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Hepatitis Delta: Immunopathogenesis and Clinical Challenges

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Key Words
Hepatitis D virus · Hepatitis B surface antigen · Interferon-α treatment

Abstract
Hepatitis delta is caused by infection with the hepatitis D virus (HDV) and is considered to be the most severe form of viral hepatitis in humans. Hepatitis delta occurs only in hepatitis B virus (HBV) surface antigen (HBsAg)-positive individuals as HDV is a defective RNA virus which requires the HBsAg for complete replication and transmission. Eight different HDV genotypes have been described with specific geographic distributions and distinct clinical courses. HDV/HBV co-infection can be associated with complex and dynamic viral dominance patterns. While HDV is frequently the dominating virus not only in HBV/HDV co-infection, but also in HBV/HCV/HDV triple-infected patients, the fluctuating courses of HDV and HBV viremia can be observed in other patients. Chronic HDV infection leads to more severe liver disease than HBV monoinfection with accelerated fibrosis progression, earlier hepatic decompensation and an increased risk for the development of hepatocellular carcinoma. However and in contrast to HCV infection, hepatic decompensation, rather than development of liver cancer, is the first clinical endpoint that develops during the course of infection. So far, only interferon-α treatment has proven antiviral activity against HDV in humans and has been linked to improved long-term outcome. Recent studies on the use of pegylated interferon showed a sustained virological response concerning HDV-RNA in about one quarter of the patients. HDV-specific immune responses might be associated with the response to treatment. Novel alternative treatment options, including prenylation inhibitors, are still awaiting clinical development for delta hepatitis. So far, only few studies have investigated immune responses against HDV and HBV in humans.

History

The hepatitis delta virus (HDV) was discovered in 1977 by Mario Rizzetto, first as a novel antigen in the nuclei of hepatocytes in liver biopsies of hepatitis B patients with a severe course of disease [1]. It was then termed 'delta antigen' and later proven to be the infectious agent of a novel form of viral hepatitis by experimental infection of chimpanzees already infected with hepatitis B virus (HBV) [2].

Virology

Accordingly, hepatitis delta only affects HBV-infected patients, as it is dependent on the hepatitis B surface antigen (HBsAg) for the packaging of its virions. It must be considered a satellite virus of the hepatitis B virus. The genome of HDV consists of RNA of about 1,700 nucleotides, thus being the smallest of any known animal virus [3]. The HDV genome is single-stranded and circular,

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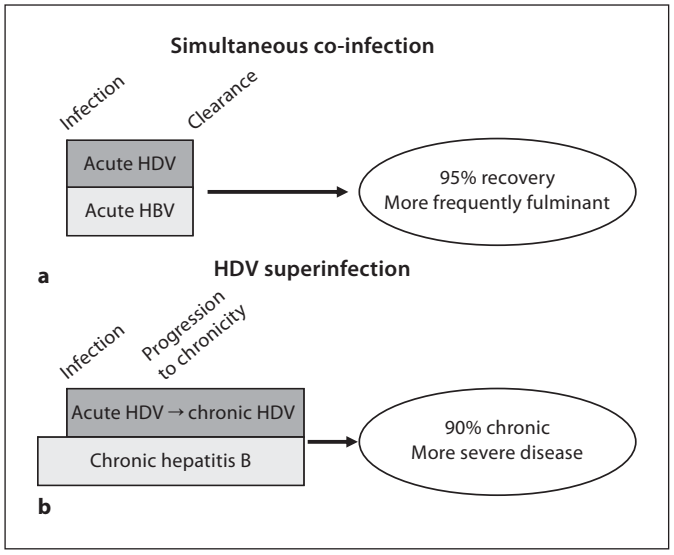

Fig. 1. Schematic comparison of course and outcome of HBV/HDV co-infection (**a**) and superinfection (**b**) of a HBV patient with HDV.

and has a high level of intramolecular base pairing. HDV does not show any similarities to other animal-pathogenic viruses, but instead resembles some features of the so-called 'viroids' from the plant world. Therefore, HDV has been classified into its own genus, Deltavirus, in which it is still the only member. HDV encodes only for one protein, the hepatitis delta antigen (HDAg). HDAg exists in two isoforms, the large and small HDAg, both encoded by the same open reading frame. The sequence of the small HDAg undergoes post-transcriptional alteration of the stop codon to create the template for the large HDAg. The small HDAg enhances genome replication. The large isoform has an inhibitory effect on replication, but is required for the morphogenesis of viral particles. Viral particles are approximately 36 nm in diameter, contain HDV-RNA and HDAg and are covered by HBs antigen [4]. Genome replication of HDV is performed by the host cell RNA polymerase II in a rolling circle manner known from other viruses or bacterial plasmids. Multimers of the HDV genome are self-cleaved by autocatalytic ribozymal activity [5].

Epidemiology

HDV is a disease with a significant impact on global health affecting 10–15 million people [6], and yet vastly underestimated [7]. The prevalence varies between different parts of the world. In some HBV-endemic regions like China, the HDV infection rate is disproportionally low; however, in other regions, like parts of Russia, Central Asia, Moldavia and Romania, southern Italy and other Mediterranean countries, Africa, and South America, it has high prevalence rates with up to 30% of HBsAg carriers being positive for anti-HDV [8]. Over the last 20 years, due to vaccination against HBV and means of human immunodeficiency virus (HIV) prevention, the prevalence of HDV infection has decreased in Italy to only 8% of HBsAg carriers [9]. A similar decline was reported for Taiwan: from 23% in the year 1983 to 4% in 1995 [10]. In Germany, a decline of HDV prevalence could also be observed between 1992 and 1997 [11]; however, this trend did not continue after 1999, with anti-HDV antibodies still detected in 8–12% of HBsAg-positive patients in the time period 1999–2006 [12].

The main risk groups for hepatitis delta infection in central Europe are migrants and intravenous drug users. Eighty percent of HDV patients in Germany migrated from South-Eastern Europe or from states of the former Soviet Union [13].

Eight genotypes of HDV have been identified so far [14]. The different genotypes have a sequence similarity of 60–70%, and within one genotype differences of 10–15% are present. Genotypes I, II and III are the most common, each with a distinct geographic distribution [6] (fig. 2). Genotype I is prevalent in Europe and central Asia [15], genotype II is mainly found in Eastern Asia and genotype III is exclusively found in South America [16, 17], where it has been reported to lead to severe outbreaks of fulminant hepatitis in the Amazonas region [18]. Other HDV genotypes can be found in various regions of Africa [14], but their pathogenicity is not well studied yet.

Clinical Course

Delta hepatitis is considered to be the most severe form of all viral hepatitis infections. Based on the mode of infection, two different courses of the disease can be distinguished (fig. 1). HDV superinfection of an already chronic HBV patient usually causes a more severe course of liver disease than hepatitis B virus or hepatitis C virus (HCV) monoinfection. Ninety percent of these cases become chronic, and spontaneous clearance of the infection is extremely rare. Chronic HDV has an accelerated progression to fibrosis, increased risk of hepatocellular carcinoma and early decompensation in the setting of cir-

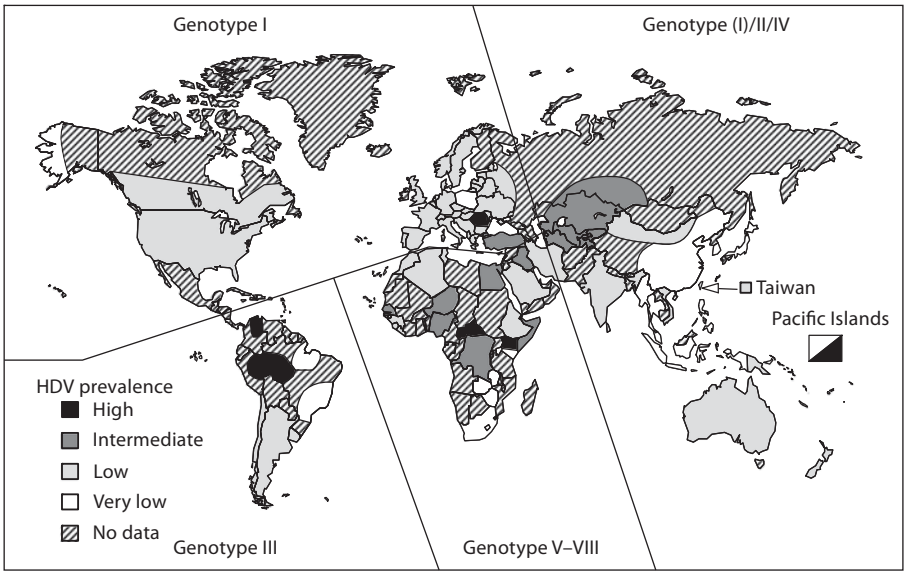

Fig. 2. Map showing the global distribution of HDV genotypes, as well as prevalence, of HDV infection in different regions of the world.

rhosis [19]. In a recent longitudinal study by Calle Serrano et al. [20], the severe course of chronic liver disease was confirmed and hepatic decompensation, rather than hepatocellular carcinoma, was identified to be the primary clinical event in European HDV patients.

Simultaneous co-infection with both HBV and HDV will often lead to fulminant hepatitis, but 95% of cases will recover spontaneously [21]. There are reports of fulminant outbreaks of HDV infections [18, 22–24], but these incidents have fortunately become less frequent due to the availability of an effective vaccination against hepatitis B. Disease severity does not depend on the genotype of HDV alone, as shown by the fulminant outbreaks of genotype III delta hepatitis in South America [17, 18], while the genotype of HBV seems to play an important role as well [25]. This study also emphasizes the poorer prognosis of HBV/HDV co-infection in contrast to HBV monoinfection alone.

Some studies have been performed to evaluate HDV infection in the context of HIV or HCV infection, with HDV usually accounting for a worse outcome. Sheng et al. [26] from Taiwan found an increased risk of hepatitis flares, liver cirrhosis, hepatic decompensation and death in patients with HIV-HBV-HDV triple infection compared to HIV-HBV-infected patients without delta hepatitis. A Spanish group found the highest rate of liver cirrhosis in HIV patients among those that had HBV-HCV-HDV triple infection in contrast to co-infection with HIV and either HCV or HBV [27]. A longitudinal study performed in Italy evaluating HBV-HCV co-infected patients found a significantly increased risk for cirrhosis in those with additional HDV infection, and cirrhosis was detected in more than 50% of triple-infected patients, but only in 33% of HBV/HCV dual-infected patients (17/30 vs. 34/103, p = 0.02) [28].

Treatment

Treatment options for hepatitis D infection are limited. None of the nucleotide or nucleoside analogs approved for the treatment of hepatitis B or hepatitis C are effective against hepatitis D [29, 30]. The current state of the art, and the only approved regimen against delta hepatitis is treatment with pegylated interferon-α [31]. Interestingly, it was shown recently that HDV interferes with interferon-signaling in vitro [32], which might explain treatment failure and low response rates. One potential new treatment option might be the use of prenylation inhibitors, which have been promising in the mouse model [33]. These novel drugs exploit the prenylation step that takes place in virus assembly [34].

Immunopathology

HDV, like HBV or HCV, is not a cytopathic virus [35, 36]. This concept is supported by the recent report that HDV viremia, despite high variability, has no correlation with biochemical activity or staging and grading of liver

Table 1. Overview of the immunological studies performed in humans

Year	Author	Finding	Reference
1997	Nisini et al.	MHC-II-restricted epitopes of the HDAg	[40]
1998	Accapezzato et al.	extracellular processing of HDAg	[41]
2004	Huang et al.	MHC-I-restricted epitopes	[45]
2006	Aslan et al.	perforin-positive CD4 T cells in HDV	[42]
2009	Grabowski et al.	strong HBV-specific T cell responses in HDV patients	[54]

disease. HDV-RNA levels are associated with HBsAg levels [37]. In HBV and HCV infections, liver damage is believed to be mainly immune-mediated, caused by cytotoxic T cells killing infected hepatocytes. Yet the T cell response and contribution of other cells of the immune system is a key mechanism controlling the infection and leading to spontaneous viral clearance [38]. Although the contribution of cellular immune responses to liver damage and elimination of the virus in hepatitis D infection has not yet been clarified due to insufficient data on this topic, it can be assumed that the underlying mechanisms are similar to those observed in other viral hepatitis infections.

Not many studies have been performed concerning the immunology of HDV infection. In acute and persistent hepatitis D infection, antibodies against both isoforms of HDAg can be detected [39]. They do not seem to contribute to viral clearance though.

In 1997, Nisini et al. [40] identified MHC-II-restricted epitopes of the hepatitis delta antigen. The analysis of T helper cell responses in eight HDV patients against 15 different HDAg-derived peptides led to the discovery of four CD4+ T cell-specific epitopes with possible importance for vaccine development. In 1998, the same group discovered that one of the aforementioned epitopes may result from extracellular processing of the hepatitis delta antigen. Whether this contributes to more severe cytotoxicity or rather enhances the presentation to specific CD4+ T cells, thus improving the virus-specific immune response, could not be clarified [41]. Similarly to HIV infection, there is a high frequency of perforin-positive cytotoxic CD4 T cells in HDV infection. 6.6% of the CD4 T cells in HDV patients express perforin, compared to only 3.2% in HBV and 3.9% in HCV patients ($p = 0.04$). The high frequency of cytotoxic CD4 cells could be correlated with elevated AST and decreased platelet count, which is an indication for the stage of liver disease [42]. Although the involvement of CD8+ CTLs in HDV pathology has been hypothesized since 1990 [43, 44], there is only one study explicitly focusing on CD8 T cell responses in hepatitis delta infection [45]. Huang et al. [45] and colleagues from Taiwan evaluated possible HLA-A2-restricted epitopes of the hepatitis delta antigen by means of bioinformatics. Cytotoxic CD8 lymphocytes specific for the predicted peptides could be detected in mice vaccinated with HDV plasmid DNA by staining with MHC-class-I-tetrameric complexes. In two of four chronic HDV patients, antigen-specific cells recognizing the same epitopes could be detected. Furthermore, these peptides were able to stimulate proliferation of PBMC (peripheral blood mononuclear cells) and trigger cytokine production in vitro.

More insight into the virology and immunopathology of HDV infection was obtained in animal models. As mentioned before, the infectivity of the delta antigen was proven by experimental exposure of chimps [2]. Four years later, a new animal model was established by the transmission of HDV to Woodchuck hepatitis virus-positive woodchucks [46]. Woodstock hepatitis virus has similarities to HBV [47] and its surface antigen allows HDV to assemble virions. One noteworthy study performed in woodchucks evaluated a DNA vaccine against HDV [48]. Though the vaccinated animals showed anti-HDV antibody production, and stimulation of PBMC with HDAg-derived peptides caused T cell proliferation, the responses mounted by this vaccine did not prevent viremia after challenge of the animals with HDV. Further animal studies on HDV were performed in the mouse model. Most of these studies are focused on vaccine development and aimed to induce cellular immune responses against the hepatitis D virus. Although mice can neither be infected with HBV nor with HDV, immune responses can be provoked by systemic administration of plasmid DNA containing the sequence of HDV. It could be shown that DNA vaccination is able to induce both CD8 T cell responses [49] and CD4 T cell responses [50] in mice. None of the immunization studies in animal models could, so far, lead to the development of a prophylactic or therapeutic vaccine against HDV applicable for humans.

Another important aspect for immunology is the interplay between the viruses in multiple hepatitis infections. We have reported cases of multiple infections with

HBV, HCV and HDV in which HDV was the dominating virus and suppressed the replication of HCV [51]. A strong induction of HDV-specific T cell responses could be shown in another case of triple infection. The patient cleared HCV during acute delta hepatitis [52]. However, in one Italian study on the interference between HBV, HCV and HDV in HIV-positive patients, HDV did not play a major role [53].

We recently investigated the strength and frequency of HBV- and HDV-specific cellular immune responses in HDV patients. PBMC of 23 HDV patients were stimulated with peptide pools derived from hepatitis D antigen, hepatitis B surface antigen, hepatitis B core antigen and hepatitis B polymerase in vitro, and proliferation was measured. While thirteen of these patients did not demonstrate any proliferative response, in the majority of the responder patients, proliferation was caused by stimulation with HBV-derived peptides. In six patient samples, HBV-specific proliferation was measured in contrast to only four positive assays resulting from stimulation with HDAg-derived peptides. The proliferation achieved by stimulation with HBV peptides was also much stronger. HBV-specific proliferative responses were mainly multispecific and in 48% of the cases triggered by hepatitis B core antigen-derived peptides. In summary, this suggests that strong HBV-specific cellular immune responses occur in the majority of HBV/HDV co-infected patients [54].

Conclusions

Hepatitis D virus infection still represents a significant global health burden with limited treatment options available. Data published so far suggest that hepatitis D is indeed a mainly immune-mediated disease and that both HDV-specific, but also HBV-specific adaptive immune responses play a key role in controlling the infection and in the pathogenesis of liver disease. More detailed studies analyzing the complex network of cellular and humoral immune responses in the context of dual or even triple infections are needed to better characterize immunological correlates of viral control. These studies could potentially be the basis for novel treatment options for HDV infection which are urgently needed.

Disclosure Statement

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