Failure of a Reinforced Triple Course of Hepatitis B Vaccination in Patients Transplanted for HBV-Related Cirrhosis

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Long-term immunoprophylaxis with anti-HBs immunoglobulins (HBIg) is used to prevent hepatitis B (HBV) reinfection after liver transplantation for HBV-related cirrhosis. This approach is highly expensive. A recent report proposed posttransplant HBV vaccination with a reinforced schedule as an alternative strategy to allow HBIg discontinuation. We investigated the efficacy of a reinforced triple course of HBV vaccination in 17 patients transplanted for HBsAg—positive cirrhosis 2 to 7 years earlier. The first cycle consisted of 3 double intramuscular doses (40 μg) of recombinant vaccine at month 0, 1, and 2, respectively. This was followed, in nonresponders, by a second cycle of 6 intradermal 10 μg doses every 15 days. All nonresponders then received a third cycle identical to the first one. Vaccination started 4.5 months after HBIg discontinuation, and lamivudine (100 mg/day) was given throughout the study. All patients were seronegative for HBsAg and HBV-DNA (by PCR) and positive for anti-HBe, and 7 were positive for anti-HDV. After the first cycle one patient (#5, 53 years old, male) developed an anti-HBs titer of 154 IU/L, another (#12) reached a titer of 20 IU/L and the remainder had titers <10 IU/L. At month 7, patient #5 reached a titer of 687 IU/L. After the second cycle only one additional patient (#9) had a slight response (an anti-HBs titer of 37 IU/L). After the third cycle patient #9 rose to an anti-HBs titer of 280 IU/L, patient #12 dropped to 10 IU/L, and no other patient responded. In conclusion, a highly reinforced HBV vaccination program is effective only in a few patients who had liver transplants for HBV—related cirrhosis. (HEPATOLOGY 2002;35:176-181.)

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HBV reinfection after liver transplantation for HBV-related cirrhosis is frequent in the absence of immunoprophylaxis and is associated with poor prognosis, resulting from the development of cirrhosis, fibrosing hepatitis, or fulminant hepatitis.1–2 The introduction of the long-term use of anti-HBs immune globulins (HBIg) has allowed the reduction of graft reinfection to acceptable levels, resulting in survival rates comparable with, or even better than, those of other indications.3–5 This passive immunoprophylaxis strategy is therefore currently considered the standard treatment to prevent HBV reinfection after liver transplantation for HBV-related cirrhosis.6 Yet, using this approach, posttransplant HBV recurrence still occurs in 10% to 30% of cases, possibly depending on a variety of factors, such as the amount of HBIg infused, the route and frequency of administration, and the minimum trough levels of anti-HBs maintained during various periods after transplant.6 According to current recommendations, HBIg should be administered indefinitely after transplant. This is associated with a number of problems, including the limited availability of HBIg, the possible emergence of HBV envelope protein mutations,7,8 and/or other adverse events,9,10 and, most importantly, the extremely high costs.6 Whatever is the prophylactic regimen used (which varies remarkably from one Center to another) and the cost differences of HBIg preparations between U.S. and most Western European countries, the expenses of post-transplant HBIg administration range between $25,000 and $120,000 per year, thus exceeding in a few years those of an uncomplicated transplant.

New prophylactic strategies to prevent posttransplant HBV recurrence are therefore needed. New approaches under evaluation include the combination of lower doses of HBIg with antivirals, such as lamivudine,11,12 and HBV vaccination, as proposed in the recent provocative study of Sanchez-Fueyo et al.13 from Spain.
HBV vaccination, however, is known to be poorly effective in immunocompromized patients, such as patients undergoing long-term hemodialysis. In addition, the seroconversion rate has been reported to be very low after HBV vaccination of patients who had liver transplants for non-HBV related cirrhosis and in patients with chronic hepatitis C, and anecdotal reports in HBV transplant recipients had been unsuccessful. In the Spanish study, a group of HBV nonreplicative liver transplant recipients with normal liver function were discontinued from HBIG administration and received a double course with a double intramuscular dose of a standard recombinant HBV vaccine. Notably, more than 80% of patients were reported to have a serological response, more often occurring after the second vaccination course. In most patients, however, the anti-HBs titer remained in the low, potentially unprotective range, and no long-term follow-up data were provided.

Because of the uncertainty of these results and the previous disappointing experience in immunocompromized patients, we planned this study to investigate, in a selected group of transplant recipients at low risk for HBV recurrence, the immunogenicity of a new reinforced schedule of HBV vaccination based on the use of high doses of vaccine given through both the intramuscular and the intradermal route. The latter route of administration was tested because of recent reports showing a greater efficacy of intradermal versus intramuscular hepatitis B revaccination in nonresponsive chronic dialysis patients.

Patients and Methods

Patients. The study included 17 patients without virological and biochemical evidence of HBV recurrence and with normal liver function who had been transplanted for HBV-related cirrhosis 25 to 85 (median 48) months earlier. All patients were HBsAg seropositive and HBV-DNA seronegative by standard hybridization methods at time of transplant; 7 (41%) were also anti-HDV–positive, and one was anti-HCV–positive. To be included in the study, patients had to be free of any type of posttransplant disease, including severe episodes of rejection. All patients received high doses of HBIG during surgery and in the first week after transplant; thereafter they were maintained on 5,000 IU intravenously per month. All patients became HBsAg negative in serum immediately after transplant. Absence of posttransplant HBV recurrence at time of the study was ascertained in all cases by the absence of HBsAg and HBV-DNA (by PCR) in at least 2 consecutive serum samples and by the absence of HBsAg and HBeAg at immunohistochemical examination of the last available liver biopsy. Liver and kidney functions were normal in all cases. Cyclosporin A microemulsion was the only immunosuppression regimen administered for at least 1 year before and during the entire study. Demographic and baseline clinical and virological features of individual patients are listed in Table 1. All patients gave a written informed consent to the study protocol, which was approved by the local ethics committee and conducted in accordance with the Declaration of Helsinki principles.

Study Protocol. Four weeks after the last HBIG administration, patients were started on lamivudine (100 mg/day), which was then continued indefinitely. HBV vaccination was started when the anti-HBs titer dropped below 10 IU/L, which occurred at a median of 4.5 months (range 2-8) after the last HBIG dose. The vaccination protocol (Fig. 1) comprised the administration of up to 3 cycles of recombinant HBsAg vaccine (Recombivax HB, Pasteur Merieux/MY, Lyon, France). Each vaccine ampoule comprised recombinant HBsAg antigen (10 or 40 μg), thimerosal (50 μg), thimerosal (50 μg), and a protein A microemulsion. Each vaccination course comprised 3 doses of recombinant HBsAg vaccine given intramuscularly at 0, 1, and 6 months.

Table 1. Demographic, Clinical, and Virological Features of Individual Patients

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<th>Anti-HCV*</th>
<th>Follow-up After OLTx (months)</th>
<th>Immunosuppression (mg/kg) *</th>
<th>Anti-HBs Titers (IU/L)</th>
<th>Anti-HBs Titers (IU/L)</th>
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Abbreviation: CyA, Cyclosporine A microemulsion.

*At time of vaccination.
†HBV-DNA status determined by liquid hybridization assay at time of transplant.
‡HBV-DNA status determined by protein-chain-reaction before the vaccination course.
μg), aluminium hydroxide (0.5 mg), sodium chloride (9 mg), formaldehyde (<20 μg), and potassium thiocyanate (<0.5 μg) in 1 mL of sterile water. The first cycle consisted of 3 intramuscular doses of 40 μg of vaccine, given at month 0, 1, and 2. Patients developing at month 3 a serum anti-HBs titer $\geq 100$ IU/L were considered as early responders and were given a fourth intramuscular 40 μg dose of vaccine at month 6. Patients with any anti-HBs titer below 100 IU/L (early non-responders) received a second vaccination cycle, consisting of the administration of 6 intradermal 10 μg dose of vaccine given every 15 days for 3 months. All patients who failed to respond according to the previous definition received a third vaccination cycle, consisting of standard immunoprophylaxis with intravenous HBIg. Anti-HBs titers were measured at monthly intervals during each vaccination cycle and one month after the completion of each individual cycle, using a third generation immunoenzymatic assay (AUSAB EIA, Abbot, Baar, Switzerland).

**Results**

**Response to the First, Second, and Third Vaccination Cycles.** No HBV recurrences nor any side effect occurred during the study. All patients were HBV-DNA negative by PCR before and at the end of the study. The anti-HBs response to the first, second, and third cycle is shown in Fig 2. After the first cycle, the overall rate of response was only 5.9%. In fact, only one patient (#5, male, 53 years old) developed an early serological response, reaching a titer of 687 IU/L at month 3, whereas another (#12, male, 48 years old) had a slight response, reaching a 3-month titer of 20 IU/L. All the remaining patients did not respond at all (titers from 0 to 8 IU/L). Thus, according to the study protocol, 16 patients underwent the intradermal second vaccination cycle. At the end of the second cycle, no further patient responded, except one (#9, female, 49 years old) who developed a slight response (reaching a titer of 35 IU/L at month 6). Thus, even after the second cycle, the overall rate of response, according to our definition, was 5.9%. All the 16 nonresponders, therefore, underwent the third, intramuscular, vaccination cycle. At the end of the third cycle none of the patients developed an anti-HBs antibody response, except patient #9, who at month 10 developed an anti-HBs titer of 253 IU/L. In contrast, patient #12 had no further increase in the anti-HBs titer, which dropped to less than 10 IU/L.

As a whole, 2 (11.8 %) of 17 patients developed a titer $>100$ IU/L and 3 (17.6 %) a titer $>10$ IU/L at the end of the 3 courses of vaccination. According to the study protocol, all patients but 2 were, therefore, returned at the end of the study to standard immunoprophylaxis with intravenous HBIg.

**Follow-up of the Early and Late Responders.** The subsequent follow-up of the only 2 responders are shown in Fig. 3. Patient #5 (A) had a slight decline of anti-HBs titer, which was still greater than 250 IU/L 14 months after the fourth 40 μg intramuscular dose of vaccine. This patient will receive an additional dose of vaccine within 3 to 6 months. Lamivudine administration was interrupted in patient #5 one month after the fourth intramuscular dose, based on the evidence of a protective anti-HBs titer, and for the same reason he has not been returned to HBIg immuno prophylaxis.

Patient #9 (B) had a more rapid decline of anti-HBs titer, which dropped to 37 IU/L 4 months after the end of the third vaccination cycle. She received a further 40 μg intramuscular dose of vaccine, which was again followed by a modest response above 100 IU/L. This patient has not yet been returned to HBIg immunoprophylaxis, but she is continuing to receive lamivudine.

**Discussion**

Although new strategies are currently under evaluation,6,11-12 the standard treatment to prevent posttransplant HBV recurrence at most transplant centers is the administration of intravenous or intramuscular HBIg on a lifelong basis. This strategy is not only cumbersome but also highly expensive and not devoid of potential adverse events.6-10 A much simpler and cheaper alternative would be the development of an effective vaccination program. This pos-

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**Fig. 2.** Anti-HBs titers during and after the first, second, and third cycle of vaccination in individual patients. Numbers indicate patients who developed an anti-HBs response.
responded to HBV vaccination. (A) Patient #5, (B) patient #9.

The epidermis is known to be rich with antigen presenting cells, making it an appropriate target for vaccine delivery.20 Abnormally. The condensation intramuscular vaccination schedule using the intradermal route is more immunogenic compared with the intramuscular route in immunocompromised patients,25,26 given that HBV reinfection is rare beyond 18 months after grafting.3 Regardless of the definition of response, alternative explanations should be considered for the divergent results between our study and that of Sanchez-Fueyo et al.13 The 2 studies used a yeast-derived recombinant vaccine containing only the major HBV envelope protein (S-protein). Both included the same number of transplant recipients (17 each) with similar demographics, all of whom were HBsAg-positive before transplant and HBe antigen– and HBV-DNA–negative at time of the study. However, our study included only patients transplanted because of HBV-related end-stage cirrhosis, whereas Sanchez-Fueyo et al.13 included 6 patients transplanted for HBV-related fulminant hepatitis. It is known that the latter subgroup of patients is at lower risk of recurrent HBV infection compared with patients transplanted for HBV-related cirrhosis, which could partially explain the observed seroconversion rate.

The observed high seroconversion rate by Sanchez-Fueyo et al.13 was attributed to the large dose of vaccine used and to the long time elapsed after transplant, which allowed them to markedly reduce the immunosuppressive therapy. However, the total dose of vaccine employed in our study was greater compared with that used by Sanchez-Fueyo et al.13 (300 μg vs. 240 μg), and the mean time elapsed between transplant and vaccination was longer (48 vs. 30 months), suggesting that the length of the antigen-free time is not an important determinant of the immune responsiveness. In addition, 5 patients in the study of Sanchez-Fueyo et al.13 were given steroids compared with none in our series. A further difference is that the 2 intramuscular vaccination courses given in our study were accelerated (3 doses in 2 months) compared with the conventional 6-month course in Sanchez-Fueyo et al.13 study, but it seems unlikely that this difference may explain the discrepancy between the 2 studies.27 Another potentially relevant difference is that Sanchez-Fueyo et al.13 started the vaccination at a median of 2 weeks after the last HBlg infusion, whereas we performed a wash-
out period of 2 to 8 months (median 4.5 months) to allow the anti-HBs titer to drop below 10 IU/L. Thus, it is possible that some patients in Sanchez-Fueyo’s study already had an unrecognized seroconversion at time of vaccination. Finally, one cannot exclude the possibility that starting the vaccine before the disappearance of anti-HBs from blood favored the formation of antigen-antibody complexes with enhanced immunogenicity, as suggested by the results of studies in mice.28,29 The latter hypothesis, however, seems unlikely as most patients responded only after the second vaccination cycle.

A further relevant difference between the 2 studies could be the use of lamivudine, which was given to all patients in our study, but to none in the study of Sanchez-Fueyo et al.13 Lamivudine has been shown to restore the hyporesponsiveness of cytotoxic T-lymphocyte in patients with chronic hepatitis B.30,31 This effect would be unlikely to cause a lower response rate to vaccination. It also seems unlikely that failure to respond to vaccination in our study was attributable to concomitant viral replication, as HBV-DNA was repeatedly negative at PCR.

HBV vaccination is highly effective in healthy individuals, leading to seroconversion rates greater than 90%.25,26,32 However, the results in patients with a suppressed immune system, have been disappointing.33 HBV vaccination has also been shown to be poorly effective in nontransplanted patients with advanced liver disease34,35 including patients with chronic hepatitis C, who showed a remarkably low seroconversion rate.17 The decreased response to HBV vaccination in patients with chronic hepatitis C was attributed to conditions potentially affecting the immune response.36,37

In conclusion, we have shown that a highly reinforced HBV vaccination program (double dose, triple course, and different routes) using the standard recombinant HBsAg vaccine is effective only in a minority of patients who had liver transplants for HBV-related cirrhosis. Alternative strategies should be explored in this setting, including the use of HBV vaccines with improved immunogenicity, such as those containing the pre-S/S recombinant antigens.

References
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