intensive care unit immediately after surgery (none was given during the anhepatic phase). After day 20, HBIg intramuscularly was continued at 2000 IU weekly for 2 years and then monthly forever. Serum levels of HBIg were measured every 3 months after surgery. The dosing of HBIg was not based on these levels however. Four patients had an episode of mild acute cellular rejection in the first 3 months posttransplant that responded to methylprednisolone 500 mg intravenous bolus given for 3 consecutive days. At that time, immunoprophylaxis with HBIg was increased to 10 mL daily for 14 days after the last dose of methylprednisolone and then resumed at the weekly interval dosing as before.

All patients had a serum HBsAg, HBeAg, and HBV-DNA done 1 week, 4 weeks, and then every 3 months after LT. A liver biopsy was done when clinically indicated, every 6 months during the first year and yearly after. Two years after LT, testing for HBV-DNA in serum

**Table 2. RESPONSE TO IFN TREATMENT IN PATIENTS WITH EVIDENCE OF ACTIVE HBV REPLICATION AT TIME OF REFERRAL TO TRANSPLANT CENTER**

Patient Number	Child-Pugh (score)	Positive HBV Serology			IFN Treatment		
		Pre-IFN	Post-IFN (time to nonreplicating status, wk)	HCC (TNM stage)	Maximum Dose (mIU/dose)	Duration (wk)	Complications of IFN Treatment
1	C (12)	HBsAg/HBeAg/HBV-DNA	HBsAg (12)	Stage I	3	28	Fever, fatigue, thrombocytopenia
2	C (14)	HBsAg/HBeAg HBV-DNA	HBsAg (20)	No	6	23	Fever, fatigue, encephalopathy
3	C (12)	HBsAg/HBV-DNA	HBsAg (12)	No	3	20	Fatigue, encephalopathy
4	C (13)	HBsAg/HBV-DNA	HBsAg (8)	No	3	8	Thrombocytopenia, fever, fatigue
5	C (12)	HBsAg/HBV-DNA/HBeAg	HBsAg (12)	No	3	42	Leukopenia, thrombocytopenia
6	B (8)	HBsAg/HBeAg	HBsAg (8)	Stage II	3	12	Fatigue
7	B (7)	HBsAg/HBV-DNA	All markers negative (12)	Stage III	3	48	Thrombocytopenia, leukopenia
8	C (10)	HBsAg/HBV-DNA	HBsAg (12)	No	1	53	Thrombocytopenia, fever, fatigue

IFN = interferon; HBV = hepatitis B virus; HCC = hepatocellular carcinoma.

and liver tissue was performed using the polymerase chain reaction (PCR) on seven and six patients, respectively.

### Posttransplant Immunosuppression Protocol

Induction immunosuppression consisted of antithymocyte globulin (ATG; Sangstat Canada, Mississauga, Canada), Cyclosporine (CsA; Sandoz Canada, Dorval, Canada), prednisolone (pred), and azathioprine (Aza; Glaxo Canada, Mississauga, Canada). Maintenance immunosuppression was CsA, Pred, and Aza. The Aza was discontinued by 1 to 3 months posttransplant. Prednisolone was discontinued by 6 to 12 months, so that by 1 year posttransplant all patients were receiving CsA alone as maintenance immunosuppression with target trough whole blood levels 100 to 150 ng/mL. All our patients who underwent LT are immunoprophylaxed against cytomegalovirus with gancyclovir intravenously at 3 mg/kg/dose twice daily for 2 weeks posttransplant.

## RESULTS

### Pretransplant Interferon Treatment

Before transplantation, the eight patients with evidence of replicating HBV converted to a nonreplicating status after treatment with IFN (Table 1). In the three patients with HBsAg, HBeAg, and HBV-DNA-positive status, IFN converted two of them to a nonreplicating state (HBsAg only

positive) by 12 weeks. Both patients continued receiving IFN until their liver transplant was performed 28 and 42 weeks after initiation of IFN therapy. The third patient required IFN to be increased to 6 million IU 3 times/week at 16 weeks after IFN was started. At that dose, a pleural effusion and decompensated liver function developed in the patient. He cleared HBeAg by 20 weeks after IFN was started (4 weeks after the dose was raised to 6 million IU) and was transplanted urgently 3 weeks later because of encephalopathy and impending renal failure. He was the only patient to have a serious adverse response during IFN therapy.

The four patients who were HBeAg negative but HBsAg and HBV-DNA positive were treated with IFN 3 million IU 3 times/week for 8, 20, 48, and 53 weeks, respectively. All four patients responded by reduction of HBV-DNA to undetectable levels by 8, 12, 12, and 12 weeks, respectively. In one patient, there was a 4.6-cm and in another an 11-cm hepatocellular carcinoma (HCC). In addition, the patient with the 11-cm HCC received a course of intra-arterial chemotherapy protocol before surgery of cisplatin and epirubicin for four monthly cycles. This patient continued receiving IFN 3 million IU 3 times/week for a total 48 weeks and became HBsAg, HBeAg, and HBV-DNA negative before LT. This is the only patient of the eight treated with IFN to convert to an HBsAg-negative anti-HB-positive status to LT.

In addition to the above two patients who were transplanted with a known HCC, two other patients had a single HCC lesion measuring <2 cm each found as an incidental finding at explantation of their livers. None of these four patients have

**Table 3. HBV-CIRRHOSIS PATIENTS AFTER LIVER TRANSPLANTATION ON IFN-HBIg PROTOCOL: TIME AFTER LIVER TRANSPLANTATION, CURRENT HBIg DOSE, LIVER BIOCHEMISTRY, HBV SEROLOGY STATUS, AND SERUM HBIg LEVELS**

Patient Number	Follow-up Post LT (mo)	Current HBIg Dose (i.m.)	Liver Function Tests at Last Visit				Latest HBV Serology Status				
			T Bilirubin ( $\mu\text{mol/L}$ )	ALT (IU)	AST (IU)	ALK P (IU)	INR	HBsAg	HBeAg	HBV-DNA	HBsAb (IU/L)
1*	56	2,000 IU/mo	19.8	22	20	91	1.0	neg	neg	neg	>1000
2	45	2,000 IU/mo	13.1	17	14	93	0.93	neg	neg	neg	258
3	42	2,000 IU/mo	9.1	10	12	79	0.97	neg	neg	neg	218
4	39	2,000 IU/mo	13.1	37	27	94	1.04	neg	neg	neg	601
5	35	2,000 IU/mo	13.8	17	18	41	0.95	neg	neg	neg	>1000
6	32	2,000 IU/mo	6.1	10	12	120	1.14	neg	neg	neg	796
7*	32	2,000 IU/mo	12.4	15	16	59	0.93	neg	neg	neg	>1000
8*	31	2,000 IU/mo	11.9	36	30	117	0.93	neg	neg	neg	>1000
9	23	2,000 IU/wk	10.4	20	22	106	1.02	neg	neg	neg	>1000
10*	20	2,000 IU/wk	13.9	58	57	95	0.85	neg	neg	neg	>1000
11	16	2,000 IU/wk	22.5	53	34	233	0.85	neg	neg	neg	>1000
12	9	2,000 IU/wk	20.7	32	22	126	0.932	neg	neg	neg	757

HBV = hepatitis B virus; IFN = interferon; LT = ; ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALK P = alkaline phosphatase; INR = international normalized ratio; neg = negative.

\* Patients with hepatocellular carcinoma at the time of liver transplantation. All patients are free of tumor at last follow-up.

evidence of HCC recurrence at a follow-up of 20, 31, 32, and 56 months posttransplantation.

Side effects from IFN therapy in seven of the eight patients treated usually were minimal and related to constitutional symptoms of fever, fatigue, and all patients had relative leukopenia and thrombocytopenia (Table 2). These complications were well tolerated and did not require dose adjustments of IFN.

### Posttransplant Results

One of the five patients who was HBsAg positive only (i.e., not treated with IFN) died 2 days after of ischemic bowel, sepsis, and a multiorgan failure. Twelve (93%) of 13 patients currently are alive and negative for all serum HBV markers with a median follow-up of 32 months (range, 9–56, Table 3). They all have a normal liver test profile. All 12 patients currently are HBsAg, HBeAg, and HBV-DNA negative. In 8 of 12 patients with a follow-up of >2 years, HBIg has been reduced from 2000 IU (10 mL) weekly to a monthly schedule (Table 3). We note that four of these eight patients continue to maintain HBIg serum levels of >1000 IU/L. Results from a yearly liver biopsy show all patients have completely normal histology on hematoxylin and eosin staining and are negative for HBsAg and HBeAg by immunoperoxidase staining (Figs. 1A, 1B). In seven of seven patients tested after the second year posttransplant, there was no detectable HBV-DNA in their serum as tested by PCR. In six of six

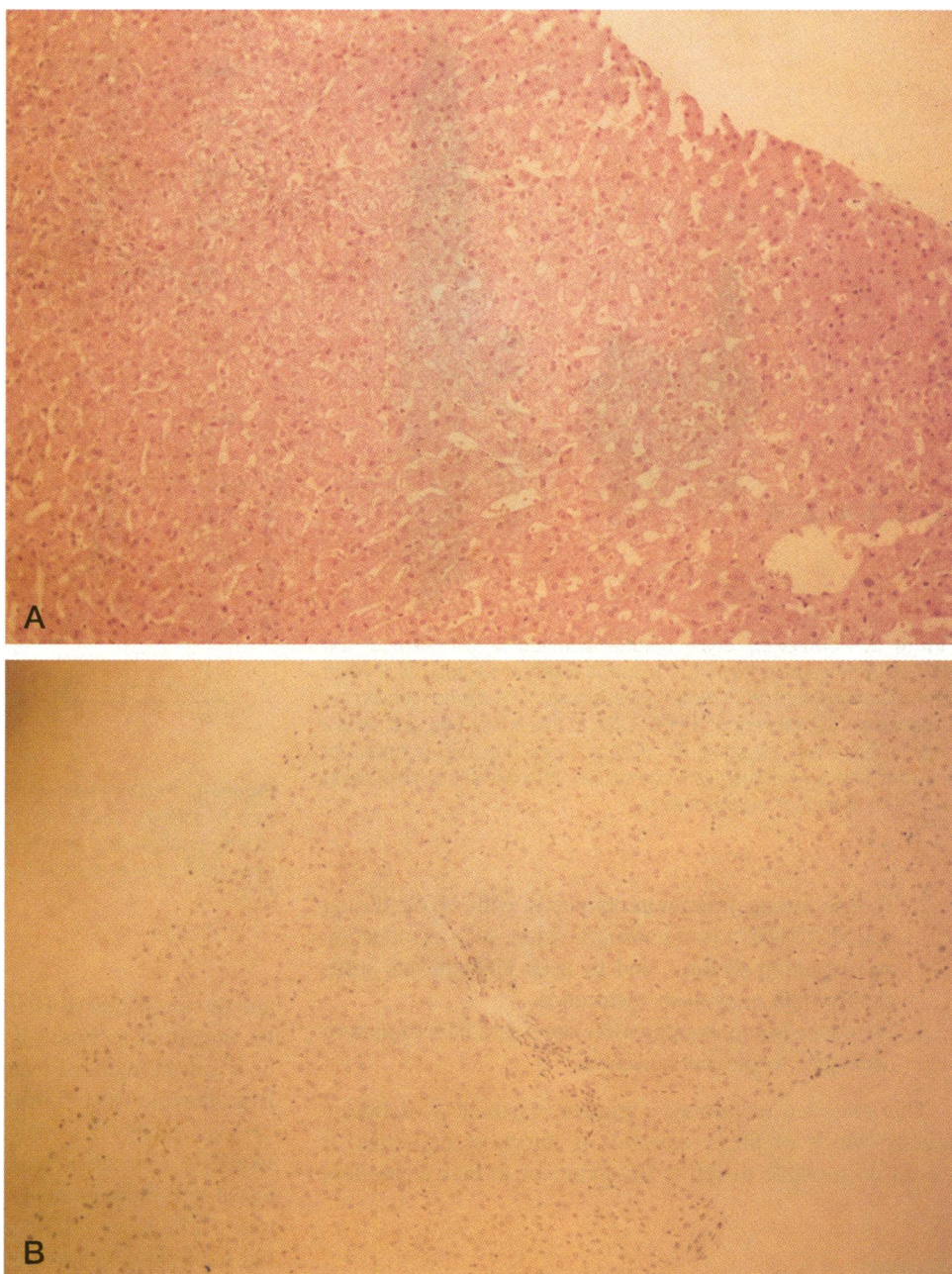
of these patients, there also was no detectable HBV-DNA in on liver biopsy specimen as tested by PCR (Fig. 2). One patient was not available for a liver biopsy at that time.

Actuarial 1-, 2-, and 3-year patient survival was 93% in the 13 HBV recipients treated with IFN and HBIg compared to 83%, 79%, and 77% in 86 recipients concurrently transplanted at our institution, with a diagnosis other than HBV cirrhosis (Fig. 3).

One patient (patient 4, Table 3) who was HBsAg/HBV-DNA positive and HBeAg negative pretransplant was re-admitted 25 months after liver transplantation with fever up to 39.0 C and a headache. This patient had undergone a lumbar discectomy 2 months before and had been receiving the nonsteroidal anti-inflammatory medication diclofenac sodium for back pain. The serum ALT and AST, which had been 64 IU/L and 40 IU/L, respectively, 3 weeks before had now risen to 3973 IU/L and 4653 IU/L, respectively, on admission. Both the total bilirubin and alkaline phosphatase also were elevated to 83  $\mu\text{mol/L}$  and 296 IU/L, respectively, up from 10 and 136 three weeks before (Fig. 4). In addition, the serum was mildly positive for HBsAg, HBeAg, and HBV-DNA and HBeAb negative at that time. HBsAb-IgG serum level at this time was 547 IU/L. The HBV-DNA level was 23.8 pg/mL. Two months before, he had been HBsAg and HBeAg negative and he also was HBV-DNA negative by PCR. Two liver biopsy specimens taken at this time (1 week apart) showed non-specific hepatitis histologically on hematoxylin and eosin



**Figure 1.** (A) Representative liver biopsy specimen from a hepatitis B virus liver transplant recipient showing normal histology at 16 months post-transplant. (B) The same liver biopsy specimen as illustrated in A, the results of which show no presence of hepatitis B surface or core antigen with immunoperoxidase.



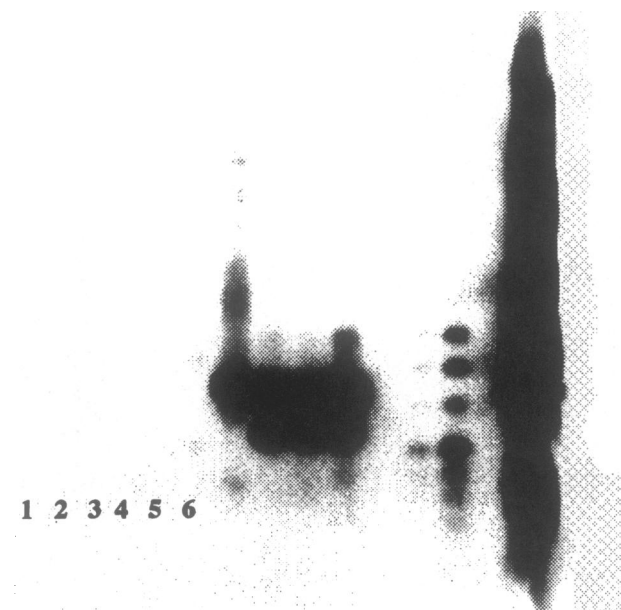
staining. Immunoperoxidase staining for HBsAg and HBcAg, however, failed to show the presence of HBV in the liver both times (Fig. 5). He started receiving gancyclovir 5 mg/kg twice daily for 2 weeks and also was given HBIg 2000 IU daily for 2 weeks. The serum transaminases and serum bilirubin returned to normal (Fig. 4). The results from the liver biopsy taken at the time when the transaminases and the bilirubin still were elevated and before gancyclovir and HBIg also were negative for HBV-DNA by PCR assays (Fig. 6). He now is 39 months post-LT and 15 months since this episode and has normal liver enzymes and is negative for HBsAg, HBeAg, and

HBV-DNA (Table 3). The most recent serum HBsAb-IgG level is 601 IU/L.

## DISCUSSION

Data presented here show that it is possible to prevent hepatitis B recurrence after LT by using some of the lessons learned from perioperative immunoprophylaxis used in surgical infections. There were three guiding principles in the treatment design:

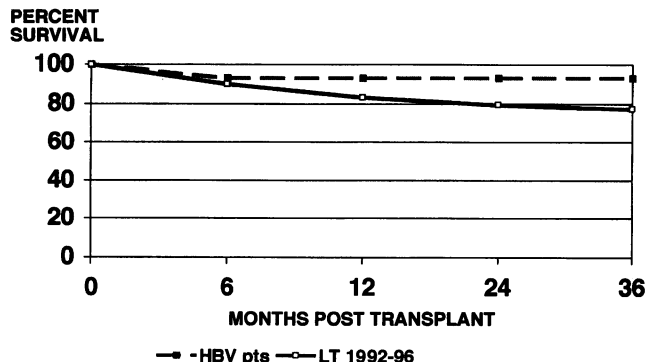
1. To reduce the viral load pretransplant with the use of IFN.



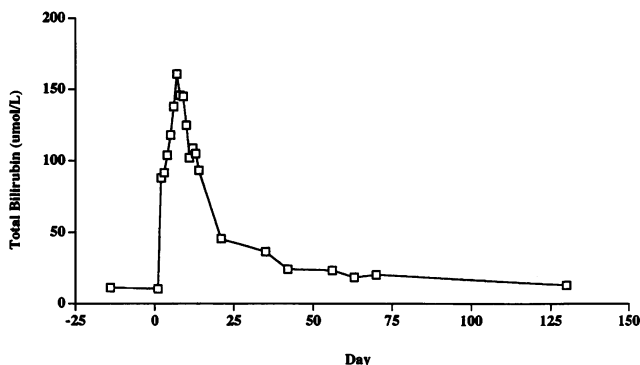
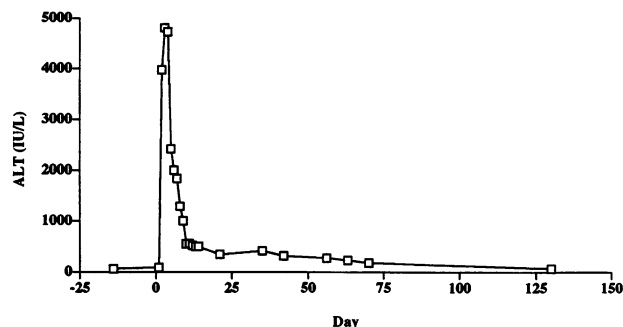
**Figure 2.** Polymerase chain reaction-assisted amplification of the HBV-DNA measurement in liver biopsies taken in six patients 2 years after liver transplantation. Note lanes labeled 1–6 demonstrating no evidence for the presence of HBV-DNA in these liver biopsies. All six patients were also negative for HBV serology and had normal liver enzymes and normal liver histology on Hematoxylin and eosin staining and imr = immunohistochemistry.

2. To use strong immunoprophylaxis with HBIG during periods of heavy immunosuppression, such as induction and rejection therapy, and to keep HBIG levels sufficiently high to prevent recurrence.
3. To reduce immunosuppressive drugs to a bare minimum necessary to prevent rejection.

This study has too limited a number of patients to delineate exactly which of these strategies is the most important. It would be possible to refine this protocol to define the dose



**Figure 3.** Actuarial patient survival in 13 HBV recipients and all 86 other patients undergoing transplants for other primary diagnoses between February 1992–August 1996.



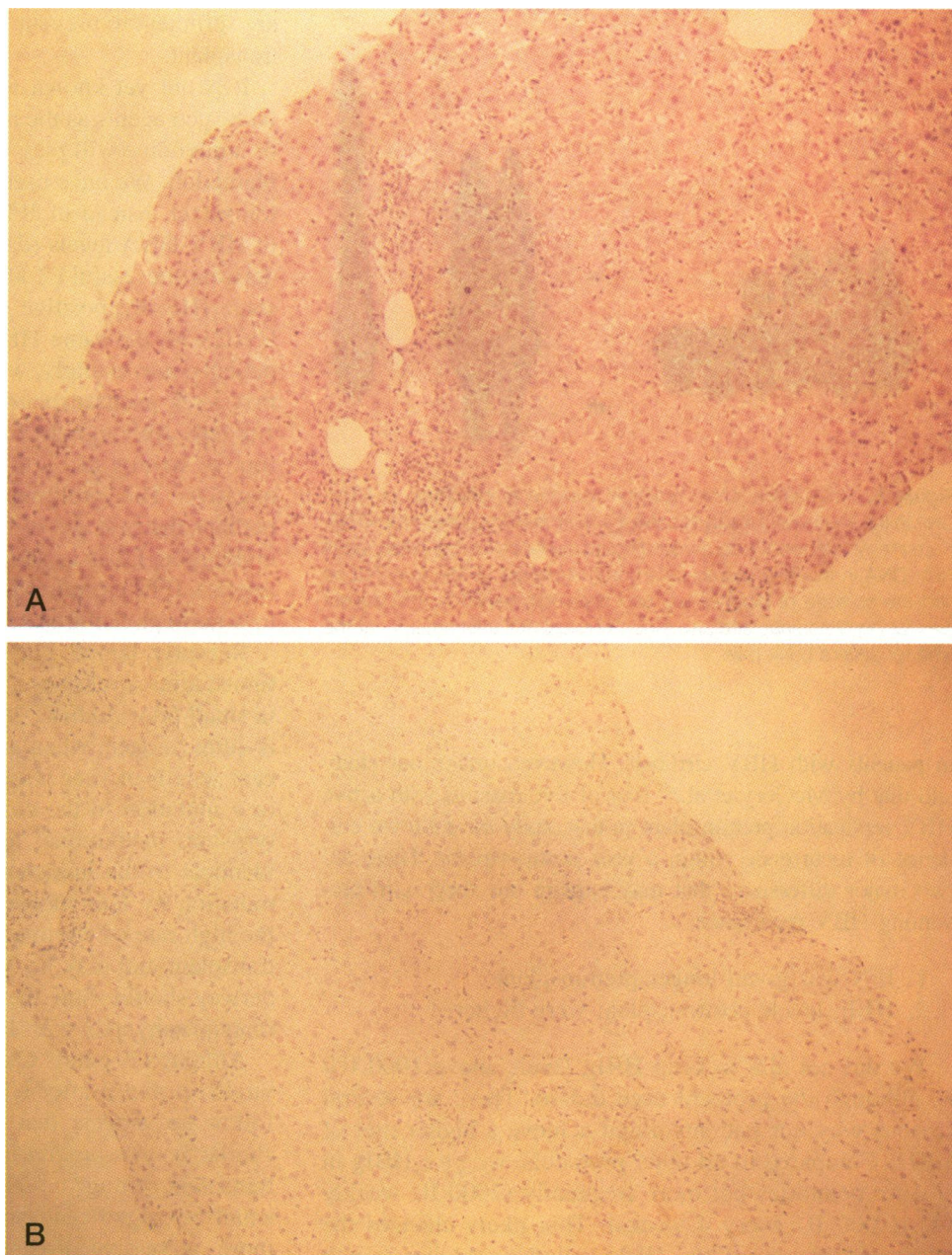
**Figure 4.** Patient 4 who developed hepatitis during a treatment course with Diclofenac Sodium for lumbar pain. His serum was transiently HBsAg positive, but both liver biopsies performed at the peak of the rise in LFT's were negative for HBV by immunoperoxidase staining and PCR.

and the frequency of HBIG used posttransplant. It also would be important to know if HBIG is needed forever or if the dose can be reduced even further and when. In one patient, liver enzymes rose 50-fold and hepatitis was seen on results of liver biopsy 25 months after LT (patient 3, Table 3). However, this patient was taking diclofenac sodium (a nonsteroidal anti-inflammatory) for back pain after lumbar discectomy. The serum HBsAg, HBeAg, and HBV-DNA were transiently low positive but became negative again 12 weeks after this incident and have remained negative since then. Immunoperoxidase staining of the biopsy specimen showing nonspecific hepatitis was negative for HBsAg and HBeAg by immunoperoxidase staining and by HBV-DNA testing of a liver biopsy specimen with PCR. This patient in retrospect had most likely an diclofenac sodium-induced hepatitis, and there is no evidence then or now that the allograft was ever actively infected with HBV.

In the past, LT for HBV cirrhosis has been unsuccessful because of a high rate of recurrence,<sup>1-7</sup> associated with premature cirrhosis and death from liver failure. This was true especially in patients with active HBV replication (i.e., HBeAg positive or HBV-DNA positive).<sup>10,11,15,16</sup> Samuel et al.<sup>10</sup> and other studies<sup>15,16</sup> now have shown that HBIG given



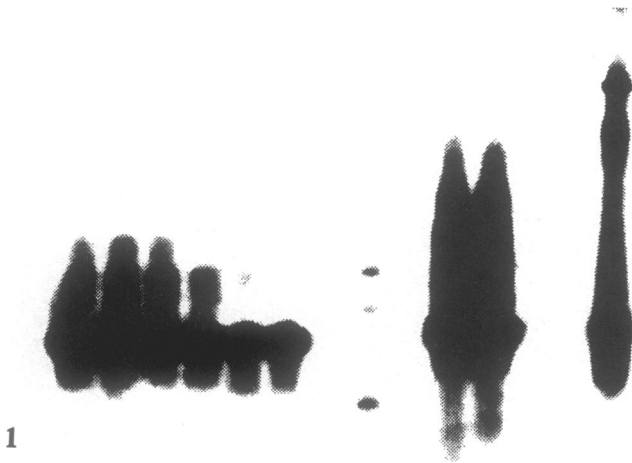
**Figure 5.** (A) A hematoxylin and eosin ( $\times 100$  amplification) of a liver biopsy of patient 4 (Table 3) at 25 months posttransplant showing significant portal and lobular hepatitis. The patient at that time was taking diclofenac sodium (a nonsteroidal anti-inflammatory) for back pain. (B) The same allograft biopsy specimen as in (A), the results of which show negative staining for hepatitis B surface and core antigen with immunoperoxidase.



for a long time, perhaps indefinitely, after LT for HBV cirrhosis prevents recurrence of hepatitis in some patients who are HBsAg positive but not in those with active HBV replication at the time of LT. Recurrence rate of HBV was  $>50\%$  in patients with cirrhosis who were only HBsAg positive but  $>85\%$  in those who had evidence of active HBV replication. The difference between these patients and ours was the serum level of HBIg achieved: 100 IU/L versus  $>1000$  IU/L. Reviewing the HBIg protocol in these studies shows that our patients received substantially less total HBIg in the first 2 years posttransplant yet achieved higher levels. The only study that has achieved a relatively low recurrence rate was

McGory et al.<sup>20</sup> They have achieved a 30% recurrence rate while keeping HBIg serum levels over 500 IU/L. To achieve this, however, very high doses of HBIg were used, especially in the first 90 days posttransplant and especially in patients with active pretransplant HBV replication. Unlike others who have used HBIg prophylaxis, our protocol uses two distinct principles of antiviral therapy: 1) an active immunostimulator, IFN, pretransplant to decrease active viral replication, and reduce it to a very low level at the time of transplant, and 2) immunoprophylaxis with HBIg posttransplant to prevent HBV recurrence and reinfection of the new allograft. Marcellin et al.<sup>21</sup> is the only other group to report a similar strategy





**Figure 6.** Polymerase chain reaction assisted amplification of HBV-DNA in the same liver biopsy in patient 4 illustrated in figures 5A and 5B. Note the lane labeled 1 demonstrating no evidence for the presence of HBV-DNA in this liver biopsy taken on this patient at the time that he had the episode of biochemical and histologic hepatitis. The patient was taking diclofenac sodium, a nonsteroidal anti-inflammatory at the time for severe back pain.

in patients with HBV cirrhosis. However, unlike our study and that by McGory et al.,<sup>20</sup> four of five patients with active HBV replication pretransplant in that study have shown evidence of recurrence within a year posttransplant. There are two major differences that may explain our success in preventing HBV recurrence:

1. IFN was given longer pretransplant.
2. HBIg levels posttransplant were higher.

We did not aim to keep HBIg levels above 1000 IU/L, although the protocol used did so. There are several reasons why our patients might achieve a higher titer of HBIg. Compared to all other protocols, we give HBIg in smaller quantities but more frequently (2000 IU weekly vs. 10,000 IU every 4 weeks). This likely changes the pharmacokinetics of the HBIg, allowing higher trough levels. In addition, our patients are all converted to a nonreplicating status at the time of transplantation. McGory et al.<sup>20</sup> found a direct correlation between the amount of HBIg required to maintain a desired high serum level and HBeAg positivity. This means that transplanting patients with active viral replication requires high doses of HBIg to prevent recurrence. Thus, our patients who are converted to a low viral load with IFN pretransplant can achieve high HBIg titers with lower doses and have a more secure HBV prophylaxis. It would be interesting to speculate whether our patients might require even less HBIg than was actually given to achieve adequate HBIg titers. Clearly our study and the one reported by McGory et al.<sup>20</sup> show that higher HBIg titers (perhaps 500 IU/L)

are sufficient to prevent HBV recurrence in patients posttransplant.

It is not yet known what role antiviral chemotherapy with such agents as the nucleoside analogues famcyclovir or lamivudine will play in patients with HBV after transplantation. We have used lamivudine in two patients pretransplant instead of IFN and have achieved a decrease of HBV-DNA levels (data not shown). Both patients underwent successful LT after converting to a nonreplicating stage (HBsAg positive only) and were immunoprophylaxed with the same HBIg protocol posttransplant. Both patients, interestingly, were HBsAg positive still after the first week posttransplant but became HBsAg negative at 1 month. Although the follow-up is not long enough at the time of writing this article, both patients still are HBsAg negative. This preliminary result lets us speculate that perhaps the best use of lamivudine in patients with HBV undergoing LT is as an alternative in patients who cannot tolerate IFN pretransplant when trying to convert them to a nonreplicating state.

Recently, lamivudine was used before surgery and continued after surgery as a single immunoprophylaxis agent in small pilot studies.<sup>22-24</sup> Recurrence with a mutant form is quite frequent when patients are observed for a significant length of time. Recurrence after lamivudine is due to a mutation in the conserved polymerase domain, the tyrosine, methionine, aspartate, aspartate locus and is identical to the human immunodeficiency virus-induced mutation by lamivudine. It is thought to be induced by the high rate of viremia due to immunosuppression posttransplant and may be 30% or more. Further studies underway should show the true efficacy of single-agent immunoprophylaxis with lamivudine after LT.

Although hepatitis C also is associated frequently with viral recurrence after LT, several studies with long-term follow-up suggest that recurrence is associated with a similar benign progression of the disease as in the nontransplant setting.<sup>25-28</sup> However, the same studies show a small but significant percentage of patients who have a more aggressive disease similar to HBV recurrence. It can be speculated, although the urgency and clinical results may not be as dramatic, that a similar approach for patients with HCV may be of benefit to some of those with more severe recurrence posttransplantation.

This study is the first to show that complete prevention of HBV recurrence with excellent long-term patient survival can be achieved by applying the principles of viral load reduction pretransplant with IFN, adequate immunoprophylaxis posttransplant with HBIg, and minimizing immunosuppression long term with CsA monotherapy.

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## Discussion

DR. J. WESLEY ALEXANDER (Cincinnati, Ohio): I congratulate the authors on their excellent presentation and on their outstanding results. This study represents what can be achieved by an accomplished surgical scientist, as discussed by Dr. Barker in his presidential address, i.e., the combination of sound surgical skills with a broad knowledge of basic science, which is in this instance, both transplant biology and infectious disease. Applications of the principles that guided this protocol, i.e., reduction in pretransplant viral load, postoperative immunoprophylaxis, and minimal long-term immunosuppression, have culminated in results heretofore not achievable.

After listening to the presentation and reading the paper, I have several questions and comments.

First of all, how were the injections of the hepatitis B immunoglobulin tolerated? This was 10 mL intramuscularly daily, which sounds awfully painful to me. We were giving this dose of antilymphocyte globulin intramuscularly in the early days of transplantation and found the patients really had a hard time. Can this be given intravenously? If so, what would be the relative titers?

Second, how would you deal with patients who are having fulminant hepatitis or rapid decompensation before the therapy with interferon could be accomplished? In particular, would lamivudine be a preferred drug in such instances, and have you had experience with this? Our experience with interferon-alpha given during the postoperative period in kidney transplants has shown that there is a relatively high loss of kidneys from rejection. In your paper, you did not mention the need for doing this, but I wonder what would be your drug of choice during the postoperative period.

Next, are there patients who would be excluded from your protocol, or would you take all patients who come?

In your paper, you mention that there were four patients with hepatocyte carcinoma and none of these patients have had evidence of recurrence over a long period of time. One of these was a relatively large tumor, as I recall, about 10 cm in size. I wonder if this protocol has any particular value in that subset of patients?