Immunotherapy of chronic hepatitis B by anti HBV vaccine

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Abstract

Vaccine therapy is now used in various infectious diseases. The hepatitis B virus (HBV) leads to chronic infection in around 5% of patients with a high risk of chronic active hepatitis which may result in cirrhosis and hepatocellular carcinoma. The partial efficacy of antiviral therapies (40% of sustained inhibition of HBV replication), their cost, their possible side effects and the immune-mediated pathology of HBV infection explain the need of new immune therapies in treating HBV infection. Experimental and clinical evidences suggest the usefulness of vaccine therapy in HBV chronic infection.

In a pilot and opened study, forty-six consecutive chronic HBsAg carriers with chronic hepatitis and detectable serum HBV DNA were given 3 standard injections of the GenHevac B® vaccine at one month interval. Six months after the first injection, 12 patients (26.1%) had undetectable HBV DNA while 8 others showed significant decrease (more than 50%) in HBV DNA titers. Six of these 12 responders received a standard course of α-Interferon (5 MU thrice weekly subcutaneously for 4 months) and all six had still undetectable HBV replication at the end of follow-up. Among the 34 non responders to vaccine, 20 were given α-interferon and 2 the monophosphate derivate of Vidarabine : 12 of these 22 patients stopped HBV replication and in all 12, vaccine therapy had induced a significant decrease of HBV replication before the antiviral treatment with a decrease of mean serum HBV DNA from 392 pg/ml before to 217 pg/ml after vaccine therapy.

In an ongoing controlled study, using the same vaccine schedule, serum HBV DNA disappeared more frequently after 6 months, in patients who were given a preS2/S vaccine (7/35) than in patients who received a S vaccine (1/21) or no vaccine (1/32). In responders to vaccine, an induction of specific proliferative responses was observed and this may contribute to the potential efficacy of anti-HBV vaccine therapy. No side-effect or vaccine-induced escape-mutants occured during the follow-up.

In summary, serum HBV DNA disappeared in 28 of the 46 patients (60.9%) who were given vaccine therapy, with (64.2%) or without (55.6%) Interferon. These results are not different at 6 months and at the end of follow-up from those of 43 HBsAg chronic carriers who were given only an antiviral treatment. Active immune therapy against HBV appears efficient and less expensive than antiviral therapies in stopping HBV replication. Such results need to be confirmed by the completed results of our controlled, randomized trial which is now conducted in our unit. (Acta gastroenterol. belg., **1998**, 61, **228-233**).

Introduction

HBV is an hepadnavirus which is transmitted parenterally, sexually and by mother to child infection. After an usually mild or asymptomatic acute infection, the risk of chronicity in immunocompetent adults is around 5% (1). It is increased in immunocompromised hosts and reaches 40% in HIV-infected, 60% in hemodialyzed patients, 90% in newborns of HBsAg-HBV DNA-positive mothers and almost 100% in renal or liver transplant recipients. Two thirds of patients with chronic HBV infection have chronic hepatitis with increased aminotransferase levels, biopsy-proven necrosis, inflammation and fibrosis and serum markers of viral replication (HBeAg and HBV DNA) (2) ; they contrast with the so-called "healthy" carriers who seem to be immunologically tolerant to HBV with histologically normal liver, normal aminotransferase levels and absence of markers of viral replication (3). Chronic hepatitis is associated with a 20% risk of cirrhosis which exposes to a 2 to 5% annual incidence of hepatocellular carcinoma (4). The epidemiological link between cirrhosis, HCC and chronic HBV infection is well demonstrated (5). This global problem highlights the need of : firstly early and efficient treatments for infected population (6) and secondly world fighting policy of vaccination against HBV which has been available for more than a decade and which should allow eradication of the infection.

Standard antiviral therapies (Vidarabine, its monosphosphate derivate ARA-AMP and alpha-Interferon (α-IFN) 5 MU subcutaneously thrice weekly for 4 to 6 months) lead to a sustained inhibition of HBV replication in around 40% of the patients (7-8) ; 5 to 10% of treated patients eventually loose HBsAg (7-9). Loss of serum markers of replication (HBeAg and HBV DNA) is usually associated with disappearance of replicative free HBV DNA in the liver and complete clearance of HBV DNA in the serum (10). The disappearance of viral replication is associated in two thirds of the patients with a flare of aminotransferase activities. These figures should be compared to a yearly 5 to 10% spontaneous rate of HBeAg to antiHBe antibodies seroconversion and HBV DNA negativation which is usually accompanied by an exacerbation of the liver disease. Positive predictive factors of response to antiviral therapies have been identified and include young age, history of acute hepatitis, short disease duration (less than 2 years), high aminotransferase levels (more than 3 times the upper normal value) and low HBV DNA levels (6).

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

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in HBV infection : immunostimulation (by thymic derivates such as thymosin, thymopentin or by growth factors such as GM-CSF (11-12), adoptive transfert of HBV immunity in experimental models and in humans (bone marrow recipients) (13) or vaccine therapy (14).

Chronic viral, bacillar and parasitic infections are common and generally show low response to antiviral therapy. Vaccines have been shown to prevent efficiently a number of infectious diseases ; in addition to this prevention, the concept that vaccines might be used to treat chronic viral infections has emerged since the beginning of the century (15-17). Indeed, vaccine therapy, in preliminary, yet promising trials, has now been used for herpes simplex virus (HSV) (18), leprosy (19), tuberculosis, leishmaniasis (20), and human immune deficiency virus (HIV) (21-22) infection. Furthermore, post-infection vaccination with recombinant herpes simplex glycoproteins, as against rabies, can modify the natural history of herpes disease in a guinea pig model (23). Despite most subjects elicit immune response to natural infectious disease, some patients develop a chronic disease probably because the host-directed reponse is not effective in neutralizing the microorganism and preventing its replication (24). This may reflect that the human host is genetically restricted to efficiently respond to critical epitopes, that the immune host response is suboptimal while present, or that critical regions for effective immunoregulation are masked, thus preventing immune recognition. Finally, microorganisms may escape immune control by their genomic variability, e.g. HIV or hepatitis virus B (HBV) and C (HCV), or may show "resistance" to immune response by binding to lipoproteins or variations in expression of adhesion molecules (HCV) (25). A HIV-specific vaccine therapy, developped in a Phase I pilot study using a baculovirus-expressed gp 160 candidate vaccine, has been highly informative (21) and raised encouraging results. Although the precise immunoregulatory mechanisms which are implicated in the post-infection vaccination are only hypothetic, numerous experimental and clinical data underline the formidable potential interest of vaccine therapy.

Various lines of evidence suggest that anti-HBV vaccine therapy may be effective in treating HBV infection. Firstly, Sylvan *et al.* have shown that peripheral T and B lymphocytes from asymptomatic HBsAg carriers are induced to secrete neutralizing anti-HBs antibodies by low concentrations of HBsAg *in vitro* (26), which strongly suggests, although debated (27), the presence of circulating B cells capable of synthesizing anti-HBs antibodies *in vivo*. Secondly, Mancini *et al.* immunized transgenic mice (which constitutively express HBsAg in the liver and secrete large amounts of HBsAg particles into the serum without producing antibodies) with recombinant HBsAg particles of a different subtype and observed a weak but significant anti-HBs response, together with a concommitant decrease in circulating HBsAg, thus indicating that autoreactive HBsAg-specific B lymphocytes are present ; no liver damage occurred in this model (28). Thirdly, vaccination of transgenic mice replicating the complete HBV genome led to HBsAg and HBeAg clearance in 78% of the animals and to a drastic fall in the amount of liver HBV DNA, as shown by in situ hybridization (29). Finally, immunization of mice with preS1 epitopes may overpass the initial non-responsiveness to preS2 or HBsAg epitopes (30) as does immunization with pre-S2 epitopes in mice non-responsive to HBsAg epitopes (31).

Three lines of clinical results of vaccine against HBV are encouraging : 1. anecdotical clearance of HBsAg has been described in HBs Ag-positive subjects who had been inadvertently vaccinated ; 2. vaccination of HBV chronic carriers led to normalization of transaminase activities in 8 of 16 patients in 1982 : conclusions of this trial were negative but detection of HBV DNA was not available at that time (32) ; 3. 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 (14,33) and these results have been confirmed by Chinese authors using the combination of vaccine and immunotherapy (34).

On this basis, we have expended our pilot study of vaccine therapy in treating chronic hepatitis B by an opened study and by an ongoing controlled study.

Patients and methods

Patients of the opened study

Forty-six consecutive chronic HBsAg carriers (38 men, 8 women, mean age : 40 years) were included in this pilot study ; they all had HBV biopsy-proven chronic hepatitis, including 11 with cirrhosis with active HBV replication, as shown by the presence of HBV-DNA in the serum by a liquid phase DNA-DNA hybridization (Genostics® or Murex® procedures) ; they had no immunosuppression or HIV or HDV associated infections. Forty-two were HBeAg-positive and 4 anti-HBe positive. Twelve patients had previously been treated by α-IFN (n = 11) and/or adenine arabinoside (n = 2) more than three years earlier without efficacy on HBV replication.

Patients had been infected by HBV for a mean time of 8.2 ± 4.2 years (time of contamination could be identified in 17 patients). The overall follow-up was 23 ± 12 months.

This "vaccine group" has been compared to an historical "antiviral group" which was made of 43 HBsAg chronic carriers with the same inclusion criteria (biopsy-proven chronic hepatitis and detectable HBV DNA). They were 36 men and 7 women with a mean age of 35 ± 10 years, with a mean 3.3 ± 3.6 years duration of HBsAg carriage (known in 32 patients) including 41 HBeAg positive and 5 patients with cirrhosis. Nine patients were given α-Interferon alone,

17 a combination of steroids and Interferon, 5 a combination of Acyclovir and Interferon and 12 patients the monophosphate of Vidarabine. Eight of these 43 patients had previously been treated without efficacy by a first antiviral treatment. The mean follow-up was 46.7 ± 29 months.

Patients of the controlled study

A randomized controlled study compared the efficiency of 6 intramuscular injections of a PreS2/S vaccine (GenHevac B®) or a S vaccine (Recombivax®) to no therapy for a 12-month period ; all the 150 included patients should receive a 6-month course of 5 MU of Interferon α-2b three times weekly subcutaneously. All the patients were naive patients without previous anti-HBV therapy with detectable serum HBV DNA and biopsy-proven chronic hepatitis. A preliminary analysis is presented after 6 months of follow-up.

Therapeutic schedule

The patients were first observed for at least a 3-month period during which serum HBV DNA was measured monthly. They were then given three standard injections of the GenHevac B® vaccine (Pasteur-Mérieux, Sérums et Vaccins, Marnes la Coquette, France) in a deltoid muscle, at one month intervals. Each 0.5 ml dose contains 20 µg of HBsAg and pre-S2 protein, with aluminium hydroxide as adjuvant. Serological tests were performed monthly after inclusion. Post-vaccination follow-up for evaluation of the vaccine efficiency lasted 6 months after the first injection. Efficacy was defined as a sustained loss of HBV DNA. Patients who had such a response were defined as responders and the other as non-responders. Six to 9 months after the first vaccine, a standard antiviral therapy (5 MU Interferon α-2b three times weekly subcutaneously for 4 months) was proposed to all patients and was accepted by 28 of the 46. The whole follow-up lasted 23 months (range 12 to 32). The analysis of the first 32 patients led to add booster vaccine injections at 6, 9 and 12 months after inclusion, in the last 14 and in the controlled study. This study was approved by the local Ethic Committee.

Results

Over the 3-month period following the complete vaccination (i.e. 6 months after the first vaccine injection), serum HBV DNA became undetectable in 12 of the 46 patients (26.1%). 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 (Table I).

Six of the 12 responders were given α-Interferon : all had still undetectable HBV replication at the end of the treatment and of the follow-up. Twenty of the 34 non-responders received α-Interferon therapy and 2 ara-AMP : 12 of these 22 patients (55%) stopped HBV replication 4.9 months after initiation of α-Interferon and another one 18 months after the end of treatment ; an additional patient, who did not receive α-IFN, lost serum HBV DNA 2 years after vaccination ; this might be a spontaneous event. In these 12 patients, vaccine therapy had previously decreased HBV replication from an average of 392 pg/ml before to 217 pg/ml after vaccination. Four other patients who did not receive α-IFN lost HBV DNA meanly 10.8 months (7 to 15) after vaccine. Finally, over the entire follow-up, serum HBV DNA disappeared in 28 of the 46 patients (60.9%) who where given vaccinotherapy, with or without α-Interferon.

During follow-up a reactivation, as defined by reappearance of HBV DNA, appeared in 7 of the 28 responders (25%) in a mean delay of 10 months (2 to 28 months) after the first HBV DNA disappearance.

Table I. — **Results of vaccine therapy and antiviral treatments**

	Vaccine group (n = 46)	Antiviral group (n = 43)
Disappearance of HBV DNA		
at six months	12 (26.1%)	17 (39.5%)
at one year (vaccine alone)	16 (36.5%)	
at the end of follow-up	INF - 10/18 (55.6%) INF + 18/28 (64.2%)	
	28 (60.9%) (23 months)	30 (69.7%) (40 months)
Percentage and mean time of reactivation	25 10 (2 to 28) months	26 22 (7 to 54) months
HBsAg clearance	0	16%
AntiHBe appearance at the end of follow-up	52%	61.0%

Reactivation was spontaneously resolutive in the 5 patients who had an available follow-up within the 3.2 months (1 to 17).

None of the patients cleared serum HBsAg. Eleven of the 42 HBeAg-positive patients (26%) developed anti-HBe antibodies 6 months after vaccination and 22 (52%) at the end of follow-up. The 4 antiHBe positive patients stopped HBV replication.

An exacerbation of hepatitis, as assessed by a marked increase in transaminase activities, preceeded the disappearance of or decrease in serum HBV DNA in 18 of the 28 (64%) patients who lost serum HBV DNA, whatever the response to vaccine, and resulted in normal transaminase activities over the whole follow-up period. None of the non-responders cleared HBeAg or exhibited an exacerbation of hepatitis or a normalization of aminotransferase activities. Vaccination was otherwise uneventful ; in particular, none of the patients had signs of immune complex-related disease. Age, gender, duration of HBV infection, level of aminotransferase activities, severity of the liver disease (cirrhosis or not) and level of HBV replication did not differ statistically between responders and non responders.

In the "antiviral group" HBV DNA disappeared in 17 (39.5%) and in 30 (69.7%) of the 43 patients six months after the treatment and at the end of follow-up, respectively (Table 1). The percentage of HBeAg clearance (22% at 6 months and 61.0% at the end of follow-up), and of reactivation (26%) did not differ from that of the "vaccine group". By contrast, an HBsAg clearance was noted in 7 of the 43 patients (16%) after a mean time of 32 months.

In the ongoing controlled study, using the same vaccine schedule, preliminary results indicate that serum HBV DNA disappeared more frequently after 6 months in patients who were given a preS2/S vaccine (7/35) than in patients who received a S vaccine (1/21) or no vaccine (1/32) ($p < 0.05$). In responders to vaccine, proliferative responses were observed and were specific of preS2 and S antigens ; this induction of specific proliferation may, at least partially, contribute to the potential efficacy of anti-HBV vaccine therapy (works submitted for publication). No side-effect or vaccine-induced escape-mutants occured during the follow-up (works in progress).

Discussion

Anti-HBV vaccination stopped or markedly reduced HBV replication in 43.5% of patients with chronic hepatitis B, whereas the rate of spontaneous HBV DNA clearance is reportedly around 7% yearly. These results seem to be confirmed by the intermediate analysis of the controlled study.

Our results establish active immunotherapy against HBV as a candidate therapeutical strategy as in other chronic infections ; furthermore, they reinforce the important point that natural infection does not define the limit of immune response to a given pathogen (23). In fact, anti-HBV vaccination showed a similar efficacy at 6 months than α-Interferon in stopping HBV replication. Among these patients who responded to vaccination, the main effect of α-Interferon appears to reinforce the antiviral effect of vaccination since all responders to the combination of vaccine and Interferon had already undergone a significant decrease or disappearance in HBV replication with vaccine alone. At six months, the efficacy of vaccine appears to be similar to that of antiviral treatment. In the absence of efficient vaccine therapy, antiviral treatment indeed leads to a disappearance of HBV DNA in 55% of case.

Main clinical rationale is that vaccine therapy, as mentionned above, appears effective in several other viral, parasitic or bacterial infectious diseases ; in addition to results of vaccine therapy in HIV infection (21) or leprosy (19), interesting results have been recently reported for herpes simplex virus infection in a placebo-controlled trial of vaccination with recombinant glycoprotein D of HSV type 2 (18). A HIV-specific vaccine therapy, developped in a Phase I pilot study using a baculovirus-expressed gp 160 candidate vaccine, has been highly informative (21) and raised encouraging results. It has demonstrated the 3 following main points : 1. natural infection with a pathogen did not define the limits of man's immune response to that particular pathogen ; 2. increase in the number of immunizations enhanced the immunogenicity of vaccine candidate in the original non responders ; 3. no evidence of systemic or immune-specific toxicity was noted.

The mechanisms involved in the response to HBV vaccination are unclear and should be further analyzed. Quantitative and qualitative variations induced by the vaccine may be hypothesized. Post-infection vaccination could bypass the inadequate initial immune response to natural infection by widing the immune repertoire against the pathogen. Vaccine therapy may induce modulations of the immune response due to differences in envelope epitopes presentation or antigens processing, post-translational modification of viral proteins, and recruitement of dendritic cells by the intramuscular injection of the vaccinal epitopes. Such an hypothesis is reinforced by the observation of the induction of specific proliferative responses to the vaccine epitopes.

Specific vaccination may theorically result in side effects. We cannot exclude a risk of immune complexes disease or fulminant hepatitis, as described in animal models. Moreover, prophylactic vaccination has been associated in rare cases with neurological disturbances, including multiple sclerosis or transversal myelitis which suggest auto-immune reaction linked to molecular mimicry between vaccinal antigens and the myelin protein. In this study, as well as in our on-going controlled study, vaccination was so far uneventful.

Another risk is an increase in the viral replication, as recently described for HIV vaccination. We did

observed in most patients a transient increase in the HBV viral load after first doses of vaccine which was unrelated to the efficacy of vaccination (data not shown).

Finally, escape mutants have been described after vaccination or immunoprophylaxis by monoclonal antiHBs antibodies (35). It has been described that the vaccine-related immune pressure may favour the emergence of mutations in the a determinant of the gene S leading to a low affinity of vaccine-induced neutralizing anti-HBs for the envelope antigens of the mutant strains. In 10 patients, however 5 responders and 5 non-responders, the sequencing of the S region before and after vaccination did not show mutations in the a determinant as described in the so-called escape mutants (Sousan *et al.*, work in progress).

Finally, altogether, our results support the hypothesis that vaccination might enhance the efficacy of alpha-Interferon therapy, which leads to a sustained loss of serum HBV DNA in 30 to 40% of patients but which is known to be more efficient when the level of HBV replication is low. We are therefore completing a controlled, prospective trial combining HBV vaccination with α-Interferon therapy to evaluate this possibility.

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