Hepatitis delta virus (HDV) is a unique defective RNA virus that requires the helper function of hepatitis B virus (HBV), which provides the essential coat protein for virion assembly and propagation. It is the smallest animal virus and the only one to possess a circular RNA genome, as seen in plant viruses. HDV has evolved into three major genotypes that differ in their geographic distribution. Infection with HDV has a worldwide distribution, although a dramatic decline in the incidence has been observed in the last decade in the Mediterranean area, likely as a result of HBV vaccination programs and improved socioeconomic conditions. HDV can be acquired either by coinfection with HBV or by superinfection of chronic HBsAg carriers. Whereas coinfection commonly resolves, superinfection progresses to chronicity in over 90% of the cases. Chronic hepatitis D is a severe and progressive disease leading to cirrhosis in about 70% of the cases. The pathogenesis of HDV-induced liver injury is still unknown. Regardless, dramatic advances have been made in the biology of HDV, as well as in the understanding of the complex interactions between HDV, HBV and the host. Currently, interferon-alfa at high doses is the only drug that can induce amelioration of the disease in about half the patients, but it has little effect on HDV viremia. Liver transplantation is the only valid therapeutic option for the treatment of end-stage HDV liver disease. Although the recent progress in molecular biology has dramatically advanced our knowledge of the biology of HDV, much remains to be understood in order to devise more effective therapies and vaccines for the control of this unique defective virus, which still remains a major cause of death or enlisting for liver transplantation.

Twenty five years ago Rizzetto et al. discovered by immunofluorescence a previously unrecognized nuclear antigen that was subsequently shown to be a specific marker of a novel human pathogen, the hepatitis delta virus (HDV). The clinical association with HBV results from the fact that HDV is a defective virus that requires a helper function specifically provided by HBV or other hepadnaviruses. Since the early studies, HDV has emerged as an important medical problem because it is highly pathogenic and causes a severe and rapidly progressive form of liver disease. The cloning and sequencing of the HDV genome in 1986 confirmed that HDV is the most unique in animal virology. It is the first animal virus to possess a circular RNA genome, a finding that has only been seen in plant viruses. Progress in molecular biology has provided the tools to further understand the unique virologic features of HDV, which continues to represent a major challenge to both virologists and clinicians.

**Hepatitis D Virus**

**Classification**

HDV does not resemble any known transmissible agent of animals, but it shares similarities with both viroids and virusoids of plants in terms of structural characteristics of the RNA genome and mode of viral replication. The International Committee on Taxonomy of Viruses has proposed to classify HDV within the floating genus Deltavirus.
Structure and Genome Organization

The virus is an enveloped, spherical particle with an average diameter of 36 to 43 nm containing in its interior a nucleocapsid of 19 nm in diameter, which consists of an RNA genome and a single structural protein, hepatitis delta antigen (HDAg), that are encapsidated by the hepatitis B surface antigen (HBsAg). Such antigen, which is the only function provided by the helper virus, is essential for HDV assembly and propagation. There are two forms of HDAg, short (HDAg-S) and long (HDAg-L), that derive from the same open reading frame.

The HDV genome consists of a single, minus-strand, circular RNA, approximately 1700 nucleotides in length, which represents the smallest known viral genome in the entire animal kingdom. It possesses several distinctive features, some of which shared with plant virus RNAs, that make HDV unique relative to other animal RNA viruses. The genome has a high degree, up to 70%, of intramolecular base-pairing that confers the potential to fold into an unbranched, rod-like structure. Moreover, HDV shares with transmissible viroids and certain nontransmissible components of eukaryotic cells the capacity of its genomic and antigenomic RNAs to function as ribozymes capable of carrying out self-cleavage and self-ligation. Although these autocatalytic RNA segments are functionally similar to the “hammerhead” ribozymes described in plant viruses, their sequence and structure are quite different from those of other known ribozymes. Another unique function described for the HDV ribozyme is general acid-base catalysis, which could allow RNA to catalyze other reactions, including peptide bond formation.

Viral Replication

After HBsAg-mediated binding to a cellular receptor that is believed to be common to that of HBV, HDV penetrates into the hepatocyte and its genome is directed to the nucleoplasm, where genome replication occurs, by the nuclear localization signal domain identified in the HDAg. The mechanism of replication of the HDV genome is one of the most complex and fascinating aspects of the biology of this virus. HDV replicates according to a rolling-circle mechanism analogous to that proposed for viroids. Three major species of RNA accumulate in infected liver cells during HDV replication: genomic RNA, its exact complement, antigenomic RNA, and a smaller form of polyadenilated RNA (0.8 kb) of antigenomic polarity, which is the messenger RNA (mRNA) containing the open reading frame (ORF) for the synthesis of HDAg. The HDV RNA genome is replicated by RNA-directed RNA synthesis operated by a host cell enzyme, RNA polymerase II. HDV has the unique ability to redirect such enzyme, which is normally DNA-dependent, to transcribe the viral RNA genome. Initially, the genomic RNA serves as a template to synthesize multimeric linear transcripts of antigenomic sense, which undergo autocatalytic cleavage and ligation to produce circular monomeric antigenomic RNA. An essential role in the replication of HDV is played by HDAg. It has been recently reported that HDAg directly binds to RNA polymerase II and stimulates transcription by displacing a 66-kd subunit of negative elongation factor (NELF) and promoting RNA polymerase II elongation.

HDAg is an internal component of the nucleocapsid. It contains several essential elements for the virus biology: a coiled coil domain that enables HDAg dimerization; a nuclear localization signal that directs HDAg to the nucleus; an RNA-binding domain consisting of two arginine-rich regions; and an isoprenylation motif, unique to HDAg-L at the C-terminus. HDAg-L differs from HDAg-S by the presence of 19 additional amino acids at the C-terminus, which result from editing of the antigenomic RNA at position 1012 operated by a cellular enzyme, double-stranded RNA adenosine deaminase. This editing changes the UAG amber termination codon of the 195 aa. HDAg-S into UGG (encoding tryptophan), allowing for a C-terminal extension that leads to the synthesis of HDAg-L. HDAg-S is...
essential for viral replication, whereas HDAg-L acts as a dominant negative inhibitor of viral replication, but is required for virus assembly \(^{21}\). Evidence is accumulating to suggest that RNA editing plays a central role in the HDV replication cycle by acting as a biological regulator of the replication and assembly of HDV infectious virions \(^{24}\).

**Viral Heterogeneity**

Genetic variation is a hallmark of RNA viruses \(^{25}\). The first description of the genetic heterogeneity of HDV was reported in 1986 \(^{4}\) when it was found that HDV circulates, within a single infected host, as a mixture of different, albeit closely related genomes that follow the model referred to as a quasispecies \(^{26}\). Subsequently, the wide application of refined molecular techniques has provided the tools to better characterize the degree of genetic heterogeneity of HDV, both within the same individual and between different isolates. The rate of HDV mutation has been calculated to be \(3 \times 10^{-2}\) to \(3 \times 10^{-3}\) base substitutions per genome site per year \(^{27}\). The genetic heterogeneity of HDV is not uniformly distributed over the entire viral genome \(^{28}\), with the genomic and antigenomic cleavage domain and the RNA-binding domain of HDAg being the most conserved.

Genetic analysis of HDV isolates collected worldwide has revealed that HDV has evolved into three major genotypes, designated I, II, and III \(^{29}\) that differ in their global distribution \(^{24}\). The sequence differences among these genotypes are significant; the most distantly related HDV isolates vary by as much as 40% over their entire genomic sequences and 35% for the amino acid sequence of HDAg \(^{24}\). Genotype I, the most common worldwide, is associated with a broad spectrum of pathogenicity. It is predominant in the United States, Europe and the Middle East. Recently, it has been associated with fulminant hepatitis in the city of Samara in Russia, \(^{30}\). Within genotype I, two subgroups, 1A and 1B have been identified; 1A is predominant in Asia, 1B in the United States and both are common in the Mediterranean area. Genotype II, which seems to be associated with milder forms of liver disease, has been found in Japan and Taiwan, as well as more recently in the area of Yakutia in Russia \(^{31}\). Genotype III, which has been identified only in northern South America, has been associated with outbreaks of severe and fulminant hepatitis \(^{29,32,33}\). Interestingly, this genotype is linked with the coinfecting HBV genotype F \(^{32}\).

**HOST RANGE**

In nature, HDV infection has been found only in humans, whereas experimentally the host range of HDV is limited to those species that support the replication of HBV-related hepadnaviruses, capable of supplying a helper function to HDV, such as chimpanzees \(^2\), Eastern woodchucks \(^{34}\) and Peking ducks \(^{35}\). In addition, different mouse models of HDV infection have been attempted, including mice injected either with serum from experimentally infected woodchuck \(^{36}\) or with naked viral DNA or RNA sequences \(^{37}\), as well as heterochimeric SCID mice engrafted with human hepatocytes \(^{38}\), but none of them has so far provided a reliable model of viral tropism and pathogenesis.

**TRANSMISSION AND EPIDEMIOLOGY**

Infection with HDV has a worldwide distribution, although there are considerable geographic differences that do not entirely mirror the prevalence of HBV infection \(^{39}\). In northern Europe and in the United States, where HDV is not endemic, the infection is mainly confined to intravenous drug users \(^{40}\), whereas it has virtually disappeared in polytransfused subjects and hemophiliacs \(^{41}\) as a result of universal blood screening for HBsAg and HBV vaccination campaigns. In areas where HDV is endemic in the general population, as in the Mediterranean basin, the inapparent parenteral route accounts for most cases of HDV transmission \(^{42-44}\). The advent of molecular epidemiology has provided new tools to
further elucidate the modes and mechanisms of HDV transmission, confirming that HDV can be transmitted through sexual contacts, as well as among family members with a trend to form clusters.

Evidence accrued in the last decade, however, is suggestive of a changing trend in the epidemiology of HDV. A decline in HDV prevalence in both acute and chronic hepatitis has been observed in the Mediterranean area, most likely due to universal HBV vaccination, measures to control human immunodeficiency virus (HIV) and socioeconomic improvements, whereas new foci of HDV infection are emerging in other parts of the world. An outbreak of acute viral hepatitis with a high incidence of fulminant hepatitis, in which HDV accounted for 39% of the cases, has been reported between 1998 and 1999 in the city of Samara, in southeastern Russia, which has remained largely isolated until 1991. This situation is reminiscent of that seen in Sweden in the early 70's, as a result of the increased use of illicit parenteral drugs. The fulminant HDV strains from Samara were of genotype I and phylogenetically closest to Far Eastern and Eastern European strains. New foci of infection have also been identified in the island of Okinawa in Japan, in Northern India and in Albania. South America, especially the subtropical area, remains an important potential reservoir for new outbreaks of HDV infection.

Overall, HDV infection is still a major public health concern. It has been estimated that approximately 5% of the global HBsAg carriers are also coinfected with HDV, leading to a total of 15 million persons infected with HDV worldwide. Thus, the absolute number of dire events on a global scale is still high and HDV remains a major cause of death or enlisting for liver transplantation.

MODES OF INFECTION AND CLINICAL EXPRESSION

In view of the obligatory dependence of HDV on HBV coinfection, the modes of HDV infection are essentially two: simultaneous coinfection with HBV or superinfection of an HBsAg carrier. Persons with anti-HBs, being immune to HBV infection, are not susceptible to HDV infection.

The clinical expression of acute hepatitis D acquired by coinfection with HBV may range from mild to severe, fulminant hepatitis. In most cases, it resembles a typical acute self-limited hepatitis that is clinically and histologically indistinguishable from the ordinary hepatitis B. The outcome is a complete recovery, as typically observed in acute type-B hepatitis, and in only 2% of cases it may progress to chronicity. Diagnosis is made on the concomitant appearance of primary markers of infection with HBV and HDV.

In the superinfection pattern, the preexisting HBV viremia provides the biological background for the full expression of the virulence of HDV. Clinically, this results in a severe acute hepatitis that may run to a fulminant course. It may present as an exacerbation of a preexisting HBV disease or as a new hepatitis in a previously asymptomatic HBsAg carrier. If the HBsAg state is unknown, it may be misdiagnosed as classical acute hepatitis B. The correct diagnosis is suggested by a negative test for IgM anti-HBc and confirmed by the detection of HDV markers. Since the HBsAg carrier permits a continuous replication of HDV, the vast majority of HDV superinfected carriers develop progressive hepatitis (over 90% of the cases), whereas in a minority the superinfection may resolve, with persistence of the original HBV infection or even clearance of HBsAg without seroconversion to anti-HBs.

The setting of liver transplantation has more recently permitted to recognize a third pattern of HDV infection, latent infection. Within few days after liver transplantation, HDV can establish infection despite low levels of HBV replication. This type of HDV infection remains latent and is associated with no signs of liver disease. However, when a shift to high levels of HBV replication occurs, the latent
infection is rapidly transformed into a florid hepatitis of the liver graft.

**PATHOGENESIS**

The pathogenesis of HDV-induced liver disease is still undefined. Although a direct cytopathic effect of the virus has been reported, this hypothesis is contradicted by the lack of liver injury observed in grafts expressing only HDAg, as well as in hepatocytes from HDV-infected humans, and transgenic mice. Of note, a deleterious effect of HDV replication on host cell proliferation has been documented using in vitro-transfected cells. A role of host immune mechanisms, such as specific CD4+ and CD8+ T lymphocytes in liver damage seems unlikely. Nevertheless, multiple types of autoimmune phenomena have been reported in chronic hepatitis D, the most common being the presence of the LKM-3 autoantibody directed against uridine diphosphate glucuronyl transferase.

The relationship between viral genotypes and clinical course of hepatitis D has represented an important area of investigation over the past few years. However, the critical question of whether genotypes play a differential role in the pathogenesis and severity of hepatitis D is still unsolved. Although the link of genotype III with outbreaks of fulminant hepatitis suggests an inherently higher pathogenicity, the association of genotype I with a wide spectrum of disease severity including fulminant hepatitis argues against a direct role of the genotype in the pathogenesis of the disease. Further studies are needed to elucidate whether there are major biological differences among genotypes in terms of efficiency of replication, packaging, infectivity, transmissibility and pathogenicity.

**NATURAL HISTORY**

The natural history of chronic HDV infection is characterized by a wide spectrum of clinical expressions. Since the earliest studies, HDV turned out to be a highly pathogenic virus causing a severe and rapidly progressive disease, with very infrequent spontaneous resolutions. Cirrhosis was shown to develop in up to 70% of the cases, in about 15% of which within 1-2 years of the disease onset. Although HDV cirrhosis, once established, may be a stable disease for many years, coinfection with HDV was shown significantly increase the risk of hepatocellular carcinoma (HCC) and death, during a median follow-up period of 6.6 years, in patients with compensated HBV cirrhosis. Liver cancer, previously considered a rare event in the course of HDV disease, developed in 42% of patients with HDV cirrhosis in Greece over 12 years of follow-up. The link of HDV with severe and progressive liver disease has been found at all ages, as suggested by the detection of markers of HDV infection in up to 40% of children with HBsAg positive cirrhosis. However, the current clinical scenario of HDV disease is changing because fresh and florid forms of chronic hepatitis D are becoming rare in the Mediterranean area, as a consequence of a dramatic decline in the prevalence of HDV infection over the past decade.

Although HDV is generally associated with a severe form of liver disease, a few studies performed in open populations in highly endemic areas, such as the island of Rhodes in Greece and the American Samoa, have documented a high proportion of anti-HDV positive individuals without liver disease. Differences in disease outcome could be related to viral, host or still undefined environmental factors. Among the viral factors, the interaction between HBV and HDV replication has been reevaluated in recent years by using highly sensitive PCR assays. Most patients with chronic HDV infection have antibodies to HBeAg and low levels of HBV DNA replication. At variance with HDV-negative chronic hepatitis B, there is no correlation between serum HBV DNA and ALT levels in patients with chronic hepatitis D, suggesting that the liver damage in these patients is mainly caused by HDV. Similarly, HBV is suppressed in asymptomatic carriers of HDV. However, a pattern characterized by decreasing levels of HDV and reactivating HBV with moderately high ALT levels has been described during the course of chronic hepatitis D in Taiwan. In intravenous drug addicts, HDV infection is most
often associated with HBeAg positivity and active HBV replication, a pattern that may increase the pathogenicity of HDV 82.

Another factor that may modify the natural history of chronic hepatitis D is co-infection with other viruses. In triple infection, several studies have shown that HDV plays a dominant role inhibiting both HBV and HCV 83-86. The course of chronic hepatitis D does not seem to be influenced by concomitant HIV infection 87-89, except in terms of a more elusive or absent antibody immune response to HDV in subjects coinfected with HIV 90.

**DIAGNOSIS**

The advent of molecular techniques has provided highly sensitive tools to diagnose HDV infection. The detection of HDV RNA by polymerase chain reaction (PCR) is presently the most reliable diagnostic method. This molecular test has overcome the limitations of the direct detection of HDAg in serum by enzyme immunoassay or radioimmunoassay due to antigen sequestration in immunocomplexes with high-titered circulating antibodies 60. Its role has been crucial not only in the early phase of infection, before antibody seroconversion, but also to investigate the molecular events during both acute and chronic hepatitis. PCR has also offered a sensitive tool for monitoring the efficacy of antiviral agents, since it can detect 10 to 100 copies of the viral genome in serum 60. Because of the genetic heterogeneity of HDV, primers from the most conserved region, the C-terminal half of the HDAg gene, are most useful in clinical practice 29,70. The HDV genotype may be determined by restriction fragment length polymorphism analysis on PCR amplification products, by sequencing analysis and, on liver biopsies, by immunohistochemical staining using genotype-specific anti-HDAg antibodies 91.

The diagnosis of HDV infection may also be indirect, based on the detection in serum of antibodies against HDAg (anti-HD) of the IgG and IgM classes 57. Testing for IgM anti-HD has been crucial not only as a marker of primary HDV infection but also for its clinical relevance in the natural history of the disease 92. As a rule, chronic hepatitis D is associated with high titers of both IgG and IgM anti-HD 56, although the IgM are monomeric (7S) and not pentameric (19S) as in primary infection 93. The decrease and disappearance of IgM anti-HD predicts impending resolution of chronic HDV-disease, either spontaneous or induced by IFN treatment 94.

**TREATMENT**

The serious nature of chronic hepatitis D and the uniqueness of the delta agent make this disease a difficult target for antiviral therapy 95. To date, only alfa interferon (IFN) was shown to be beneficial, while other antiviral agents, including acyclovir 96, ribavirin 97 and famciclovir 98, failed to show any efficacy in chronic hepatitis D. Similarly, lamivudine, a nucleoside analog that potently inhibits HBV replication, has shown no effects on HDV replication nor on the disease activity 99. Experience with combination therapy with lamivudine and IFN is still limited, although preliminary data are not promising 100.

Pilot studies as well as randomized controlled trials have shown that IFN-alfa can induce an amelioration, albeit transient, of chronic hepatitis D. However, its efficacy is related to the dose and duration of therapy. When an initial dose of 5 megaunits (MU) per m² was given thrice weekly for 4 months followed by 3 MU per m² for 8 additional months, the dose reduction was associated in most patients with a relapse in ALT levels, which became normal at the end of treatment in 25% of patients,
but in only one patient at the end of the 12 months of follow-up \(^{101}\). Higher doses (9 MU three times weekly) given for up to one year induced ALT normalization in 71% of patients at the end of treatment and in about 50% after 6 months of follow-up \(^{102}\). This biochemical response correlated with an improvement in liver histology, but not with a loss of HDV viremia, as measured by PCR. The results of several studies confirmed that IFN has no or little effect on HDV replication \(^{95}\). Clearance of HDV RNA, which is associated with a progressive decline in IgM anti-HD titer, was documented in less than 10% of treated patients. Individuals who clear HDV RNA may ultimately lose HBsAg as well \(^{103,104}\). In chronic hepatitis D there are no reliable features that can predict which patients will have long-term benefits from the use of IFN, although patients with a short disease duration are the most likely to respond \(^{105}\), underscoring the importance of early treatment. In patients treated with a high dose and for a prolonged time, side effects are common and therefore a dose monitoring is essential for detecting major psychiatric and medical complications \(^{95,106,107}\).

The efficacy of IFN in chronic hepatitis D has been primarily evaluated upon evidence on short-term outcomes \(^{95}\). Recently, however, a long-term prospective study (up to 14 years) of patients treated with high doses of IFN (9MU) for one year has provided important information on the long-term effects of IFN \(^{108}\). Remarkably, half the patients who had a biochemical response at the end of treatment still had normal ALT values after 14 years of follow-up. But the most striking finding of this study was the complete regression of liver fibrosis documented in some patients with persistent biochemical response, loss of IgM anti-HD and reduced levels of HDV replication: all these patients had an initial diagnosis of active cirrhosis that was confirmed in two subsequent biopsies performed at the end of treatment and one year thereafter. Thus, these data have challenged the belief that active cirrhosis is irreversible. The reversion of hepatic fibrosis despite continuous HDV viremia detected by PCR, suggests that IFN may dramatically influence the natural history of the disease, beyond its antiviral effects. In only two patients was HDV RNA eventually cleared. Interestingly, eradication of both HBV and HDV, associated with a complete reversion of liver fibrosis, was achieved in a single patient with active HDV cirrhosis treated with continuous therapy, 5MU of IFN daily for up to 12 years \(^{109}\).

In conclusion, IFN-alfa is currently the only effective treatment for chronic hepatitis D. High doses (9MU thrice weekly or 5MU daily) for at least one year are required for achieving both short- and long-term effects on the disease outcome. In responders, therapy should be continued as long as possible, until eradication of HDV RNA and eventually loss of HBsAg. An individualized regimen in which the dose is titered according to tolerance and serum ALT levels is recommended. Despite the encouraging results, however, many problems - mainly related to the low rate of response and the high rate of relapse - remain to be solved. These limitations emphasize the need for improving the efficacy of IFN treatment, as well as for identifying innovative approaches and new antiviral agents that may benefit patients with chronic hepatitis D.

Orthotopic liver transplantation is the only valid therapeutic option for end-stage HDV-related liver disease. The risk of HDV reinfection of the graft has been found to be lower than that of HBV reinfection and can be prevented by the continuous administration of anti-HBs immunoglobulins \(^{64,110}\).

**PREVENTION**

Because of the critical contribution of HBV to the life cycle of HDV, immunoprophylaxis of HDV infection can be successfully achieved by vaccination against HBV. To date no effective vaccine specific for HDV has been developed \(^{111-114}\), which would represent the only means to eliminate the risk of HDV superinfection for over 300 million chronic carriers of HBsAg in the world.

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