Immune therapy of hepatitis B virus (HBV) chronic infection. European experience

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Treatment of HBV chronic infection is mandatory because chronic hepatitis is associated with a 20% risk of cirrhosis which exposes to a 2 to 5% annual incidence of hepatocellular carcinoma (1).

Standard antiviral therapy (alpha-Interferon (α-IFN) 5 MU subcutaneously thrice weekly for 4 to 6 months) leads to a sustained inhibition of HBV replication in around 40% of the patients and eventually loss of HBsAg in around 15% (2-3). Discontinuation of HBV replication is usually associated with a significant clinical improvement including survival of patients (4). Lamivudine, a nucleoside analogue, is highly efficient in controlling HBV replication but its long term efficacy is limited by the risk of both relapse after lamivudine discontinuation (85%) and breakthrough when treatment is continued (15 to 20% yearly) (5). The partial efficacy of antiviral therapies, their cost (around 3 000 US $ for each of them), their side effects explain the emergence of new therapeutical approaches in HBV infection including immunostimulation (by thymic derivates such as thymosin, thymopentin or by growth factors such as GM-CSF), adoptive transfer of HBV immunity in experimental models and in humans (bone marrow recipients) or vaccine therapy.

Indeed, vaccine therapy, in preliminary, yet promising trials, has now been used in various infectious disease (6-7). Various lines of experimental and clinical evidence suggest that anti-HBV vaccine therapy may be effective in treating HBV infection (8-10). In a previous pilot study in HBsAg chronic carriers with chronic hepatitis, we have established that specific vaccine therapy by a standard anti-HBV vaccination may be efficient in reducing HBV replication and cancelling the immune tolerance of HBsAg particles in half of patients : over the 3-month period following a complete 3 injections vaccination-shedule (i.e. 6 months after the first vaccine injection), serum HBV DNA became undetectable in 12 of the 46 patients (26.1%) and eight additional patients (17.4%) showed a significant decrease (more than 50%) in HBV replication ; these 8 patients finally lost serum HBV DNA replication, one 12 months after the first vaccine without other treatment and 7 after starting α-IFN within a mean time of 2.8 months (11). These results have been confirmed by Chinese authors using the combination of vaccine and immunotherapy (12). On this basis, we have performed a multicenter controlled study of vaccine therapy in treating chronic hepatitis B to evaluate the efficacy and potential side effects of HBV vaccination in HBV-related chronic hepatitis (submitted for publication).

The 118 included patients were "naive" subjects who had never received any previous anti-HBV therapy, showed detectable serum HBV DNA and had biopsy-proven chronic hepatitis. In a 12-month follow-up they were given either 5 intramuscular injections of 20 µg of a PreS2/S (GenHevac B®, Pasteur-Mérieux) (n = 47) or an S vaccine (Recombivax® Merck, Sharp and Dhome)(n = 34) or no treatment as control (n = 37). The efficacy of vaccination was evaluated by testing for serum HBV DNA negativation using a standard liquid hybridization assay. At 6 months, i.e., 3 months after the first 3 vaccine injections, the percentage of serum HBV DNA negativation was higher in the vaccine groups (19.1 and 11.8% in GenHevac B® and Recombivax® groups, respectively) than in the control group (2.7%) ; there was no significant difference between the two vaccines but a significant difference between pooled vaccinated (15.5%) and unvaccinated patients ($p = 0.038$, by the chi-square Pearson test). Negativation of serum HBV DNA at the sixth month was more frequently observed in patients who had pretreatment viremia ≥ 200 pg/ml (none in the control group vs. 16.7% in the vaccinated groups) ($p = 0.025$), whilst there was no difference between non-vaccinated and vaccinated

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Presented at the International Symposium on Viral Hepatitis beyond the Millennium Session of December 10, 1999.

subjects with pretreatment viremia below 200 pg/ml. After one year follow-up and 5 vaccine injections, there was no difference in the rate of serum HBV DNA negativation (around 25 and 30%) between vaccinated and unvaccinated subjects. However, in those patients who maintained HBV replication during the study period, HBV vaccines significantly decreased the HBV viral load between the sixth and twelfth months ($p = 0.04$) in contrast with the control group. The rate of HBe/antiHBe seroconversion did not statistically differ between the vaccinated and unvaccinated groups, but early HBeAg negativation and anti-HBe detection after 6 months of follow-up occurred only in vaccinated patients (8 and 15% in vaccinated patients compared to 0% in the controls). Disappearance of serum HBsAg was not observed in any of the patients.

This study offers the first direct evidence, based on a controlled study, that the HBV vaccine may decrease HBV replication without side effects in chronic hepatitis B patients, and paves the way for new therapies based on the concept of specific immunomodulation. However, the limited efficacy of current HBV vaccines suggests that different HBV antigenic preparations and immunization protocols should be considered in the future.

To better understand the mechanisms of action of vaccine therapy, we analyzed the vaccine-induced immune responses in 40 patients of the controlled trial (13). Vaccination elicited PBMC proliferative responses specific for envelope antigen in 7 among 27 patients who received and in none of the 13 who did not receive HBsAg. The responses induced by the vaccines were mediated by the $CD4^+$ T lymphocytes and at least 3 different epitopes were recognized. HBV-specific $CD4^+$ T lymphocytes produced high levels of gamma interferon and belonged to the T helper-1 subset. Reduction of serum HBV-DNA in some of these patients suggests that induction of $CD4^+$ T cell responses could be important in controlling viremia during vaccine therapy of HBV chronic carriers. Indeed, immune clearance of HBV depends on T-helper cells which secrete cytolytic cytokines and activate specific CTL resulting in cytolysis of infected cells and direct secretion of antiviral cytokines. Recently, a pilot study of a CY-1899 T-cell vaccine in subjects chronically infected with HBV has been reported (14). Administration of this single-epitope vaccine initiated cytotoxic T lymphocyte lymhocyte (CTL) activity by using a ^{51}Cr release assay to test cytolytic activity of restimulated T-cell cultures in majority of patients, including asymptomatic carriers, who had no CTL response at baseline ; but the magnitude of the CTL activity was lower than that observed during spontaneous HBV clearance and not sufficient to induce viral clearance. Although this is a negative study with respect to therapeutic efficacy, authors suggest that further studies using the CY-1899 vaccine are required in combination with an antiviral and/or a more potent multiepitope vaccine.

In summary, HBV vaccine therapies have shown some promises. Antigen vaccine therapy may induce specific proliferative (and CTL) response which may result in decrease or withdrawal of HBV replication in some patients. Immunization strategies should to be reinforced (new vaccines including pre-S1/pre-S2/S, repeated doses, higher amount of vaccine antigens, DNA vaccine) and combined with multiple nucleoside analogues to associate synergistically immunomodulatory and antiviral approaches.

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