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Successful Treatment of Decompensated Chronic Viral Hepatitis by Bursal Disease Virus Vaccine

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Abstract. *Three cases of women with chronic liver inflammation caused by hepatitis B (two) and C (one) viral infections, were followed up to twelve years after diagnosis. As conventional therapy was ineffective and the patients progressed into decompensated liver disease, they were superinfected with massive doses of an attenuated variant (MTH-68/B) of the apathogenic avian Bursal Disease virus (a double-stranded RNA virus from the Birnaviridae family). Clinical symptoms and biochemical abnormalities were resolved in two patients following few months of virus treatment. Cirrhosis was stabilized and significant clinical improvement was achieved in the third patient - who before the virus therapy was moribund with recurring, diuretic-resistant ascites, variceal bleedings, portal encephalopathy and renal failure. To our knowledge, these are the first recorded cases of decompensated chronic viral hepatitis which went to long-lasting remission or were stabilized by superinfection with an apathogenic virus.*

Viral hepatitis is a necroinflammatory disease caused by at least six different, mostly unrelated, hepatotropic viruses (hepatitis A, B, C, D, E and G respectively) (1,2). While the hepatitis A and E viruses cause acute, self-limited disease only, the hepatitis B virus (HBV), C virus (HCV), D virus (HDV) and probably G virus (HGV) can cause persistent infection and chronic hepatitis (3). Chronic viral hepatitis is the principal cause of chronic inflammation of the liver, liver cirrhosis and hepatocellular carcinoma (HCC). Chronic infection may follow an acute attack, when the virus does not resolve, but also it can occur without clinical symptoms. Five to 10% of acute HBV infections (1,4,5) but about 85% of acute HCV infections progress to chronic disease (6). Of those that become chronic, HCV accounts for nearly 50% of cases and is the most common cause of cryptogenic cirrhosis (7). Ten and 20% of chronic HBV and HCV infections respectively progress to chronic liver disease and cirrhosis (3,6). HBV infection, mainly in infants and children (8), but most cases of HCV infections are asymptomatic (9). The World Health Organization estimates that the number of HBV carriers will reach 400 million by the year 2000 (8) while over 100 million people worldwide are infected with HCV (10). Such a vast infected population continuously supplies new cases of chronic hepatitis. Not unexpectedly, HCC ranks as one of the top 10 most common cancers (1).

At present the only therapy of proven benefit for chronic viral hepatitis is interferon-α (3). The likelihood of a response with interferon-α is however, inversely correlated with the duration of disease before therapy and patients with decompensated liver disease have a poor prognosis, thus interferon-α therapy in such patients is not only rarely successful but could be dangerous.

Recently an attenuated variant (MTH-68/B) of the Bursal Disease Virus (BDV), a double-stranded avian RNA virus, a member of the Birnaviridae family, which is known to be apathogenic for humans (11) was successfully used in patients with acute HBV and HCV infections in a Phase II trial (12).

Here we report three patients with chronic active hepatitis (two HBV and one HCV respectively) who responded to intranasal or oral MTH-68/B virus vaccine treatment. Each patient had exhausted conventional therapy and had parenchymally decompensated liver disease, two patients also had diuretic-resistent ascites, one of them was moribund with portal encephalopathy, variceal bleedings and renal failure when the vaccine therapy was started. The patients gave their informed consent to the experimental therapy according to

Abbreviations: Anti-HBc, anti-hepatitis B virus antibodies; anti-HCV, anti-hepatitis C virus antibodies; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BDV, Bursal Disease Virus; ChE, cholinesterase; GGT, gamma glutamyltransferase; HBeAg, hepatitis B envelope antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV hepatitis C virus; PCR, polymerase chain reaction.

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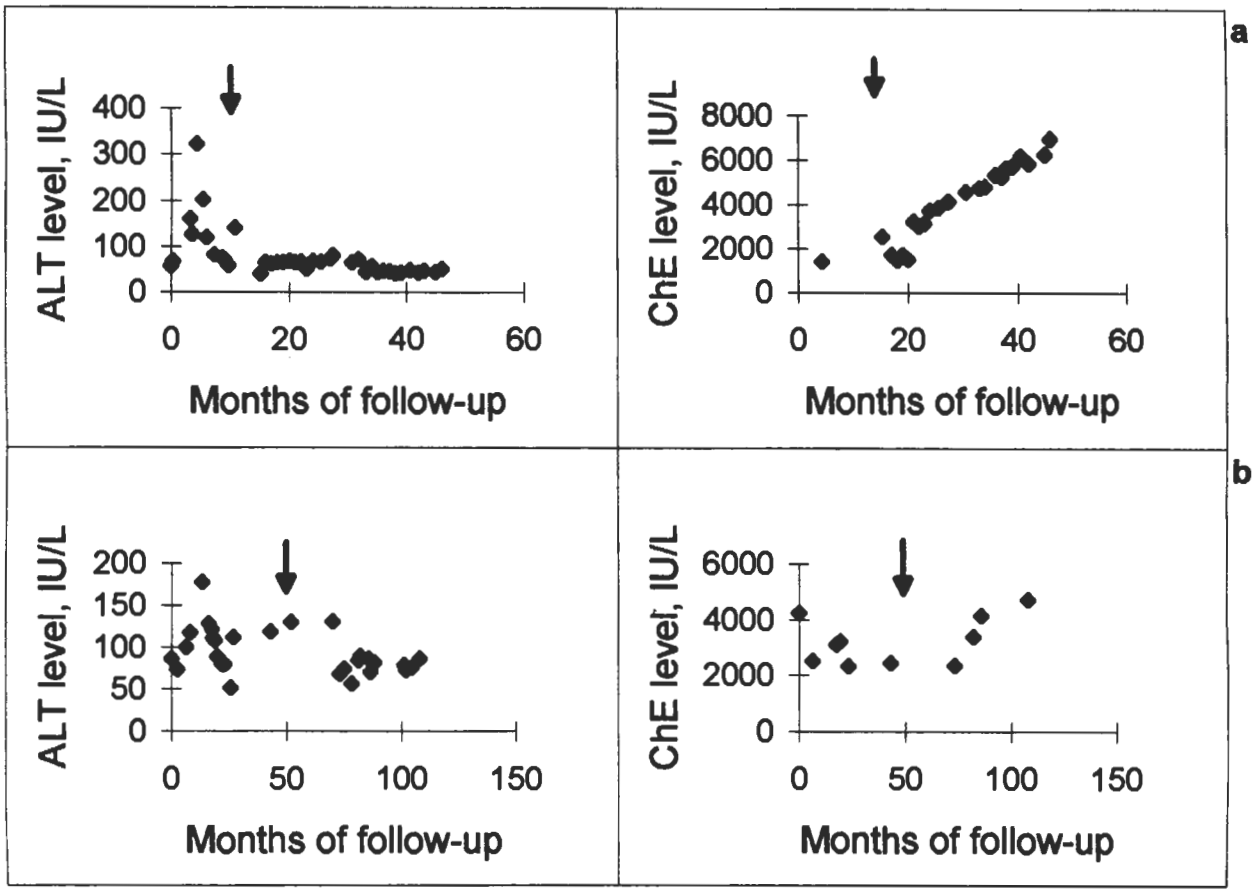

Figure 1. *Alanine aminotransferase (ALT) and Cholinesterase (ChE) activity levels in two patients (a Case 1, b Case 3) with chronic hepatitis C and B during treatment with MTH-68/B virus vaccine (start of treatment is labelled by arrow, cummulative virus dosages were 600000 and 390000 U in Cases 1 and 3 respectively).*

the requirements of the Helsinki Declaration of Human Rights.

Case Reports

Case 1. A 67-year-old female patient was hospitalized in June 1994 for further management of her pruritus and exanthemas, the aetiology of which was not found for more than a year. She also complained of decreased appetite, increasing fatigue, drowsiness, felt fullness and pain in the right upper abdominal quadrant which increased upon physical activity. Her stool became irregular (diarrhea and constipation alternated). Development of jaundice, a high serum bilirubin level (SeBi: 36.7 μmol/l), increased serum aspartate aminotransferase (AST: 189 IU/L), alanine aminotransferase (ALT: 322 IU/L), gamma glutamyltransferase (GGT: 174 IU/L), alkaline phosphatase (ALP: 447 IU/L) and decreased cholinesterase (ChE: 1400 IU/L) activity demonstrated that her pruritus was due to hepatic failure. Ascites was found on abdominal ultra-sound and on liver biopsy histological features of chronic hepatitis, including portal inflammation, interface hepatitis (piecemeal necrosis) and lobular injury were present. The HCV RNA homology analysis by polymerase chain reaction (PCR) amplification proved hepatitis C virus replication. She was treated by steroids, which induced a steroid diabetes, but did not stop the progression of hepatitis. Her physical performance decreased rapidly, a severe malaise developed and eventually she was confined to bed, unable to support herself.

As conventional treatment failed, an oral Bursal Disease Virus (BDV) vaccine therapy was started in November 1994 (2000 U/day for a month). The pruritus soon decreased, the patient gradually recovered her appetite and physical activity. While the biochemical abnormalities (SeBi, AST, ALT, and GGT) returned to normal range within few months, the ChE activity remained increasing over an observation period of 40 months (Figure 1a). As accidental interruption of vaccine therapy resulted in the recurrence of pruritus, the virus therapy was continued for more than two years (1000 U/day

for a year, then 1000 U/every other day the following year, amounting to a cumulative virus dosage of 600000 U). Currently no hepatitis C virus RNA can be detected by PCR amplification test, the patient is well and capable of light work.

Case 2. A 57-year-old female physician acquired a subclinical acute viral hepatitis infection probably during her medical practice. Symptoms occurred about one year before her first hospitalization (in 1985) for loss of appetite, meteorism, pruritus, abdominal discomfort and pain in the right upper abdominal quadrant which was independent of meals. She alternatively had diarrhea and constipation, her liver was enlarged and painful. A week before hospitalization jaundice and dark colored urine developed. The laboratory analysis found biochemical abnormalities (SeBi: 50.9 μmol/l, AST: 57 IU/L, ALT: 91 IU/L, GGT: 66 IU/L). She was found positive for hepatitis B surface antigen (HBsAg), hepatitis Be antigen HBeAg) and anti-HBc antibody (IgM) respectively. At the time of her first hospitalization the PCR technology was not available, thus no firm evidence for viral replication could be proven. Due to her coagulopathy she had not been assessed by liver biopsy, thus the diagnosis of chronic active hepatitis was based on the persistently high serum bilirubin, aminotransferase concentrations and serological features of the disease. The developing portal hypertension, which was demonstrated by abdominal ultra-sound, suggested a very poor prognosis. Consistent with these findings, plus that the patient also complained of breathing difficulties and had swollen legs. As there was no hope that she would be able to return to work, early retirement was required.

Since conventional treatment failed she began the BDV vaccine therapy in 1985 - months after her first hospitalization - 1000 U/day for three months, then 3x1000 U/week for further six months, then 1x1000 U/week for three years (approximate cumulative virus dosage of 340000 U). SeBi, AST, ALT, and GGT activities returned to normal range within three months (not shown). Along with improvement in the laboratory values her clinical symptoms and physical conditions improved, indeed after four months of BDV therapy she returned to work. It is significant that after five years of remission, during which she gradually reduced the dose of vaccine therapy, the hepatitis recurred in 1991 (ALT being 155 IU/L). BDV therapy was reinstituted in full dose (1000 U/day) resulting in a second remission. Twelve years after starting of the virus therapy HBV replication is demonstrated by the PCR test, however, the patient is currently well and working.

Case 3. A 24-year-old female patient was repeatedly hospitalized from 1987 on for the management of fatigue, loss of appetite, jaundice, recurring, diuretic-resistant ascites and variceal bleedings from the oesophagus. Laboratory analysis showed extremely high SeBi concentrations (upto 131 μmol/l) and strongly increased liver enzyme values (highest of AST: 240, ALT: 177, GGT: 408, ALP: 950 IU/L respectively) and decreased ChE activity (lowest being 2316 IU/L). She was found positive for HBsAg, HBeAg and anti-HBc antibody (IgM) respectively. The hepatitis B virus replication was demonstrated by PCR amplification test. The histological examination showed chronic active inflammation of the liver with signs of cirrhosis. Her diuretic-resistant, generalized ascites had to be resolved by total paracentesis, and severe secondary anaemia by transfusion. To prevent further variceal bleedings the esophageal veins were sclerotized. Despite the decompensated cirrhosis, where interferon therapy is usually not indicated, a careful trial with leukocyte interferon-alfa therapy (2 MU per day for four months) was conducted without any improvement. Progressive jaundice, generalized edema and hepatic encephalopathy developed, and interferon treatment was stopped. Conventional therapy also failed to stabilize her condition, and a request for liver transplantation was refused because of the high-level viremia.

Subsequently she was started on the BDV vaccine therapy (2000 U/ every other day), first from November 1991 (with brief interruptions) until the end of 1993, receiving a total of approximately 270000 U of virus dosage. Standard therapy with diuretics, spironolactone, beta-receptor blockers, low-salt diet with restricted protein and high calory intake, free radical inhibitors, etc had been continued. Soon after the vaccine therapy was started her clinical symptoms improved considerably, *i.e.* her appetite increased, pruritus decreased and gradually she regained physical activity.

Unfortunately the patient interrupted the BDV therapy (first in 1994) because of nasal bleeding. A deterioration was observed, a severe cholestasis occurred (SeBi concentration elevated to 383 μmol/l, AST: 257, ALP: 1550 IU/L respectively) which was accompanied by portal encephalopathy and repeated bleedings and edemas. Abdominal ultra-sound scanning demonstrated that liver and spleen were considerably enlarged (5 and 4 cm respectively), and the liver had multiple (few cm size) echodense nodules. Her secundary anaemia was treated by transfusions, but her conditions worsened rapidly.

Upon reinstitution of the BDV vaccine therapy (starting with 2000 U virus/day, and receiving a cumulative virus dosage of 120000 U) biochemical abnormalities improved moderately (see ALT and ChE in Figure 1b), jaundice decreased, the progression of cirrhosis stopped (indeed her Child-Pugh class C stage returned to class B stage) and ascites did not recur. Viral serology showed that B virus replication stopped: she was HBsAg positive but HBeAg negative and anti-HBe antibody positive (partial seroconversion), HBV DNA negative by the nested PCR amplification test. Currently the patient is sufficiently well to be capable of light work, her disease seems to be stabilized in Child B stage since two years. Although she tested positive by PCR, an improvement in prothrombin index and serum pseudocholinesterase level show some degree of regeneration of the liver tissue.

Discussion

Since the response rate to interferon alfa in chronic viral hepatitis is not satisfactory, side effects of treatment are frequent and retreatment is rarely helpful, new therapies are needed to prevent cirrhosis, liver failure and hepatocarcinoma (3,13). The greatest promise for treatment of HBV infection are several new nucleosid analogues, such as famciclovir, lamivudine, lobucavir and adefovir dipivoxil, however, after short treatment courses a rapid return of viral replication and liver disease is observed. Currently, the only antiviral drug of promise in chronic HCV is ribavirin, but serum ALT concentrations return to pretreatment values in nearly all patients after therapy is stopped (3). To stimulate MHC class I, cell-mediated immune response to clear the infection, DNA vaccines are also being investigated in clinical trials for treatment of chronic infection with HBV (14).

Isolated case reports demonstrated that chronic hepatitis induced by a B virus resolved during an intermittent infection with an acute type hepatitis A (15), hepatitis C or D viruses (16). Indeed, HCV superinfection can suppress HBV replication or terminate the HBsAg carrier status, (17,18) but also HCV replication was suppressed by HBV replication among patients with chronic hepatitis B (19). It was suggested that in patients with dual hepatitis infections, viruses show alternative dominance in replication (20). More importantly, coinfection or superinfection of the HBV infected liver by a non-hepatitis virus may facilitate HBV clearance by inducing the local production of cytokines such as IFNα/β, IFNγ or TNFα (21).

Interestingly the proof of concept for a therapeutically effective viral superinfection was tested by Csatary *et al.*, some eight years earlier prior to elucidation of the mechanism of beneficial effect. Human A virus induced hepatitis of marmoset monkeys was cured by superinfection with a replication-competent, apathogenic BDV (22).

Introducing self-replicating agents into the patient and environment - even if only with therapeutic intent for a limited number of patients - requires the highest levels of ethical and medical review (23). Unlike many pathogenic virus vectors, the avian BDV poses no danger to the general population (24), thus BDV was safely and effectively used in a clinical trial for the treatment of acute B and C hepatitis (12).

The patients presented in this report had decompensated liver disease, thus interferon therapy was not a realistic option (indeed, case 2. contracted her hepatitis before interferon therapy was available). Our working hypothesis was, that superinfection of the patients by an apathogenic virus may facilitate the hepatitis virus clearance by inducing the local production of cytokines (such as IFNγ or TNFα) in the infected liver. Considering, however, the severe clinical conditions of patients it was unexpected that two patients would be essentially cured and the clinical symptoms of the third, moribund patient was significantly improved by the BDV therapy. In a group of patients with well-compensated chronic hepatitis B loss of HBsAg and HBV DNA was found following up to 7 years of interferon-alfa therapy (25). Such a response, however, is less likely in patients with decompensated hepatitis.

A common experience with the MTH-68/B vaccine treatment is that in order to be therapeutically effective, the virus must be given in massive doses over a long period. As we are not aware of spontaneous remission in decompensated chronic viral hepatitis, furthermore symptoms recurred when treatment was interrupted, we believe that the positive changes presented in this report can most realistically be ascribed to the repeated courses of MTH-68/B (BDV) vaccine treatment. Indeed, soon after the beginning of the virus therapy, the patients experienced an improvement in symptoms like pruritus and malaise, which was accompanied by a gradual increase of physical activity. Liver enzyme levels (ALT, AST, GGT) returned to normal range within a few months of MTH-68/B therapy, demonstrating the cessation of liver cell damage. By contrast, the normalization of the ChE activity (a sign of liver regeneration) continued over the entire follow-up period. Since we repeatedly found normal ALT 12 months (or more) after the completion of BDV therapy in two patients, we believe that their hepatitis went into long-lasting remission. According to histological assessment, the chronic progressive hepatitis of the moribund patient (who had very slim life expectancy before the virus therapy) was stabilized and she is now capable of light work.

Most patients with chronic HBV and HCV requiring liver transplantation have reinfection of the hepatic graft, (9,26) where recurrence of a more aggressive course of the infection is very commonly followed by graft failure (27). At a recent International Liver Transplantation Society congress, it was suggested, that to use prophylactic interferon-α-2b and ribavarin be given very early after transplantation when the viral load is low (26). To this end, we believe, a safer approach could be the use of MTH-68/B vaccine as it has no side effects in humans.

The efficacy and safety of BDV therapy in chronic active hepatitis B and C can only be properly evaluated in randomized clinical trials. The presented cases are, however, both remarkable and instructive since they challenge the current assumption that patients with decompensated liver disease, who are refractory to interferon therapy, cannot be successfully treated.

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