Hepatitis D Virus Coinfection and Superinfection

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Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

HDV is a defective RNA pathogen requiring the simultaneous presence of HBV to complete its life cycle. Two major specific patterns of infection have been described: the coinfection with HDV and HBV of a susceptible, anti-HBs-negative individual, or the HDV superinfection of a chronic HBV carrier. Coinfection mostly leads to the eradication of both agents, whereas the majority of patients with HDV superinfection evolve to chronic HDV infection and hepatitis. Chronic HDV infection worsens the preexisting HBV-related liver damage. HDV-associated chronic liver disease (chronic hepatitis D) is characterized by necroinflammation and the relentless deposition of collagen culminating, within a few decades, into the development of cirrhosis and hepatocellular carcinoma.

Hepatitis D virus (HDV) infection characterizes a subgroup of hepatitis B surface antigen (HBsAg)-positive patients affected by a frequently aggressive form of chronic liver damage (hepatitis D). Because HDV particle assembly and release are dependent on the obligatory presence of HBV within the same hepatocytes, a productive HDV infection is invariably associated with HBV infection. Two major patterns of infection have been described: coinfection with HBV and HDV, or superinfection with HDV of a person chronically infected with HBV. A third minor pattern or helper-independent HDV infection has been reported in the liver transplant setting. Although its existence is questioned, it will be briefly discussed in view of its historical interest.

ACUTE HEPATITIS D

Acute hepatitis D caused by HBV/HDV coinfection occurs on the simultaneous infection with both HBV and HDV of an individual who is susceptible to HBV (and therefore anti-HBs negative) (Fig. 1). From a clinical standpoint, this entity is indistinguishable from an acute hepatitis B (Smedile et al. 1982). Acute hepatitis D occurs after an incubation time of 1–2 mo. The preicteric phase is characterized by nonspecific symptoms such as fatigue, lethargy, digestive symptoms (anorexia, nausea), and the appearance of the usual biochemical markers, such as elevated serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The icteric phase, which is not always observed,

Editors: Christoph Seeger and Stephen Locarnini
Additional Perspectives on Hepatitis B and Delta Viruses available at www.perspectivesinmedicine.org

 doi: 10.1101/cshperspect.a021550
Cite this article as *Cold Spring Harb Perspect Med* 2014;4:a021550

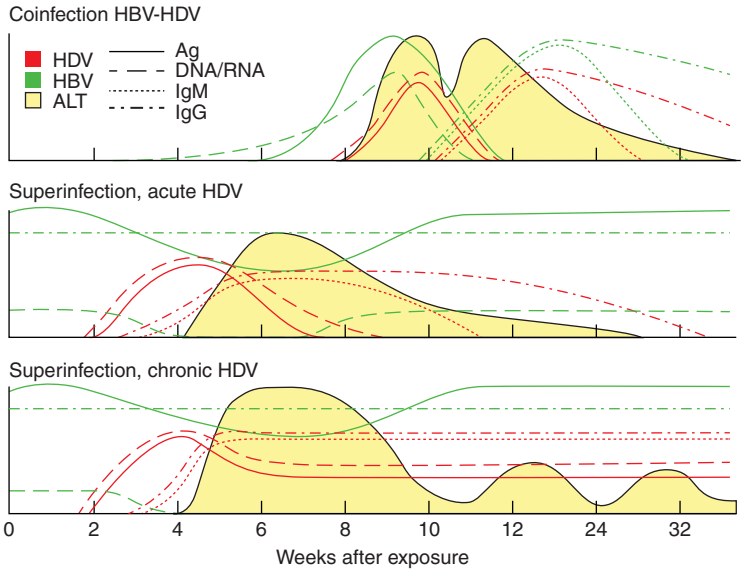

Figure 1. Serologic patterns of hepatitis D. Coinfection with HBV and HDV leading to eradication of both viruses (*top* panel), self-limited superinfection with HDV of a chronic HBV carrier (*middle* panel), and superinfection with HDV of a chronic HBV carrier leading to persistent HDV infection (*bottom* panel). The expression levels of antigens, DNA or RNA, IgM, and IgG for both HDV and HBV and ALT are indicated. (From Pascarella and Negro 2011; reprinted, with permission, from Wiley © 2011.)

is characterized by elevated levels of serum bilirubin. In adults, HDV/HBV coinfection is usually transient and self-limited, as the rate of progression to chronicity is the same as that of HBV monoinfection (i.e., less than 5%) (Caredda et al. 1983). A more severe clinical course is frequent, however, and two peaks of serum ALT and AST may be observed. An increased risk of acute liver failure has been reported among patients with HBV monoinfection, especially in drug addicts (Smedile et al. 1982). This is characterized by a massive hepatocyte necrosis that leads to death in 80% of the patients, unless urgent liver transplantation is performed.

To establish diagnosis, assessing the presence of HBsAg is necessary before investigating any other marker because HDV is dependent on HBV (Table 1). HDAg, the only antigen encoded by HDV genome, is detected early but also vanishes quickly. Thus, its transient appearance requires repeated testing (Buti et al. 1986). Serum HDAg lasts longer only in immunodeficient patients because of their slow and weak immune response (Grippon et al. 1987). HDV genomic RNA is an early and sensitive marker of HDV replication in acute HDV infection (Tang et al. 1993). Serum HDV RNA can be detected by RT-PCR testing, which has superseded classical hybridization-based assays owing to its increased sensitivity, with a lower detection limit of 10 genomes per mL (5–11). However, a crucial diagnostic marker is represented by high-titered IgM anti-HBc antibodies, which disappear along with the clinical resolution. IgM anti-HBc are absent in chronic HBV infection, thus enabling to distinguish the acute HBV/HDV coinfection from an acute HDV superinfection of a chronic HBV carrier or from an established chronic HDV infection. On the other hand, the antibody response to HDAg can be detected in coinfected patients. However, anti-HD antibodies of the IgM class are not specific of acute hepatitis D, and anti-HD IgG are low titered and appear late. They may be the only detectable marker in patients who present late because of paucity of symptoms, thus rendering a proper diagnosis difficult. Serum HDAg, anti-HD IgM, and IgG can be detected by enzyme-

Table 1. Comparison between the clinical and diagnostic features of HDV infection according to the two patterns of coinfection and superinfection

	Coinfection	Superinfection
HBV infection	Acute	Chronic
Outcome	Usually recovery with viral eradication (<5% chronicity)	Usually persistent infection
HBsAg	Present, early, and transient	Preexisting and persistent
IgM anti-HBc	Positive	Negative
Anti-HBs	Appears during the convalescence phase	Negative
HDV infection	Acute	Acute or chronic
Outcome	Usually recovery with viral eradication (<5% chronicity)	Usually persistent infection (80% progress to chronicity)
Serum HDAg	Early and short-lived	Transient and later undetectable because of complexing with antibodies
Liver HDAg	Positive and short-lived	Positive but 50% sensitivity at late stages
Serum HDV RNA	Early positive and transient	Early positive and persistent
Anti-HDV	Late and low titered	Rapidly increasing titers and persistent
IgM anti-HDV	Positive, transient	Positive, high titered

linked immunosorbent assay (ELISA) (Shattock and Morgan 1984) or radioimmunoassay (RIA), but testing for all of these markers are not available in all countries.

Superinfection is the HDV infection of an individual chronically infected with HBV. This pattern of infection causes a severe acute hepatitis that may be self-limited (Fig. 1) but that in most cases (up to 80%) progresses to chronicity (Smedile et al. 1982). Once chronic HDV infection is established, it usually exacerbates the preexisting chronic hepatitis B (Smedile et al. 1981). On the other hand, HBV replication is usually suppressed by HDV, and this suppression becomes persistent in the case of a chronic HDV infection (Table 1) (Krogsgaard et al. 1987; Farci et al. 1988). In the setting of superinfection, the serum level of HDV RNA can reach 10^{12} copies/mL within a few weeks from infection. Increasing titers of anti-HD IgG appear late, but the seroconversion confirms the diagnosis in the absence of other tests. IgM anti-HBc are typically absent (Table 1).

HELPER-INDEPENDENT HDV INFECTION

Helper-independent infection of HDV was initially reported after liver transplantation (Ottobrelli et al. 1991). HBV infection of the transplanted liver is usually prevented by the administration of anti–hepatitis B surface antigen immunoglobulins. When poorly sensitive molecular hybridization assays were used to monitor HDV RNA in serum, it was observed that patients were HDV RNA–negative in serum despite the rare occurrence of HDAg in the hepatocyte nuclei—as detected by immunohistochemistry—well before HBV recurrence. This was interpreted as consistent with the infrequent evasion of neutralizing antibodies by some HDV particles, thus capable of infecting hepatocytes. Without the concomitant infection of HBV, a productive cycle of HDV could not be completed. This view was corroborated by the notion that the helper virus is necessary for particle formation but not for viral replication (Kuo et al. 1989). In these patients, circulating HDV RNA was only detected—by molecular hybridization assays—several months after transplantation, for instance, when residual HBV had evaded neutralization and coinfected hepatocytes harboring replicating HDV, thus allowing for HDV rescue and cell-to-cell spread (Ottobrelli et al. 1991). This pattern of infection, however, has been revisited with the use of more sensitive RT-PCR–based techniques for detecting HDV RNA. In addition, experiments on chimpanzees first infected with HDV

and later challenged with HBV have shown that the rescue of HDV by HBV is possible only when the challenge with the helper virus is performed very early (i.e., after 1 wk from HDV infection) but not later (i.e., after 1 mo) (Smedile et al. 1998). Thus, helper-independent HDV infection is now believed clinically irrelevant and probably of limited virological significance.

CHRONIC HEPATITIS D

HDV induces a usually severe form of chronic hepatitis, although the range of clinical manifestations is very broad, and HDV infection can be associated with asymptomatic cases as well as rapidly progressive hepatitis (Smedile et al. 1981; Rizzetto et al. 1983; Govindarajan et al. 1986a). The diagnosis is often fortuitous, or may follow the appearance of late complications at the cirrhosis stage. ALT and AST levels are persistently elevated in most patients. In chronic hepatitis D, HDAg is complexed with its corresponding anti-HD antibodies, typically present at high titer. Thus, HDAg cannot be detected unless under denaturing conditions (e.g., by immunoblot assay) (Bonino et al. 1986), which is not practical for routine testing, albeit very sensitive (Buti et al. 1989). Even the detection of HDAg by immunohistochemistry in infected livers is out of reach of most routine pathology laboratories. And it is poorly sensitive as the nuclei stained positive only in ~50% of patients, especially at late stages of the disease (Negro et al. 1988; Wu et al. 1995a). Conversely, HDV RNA is easily detectable in the serum by sensitive RT-PCR-based assays, currently used not only for diagnostic purposes but also to follow the viral RNA titer and kinetics during antiviral treatment (Castelnau et al. 2006; Erhardt et al. 2006; Niro et al. 2006; Mederacke et al. 2010; Schaper et al. 2010; Wedemeyer et al. 2011). Interestingly, anti-HD of the IgM class also persists after the acute infection throughout the chronic phase, at variance with other viral infections (Table 1). Thus, to establish the diagnosis of chronic hepatitis D, the screening assay used should be the detection of anti-HD antibodies by ELISA. The diagnosis of ongoing infection is then confirmed by the immunohistochemical staining for HDAg in the liver or the detection of HDV RNA in the serum. If the HDV infection is confirmed, the next step is to evaluate liver grading and staging to determine whether the patient may be a candidate for antiviral treatment.

Clinically, once chronic HDV infection is established, it usually exacerbates the preexisting liver disease associated with HBV (Smedile et al. 1981). Progression toward cirrhosis may be rapid (Rizzetto et al. 1983; Govindarajan et al. 1986a), although HDV-associated chronic liver disease may run an indolent course (Bonino et al. 1987), and even asymptomatic HDV carriers have been reported in some geographical areas (Hadziyannis et al. 1991). Older studies had reported that 70%–80% of chronic hepatitis D patients developed cirrhosis within 5 to 10 yr (Rizzetto et al. 1983; Govindarajan et al. 1986a) and 15% within 1–2 yr (Saracco et al. 1987). A recent retrospective study (Romeo et al. 2009) followed 299 patients for a mean period of 233 mo. At enrollment, seven had acute hepatitis and 104 had cirrhosis. Among the noncirrhotic, 82 patients developed cirrhosis during follow-up at a yearly rate of 4%. Persistent HDV replication predicted the development of cirrhosis. Overall, the relative risk of developing cirrhosis during follow-up in patients coinfected with HBV and HDV seems twofold compared with patients infected with HBV alone (Fattovich et al. 2008). Once established, however, cirrhosis caused by HDV may remain stable for years before progressing to liver failure or developing a hepatocellular carcinoma (HCC). Patients with HDV-associated cirrhosis have a probability of survival at 5 and 10 yr of 49% and 40%, respectively (Rosina et al. 1999).

The impact of HDV infection on the rate of HCC development in HBV-positive patients is controversial. A retrospective study conducted in Western Europe on 200 patients with compensated HBV-related cirrhosis, of whom 20% were anti-HDV positive, found that HDV infection increased the risk of HCC threefold and mortality twofold (Fattovich et al. 2000) compared with HBV monoinfection. After adjustment for clinical and serological features

at baseline, the estimated 5-yr risk for developing HCC was 13%, 4%, and 2% for anti-HDV positive/HBeAg negative, antiHDV negative/HBeAg negative, and anti-HDV negative/HBeAg positive patients, respectively. The corresponding figures for hepatic decompensation were 18%, 8%, and 14%, respectively, and for survival 90%, 95%, and 93%, respectively (Fattovich et al. 2000). The incidence of HCC according to the retrospective study mentioned above was also 2.8% per year (Romeo et al. 2009). Thus, persistent HDV replication predicts the development of HCC and liver-related mortality (Romeo et al. 2009).

FACTORS INFLUENCING HEPATITIS D PROGRESSION

Many factors can influence the outcome of hepatitis D, the single most important being the pattern of infection with HBV, for instance, coinfection versus superinfection as described above.

The severity of hepatitis D is influenced by the HDV genotype. HDV isolates show up to 39% heterogeneity, and the different sequences have been classified into eight HDV genotypes (Dény 2006). Although infection with multiple genotypes may occur in patients at high risk of repeated exposure such as injecting drug users, a single genotype usually predominates with >10% of the viral load being represented by the minor strain (Wu et al. 1999). In the Western world, where most natural history studies detailed in the previous paragraph have been conducted, HDV genotype 1 is prevailing (Dény 2006). In Taiwan, where the predominant genotype is 2, acute HDV infection evolves less frequently toward acute liver failure, and even chronic hepatitis D seems less rapidly progressive (Wu et al. 1995a,b). On the other hand, infection with genotype 3, which is prevalent in South America, induces a severe form of hepatitis. Indeed, severe outbreaks of acute hepatitis D with a high incidence of liver failure have been reported among the Yucpa Indians of Venezuela (Hadler et al. 1992), the Sierra Nevada de Santa Marta in Colombia (Popper et al. 1983), and some areas of the Brazilian (Bensabath et al. 1987) and Peruvian (Casey et al. 1993) Amazonian forest. A viral factor potentially involved in influencing disease outcome is the occurrence of specific HDAg species that have been reported in acute liver failure cases (Tang et al. 1993).

Among the factors related to the helper virus, the HBV genotype modulates the HDV viral load and correlates with adverse outcomes (Su et al. 2006; Kiesslich et al. 2009). High levels of HBV replication are associated with more severe liver damage, and a more ominous course toward liver decompensation has been documented in patients with ongoing replication of both HBV and HDV (Smedile et al. 1991).

PATHOGENESIS OF HEPATITIS D

HDV only replicates in the liver, and therefore pathologic changes are limited to this organ. Liver damage in HDV infection is thought to be mostly immune mediated, although initial data from experimentally infected chimpanzees had suggested a direct cytopathic effect of HDV on hepatocytes, particularly during the primary infection (Kamimura et al. 1983; Canese et al. 1984; Govindarajan et al. 1986b). It was observed that in acute hepatitis D, infected hepatocytes were undergoing degenerative changes characterized by shrunken eosinophilic cytoplasm and pyknotic nuclei, with minimal inflammatory cells in the liver parenchyma, consistent with a cytopathic hepatocellular damage. These findings were reported both in vitro (cell culture system) (Cole et al. 1991) and in human studies (Popper et al. 1983; Lefkowitch et al. 1987). The small isoform of HDAg expressed was suggested to be responsible for this direct cytopathic effect of HDV (Cole et al. 1991). However, other results and in vivo observations, such as the presence of inflammatory cells surrounding the infected hepatocytes and the presence of various autoantibodies in the serum of patients, argue for a mostly immune-mediated liver damage. On the other hand, because HBV replication is usually suppressed by HDV, liver damage is believed to be induced mostly by HDV rather than by HBV.

Variation in immune-mediated responses during acute and chronic HDV infection has

been noticed (Casey et al. 2006; Fiedler and Roggendorf 2006), which may explain the variability of the clinical course of HDV infection. Cytotoxic T lymphocytes are mainly responsible for clearing the virus by destroying HDV-infected cells. Delayed and insufficient immune response with the ability to recognize only limited viral epitopes has been implicated in failure to clear the infection coupled with establishment of chronic infection. An exaggerated immune response, particularly a cell-mediated one, is proposed to be involved in causing massive hepatocyte necrosis and liver damage in acute liver failure (Hansson et al. 1991). Thus, a vigorous immune response involving HDAg-specific T-cell response and cytotoxic killing of HDV-infected cells leads to both viral clearance and increased liver damage.

The fine details of the pathogenesis of HDV-induced immune damage are largely unknown, as they have been addressed by only a few studies. Response to HDV involves the activation of antigen-specific helper T cells secreting a variety of cytokines, including interleukin (IL)-2, IL-2 receptor, IL-10, and interferon (IFN)-γ (Magrin et al. 1989; Nisini et al. 1997; Grabowski et al. 2011). IL-2, in turn, stimulates both additional HDV-specific helper T cells and $CD8^+$ cytotoxic T cells that target infected hepatocytes. HDV-specific Th1 and cytotoxic T cells produce large amounts of IFN-γ (Nisini et al. 1997), which, in addition to its known immunological effects (among others, the induction of class I and class II MHC proteins at the surface of hepatocytes) (Franco et al. 1988), may also inhibit viral replication (Magrin et al. 1989). HDAg-specific T-cell responses in peripheral blood of HDV-infected individuals is associated with reduced HDV replication levels (Nisini et al. 1997). IFN-γ also stimulates the secretion of IFN-γ-induced protein-10 (CXCL-10), a chemoattractant that recruits natural killer (NK) cells, which add to the cell damage.

In addition to the immune mediated liver damage, several interactions between HDV and the cell machinery have been reported that may have pathophysiological consequences and clinical impact. Like many viruses capable of establishing persistent infections, HDV has developed a strategy to counter endogenous IFN-α. HDV directly inhibits the activation of the IFN-α signaling pathways by interfering with the early steps of the Janus kinase (JAK)/signal transducers and activators of transcription (STAT) signal transduction pathway. There is an inhibition of the tyrosine kinase 2 (Tyk2), STAT1, and STAT2 phosphorylation and a transcription impairment of several interferon-stimulated genes, including the myxovirus resistance-A (MxA), the 2′,5′-oligoadenylate synthetase (2′,5′-OAS) and protein kinase R in the presence of the virus (Pugnale et al. 2009). Furthermore, the large isoform of HDAg upregulates MxA transcription, and this may account for the suppression of HBV replication (Williams et al. 2009). In addition, HDV seems to sensitize cells to inflammatory stimuli. The large isoform of HDAg increases the tumor necrosis factor (TNF)-α-induced nuclear factor (NF)-κB signaling, probably via a direct interaction with the TNF-receptor-associated factor 2 (TRAF2), a factor involved in early signal transduction (Park et al. 2009). More generally, HDV products—encompassing the two HDAg isoforms and the various RNA species—have been shown to interact at several levels with the host cell proteome (Mota et al. 2008, 2009), including factors involved in the regulation of cell metabolism and energetic homeostasis, nucleic acid and protein metabolism, and apoptosis and cell growth. Both HDAg isoforms enhance clusterin gene expression via an increased histone H3 acetylation within the clusterin promoter (Liao et al. 2009). These epigenetic changes, common to several other viral infections, may play a role in oncogenesis and thus favor the development of HCC in HDV-infected individuals.

CONCLUSIONS

Because HDV requires the simultaneous presence of HBV to complete its life cycle, two specific patterns of infection may occur: a coinfection with HDV and HBV of a susceptible, anti-HBs-negative individual, or the HDV superinfection of a chronic HBV carrier. Although coinfection mostly leads to the eradication of

both viruses, the majority of patients with HDV superinfection progress to chronic HDV infection and hepatitis, usually worsening the preexisting HBV-related liver damage. Chronic hepatitis D is characterized by typical hallmarks of all chronic hepatitides, for instance, necroinflammation and fibrosis. This process may lead to the development of cirrhosis, liver failure, and HCC within decades.

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