

DNA vaccine: a promising new approach for chronic hepatitis B therapy

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Why is there an urgent need for development of therapeutic alternatives for chronic hepatitis B?

Despite of existence of an effective vaccine, chronic hepatitis B virus (HBV) infection is still a global public health problem with at least 370 million virus carriers, who are of a high risk of cirrhosis and hepatocellular carcinoma development. The efficacy of current anti-HBV therapies relying on IFN- α and nucleoside analogues (lamivudine, adefovir) is limited by the emergence of drug-resistant mutants and the persistence of intranuclear covalently closed circular viral DNA (cccDNA) form, responsible for viral relapse after treatment withdrawal (1). Therefore, this cccDNA pool, which can be considered as a viral minichromosome, plays a crucial role in the persistence of HBV infection and its elimination remains a major clinical challenge.

In this regard, increasing number of evidence from different animal models such as HBV-replicating transgenic mice, HBV-infected chimpanzee, woodchuck HBV (WHV)-infected woodchucks and duck HBV (DHBV)-infected ducks indicate that at least partial viral RNA and cccDNA clearance can be achieved by two not mutually exclusive mechanisms: i/ cytolytic death of infected cells ii/ non cytolytic mechanisms depending of CD8+T cell response via production of TH-1 cytokines such as gamma interferon (IFN- γ) in the liver. It is now evident that chronic hepatitis B is characterized by a weak and narrow HBV-specific T cell response, whereas resolution of infection requires vigorous immune responses, especially polyclonal cytotoxic T-cell (CTL) and T helper 1 (Th1) cell response directed towards different viral epitopes (2). Therefore, immunotherapeutic approaches aimed at restoring or reactivating HBV-specific immune responses are considered of particular value for chronic hepatitis B therapy (2).

Why is DNA vaccination pertinent for immunotherapy of HBV-carriers?

DNA vaccination (or genetic vaccination) is an exciting novel immunization approach which was introduced a more than decade ago and became an extremely fast growing field in vaccine technology. The principle of DNA vaccine is very simple since it is based on the immunization of the host with plasmid DNA encoding a given antigen, instead of conventional vaccines consisting on recombinant antigens obtained in bacteria or viruses (3). Genetic vaccination has been applied to variety of disease models and their corresponding pathogens, including influenza B, malaria, tuberculosis, SIV, HSV, HIV, HCV, HBV....and various cancers (for review 3). Because antigens encoded by plasmid DNA are directly expressed and processed in the transfected cells (myocytes, APCs), the body of the host is its own vaccine factory. This leads in the activation of both MHC-I and MHC-II pathways resulting in the induction of both CD8+ and CD4+ cells, thus mimicking some aspects of natural infection of the hosts and contrasting with traditional antigen-based vaccines that generally induce only antibody response (3). This is also a main advantage of DNA vaccination for chronic hepatitis B for immunotherapy, since it is able to activate not only B but, importantly, also T arm of specific antiviral immune responses, which is crucial for resolution of HBV infection (4). In addition, the potency of genetic vaccine can be greatly enhanced via ; optimization of plasmid DNA vector, introducing trans-membrane sequences, designing truncated DNA vaccine which encode only defined epitopes, adding immunostimulatory sequences such as CpG-oligonucleotides, co-delivery of cytokine encoding genes and improvement of plasmid delivery (3). Finally, DNA vaccine storage is not dependent upon maintaining of cold chain, which facilitates its distribution in developing countries.

What did we learn from pre-clinical studies in animal models?

The ability of DNA vaccine to induce specific immune responses to HBV envelope proteins has been initially demonstrated in naïve mice, which elicited after already a single DNA injection high levels of long-lasting anti-HBsAg antibodies associated with strong CTL response induction (4). Moreover, studies in nonhuman primates demonstrated that DNA vaccine encoding HBV proteins induced high antibodies titers reaching levels required for HBV protection in humans (4).

The woodchuck and Pekin duck models of hepadnavirus infection played an important role in studies aiming to understand the protective immune response induced by DNA vaccines to viral structural proteins. Immunization of naïve woodchucks with plasmids expressing WHV surface antigen has not resulted in measurable anti-WHs antibodies, whereas DNA immunization against core led to the induction of potent lymphoproliferative response which was further enhanced by co-administration of IFN- γ expressing plasmid and protected animals against subsequent virus challenge (5). In a recent study, a bicistronic DNA vaccine encoding both WHV core and IFN- γ was shown to be considerably more effective in preventing hepatitis and serologically detectable infection in woodchucks following

high-titer WHV challenge, highlighting the potent adjuvant role of IFN- γ (6). Interestingly, the apparently protected animals have carried low levels of virus in both lymphoid cells and livers. The lack of sterilizing antiviral immunity induced by such DNA vaccine, targeting viral core only, could be related to; i/ the absence of neutralizing antibodies against viral envelope, playing a key role in the prevention of early virus-host cell interactions and/or ii/ the persistence of residual WHV replication in both hepatocytes and lymphoid cells, following initial exposure to high-dose virus challenge and leading to the occult hepadnavirus carriage (6).

In the duck model, DNA vaccines targeting DHBV small (7) or large preS/S (8) envelope proteins were able to induce high-titer anti-S or anti-preS antibodies respectively, which were highly protective in vivo and neutralized viral infectivity in vitro in primary duck hepatocytes (PDH). We provided first evidence that maternal anti-preS antibodies elicited by DNA vaccination were vertically transmitted, protecting progeny of vaccines against high-titer hepadnavirus infection (9). Our recent data strongly suggest that neutralization capacity of anti-preS antibodies induced by DNA can be considerably enhanced by co-delivery of duck IFN- γ encoding plasmid. Comparison of DNA and protein vaccines targeting DHBV core revealed that antibodies elicited by DNA immunization recognized broader epitope pattern, which was closer to the one observed in chronic viral infection.

Taken together these different studies convincingly demonstrated the ability of DNA vaccine to HBV, WHV and DHBV structural proteins to induce potent, specific, sustain and protective immune responses in naïve animals. However, therapeutic DNA immunization of chronic hepatitis B was less investigated. Studies in the HBV transgenic mouse lineage, E36, demonstrated for the first time the therapeutic potency of DNA vaccine to HBV envelope, which was able to decrease viral replication and clear circulating HBsAg (4). In addition, adoptive transfer of spleen cells from DNA-immunized mice highlighted the role of T cells in the down-regulation of HBV mRNA in transgenic mice livers (4). However, the ultimate question of whether DNA vaccination can induce viral cccDNA clearance cannot be answered in this model, since transgenic mice do not produce cccDNA.

In this regard, DHBV-infected duck is an attractive model for therapeutic DNA vaccination studies, since it is a reference for evaluation of novel anti-HBV approaches and their impact on intranuclear cccDNA clearance. We initially demonstrated that DNA immunization of DHBV-carriers ducks to virus large envelope protein resulted in a marked drop of viremia, associated with significant decrease in intrahepatic viral replication and even viral cccDNA clearance in some animals (8). Interestingly, viral clearance was observed in those animals having low pre-treatment viremia levels, suggesting that approaches aimed at decreasing viral load can be beneficial for association with an effective DNA-based immunotherapy.

Combination therapy associating antiviral drug treatment with DNA vaccine

Such combination therapy relies on initial observations in patients, indicating that antiviral drug treatments lowering viremia can transiently restore anti-HBV immune responses, which can be stimulated in a sustain manner by an effective vaccination. Combination of DNA vaccine targeting viral proteins with antivirals was explored in DHBV infection model with variable results. Treatment of DHBV-carriers with entecavir (ETV) and DHBV DNA vaccine showed a potent antiviral effect of drug, however DNA vaccine mono- or combination therapy have not resulted in reduction of viral replication (10). We showed an additive effect of adefovir and DNA immunization in term of more pronounced decrease in serum and liver viral DNA (11), whereas such synergy was not observed for lamivudine-DNA vaccine combination, probably due to the low antiviral pressure of lamivudine in this model (12). Interestingly, in a lamivudine-DNA study a potent effect of DNA immunization was observed, since 30% of animals on DNA vaccination combined or not with lamivudine showed viral cccDNA clearance, which was tightly associated with restoration of anti-preS responses (12).

Because these three studies differed not only by the choice of an antiviral drug but, importantly, by the design of DNA immunization protocol, in our view, number of factors such as: i/ plasmid construct ii/ larger amount of plasmid DNA ii/ higher number of DNA injections and ii/ longer DNA immunization schedule, may play a key role in the potent antiviral efficacy observed in lamivudine-DNA study. The role of T cell response in viral clearance was not examined in these studies since the tools for duck cellular response analysis are still lacking and urgently need to be developed. In this regard, WHV-carrier woodchuck represents a pertinent model to study the impact of therapeutic DNA vaccine on cellular immune response restoration. Surprisingly, in spite of numerous studies in naïve animals, to date, there is no published reports evaluating genetic vaccines, combined or not with antiviral drugs, for chronic WHV-infection, probably because it is much more difficult to break the immune tolerance status in the woodchuck as compared to the duck infection model.

Results of first clinical trials

Based on the results generated in animal models, the clinical trials of anti-HBV DNA vaccine have been recently initiated. DNA vaccination was first tested in healthy seronegative volunteers, showing its safety and ability to induce anti-HBs-specific humoral and cellular responses. In a phase I trial conducted in France, patients with chronic active hepatitis B, who were nonresponders to current antiviral treatment received DNA vaccine encoding HBV small and middle envelope proteins. The results demonstrated for the first time its safety and ability to activate T-cell responses in some HBV patients with lamivudine resistance, although no sustained serum HBV DNA clearance was achieved (13). A recent proof-of-concept study carried in Lithuania by a Korean group evaluated a combination of lamivudine treatment with DNA vaccine comprising HBV genes plus interleukin-12. DNA vaccination was well tolerated and was

associated with a detectable HBV-specific Th1 cell response and a marked and a decrease of viremia in some patients (14). Although these results are promising, the ability of such DNA vaccine-based immunotherapies to induce a sustain elimination of circulating virus and intrahepatic HBV cccDNA clearance is actually unknown and awaits to be tested in further clinical trials.

In vivo DNA electroporation: a breakthrough for DNA vaccination field

Improvement the potency of DNA vaccine is actually a key issue for immunotherapy of chronic HBV carriers. In this view, recent data presented few months ago at DNA Vaccine 2007 conference held in Malaga, Spain strongly suggest that in vivo DNA electroporation (EP) may be a breakthrough for the DNA vaccination field, able to increase 10- to 1000-fold gene expression in muscle and skin. The basis of electroporation is permeabilization of cell membranes by electric field leading to the increased uptake of plasmid DNA molecules. In addition, DNA EP induces the acute local inflammatory response (up-regulation of cytokines, heat shock proteins, co-stimulatory molecules), which combined with enhanced gene expression results in increased immune responses specific to plasmid-encoded antigen. Delivery of plasmid DNA by EP spectacularly enhanced both humoral and cellular responses against different viral and bacterial antigens not only in mice but, importantly, in larger species such as pigs, sheeps and nonhuman primates in which conventional DNA vaccination had only limited efficacy. Furthermore, EP showed a benefit for therapeutic DNA vaccination of macaques chronically infected with SIV, in term of long lasting decrease of viremia and dramatic increase in cellular immune responses. The first clinical trials using DNA EP showed already promising results, especially for human melanoma patients.

It is of interest that following HBV DNA vaccine electroporation to naïve mice and rabbits, an enhancement of both humoral and cellular responses to HBsAg and HBcAg has been recently reported (15). In addition, a single HBsAg DNA immunization of sheep using EP elicited long-term antibody response of a magnitude considered to be protective, indicating its efficacy in larger species (16). In my view, DNA electroporation can be a valuable approach for therapeutic HBV DNA vaccine development, which needs to be evaluated and optimized in animal models of chronic hepatitis B in order to obtain a complete and sustain recovery from viral infection for clinical development in a near future.

References:

Bibliography

1. Zoulim F. Antiviral therapy of chronic hepatitis B: can we clear the virus and prevent drug resistance? . *Antivir Chem Chemother* . 15 : 299 - 305 2004 ;
2. Wieland S , Chisari F . Stealth and cunning: Hepatitis B and Hepatitis C viruses . *J Virol* . 79 : 9369 - 9380 2005 ; Review
3. Lai WL , Bennett M . DNA vaccines . *Critical Reviews in Immunology* . 18 : 765 - 777 1998 ; Review
4. Michel ML , Loirat D . DNA vaccines for prophylactic or therapeutic immunization against hepatitis B . *Intervirology* . 44 : 78 - 87 2001 ; Review
5. Siegel F , Lu MJ , Roggendorf M . Coadministration of gamma interferon with DNA vaccine expressing core antigen enhances the specific immune response and protects against WHV infection . *J Virol* . 73 : 281 - 289 2001 ;
6. Wang J , Gujar SA , Cova L , Michalak TI . Bicistronic woodchuck hepatitis virus core and gamma interferon DNA vaccine can protect from hepatitis but does not elicit sterilizing antiviral immunity . *J Virol* . 2 : 903 - 916 2007 ;
7. Triyatni M , Jilbert AR , Qiao M , Miller DS , Burrell CJ . 1998 ; Protective efficacy of DNA vaccines against duck hepatitis B virus infection . *J Virol* . 1 : 84 - 94
8. Rollier C , Sunyach C , Barraud L , Madani N , Jamard C , Trepo C , Cova L . 1999 ; Protective and therapeutic effect of DNA-based immunization against hepadnavirus large envelope protein . *Gastroenterology* . 116 : 658 - 665
9. Rollier C , Charollos C , Jamard C , Tepo C , Cova L . 2000b ; Maternally transferred antibodies from DNA-immunized avians protect offspring against hepadnavirus infection . *J Virol* . 74 : 4908 - 4911
10. Foster WK , Miller D , Marion PL , Colonna RJ , Kotlarski I , Jilbert AR . 2003 ; Entecavir therapy combined with DNA vaccination for persistent duck hepatitis B virus infection . *Antimicrob Agents Chemother* . 47 : 2624 - 2635
11. Le Guerhier F , Thermet A , Guerret S , Chevallier M , Jamard C , Gibbs CS , Trepo C , Cova L , Zoulim F . 2003 ; Antiviral effect of adefovir in combination with a DNA vaccine in the duck hepatitis B virus infection model . *J Hepatol* . 38 : 328 - 334
12. Thermet A , Burnfosse T , Le Guerhier F , Pradat P , Trepo C , Zoulim F , Cova L . Editor: Willis AP . Immunotherapeutic efficacy of DNA vaccine alone and combined with antiviral drugs in the chronic DHBV infection model . *Hepatitis B research advances* . NOVA publishers ; New York 2007 ; in press
13. Mancini-Bourguin M , Fontaine H , Scott-Algara D , Pol S , Brechet C , Michel ML . 2004 ; Induction or expansion of T-cell responses by a hepatitis B DNA vaccine administered to chronic HBV carriers . *Hepatology* . 40 : 874 - 882
14. Yang SH , Lee CG , Park SH , Im SJ , Kim YM , Son JM , Wang JS , Yoon SK , Song MK . 2006 ; Correlation of antiviral T-cell responses with suppression of viral rebound in chronic hepatitis B carriers: a proof-of-concept study . *Gene Ther* . 13 : 1110 - 1117
15. Luxembourg , Hannaman D , Ellefsen B , Nakamura G , Bernard R . Enhancement of immune responses to an HBV DNA vaccine by electroporation . *Vaccine* . 24 : 4490 - 4493 2006 ;
16. Babiuk S , Tsang C , van Drunen Littel-van den Hurk S , Babiuk LA , Griebel PJ . A single HBsAg DNA vaccination in combination with electroporation elicits long-term antibody responses in sheep . *Bioelectrochem* . 70 : (2) 269 - 74 2007 ;