Immunomodulatory drugs and therapeutic vaccine in Chronic hepatitis B infection

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Abstract

The main risk of patients with chronic HBV infection is the occurrence of cirrhosis resulting in overmortality related to the development of hepatocellular carcinoma or non carcinomatous complications of cirrhosis (portal hypertension and liver failure). Control of viral replication significantly reduces these risks since pathology of HBV infection is mainly immune-mediated.

The two approved therapies for chronic hepatitis with a clear clinical beneficial effect are interferon-alpha and lamivudine but they have some limitations. Interferon-alpha therapy, which combines antiviral and immunostimulant properties, results in a sustained suppression of HBV replication in only one third of patients. Lamivudine leads to a rapid and almost absolute discontinuation of HBV replication but short-term treatment leads to a frequent relapse of HBV replication and long-term treatment to virological breakthrough with a yearly incidence of 15 to 25%. These limitations of both antiviral therapies of HBV underline the need for alternative therapeutic approaches, including new nucleotide analogues and specific or non specific immunotherapeutic strategies in order to enhance or to broaden the defective T cell responses in chronically infected patients. These immunotherapeutic strategies are mainly the passive transfer of specific HBV immunity, immunomodulatory drugs (cytokines, thymic derivates, growth factors, polyadenur…) and therapeutic vaccines (recombinant anti-HBV vaccine, T-cell vaccine and DNA vaccine). We report the results of these unusual strategies based on limited number of patients. Thymosin treatment seems to be beneficial and vaccine therapy appears to be potentially efficient but have some limitations including safety issues associated with the injection of DNA and potential adverse effects (too strong cytotoxic response).

These results point to the possibility of designing new ways for the treatment of HBV infections and a potential synergistic action of combined antiviral (lamivudine, adefovir), immunomodulatory (interferon-alpha, thymic derivates) and vaccine (DNA vaccines) therapeutic approaches.

Introduction

Around 350 million subjects are chronically infected by the hepatitis B virus (HBV) worldwide (1). The main risk of patients with chronic HBV infection is the occurrence of cirrhosis resulting in overmortality related to the development of hepatocellular carcinoma or non carcinomatous complications of cirrhosis (portal hypertension and liver failure) (2, 3). Most of acutely infected adults recover spontaneously and completely from acute HBV infection. Only a small fraction (3 to 5%) of HBV-infected immunocompetent adults becomes chronic carriers of hepatitis B surface antigen (HBsAg), while 40 to 90% of immunocompromised infected subjects (children born to HBV-infected mothers or children infected in the first years of life, HIV co-infected subjects or allograft recipients) become chronically infected (4). One third of chronically HBV-
infected subjects are healthy carriers while the other two thirds develop chronic liver disease.

HBV is a non-cytopathic virus and liver injury is mainly mediated by the host immune response against virus-infected liver cells and by the production of inflammatory cytokines. A vigorous, polyclonal and multispecific cytotoxic (CTL) and helper T (Th) cell response to HBV is readily detectable in the peripheral blood of patients with acute self-limited hepatitis B, but is weak, antigenically restricted or undetectable in patients with chronic infection (5) (Figure 1). This T cell response is believed to be responsible for the elimination of the hepatitis B virus: by opposition with what is observed during acute hepatitis B, Th1 cytokines (interferon-γ, Interleukin-2) production following antigen stimulation of T cells is weak or absent in chronic hepatitis B (6-8)(Figure 1). Therefore, skewing the T-cell response into a predominant Th1 pathway could facilitate eradication of chronic HBV infection. Indeed, resolution of acute hepatitis B virus (HBV) infection requires adequate T and B cell responses.

Currently, the two approved therapies for chronic hepatitis with a clear clinical beneficial effect are interferon-alpha and lamivudine. Interferon-alpha therapy, which combines antiviral and immunostimulant properties, results in a sustained suppression of HBV replication in only one third of patients (3, 9-12). Lamivudine leads to a rapid and almost absolute discontinuation of HBV replication (13) and to a frequent improvement of the necro-inflammatory activity of the liver disease and to a less extent of fibrosis. However short-term treatment leads to a frequent relapse of HBV replication (14) and same results are expected with adefovir dipivoxyl; long-term treatment (to avoid the risk of relapse) may result in virological breakthrough related to the selection of resistant viral variants with a yearly incidence of 15 to 25% (15). These limitations of antiviral therapies of HBV underline the need for alternative therapeutic approaches, including specific and non specific immunotherapeutic strategies in order to enhance or to broaden the defective T cell responses in chronically infected patients. These immunotherapeutic strategies are the passive transfert of specific HBV immunity, immunomodulatory drugs (thymic derivates, growth factors, polyadenur…) and specific vaccine therapies using either currently available recombinant anti-hepatitis B vaccines (16), a lipopeptide-based T-cell vaccine designed to induce a nucleocapside-specific CTL response (17) or newly developed genetic vaccines (18-19) that have been recently studied in animal models or during clinical trials (Figure 2).

Adoptive transfer of immunity to HBV

Adoptive transfer of immunity to HBV consists to transfer immune memory through bone marrow transplantation (BMT) or peripheral blood lymphocytes (PBLs) in order to induce secoconversion to anti-HBs. It has been performed in immuno-suppressed patients or in non-responders to active immunization as a prophylaxis therapy and in patients with persistent viral infection to clear HBV infection as an antiviral therapy.

1. Prophylaxis therapy: adoptive transfer of immunity to HBV in immune-suppressed patients.

The first report concerns 12 pairs of anti-HBc-/anti-HBs- recipients who received bone marrow from anti HBC+/anti HBs+ donors: they developed protective anti HBs levels after BMT (20), suggesting that transplantation of HBV immune bone marrow cells led to acquisition of humoral (and possibly cellular) immunity to HBV. Then, the authors demonstrated adoptive transfer of immunity in mice through active immunization of bone marrow donors against HBV with recombinant HBV vaccines (21): seroconversion to anti-HBs occurred in more than 80% of BMT recipient mice within 3 weeks after transplantation and up to 90% responded to a booster vaccination with homologous vaccine.

To confirm the adoptive transfer of immunity to HBV in immune-suppressed humans (bone marrow recipients) by active immunization of donors, Shouval et al. performed a prospective study in which 31 blood marrow donors were immunized with a recombinant HBV vaccine : seroconversion to anti-HBs occurred in 20/31 (65%) of bone marrow recipients within a mean of 24 days after BMT; a moderate secondary humoral immune response was observed after booster injections of vaccine in BMT recipients (22).

Finally, because of significant morbidity and mortality associated with BMT, less invasive procedures were tested using peripheral blood lymphocytes (PBLs) from donors actively immunized against HBV (23). Eight recipients received bone marrow from 8 anti HBC-/anti HBs- donors. Therefore, donors were actively immunized against HBV and bone marrow recipients then received PBLs from their post-BMT immunized donors: seroconversion to anti-HBs occurred in 7/8 patients.
Anti-HBs remain detectable (>10mIU/ml) after 4 years in 50% of bone marrow recipients (24). Adoptive transfer of immunity to HBV was obtained in 22 of 35 (62%) BMT anti-HBc-/anti-HBs- recipients who received their bone marrow from actively immunized donors and in 6 of 8 anti-HBc-/anti-HBs- patients who received PBLs from HBV immune donors. Anti-HBs antibodies were detected in 7 of the 9 patients still alive 25 months following BMT and in 3 patients still alive 18 months after transplantation of PBLs.

**Antiviral therapy: Adoptive transfer of immunity to HBV in patients with chronic HBV infection.**

The clearance of HBV infection has been reported in an HBsAg+/HBV-DNA+ carrier with leukemia following BMT from an anti HBC+/antiHBs+ donor (25): Ag HBs and HBV-DNA disappeared within 28 days after BMT and seroconversion to anti HBs occurred with a minimal rise of alanine aminotransferase. Similar results were reported from Hong Kong where 226 patients who received BMT were retrospectively studied (26-27). Twenty-one patients were HBsAg-positive before BMT and 5 received anti-HBs/anti-HBc positive bone marrow. Two of the 5 patients (HBV-DNA negative, HBeAg negative and anti-HBe positive) had persistent clearance of HBsAg and seroconverted from HBsAg to anti-HBs. The seroconversion was accompanied by a flare in ALT level (one anicteric and one icteric with hepatic failure). A third patient (HBV-DNA and HBeAg positive) had transient HBsAg to anti-HBs seroconversion. After reversion to HBsAg, he remained HBeAg, HBV-DNA-negative and anti-HBe positive. Finally, cases of adoptive transfer of immunity to HBV have been reported in rats after liver or kidney transplantation (28-29).

**Antitumoral therapy: Adoptive transfer of immunity to HBV in mice transplanted with Hep3B human hepatoma cells.**

Growth of human hepatoma cells may be suppressed in vitro and in vivo in mice through passive administration of anti-HBs antibodies (30). Human HCC xenografts expressing HBsAg in athymic mice may be suppressed through BMT from donor mice which have been actively immunized against the hepatitis B small envelope protein: a marked reduction in tumor volume and serum AFP levels may be achieved using immune bone marrow from donor mice actively immunized (31).

**Potential risks associated with adoptive transfer of immunity to HBV.**

Risks of adoptive transfer are those of BMT and the absence of immune control that may result in fulminant hepatitis. BMT is a major invasive procedure and is associated with significant morbidity and mortality, especially because infections, veno-occlusive disease and graft versus host disease (GVHD)(32).

Several cases of hepatic failure have been reported following the adoptive transfer of immunity to HBV (25-27), including a case of fatal fulminant hepatitis in a 27-year-old man inactive HBsAg carrier (anti-HBe positive and HBV-DNA negative) who received allogeneic BMT from his HLA-matched anti HBc+/anti HBs+ brother (33).

In summary, the technique of adoptive transfer of immunity to HBV is an interesting prophylactic, antiviral or antitumoral procedure but it is restricted to the bone marrow transplantation setting and is limited by the potential serious adverse events (GVHD, fulminant hepatitis).

### 2. Immunomodulatory treatments of chronic hepatitis B

#### 2.1. Cytokines

Interferon-gamma, tumor necrosis factor-alpha and interleukin 1-beta suppress HBV replication. Cytokines like IL-2 and IL-12 in human or recombinant form have been studied as immune modulators activating antiviral response against HBV (34).

##### 2.1.1. Interleukin 2 (IL-2)

Despite HBsAg clearance and anti-HBs antibodies appearance were reported in a human immunodeficiency virus-infected patient after a recombinant IL-2 therapy (35), a pilot study of natural human IL-2 in patients with chronic hepatitis B did not conclude to either a biochemical or an immunological response (36). Two randomised controlled trials of IL-2 versus placebo (one in HIV co-infected patients) did not find a significant benefit in decreasing HBV-DNA (37-38). A randomised,
prospectively controlled multicenter trial comparing IL-2 and interferon alpha-2b versus interferon alone in 37 patients did not conclude to significant differences in the rates of HBV-DNA decrease, HBeAg seroconversion or normalisation of transaminase activities while side effects were more pronounced during combination therapy (39).

Thus, IL-2 is probably not efficacious for the treatment of chronic hepatitis B and is not recommended at the doses and schedules used.

2.1.2. Interleukin 12 (IL-12)
Interleukin-12 is produced by antigen-presenting cells. It promotes Th1 cell development, cell-mediated cytotoxicity, interferon-gamma production and inhibited autoantibody production by shifting the Th2-mediated response toward Th1 predominance (40).

Experimental data found that Interleukin-12 inhibited hepatitis B virus replication in transgenic mice (41). Amongst patients with chronic hepatitis B undergoing interferon-alpha treatment, only those who clear hepatitis B virus show a substantial increase in the production of biologically active IL-12 and an inverse ratio between serum levels of IL-12 p40 subunit and IL-12. The peak of serum IL-12 occurs after the hepatitis flare and precedes or coincides with the time of HBe seroconversion.

Preliminary data reported elimination of HBV-DNA and HBeAg during IL-12 therapy in a patient with chronic hepatitis B virus infection who had not responded to three previous therapies (42). A multicenter open label phase I/II trial was assessed in the treatment of chronic hepatitis B (n=46) with recombinant human interleukin-12 (rHuIL-12) (43). Human recombinant IL-12 was generally well tolerated, although associated with transient decreases in neutrophils and lymphocyte counts, and with elevations in serum transaminases and bilirubin. At the end of treatment, the rate of HBV DNA clearance was higher in patients treated with 0.50 µg/kg (25%) or 0.25 µg/kg (13%) than 0.03 µg/kg.

Nonetheless, antiviral activity of rHuIL-12 did not appear to be advantageous in comparison to other currently available treatments

2.2. Thymosin α1
Thymosin alpha-1 accelerates the replenishment and maturation of thymocytes, stimulates differentiation and conversion of lymphocytes in active T cells. Thymosin alpha1 restored also the T-cell function by T cell-mediated antibody production. Thymosin alpha-1 concentrations are low in patients chronically infected with the hepatitis B virus (44).

A recent meta-analysis, compelling 353 patients from five trials, evaluates the efficacy of thymosin alpha-1 (Table 1). Trials compared thymosin with placebo for two and with interferon therapy for three. Thymosin alpha-1 dose was 900 µg/m2 or 1.6 mg twice weekly in subcutaneous injections for at least 24 weeks. Tolerance was good in all trials. There was no difference in the biochemical response but odds ratio for a virological response to thymosin at the end of treatment, 6 and 12 months post-treatment were 0.56 (0.2-1.52), 1.67 (0.83-3.37) and 2.67 (1.25-5.68), respectively. Finally, there was an increase in virological response overtime after discontinuation of thymosin treatment (P = 0.02). Thymosin appears to be potentially effective in suppressing viral replication and its effects are delayed until 12 months after treatment discontinuation (45-51).

Few studies have focused on combination therapy. Combination of low-dose lymphoblastoid interferon and thymosin alpha 1 therapy was evaluated in 15 patients, including 11 patients previously treated with Interferon-alpha 2b. After 12 months, nine patients (60%) were responders (negativation of serum HBV DNA and normalisation of ALT) and 6 exhibited HBsAg clearance (52). A recent trial analysed the efficacy of thymosin alpha-1 plus farnesiclovir for 26 weeks versus farnesiclovir alone (500 mg thrice daily) or placebo in 96 patients with wild type chronic HBV infection in immune tolerant phase. No significant difference in the rate of adverse effects was observed among the 3 groups during the 78 weeks of follow-up. The combination group showed a significant higher HBV DNA reduction and 15.6 % of HBe seroconversion versus none in others groups (53).

2.3. Granulocyte-macrophage colony-stimulating factor (GM-CSF)
GM-CSF is used to improve the immunological function or hematological disorders. GM-CSF changes cytokine production
In a preliminary study with GM-CSF (500 micrograms subcutaneously twice weekly) plus interferon (5 MU daily) for 4 months in patients with HBV resistant to interferon therapy, five of the eight hepatitis B patients responded to combined therapy. Factors associated with response were low baseline ALT, AST and gamma GT levels (55). Another pilot study analysed daily doses of recombinant human GM-CSF (3, 1 or 0.5 µg/kg) to nine patients with chronic hepatitis B alone or in combination with 5 MU of interferon-alpha 2b. GM-CSF reduced significantly hepatitis B virus DNA levels (p < 0.02). Four patients became negative for HBV DNA and HBeAg (two of them seroconverted to HBe antibodies) and had an histopathological improvement. The three doses used were equally effective (56).

2.4. Polyadenylic-Polyuridylic Acid

Efficacy of the polyadenylic-polyuridylic acid in the treatment of chronic active hepatitis B was studied in a pilot study with 19 patients. One hundred to 150 mg of poly (A), poly(U) were given weekly for six weeks. Serum ALT levels were decreased gradually and 2’5’-AS activities were significantly increased after initiation of poly(A),poly(U) injections. At the end of this trial (24th week) a normalisation of ALT levels was observed in 14 (73.7%), a seroconversion of HBeAg in 11 (57.9%) and a loss of HBV-DNA in 12 out of 19 patients (63.1%). No adverse effects were observed during the treatment (57). A combination of polyadenur with interferon versus interferon alone for 24 weeks was studied in 86 patients: the percentage of HBe seroconversion (33 vs. 9%) and HBV DNA clearance (43 vs. 18%) were significantly higher in the association group at the end of the 6-month follow-up (58).

In summary, non specific immunomodulatory treatments of chronic hepatitis B are conceptually attractive and result in encouraging results, but to date, except thymosin, they have not demonstrated sufficient efficacy for widespread use or recommendations out of therapeutic protocols.

3. Vaccine therapy of HBV

3.1. Immunotherapy using recombinant anti-HBV vaccine

In a first step, immunization of transgenic mice that constitutively express the HBsAg in the liver have evidenced the ability to overcome functional tolerance to HBsAg by inducing a specific immune response (59). In a second step, pilot clinical studies established that specific vaccine therapy by standard anti-HBV vaccination could cancel or reduce HBV replication in around 50% of chronic carrier subjects (16, 60-61). Finally, a multicentric controlled trial showed both the efficacy and the limitations of vaccine therapy in HBV chronic infection (62). In a 12-month follow-up, 118 “naïve” patients 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 Dohme, n = 34) or no treatment as control (n = 37). They had never received any previous anti-HBV therapy, showed detectable serum HBV DNA using a standard liquid hybridization assay and had biopsy-proven chronic hepatitis. 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 (16.3%) than in the control group (2.7%) (p = 0.033, by the chi-square Pearson test), especially in patients who had pre-treatment viremia ≥ 200 pg/ml (none in the control group vs. 16.7% in the vaccinated groups) (p=0.025) and who are known for their poor response to Interferon-α (63).

After one year follow-up and 5 vaccine injections, there was no difference in the rate of serum HBV DNA negativation but HBV vaccine injections 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 (13.3%) as compared to 3.6% in the controls (62). The vaccine-induced immune responses have been tested in 40 patients with HBV chronic hepatitis (64): vaccination elicited PBMC proliferative responses in 7 among 27 patients who received and in none of the 13 who did not receive HBsAg. These responses specific for envelope antigen (at least 3 different epitopes were recognized) are mediated by the CD4+ T lymphocytes. HBV-specific T lymphocytes produced high levels of gamma interferon and belonged to the Th-1 subset. The 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. These results offered the first direct evidence, based on a controlled study, that the HBV vaccine may decrease HBV replication; but the efficacy of current HBV vaccines appeared however limited suggesting that...
different immunization protocols should be considered in the future and combined with multiple nucleoside analogues to associate synergistically immunomodulatory and antiviral approaches.

3.2. T-cell vaccine for chronic hepatitis B
An other approach based on injection of a T-cell vaccine in subjects chronically infected with HBV has been reported (65). This vaccine is a lipopeptide consisting of covalently linked components: a cytotoxic T-cell epitope derived from the hepatitis B core protein amino acids 18-27, a T-helper epitope derived from tetanus toxoid and 2 palmitic acid molecules. In a dose escalation trial carried out in 26 normal subjects this vaccine was shown to be safe and able to induce a primary HBV core-specific CTL response (17). A dose-response curve was observed and five out of five subjects responded to the highest dose (500 mg). In a pilot study, this vaccine was given to 19 patients with chronic hepatitis B infection in order to initiate in vivo a CTL response that could mediate viral clearance (65). Vaccination with the highest dose (5 mg on 6 occasions) induced CTL that were 10-fold weaker as compared with CTL responses induced in previous studies in healthy volunteers with no exposure to hepatitis B (66). No significant changes in liver biochemistry or viral serology (HBeAg, HBeAb, HBV-DNA) nor serious adverse events were noted during the follow-up. In summary administration of this single epitope vaccine initiates CTL activity in HBV chronic carriers but with a magnitude being well below that seen in patients following resolution of acute hepatitis B.

3.3. DNA vaccines for HBV infections
New approaches of vaccination for hepatitis B infections are now based on intramuscular injection of plasmid DNA encoding hepatitis B virus antigen (67). The so-called “DNA vaccines” induce immune responses against antigens synthesised in vivo after direct introduction of DNAs encoding antigen sequences. This novel approach to immunization may overcome failures of the traditional antigen-based approach and provide safe and effective prophylactic and therapeutic vaccines. Owing to the endogenous synthesis of antigen, the processing into relevant epitopes, and the induction of CD8+ CTLs, DNA vaccines may be useful for treatment of individuals chronically infected with HBV.

Systemic immunization of mice by intramuscular injection of plasmid DNA expressing HBV envelope proteins induces rapid, strong and sustained humoral and cell-mediated immune responses. Antibodies, which are initially of the IgM then IgG isotype (predominantly IgG2a), recognize several of the B-cell epitopes present on the S, preS2 or preS1 domains of the envelope proteins (68). High titers of anti-HBs are present by 4-8 weeks and persist for at least 17 months after a single DNA injection, although they can be boosted ten-fold by a second injection of DNA, or somewhat less, by injection of a recombinant HBsAg protein. A strong cellular immune response is induced by the DNA-based immunization with high level of CTL and CTL precursors being detected in one week and maintained for several months (69). These results has been confirmed in other animal models for hepadnavirus infection. In Pekin ducks, a model for duck hepatitis B virus (DHBV) infection, DNA vectors encoding DHBV large (L) or small (S) envelope proteins were tested after multiple injections. Anti-DHBsAg antibodies induced after DNA vaccination with the plasmid encoding the S protein only, display neutralizing activity in vitro in primary duck hepatocytes culture and in vivo in preventing ducklings infection (70). However in this study, immunization of ducks with plasmids encoding the DHBV L protein induced only very limited neutralizing responses. This contrasts with a study by Rollier et al. (71) showing that high titer anti pre-S response was induced with plasmids encoding the DHBV L which almost completely abolish DHBV infectivity for hepatocytes in vitro and in vivo in ducklings. Moreover, a recent study has demonstrated that maternal antibodies induced in breeding ducks after DNA immunization to DHBV L protein are vertically transmitted and protect progeny against high titer DHBV challenge (72). The outcome of challenge was directly correlated with anti-preS titers.

In contrast, in the woodchuck model for woodchuck hepatitis virus (WHV) infection, no measurable antibodies against WHBsAg have been detected even after 3 injections of plasmid encoding envelope proteins. Nevertheless, the woodchucks were protected from an infectious challenge and the rapid appearance of anti-WHsAg after challenge demonstrated that priming of the B-cell response took place as a result of DNA vaccination (73).

Ultimately, the chimpanzee is the best animal model to evaluate DNA vaccines for their potential use in humans. In non human primates, the use of plasmid encoding HBV proteins induce high antibody titers that reach levels required for HBV protection in humans (74-75). Intramuscular immunization using 2 mg DNA encoding HBsAg resulted in high anti-HBs levels (>100 mUI/ml) in a female chimpanzee, however at least two boost injections of DNA were required to prevent antibody levels from diminishing over time. Nevertheless, extremely high antibody titers were ultimately attained (>14000 mUI/ml),
with high level being sustained for at least one year. In a second chimpanzee (male) injected with a lower dose of DNA (400 µg), no anti-HBs was detected until after the second injection of DNA. Although antibody titers of 60 mU/ml were attained these were transient even after three DNA boosts. This underlines the difficulties of transposing results obtained in mice or in other small animals to animal models more closely related to humans. We have no data of DNA vaccine therapy in humans but trials are in progress.

In summary, vaccination may be a therapeutic procedure with the lowest cost and the potentially greatest benefit. However many basic questions of this vaccine approach need to be answered, namely the potential efficacy of DNA vaccines in humans, safety issues associated with the injection of DNA vaccines and potential adverse effects of a too strong cytotoxic response against the infected liver cells.

### Conclusion

These data point to the possibility of designing new ways for the treatment of HBV infections and a potential synergistic action of combined antiviral (lamivudine, adefovir dipivoxyl), immunomodulatory (interferon-alpha, thymosin) and vaccine (DNA vaccines) therapeutic approaches. The secretion of the Th1-associated lymphokines (IFN-γ and IL-12) is impaired during chronic hepatitis B infection favouring production of the Th0/Th2-associated lymphokine IL-5 (76). Since lamivudine treatment can restore Th cell responsiveness in patients with chronic HBV (77), combination of therapy with either specific or non specific immunostimulants or antiviral treatment might reverse the altered Th function and participate in HBV clearance.

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**Table 1**: Meta-analysis of monotherapy randomized controlled trials with Thymosin 1-α in chronic HBV carriers: Description and end of follow-up results.

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Figure 1: Immune responses associated with recovery or chronic disease after hepatitis B virus infection.
Figure 2: Potential therapeutic approaches for the treatment of HBV chronic infection which pathology is mainly immune-mediated. Currently available treatments (interferon-a and lamivudine) can be combined with new specific immunotherapeutic approaches.

Figure 3: Summary of the immune effects obtained with the DNA based immunization to the hepatitis B surface antigen in mice (reference 68).
Figure 4: Summary of the therapeutic effect of DNA-based immunization against hepadnavirus large envelope protein of duck hepatitis B virus infection (reference 71).

CD4-proliferative response

Th1 cytokine release

Mean viremia titers of chronically infected ducks

REFERENCES