

Hepatitis delta virus: A fascinating and neglected pathogen

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Abstract

Hepatitis delta virus (HDV) is the etiologic agent of the most severe form of virus hepatitis in humans. Sharing some structural and functional properties with plant viroids, the HDV RNA contains a single open reading frame coding for the only virus protein, the Delta antigen. A number of unique features, including ribozyme activity, RNA editing, rolling-circle RNA replication, and redirection for a RNA template of host DNA-dependent RNA polymerase II, make this small pathogen an excellent model to study virus-cell interactions and RNA biology. Treatment options for chronic hepatitis Delta are scarce and ineffective. The disease burden is perhaps largely underestimated making the search for new, specific drugs, targets, and treatment strategies an important public health challenge. In this review we address the main features of virus structure, replication, and interaction with the host. Virus pathogenicity and current treatment options are discussed in the light of recent developments.

Key words: Hepatitis delta virus; Hepatitis B virus; RNA replication; Pathogenesis; Treatment

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Core tip: Hepatitis delta virus (HDV) is the etiologic agent of probably the most severe form of virus hepatitis. HDV replication and spread depends on the presence of hepatitis B virus which provides the envelope proteins coded exclusively by its own genome. About 20 million people are currently chronically infected with HDV and no specific therapy is still available. Here, we review the current knowledge on HDV biology, epidemiology, pathogenesis, and treatment. Future trends and perspectives are discussed in the light of recent developments on HDV biology and its interaction with the host.

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INTRODUCTION

Over 35 years have passed since Rizzetto *et al.*^[1] reported the discovery of what has been called Delta antigen in a patient with diagnosis of severe hepatitis B infection. Subsequent research on the nature of this antigen led to the identification, in 1980, of a new hepatotropic virus, hepatitis delta virus (HDV)^[2,3]. This new infectious agent was later found to be a sub-viral agent dependent on the presence, in infected cells, of hepatitis B virus (HBV) to accomplish the replication cycle^[3,4]. In nature, both viruses, HBV and HDV, share the same envelope proteins coded exclusively by the HBV genome^[5,6].

Today, the World Health Organization estimates that about 400 million people are chronically infected with HBV worldwide^[7,8], of which approximately 20 million are co-infected with HDV^[9,10]. The Amazon basin and some central African and east European countries are among the regions with higher prevalence. However, there is still a considerable lack of information concerning a significant number of countries mostly situated in Africa, Asia, and Latin America (Figure 1). The geographic distribution of the so far identified eight HDV clades is also far from being uniform. Clade 1 may be found worldwide, in contrast with clade 3 which seems to be confined to the Amazon region (Figure 1). The most frequent outcome of the acute co-infection with HDV is virus clearance and patient's recovery. However, in up to 5% of the infected individuals a chronic form of HDV infection will develop^[11]. In the case of super-infection, when a chronic HBV carrier gets super-infected with HDV, the outcome is distinct. About 70%-90% of super-infected individuals will become chronic carriers for both viruses, HBV and HDV^[12].

As compared to the individuals that are chronic carriers of HBV alone, HDV additionally increases the risk of hepatocellular carcinoma (HCC) and mortality threefold and twofold, respectively, in HDV/HBV carriers^[13,14]. Currently, in clinical practice, there are no drugs used that directly and specifically target HDV. None of the currently approved anti-HBV drugs efficiently blocks HDV infection^[7,9,14-17].

All of the above, given additional HDV-inflicted liver pathogenesis, and inability to efficiently circumvent HDV infection by anti-HBV drugs, makes HDV a very serious pathogen, and it does call for additional attention to HDV and development of specific anti-HDV interventions.

HDV is mostly endemic in low income countries in which the budget for new, potentially expensive drugs is, of course, not the first priority. Accordingly, development of new treatment options based on specific drugs has not only proved to be difficult (the virus apparently does not code for any specific enzymatic activity that could be

targeted) but may also represent an uninteresting option for pharmaceutical companies, speaking from a strictly financial point of view.

Nevertheless, this small human pathogen bears a set of features that make it a formidable model to study fundamental aspects of host-pathogen interactions and RNA biology including mechanisms of transcription, replication, and genome evolution^[18,19]. The small size and structure of the genome bearing only one open reading frame (ORF), which is edited by host enzymes, its ribozyme activity and still largely undeciphered mechanism of RNA-directed RNA replication, are prominent examples of the uniqueness of this human pathogen^[19].

In this review, we will address the specific features of HDV structure and replication, its interaction with host cells and HBV. Future perspectives of research based on recent important developments will be discussed.

The virus and its replication

The virus: HDV is an enveloped spherical subviral agent about 36 nm in diameter^[19]. The virus particle contains a ribonucleoprotein (RNP) core consisting of one copy of the RNA genome and approximately 200 copies of the only virus encoded protein, the Delta antigen (HDAg)^[20]. The HDV envelope contains hepatitis B virus surface antigens (HBsAg), provided solely by HBV. In accordance, the two viruses share virtually indistinguishable envelopes^[6].

The virus genome is a circular single-stranded RNA molecule of around 1.7 kb and negative polarity^[21,22]. A significant degree of internal base-pairing (about 70% of all nucleotides) is an important feature, with potential not yet unveiled functional implications, observed in this molecule^[23,24]. This structure is similar to that described for plant viroids, albeit the latter have a smaller size and do not code for any protein (Table 1). On the contrary, the HDV genome displays one ORF which codes for the only viral protein, the Delta antigen^[25-27]. This protein can be found in virions under two distinct forms: Small (S-HDAg, 195 aa) and large (L-HDAg; 213 or 214 aa, depending on the genotype). L-HDAg is synthesized mainly later in the replication cycle^[28,29] as a consequence of an editing mechanism that takes place in the so-called anti-genome, an exact copy of the genome that arises as a replicative intermediate during RNA replication. The editing reaction is catalyzed by cellular adenosine deaminase 1 which converts an amber stop codon into a tryptophan codon (UGG) allowing a 57 nucleotide and consequently 19 aa extension of the ORF^[30,31].

Both L-HDAg and S-HDAg share the same functional domains with the exception of the L-HDAg-specific C-terminal extension, which bears an isoprenylation signal present in cysteine residue 211^[32]. Farnesylation of this residue is reported to be crucial albeit not sufficient for interaction with HBsAg and subsequent virion packaging and release from the cells^[33,34]. The common functional motifs are a nuclear localization signal (NLS; aa 66-75), a coiled-coil domain (aa 12-60), and a

Table 1 Similarities and differences between hepatitis delta virus and plant viroids

HDV (1700 nt)	Pospiviroidae (200-400 nt)	Avsunviroidae (200-400 nt)
Circular ssRNA	Circular ssRNA	Circular ssRNA
Extensive intramolecular base pairing	Extensive intramolecular base pairing	Extensive intramolecular base pairing
A DNA-directed RNA polymerase makes both plus and minus strands	A DNA-directed RNA polymerase makes both plus and minus strands	A DNA-directed RNA polymerase makes both plus and minus strands
Encodes for protein	No proteins encoded	No proteins encoded
Virion maturation depends on a helper virus	Replication does not depend on the presence of a helper virus	Replication does not depend on the presence of a helper virus
Symmetric rolling circle RNA replication	Asymmetric rolling circle RNA replication	Symmetric rolling circle RNA replication
Replicates in the nucleus	Replicates in the nucleus	Replicates in chloroplasts
Ribozyme activity	No ribozyme activity	Ribozyme activity

HDV: Hepatitis delta virus.

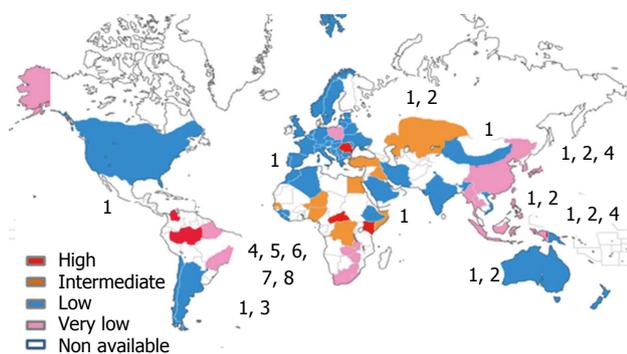


Figure 1 Prevalence and geographical distribution of eight hepatitis delta virus clades in the world.

bipartite arginine-rich RNA binding domain (aa 97-107 and 136-146; ARM1 and ARM2, respectively)^[35-37]. More recently, however, it was shown that mutation in the core arginines of both ARM1 and ARM2 did not impair the RNA-binding ability of a C-terminal HDAG-160 truncated form of HDAG^[38]. The authors suggested that HDAG establishes numerous contacts with HDV RNA to assemble ribonucleoprotein complexes.

Delta antigens: Several properties have been assigned to S-HDAG but none related to any known enzymatic activity. Among the reported putative and observed functions are the promotion of nuclear import of HDV RNPs^[39], regulation of HDV RNA editing^[40], facilitation of ribozyme cleavage (chaperone)^[41,42], and facilitation of accumulation of processed RNA transcripts^[43,44]. Both Delta antigens are post-translationally modified by host enzymes. Several post-translational modifications (PTM) have been described in HDAG and these include phosphorylation, methylation, acetylation, and sumoylation^[45-48]. Phosphorylation occurs at multiple sites and can be mediated by different host kinases, dsRNA-activated protein kinase R, protein kinase C, and ERK1/2^[49-51]. All these modifications may have distinct functional significance but it seems consensual that they are all involved in promoting virus RNA replication^[52].

Methylation of Arg 13 on S-HDAG by arginine methyltransferase I was reported and proposed to be important to enhance both genomic RNA and mRNA

synthesis^[46]. Additionally, cellular p300 acetyltransferase was found to acetylate Lys72 on the NLS of S-HDAG^[53]. Although speculative, this modification may have impact on the efficiency of nuclear import.

Finally, sumoylation was the most recent PTM to be reported on S-HDAG. It occurs at multiple lysine residues and is catalyzed by host small ubiquitin-related modifier isoform 1. Sumoylation was proposed to be important to promote genomic RNA and mRNA synthesis^[48].

Undoubtedly, these observations represent only a tiny part of the whole picture drawn by HDAGs inside the cell. In fact, Delta antigens can also be found as peptides of different smaller sizes in the nucleus of HDV replicating cells^[54]. Do these additional smaller forms correspond to distinct functional features? The answer is still far from being clear as no evidence supporting this point of view have been reported. In addition, it has been shown that S-HDAG can form multimers in HDV replicating cells^[20,55,56]. These multimers may play an important role in virus replication by facilitating the accumulation of virus RNAs. Moreover, it is known that HDAGs are basic proteins with an estimated overall + 12 charge^[57]. Thus, it is not surprising that, at least *in vitro*, the protein can bind nonspecifically to several types of nucleic acids including dsDNA and several distinct RNAs^[58].

Furthermore, S-HDAG may also be involved in sequestering and manipulating host cell components to facilitate HDV replication. In this context, it is not surprising that the search for S-HDAG interacting proteins unveiled a considerable number of potential partners. First Cao *et al.*^[59] used an immunoprecipitation followed by mass spectrometry approach being able to identify more than 100 host proteins in the assay. Later, Gowans *et al.*^[60] performed a yeast two-hybrid screen using a human liver cDNA library and identified 30 host candidate proteins capable of specifically interacting with S-HDAG. Making use of RNA silencing strategies some of these candidate interactions were found to be of potential functional significance. However, the above mentioned strong positive charge of HDAGs compels one to be careful when analyzing the specificity and role of these interactions in the HDV replication cycle.

S-HDAg is predicted to be an intrinsically disordered protein, a property already assigned to several other virus and cellular proteins^[61]. This feature may be responsible for the lack of success in all, to our knowledge at least in three different laboratories, attempts to crystalize and solve the 3D structure of the Delta antigen. These properties of the Delta antigen make the study of HDV biology much more complex than perhaps initially believed. However, as we shall discuss below, they are not the only most important ones.

HDV replication: HDV replication takes place in the nucleus of infected cells^[60,62,63]. The study of the HDV replication has long been difficult due to the lack of an appropriate cell culture system capable of supporting all steps of the virus life cycle, from attachment to release from the cells. Primary human hepatocytes have been long the only cells known to support the complete life cycle of HDV^[64]. These are expensive and not easy to cultivate. Thus, other approaches needed to be developed and a number of alternatives arose with time. Among them are the Hepa RG cell line and the stably transfected HEK-293 cells expressing S-HDAg under the control of a tetracycline inducible promoter^[65,66]. Although not representing ideal models, they became important tools for HDV research. The recent identification of the sodium-taurocholate co-transporting polypeptide (NTCP, encoded by SLC10A1) as the bona fide receptor for HBV and HDV culminated a long run that included a number of tested hypothesis and putative isolations^[67,68]. It represented an important breakthrough since it allowed engineering cell lines overexpressing it and consequently also supporting the initial steps of virus attachment and entry. So far, these human NTCP-expressing cell lines include human HepG2 and Huh7 as well as mouse Hepa1-6, AML-12, and primary mouse hepatocytes^[69].

After the uncoating of virus particles, HDV RNPs are transported to the nucleus, where RNA replication takes place^[70]. The existing data indicates replication of the virus genome involves a double rolling-circle mechanism with formation of multimeric anti-genomic and genomic molecules^[71]. These RNA multimers are cleaved at precise monomeric intervals by a ribozyme activity present in both genomic and antigenomic molecules^[72,73]. The presence of ribozymes in HDV RNAs is a feature shared with the viroid family of Avsunviroidae^[74] (Table 1).

Although it is well established that the presence of S-HDAg stimulates virus RNA accumulation, the precise role of this virus antigen in the mechanism of HDV RNA replication remains elusive. Controversy on which host polymerase or polymerases are involved in synthesis of genomes and antigenomes lasted, for a long time. Some groups claimed that both RNA pol I and pol II are involved in genome and antigenome synthesis, respectively^[75]. Mainly, these evidences were obtained in *in vitro* assays using different inhibitory concentrations of α -amanitin and on reports showing the presence of virus RNA in the nucleolus^[75,76]. By

contrast, other groups, using different types of transcription inhibitors, actinomycin D, 5,6-dichloro-1- β -D-ribofuranosylbenzimidazole, α -amanitin, provided data suggesting the involvement of solely RNA pol II^[77]. Furthermore, the presence of virus RNA in the nucleolus could not be observed in the absence of Delta antigen suggesting that this presence lacks functional relevance^[62,63]. In recent years, the use of immunoprecipitation and proteomic approaches, among others, led to the identification of several pol I, pol II, and pol III subunits as binding partners for HDV RNA^[59]. These results need to be interpreted with care since the observed binding to HDV RNA could be a result of indirect interaction through other non-identified partners. However, independently of the host polymerase(s) involved in replication of virus RNAs a striking question is still hanging in the air: How does the virus redirect a host DNA-dependent RNA polymerase to use an RNA template? Here, the eventual participation of the S-HDAg, which as mentioned before displays a net positive charge and intrinsic disorder, may play a crucial role allowing the virus to overcome obstacles posed by the host environment for its replication.

The search for promoter sequences in virus RNA has also been followed by a few groups with inconclusive results. Yet, there is evidence from *in vivo* models supporting that mRNA synthesis initiates at nt 1630^[78,79]. It may additionally be possible that multiple binding sequences for host RNA polymerases are present both in the virus genome and antigenome. This "nonspecific" binding could be a consequence of the RNA secondary structure bearing an extensive base-pairing with a number of predicted internal loops. Additionally, S-HDAg could also play an important role since it can bind nonspecifically to several nucleic acids, from dsDNA to ssRNA. It could be possible that S-HDAg plays a role as mediator between the virus RNA and a host RNA polymerase promoting its binding to several sequences in the genome and antigenome. Alternatively, S-HDAg could simply act as a chaperone, stabilizing RNA molecules and making them available for transcription. Assembly of HDV virions takes place in the cytoplasm. In this cellular compartment HBV-derived HBsAgs interact with HDV RNPs that are exported from the nucleus^[80,81]. This interaction was shown to be mediated by L-HDAgs^[82,83]. Tavanez *et al.*^[81] used heterokaryon assays to show that HDV RNPs shuttle between the nucleus and the cytoplasm. The authors claimed that nuclear import is mediated by an NLS located in Delta antigens (aa 66-75) and provided evidence that export to the cytoplasm is mediated by a cis-acting sequence in virus RNA^[35]. However, Lee *et al.*^[84] (2001) have shown a year before that aa 198-210 in L-HDAg were able to promote the export of a reporter protein. More recently, Freitas and Cunha used a well-established CAT reporter system to investigate a possible presence of nuclear export elements (NEEs) in HDV RNAs^[85]. The authors showed that NEEs may be present in both genomic and antigenomic molecules and that nuclear export is, at

least in part, sensitive to leptomycin B, an inhibitor of the host CRM1-mediated export pathway. Whether a NES present in L-HDAg or a NEE in virus RNA are responsible for promoting HDV RNP export may be considered still controversial. Consequently, further research is mandatory to unequivocally answer this question.

Clinical manifestations and therapy: It is widely and for a longtime known that HDV infection is associated with a broad range of clinical manifestations, from asymptomatic to fulminant hepatitis. In the latter cases, mortality often reaches 80% of the affected individuals^[86,87].

Concomitant infection of HBV and HDV usually displays more severe symptoms when compared with a single HBV infection. Nevertheless, the most frequent outcome is virus clearance, a situation reported in about 95% of the cases^[88]. In contrast, HDV super-infection of chronic HBV patients results in progression to chronicity in up to 80% of patients. Moreover, about 60%-70% of these patients will develop cirrhosis^[89]. These patients usually progress more rapidly to cirrhosis, show increased liver decompensation, and eventually death when compared with those chronically infected with HBV alone^[90,91].

The factors influencing the distinct clinical course in coinfecting and superinfected patients are still poorly understood. In both cases the organism produces a strong anti-HDAg antibody response which is, unfortunately, unable to modulate the course of infection^[92-94]. The majority of superinfected patients progresses to chronic disease independent of the presence of high titers of anti-HDV antibodies. Despite the limited number of studies there are evidences supporting a role of cytotoxic T cells in HDV infection including the destruction of infected hepatocytes^[95]. In any case, immunology of HDV infection is perhaps one of the most poorly understood aspects of the disease.

From the histologic point of view there are no detectable differences between anomalies observed in the liver of HDV-infected patients and patients with other acute or chronic virus liver disease^[96,97]. These anomalies mostly consist of hepatocellular necrosis and inflammation and may represent, at least in part, a consequence of the immune response of the host. Proteomic and systems biology approaches have more recently been used to investigate changes in protein expression patterns and metabolic pathways altered during HDV replication. Although the model systems used can hardly be considered ideal, the obtained results provided consistent evidence that HDV replication results in significant alterations in pyruvate and glycolysis metabolism^[98-100]. Of note, these studies have shown that cancer was the most likely disease associated with HDV replication and provided evidence that the G2/M cell cycle checkpoint is altered as a consequence of the presence of the virus^[100]. Definitely, these observations, of which a significant number of arise from proteomic

experiments and analysis, need to be interpreted and handled with care. In any case, it seems uncontroversial that further research on liver biopsies of infected patients may possibly help confirming these findings.

There is no efficient therapy for chronic HBV/HDV infection. Pegylated interferon- α (PEG-IFN- α) is perhaps the most popular therapy and the one that has shown some antiviral activity against HDV^[15,101]. However, the efficacy is limited - a temporary reduction in virus titers is usually observed in 15%-40% patients - and the need for prolonged administration often results in severe adverse effects^[101,102]. These effects include fatigue, weight loss, and psychiatric disturbances. Ribavirine, lamivudine and other nucleotide analogues have also been tested but have shown a very limited, if any, efficacy^[103-106]. The Hep-Net International hepatitis D intervention trial included 77 patients from Germany, Greece, and Turkey. In this study a PEG-IFN- α 2a therapy was compared with adefovir and a combination of PEG-IFN- α 2a and adefovir^[107]. Adefovir showed a very limited efficacy and the combination therapy based on PEG-IFN- α 2a and adefovir was only superior in reducing HBSAg levels but not in HDV RNA^[17]. In any case, HDV RNA relapses were often observed in a long-term follow-up (median time 4.5 years). The nucleoside analog entecavir, which showed antiviral efficacy in the woodchuck model of hepatitis B, was assayed in thirteen chronic hepatitis D patients for one year also proving to be ineffective^[17]. It thus seems evident that current anti-HBV drugs are unable to efficiently circumvent HDV infection.

Today, it is usually recommended to treat chronic hepatitis D with PEG-IFN- α for at least one year if the patient tolerates the eventual adverse effects. However, in patients with advanced liver disease, liver transplantation may represent the only available option^[108]. It is thus clear that current therapeutic options are unsatisfactory and there is an urgent need for more effective and specific anti-HDV drugs that will directly target HDV. Prenylation inhibitors may become an interesting and effective option and have been shown to be safe when used to treat neoplasias^[109,110]. As discussed before, prenylation of L-HDAg is essential for interaction with HBSAg and virion assembly, and thus may be regarded as a potential target for therapeutic intervention.

Most recently, and as a consequence of the identification of NCTP as the host cell HDV receptor, inhibitors of viral entry have been tested and proposed as potential anti-viral drugs. Namely, Myrcludex B, a synthetic N-acylated preS1 lipopeptide and cyclosporine A were shown to inhibit virus entry by interfering with the receptor functions of NCTP, however, currently there is no data available regarding the performance of this drug in actual HDV-infected individuals^[111,112].

However, it is clear that a higher investment in research of fundamental aspects of HDV biology as well as of anti-HDV specific compounds is crucial in order to improve the quality of life and life expectancy of chronic HBV/HDV carriers.

Recent trends in HDV research

In the past few years a number of interesting developments have occurred in the field of HDV research and its interaction with HBV.

Using super-infection with WHV-enveloped HDV of the woodchucks that were chronic carriers of WHV and already developed HCCs, it was found that HDV was able to infect fractions of the cells of WHV-induced HCCs. These results suggest that at least a certain percentage of HCC cells *in vivo* express functional WHV receptors and support the attachment, entry, trafficking, and complete replication cycle of HDV^[113]. The data also opens new avenues of research that will further address the mechanisms of the relationship between established HCCs and ongoing virus infection.

A second study compared several types of HDV that differed only by the envelope proteins of HBV that coated the virions^[114]. Twenty five different types of HBV envelope proteins that belonged to twenty five different HBV variants of nine genotypes A-I were analyzed. It was found that all nine HBV genotypes tested were able to support the production of infectious HDV virions that contained HDV genome of genotype I. Significant differences in infectivity were found for the envelope proteins of different HBV variants. The data generated strongly suggest that HBV envelope proteins facilitate not only attachment and entry, but also at least one additional immediate post-entry step of the HDV life cycle. In addition, testing of infectivity suggested that it cannot be concluded that the envelope proteins of HBV produced during chronic stage of HBV infection are mainly responsible for assembly of the virions with diminished infectivity. The study also suggested that correctly regulated disassembly of HDV RNP from the HBV envelope proteins after entry is critical for the overall infectivity of HDV particles^[114].

Finally, a third recent study demonstrated that infectious HDV virions can be assembled by the envelope proteins derived from the naturally integrated HBV DNA in the absence of ongoing HBV replication^[115]. These findings suggest that HDV can possibly persist *in vivo* in the absence of HBV replication (or when HBV replication is suppressed by a drug), when functional HBV envelope proteins are supplied from integrated HBV DNA. Such a mechanism of HDV persistence was not explored previously. The results obtained explain, at least in part, inability of anti-HBV drugs to efficiently block HDV infection *in vivo*. Additionally, they also suggest that HDV can be actually a more independent and more significant pathogen than it is currently assumed^[116].

Origin of the virus

As discussed earlier, HDV bears a number of characteristics similar to those found in plant viroids (Table 1).

These similarities may allow speculation on a possible HDV origin from the plant world. According to this hypothesis, HDV could have evolved to encode the Delta antigen thus providing an explanation for its larger genome when compared with viroids^[117]. However, a

deeper analysis of this homology was evaluated as non-significant and this hypothesis seems to be, at least for the time being, ruled out.

One of the key features of HDV genomic and anti-genomic RNA molecules is their ribozyme activity. Ribozymes are considered to be characteristic of viroids. However, the two HDV ribozymes are not only structurally different from those of Avsunviroidae but also display similarities to several HDV-like ribozymes found in eukaryotes^[74,116]. This finding rather supports the hypothesis of a human transcriptome origin of HDV.

We can thus conclude that the plant or animal origins of HDV are still questionable and highly speculative. But this is one of the many fascinating questions that still remain to be unveiled for this awkward and awesome virus.

CONCLUSION

Almost 40 years after its discovery, HDV remains a challenge for clinicians and researchers. It is disconcerting simplicity, with a small RNA genome and a single protein, the Delta antigen, make it an excellent model not only for virologists but also for those interested in RNA and cell biology. The virus bears a number of unique features including a RNA-directed RNA replication mechanism of the genome catalyzed by host RNA polymerase II. Enzymatic activities were not identified in Delta antigens thus making difficult the identification of potential targets for specific and effective therapies. Development of such therapies is crucial to reduce the number of chronic patients progressing to cirrhosis and hepatocellular carcinoma. The burden of disease caused by HDV is most probably underestimated since there is a considerable lack of epidemiologic data from several countries where HBV is highly prevalent.

In conclusion, despite considerable progress made in HDV research a significant number of questions remain to be answered concerning fundamental aspects of its biology, pathogenesis, and interaction with the host. The next few years will hopefully bring to light new answers but also new exciting questions, helping understand this fascinating pathogen, and contributing to reducing morbidity and mortality among infected individuals.

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