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Review

Current status of immunomodulatory therapy in chronic hepatitis B, fifty years after discovery of the virus: Search for the "magic bullet" to kill cccDNA

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ABSTRACT

Chronic hepatitis B (CHB) is currently treated with IFN-α and nucleos(t)ide analogues, which have many clinical benefits, but there is no ultimate cure. The major problem consists in the persistence of cccDNA in infected hepatocytes. Because no antiviral drug has been evaluated which significantly reduces copies of cccDNA, cytolytic and noncytolytic approaches are needed. Effective virus-specific T- and B-cell responses remain crucial in eliminating cccDNA-carrying hepatocytes and for the long-term control of HBV infection. Reduction of viremia by antiviral drugs provides a window for reconstitution of an HBV-specific immune response. Preclinical studies in mice and woodchucks have shown that immunostimulatory strategies, such as prime-boost vaccination and PD-1 blockade, can boost a weak virus-specific T cell response and lead to effective control of HBV infection. Based on data obtained in our preclinical studies, the combination of antiviral drugs and immunomodulators may control HBV viremia during a patient's drug-off period. In this article, we review current immune-modulatory approaches for the treatment of chronic hepatitis B and the elimination of cccDNA in preclinical models. This article forms part of a symposium in *Antiviral Research* on "An unfinished story: from the discovery of the Australia antigen to the development of new curative therapies for hepatitis".

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Abbreviations: HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; IFN, interferon; CHB, chronic hepatitis B; NUC, nucleos(t)ide analogues; cccDNA, covalently close circular DNA; DHBV, duck hepatitis B virus; WHV, woodchuck hepatitis virus.

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1. Introduction

Since the discovery of the Australia antigen by Blumberg 50 years ago, and the subsequent detection of the complete hepatitis B virion by Dane, rapid developments have occurred in the field. Early diagnostic tools were developed to identify chronic carriers of hepatitis B virus (HBV), by detection of HBsAg and corresponding antibodies to the surface and core protein, and the molecular, biochemical and immunological characteristics of the virus and its replication were established in parallel. Serological tools were used to study epidemiological patterns of HBV infection worldwide, which revealed that sexual transmission, mother-to-child transmission and direct blood contacts were the major sources of infection.

In the 1970s, the DNA genome of HBV was identified with a DNA polymerase activity in Dane particles (Robinson and Greenman, 1974) and a reverse transcriptase in core particles was identified in 1982 (Summers and Mason, 1982). In the 1980s, the HBV DNA genome was cloned from viral particles and was shown to be infectious on injection into the liver of chimpanzees (Will et al., 1982). The envelope of HBV was found to consist of three proteins with identical c-termini (Seifer et al., 1990; Stibbe and Gerlich, 1983).

Many steps were undertaken early on to develop a vaccine to prevent HBV infection worldwide. Progress in diagnostic procedures, molecular biology, prevention by vaccination, and treatment should eliminate this major public health problem soon. However, there are still an estimated 240 million HBV carriers worldwide (Ott et al., 2012). Most of them result from the pre-vaccination era or lack of vaccination programs.

Persistently HBV-infected patients are at risk of developing chronic liver disease, cirrhosis and hepatocellular carcinoma (HCC). An effective and affordable therapy to achieve sustained suppression of HBV replication and remission of liver disease is urgently needed. Pegylated interferon-alpha 2a (IFN-α) is recommended for the treatment of chronic hepatitis B (CHB) in the current consensus guidelines of many countries. Compared with conventional recombinant IFN-α, however, pegylated IFN-α alone or in combination with nucleoside analogues does not significantly increase the rate of sustained response (Janssen et al., 2005; Lau et al., 2005).

Nucleos(t)ide analogues (NUCs), such as entecavir and tenofovir, suppress HBV replication and result in the improvement of liver histology and function. However, these agents cannot eradicate HBV genomes from the liver, and may be further limited by the development of drug-resistant mutants with prolonged use (Locarnini and Mason, 2006; Zoulim and Locarnini, 2009). Therapy with additional antiviral drugs targeting other steps in the viral life cycle, in combination with immunomodulatory options, might be more beneficial and effective. Antiviral treatment with NUCs is also expensive and live long which cannot be effort by the health system of many countries. New approaches have to be envisioned to develop therapeutic vaccines which may be effective and at low costs to treat chronic carriers even in developing countries.

In this review, we describe new immune-modulatory approaches for the treatment of CHB, having in mind to reduce covalently close circular DNA (cccDNA) by the elimination of infected hepatocytes. A number of studies in preclinical models, such as transgenic mice, woodchucks and chimpanzees, have demonstrated possible immunomodulatory approaches, which will be discussed in detail.

2. Persistence of cccDNA is the major problem in CHB treatment

The elimination of HBV during treatment is difficult, due to the presence of a cccDNA, which persists in the form of a mini-chromosome in hepatocytes (Gish et al., 2015). Cytokine release e.g. IFN-γ described by Guidotti et al. in HBV-infected chimpanzees, reduced cccDNA in the early stage of infection (Guidotti et al., 1999). However, this reduction may be ineffective in an established, persistent HBV infection. The intracellular circle of core particles filling up the cccDNA pool may be important during acute infection, but seems to play a minor role in persistent infection (Guo and Guo, 2015). So far there is very limited success in eliminating this form of transcriptional template of the virus, either by destruction of existing cccDNA molecules or inhibition of the generation of new molecules.

Antiviral drugs that have been developed in recent years act as inhibitors of reverse transcription, inhibition of core particle assembly or the release of viral or subviral particles, but their impact on reduction of copies of cccDNA has not yet been carefully evaluated (Block et al., 2015). The pool of cccDNA molecules in hepatocytes seems to be very stable. Cell turnover may dilute the number of copies, but it may take many years to completely eliminate cccDNA by cell division. In addition, the integration of HBV DNA into the genome of hepatocytes or hepatoma cells has been described, and may result in the persistent generation of viral or subviral particles (Bertoletti et al., 2015). In the woodchuck, an important model for hepatitis B, integration of hepadnaviral genomes is a frequent event, resulting in a high frequency of hepatocellular carcinoma (Gerin et al., 1989; Roggendorf et al., 2010)

HBV proteins are translated from viral mRNA in infected hepatocytes and are presented after processing by proteasomes on the cell surface by MHC class I. Infected cells can then be recognized by T cells. Similar to many other viral infections, virus-specific CD4 and CD8 T cells are capable of eliminating HBV-infected cells and cccDNA in about 90% of patients infected as adults. By contrast, only 10% of infected newborns mount a vigorous, multi-specific immune response to HBV proteins. Recovered individuals have circulating antibodies which prevent reinfection of uninfected hepatocytes and CD8 T cells which may limit the number of hepatocytes carrying residual cccDNA (Penna et al., 1996). In a resolving acute infection, however, some cells carrying cccDNA survive T cell elimination and may cause reactivation of the virus under immunosuppression. Patients with CHB tend to have delayed, transient or narrowly focused T-cell responses. Treatment which destabilize core particles e.g. IFN-γ or assembly inhibitors have obviously no strong influence on degradation of the cccDNA pool.

The difficulty of therapy in CHB due to the persistence of mini-chromosomes of cccDNA is similar to HIV infection, when the virus fully integrates. In the case of HIV, it is even possible that cells with