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REVIEW

Viral hepatitis update: Progress and perspectives

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Abstract

Viral hepatitis, secondary to infection with hepatitis A, B, C, D, and E viruses, are a major public health problem and an important cause of morbidity and mortality. Despite the huge medical advances achieved in recent years, there are still points of conflict concerning the pathogenesis, immune response, development of new and more effective vaccines, therapies, and treatment. This review focuses on the most important research topics that deal with issues that are currently being solved, those that remain to be solved, and future research directions. For hepatitis A virus we will address epidemiology, molecular surveillance, new susceptible populations as well as environmental and food detections. In the case of hepatitis B virus, we will discuss host factors related to disease, diagnosis, therapy, and vaccine improvement. On hepatitis C virus, we will focus on pathogenesis, immune response, direct action antivirals treatment in the context of solid organ transplantation, issues related to hepatocellular carcinoma development, direct action antivirals resistance due to selection of resistance-associated variants, and vaccination. Regarding hepatitis D virus, we describe diagnostic methodology, pathogenesis, and therapy. Finally, for hepatitis E virus, we will address epidemiology (including new emerging species), diagnosis, clinical aspects, treatment, the development of a vaccine, and environmental surveillance.

Key Words: Viral hepatitis; Hepatitis A virus; Hepatitis B virus; Hepatitis C virus; Hepatitis D virus; Hepatitis E virus

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Core Tip: Viral hepatitis is a global public health concern that affects millions of people and causes thousands of deaths due to acute and chronic infections, cirrhosis, and liver cancer. Although clinical and epidemiological characteristics of hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, and hepatitis E virus infections are widely known, there are still other critical points that need to be discussed. This review focuses on the most important research topics, dealing unsolved issues and future research directions that can maximize practical impact in the field of viral hepatitis.

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INTRODUCTION

The term viral hepatitis refers to liver inflammation related to a viral infection. As of today, five viruses (hepatitis A, B, C, D, and E) that selectively infect the liver, usually by different routes, have been recognized. In some of these viral infections, acute hepatitis can resolved without intervention, whereas, sometimes, the process turns into a chronic infection[1]. Huge medical advances made in recent decades led to the implementation of preventive measures, the development of vaccines and passive immunization strategies, and, more recently, the development of promising and effective treatments, at least for some forms of viral hepatitis. The results obtained by basic research on viruses and on viruses-cell interaction made it possible to struggle with what a century ago seemed an insurmountable scourge on humanity. Achievements in hepatitis prevention and treatment are perhaps the paradigm of successful translational research[1]. Nonetheless, viral hepatitis is still a global public health concern that affects millions of people and causes thousands of deaths due to acute and chronic infection, cirrhosis, and liver cancer[1,2]. This review focuses on the currently most important research topics and future research directions that can maximize practical impact in the field of viral hepatitis. Table 1 summarizes the principal characteristics of these hepatotropic viruses and Tables 2-6 highlight the main topics of viral hepatitis addressed in the present review.

HEPATITIS A VIRUS

According to the World Health Organization (WHO), 1.4 million new cases of hepatitis A are reported worldwide each year, with a consequent nearly 7000 deaths[3]. Hepatitis A virus (HAV), a member of the *Picornaviridae* family and the only species from the *Hepatovirus* genus that infects humans, is a non-enveloped single-stranded RNA virus[4]. HAV is classified into six genotypes, three infecting humans and three affecting simians, but there is only one known serotype[5].

Despite HAV being discovered more than 4 decades ago, it has been well characterized, and its detection and diagnosis have been widely implemented; changes in the socio-economic conditions and the control mechanisms of the virus have triggered new circulation and transmission scenarios that have generated new targets for its assessment. Some of them are the epidemiology and molecular surveillance of the virus, the different vaccination schemes and immune responses, the new susceptible populations (after the implementation of massive vaccination), and the study of the virus in environmental and food matrices.

Epidemiology and transmission: Old and new challenges

Although HAV epidemiology is complex, it is changing in those countries that are improving their public health and sanitation policies, considering that the most usual routes of HAV transmission are contaminated water ingestion and the contact with infected individuals[6]. Three circulation patterns have historically been described for

Table 1 Features of different types of hepatitis virus

	HAV	HBV	HCV	HDV	HEV
Family	*Picornaviridae*	*Hepadnaviridae*	*Flaviviridae*	Undefined[1]	*Hepeviridae*
Genus	*Hepatovirus*	*Orthohepadnavirus*	*Hepacivirus*	*Deltavirus*	*Orthohepevirus*
Genome	Positive single-stranded linear RNA	Double stranded gapped DNA	Positive single-stranded linear RNA	Negative single-stranded circular RNA	Positive single-stranded linear RNA
Genome length (kb)	7.5	3.2	9.6	1.7	7.2
Genotype	6 genotypes: I, II and III infect humans, and IV, V and VI infect non-human primates	10 genotypes (A to J)	8 (1 to 8)	8 (1 to 8)	8 (1 to 8)
Transmission	Fecal-oral	Parenteral, sexual, and perinatal	Exposure to infected blood	Exposure to infected blood and body fluids	Fecal-oral; zoonotic; blood transfusion
Treatment	None. In case of severe hepatitis, treatment of symptoms	Pegylated interferon-alpha and nucleoside/nucleotide analogues	DAA	Pegylated interferon-alpha	Ribavirin (in chronic HEV infection)
Prophylaxis	Yes (inactivated vaccine)	Yes (recombinant vaccine)	No	Yes (HBV vaccine)	No[2]
Clinical outcome of infection	Self-limited	Self-limited and chronic	Self-limited and chronic	Self-limited and chronic	Self-limited
Chronic infection rate	No	Depends on the age of acquisition of the infection. Birth or in infancy 90%, 1 yr and 5 yr of age 30%-50%, adulthood 5%. Hemodialysis patients 40%. Immune deficient patients 20%	80%	More frequent in HBV/HDV superinfection than coinfection	Acute infection in most of the cases. Chronic infection in immunosuppressed populations

[1]It is not defined yet in any of the established viral families.
[2]There is only one vaccine, approved and used only in China. HAV: Hepatitis A virus; HBV: Hepatitis B virus; HCV: Hepatitis C virus; HDV: Hepatitis D virus; HEV: Hepatitis E virus; DAA: Direct antiviral agents.

Table 2 Hepatitis A virus highlights

	Hepatitis A virus
1	The risk of HAV infection is associated with the lack of safe water and poor and sanitation
2	Due to the vaccine introduction in childhood, young adults are becoming more susceptible to HAV infections
3	In countries where waterborne transmission is rare, outbreaks occur among men who have sex with men, injecting drug users and contaminated food
4	Since molecular detection is not routinely performed for diagnosis, surveillance programs, including viral amplification and sequencing, are needed to know the strains that circulate in a certain place
5	One of the greatest challenges for HAV is to increase vaccination coverage globally, still implementing the single-dose schedule, to decrease the new infections, and, in the long term, to achieve its eradication

HAV: Hepatitis A virus.

HAV: (1) In high endemicity areas from low- and middle-income countries, where the incidence varies from low to high over time and between different regions, there is a peak age of infection in early childhood that is frequently asymptomatic, the transmission pattern is person-to-person, and outbreaks are uncommon due to high rates of immunity from previous childhood infection; (2) In moderate endemicity areas, from middle-income countries (regions where sanitary conditions are variable), the incidence is high, the peak age of infection is in late childhood/adolescence or in young adults that is frequently symptomatic, the transmission pattern is also from person-to-person, related to food and water, and therefore outbreaks are common due to low rates of immunity from previous childhood infection; and (3) In low endemicity

Table 3 Hepatitis B virus highlights

	Hepatitis B virus
1	Several host factors, such as male gender, alcohol intake, and obesity have been associated to worse disease progression. Current challenge implies finding genetic markers to predict the course of HBV infection. In this line, different SNPs associated with the outcome of HBV infection have been recently identified
2	In the last years, new diagnostic assays have been developed in the framework of the diagnosis of HBV infection. The implementation of quantitative HBsAg, HBcAg, and HBV-RNA in routine clinical practice could probably improve the management of patients with CHB
3	Current antiviral treatments have some shortcomings, such as poor SVR or prolonged schedules. Direct antiviral agents against different HBV targets, including HBV cccDNA, are under evaluation. Moreover, immunemodulatory therapies to overcome host immune impairment observed in chronic infections are being investigated
4	Although a safe and cost-effective vaccine is available since the 1980s, an inadequate response is achieved in particular settings. New and more potent adjuvants, as well as formulations that include alternative viral antigens could improve the response rate vaccination
5	The development of new antiviral therapies that enables achieving functional cure as well as accurate diagnostic methods and more effective vaccines will contribute with the purpose of the WHO to eliminate by 2030 hepatitis as a global health problem

HBV: Hepatitis B virus; SNP: Single nucleotide polymorphisms; HBsAg: Hepatitis B surface antigen; HBcAg: Hepatitis B core Antigen; CHB: Chronic hepatitis B; SVR: Sustained virological response; WHO: World Health Organization.

Table 4 Hepatitis C virus highlights

	Hepatitis C virus
1	WHO global hepatitis elimination strategy aims to reduce 90% of new HCV incidence, 65% of mortality and treat at least 80% of patients
2	DAA treatment leads to regression of clinical symptoms and liver disease complications even in those patients with other comorbidities, co-infections, or advanced liver disease
3	The immune response plays a central role in viral elimination. The understanding of the relationship between achieving protection and activation of immune responses is mandatory for the development of an effective prophylactic vaccine
4	Immune response restoration after DAA treatment is also under debate, certain immune features are reinvigorated, but many immune exhaustion signs may persist
5	SVR after DAA rates higher than 97% are usually attained, but still, a minor group of patients (4%-5%) fails to eradicate HCV due to resistance-associated variants, some of them arising after treatment but others naturally occurring in treatment naïve individuals
6	DAA efficacy impacts on transplantation from HCV-infected donors into infected or uninfected recipients; however, early outcome data are encouraging, experience is limited, and many issues remain under debate
7	HCC risk after DAA treatment has been extensively discussed; however, recent seminal reports support the notion of a reduced rate for occurrence or recurrence of HCC after DAA SVR
9	There are numerous HCV vaccine approaches including a few candidates who accomplished phase I trials, but a prophylactic HCV vaccine that can contribute to the eradication goal remains a pending issue

DAA: Direct antiviral agents; SVR: Sustained virological response; WHO: World Health Organization; HCV: Hepatitis C virus; HCC: Hepatocellular carcinoma.

areas from high income-countries, the incidence is low, the peak age of infection is in young adulthood, the transmission pattern is from person-to-person and also *via* food and water; and outbreaks are common due to low rates of immunity from previous childhood infection[3].

Nowadays, 34 countries have included vaccination against HAV in routine immunization programs among children[3]. Many countries use an inactivated HAV vaccine with a two-dose regimen, while other countries have successfully implemented it in their immunization programs in a single-dose[3,7]. However, long term results of the single-dose schedule have been only partially studied. The most recent investigation showed sustained immunologic protection for up to 9 years, with high levels of antibody titers, when children were vaccinated at 12 mo[8]. More studies that assess long-term seroprotection against HAV after single-dose vaccination scheme are necessary to monitor the effectiveness of this innovative strategy. In some territories vaccination is also recommended for people at risk of HAV infection, like those who travel to regions where HAV is endemic, drug users, men who have sex with men, and individuals with chronic liver disease[3].

The recent improvement of socio-economic, hygienic, and sanitation measures may translate into an increase in the number of adults who have never been infected in

Table 5 Hepatitis D virus highlights

	Hepatitis D virus
1	The natural course and outcome of acute hepatitis D differ according to HBV and HDV co-infection or superinfection
2	HDV and HBV genotypes in addition to host factors influence the course of chronic hepatitis
3	The implications on liver disease of HVD, HBV, and innate immunity interplay remain to be understood
4	Chronic setting leads to more severe hepatitis associated with higher rates of HCC and a faster progression to cirrhosis compared with HBV monoinfection. HDV pathologic changes are limited to the liver with histopathologic features that are not specific for it
5	HDV remains difficult to treat with the current available therapies, and although, several promising new therapies have been described treatment is still the greatest challenge in HDV infection

HDV: Hepatitis D virus; HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma.

Table 6 Hepatitis E virus highlights

	Hepatitis E virus
1	HEV is transmitted by the fecal-oral route (involving contaminated waters) and also as a zoonosis
2	In the last years, many studies have focused on HEV detection in environmental and food matrices, and blood products as alternative sources of infection
3	A new etiological agent of human hepatitis E, Orthohepevirus C, previously known to infect rats, has been recently described
4	Although most cases of HEV infection produce acute hepatitis, chronic infections seem to be an increasing problem, particularly in Europe
5	Complications and extrahepatic manifestations are also increasingly recognized
6	Only one vaccine for HEV has been licensed in China, with little known data, which limits its use

HEV: Hepatitis E virus.

childhood and therefore lack immunity. Furthermore, despite pediatric immunization programs, many young adults may have been above the cut-off ages to be included when such social programs were introduced. Therefore, young adults are now becoming more susceptible to HAV infections, so in areas of low and middle-endemicity, the prevalence of symptomatic cases in this age group has increased[6]. In this sense, between the middle of 2016 and the beginning of 2018, several hepatitis A outbreaks were reported in Europe, the United States and South America, which disproportionately affected HAV unvaccinated young adult men, mainly men who have sex with men. This group presents an increased risk of infection same as persons, regardless sex orientation, who have oral-anal sexual contact[4]. Interestingly, through phylogenetic analysis accompanied by detailed questionnaires to capture the sexual history of the patients, it was possible to establish epidemiological links between cases, demonstrating that the viruses responsible for these outbreaks belonged to HAV genotype IA and grouped with one of these strains: VRD_521_2016, RIVM-HAV16-090, and V16-25801[6]. This highlights the importance of carrying out a more detailed epidemiological record of cases, including sexual history, which will help to establish the source and chain of infection.

Regarding travelers to endemic regions, although the WHO has recommended their vaccination, it does not always happen, increasing hepatitis A cases among this group. Furthermore, the movements of immigrants in some areas of the world (*e.g.*, in South America) led the virus to be transported from endemic areas (often without vaccination coverage) to non-endemic areas, introducing new viral strains[3,6]. Screening for immunoglobulin (Ig) G anti-HAV should be offered to this group; therefore, patients who test negative should be offered vaccination[4].

Detection and surveillance

Diagnosis of hepatitis A is performed by the detection of HAV IgM with serological assays. Specific antibodies are present in sera for at least 7 mo after infection, although in some individuals they remain for up to a year[4]. During acute infection, IgG anti-HAV appears, and it remains present in serum for life[3,4]. Serological surveillance is assumed as the main monitoring strategy for the infection. Since molecular detection is

not routinely performed for diagnosis, surveillance programs, including viral amplification and sequencing, are needed to understand the strains that circulate in a certain place or that are introduced by travelers; however, it is seldom carried out. Molecular surveillance includes the detection and study of HAV in environmental and food matrices, an area of study that has been carried out in recent years. For the purpose of molecular surveillance, the HAV Network (HAVNET) was created in 1999 [9]. This is an international HAV network of scientists who work in reference laboratories of hepatitis A and share molecular and epidemiological data on this virus, information that is useful for the scientific community. The HAVNET aims to increase the knowledge of HAV infections and map the worldwide distribution of HAV strains. As there is a strong geographical signal in the sequences, this can be used for source tracking.

The study of HAV in environmental and food matrices is a valuable tool for monitoring circulating HAV strains, to know the sources of infection and to take sanitation and prevention measures. After a large outbreak of foodborne hepatitis A in Europe in 2013-2014, the crucial role of sequence data analysis to investigate outbreaks and define transmission pathways was recognized, as well as the need of the agreement on a common genomic region for sequencing and a common protocol to perform HAV detection in food[10]. In this sense, with the aim of harmonizing the existing protocols for HAV detection in food, the European Committee for Standardization and the International Standards Organization developed and published a standard methodology for quantitative and qualitative determination of HAV (together with norovirus) in seven food matrices, using real-time (RT) polymerase chain reaction (PCR), which has allowed to obtain comparable results between laboratories[5]. Furthermore, the sequencing of a common consensus region was agreed to target the HAV VP1/2A junction and thus promote the protocol described in the HAVNET[10]. In this context, collaborations between the public health sector, the food sector, HAVNET, and other organizations, together with government dependencies, are highly recommended.

Although there is no legislation about the presence of HAV in environmental matrices at a global level, some countries have adopted measures for the surveillance of cases of food outbreaks due to HAV, which has led to strict controls of imported food, incorporating the mandatory control of this virus in some cases[11]. Foodborne HAV clinical cases and outbreaks are difficult to identify, track, and assess their magnitude due for many reasons: (1) The difficulty for patients to remember food consumption history before the onset of the disease; (2) The asymptomatic nature of many cases, which are not reported (in the case of outbreaks); (3) The long incubation period of HAV; (4) Viral contamination levels of a food item may be low and focal and, therefore, hard to detect; and (5) The scarce knowledge of health care teams about foodborne viral diseases[5].

The above issues highlight the new epidemiological scenarios of this virus, showing the targets to whom control and prevention actions should be directed. The main goal for the next years should be to increase vaccination coverage globally, implementing the single-dose schedule, so to decrease the new infections, and, in the long term, to achieve eradication.

HEPATITIS B VIRUS

The hepatitis B virus (HBV) was discovered by serendipity in the 1960s and subsequently several milestones were achieved such as the development of diagnostic tests in the early 1970s or the implementation, in the 1980s, of a safe and cost-effective vaccine with subsequent different therapies for the treatment of chronic hepatitis B (CHB) infection[12].

Despite these advances, the landscape is still far from satisfactory. Currently, an estimated 257 million people are living with CHB, and around 887000 deaths occur annually as a consequence of infection progression, mainly due to cirrhosis and hepatocellular carcinoma (HCC)[13]. Furthermore, it is expected in the coming decades that the problem of HBV infection might increase, particularly in developing countries, as a consequence of the limited access to diagnosis and treatment, in addition to the subclinical characteristics of the infection[14]. In fact, the WHO has proposed strategies to eliminate viral hepatitis as a Public Health problem by 2030. To achieve this goal, it will be necessary to implement prevention, diagnosis, and treatment measures, as well as to raise awareness among the population and primary care physicians from the infections caused by HBV[15].

Among the current challenges to overcome are the identification of host markers that would allow to predict accurately the evolution of infection and the implementation of a personalized medical approach, the development of anti-HBV therapies that enables achieving functional cure in chronically infected patients, as well as the restoration of the host's immune response, the implementation of new diagnostic methods, and the development of more effective vaccines that would lead to improving prevention policies in order to reduce the global burden of HBV disease.

Host factors

HBV infection has a wide range of clinical presentations, from subclinical to symptomatic in the acute stage, and from inactive carrier state to active chronic hepatitis with different degrees of severity[16]. Epidemiological data early established that the course of the infection is closely related to the age at which the infection is acquired, being the evolution to chronicity much more frequent in individuals infected at birth or in childhood[17]. Additionally, male gender, heavy alcohol consumption (more than 60 g/d), obesity, and comorbidities, such as co-infections with human immunodeficiency virus (HIV), hepatitis C virus (HCV), or hepatitis D virus (HDV), have also been reported to contribute to progression to end-stage liver disease[18-22]. Recently, genome-wide association studies have shown that the host genetic background may also affect the natural history of infection[23,24]. Several studies have identified single nucleotide polymorphisms (SNPs) in human leukocyte antigens (HLA) that have been associated with the outcome of HBV infection, either with clearance or progression of chronic infection, although some findings were not subsequently supported in other manuscripts. Among the more in depth characterized, it has been found that HLA-DP (rs3077 and rs9277535) and HLA-DQ (rs7453920 and rs2856718) SNPs were associated to HBV persistence[25-27] Notably, different studies have also identified several HLA polymorphisms associated with the response to the HBV vaccine[28,29]. Additionally, it was also reported that cytokine, chemokine, toll like receptor, sodium taurocholate cotransporting polypeptide, and vitamin D-related genes may influence the clinical outcomes of HBV infection[24,30,31]. Beyond the controversies observed among studies addressing the genetic polymorphisms involved in the outcome of HBV infection, mainly probably due to ethnic differences (haplotype structures and allele frequencies), these findings will undoubtedly help to individualize the risk of infection progression and to improve the effectiveness of HBV vaccination campaigns, contributing to the implementation of a personalized approach and a greater chance of accomplishing the achievement of eliminating HBV infection as a public health problem by 2030.

Diagnosis

In order to achieve global control of HBV infection, one of the main obstacles to overcome is the limited access to diagnostic resources. Since the 1980s, classical serological markers have been available, including detection of antigens s and e (HBsAg, HBeAg) and antibodies against antigen e and core (anti-HBe and anti-HBc), along with the later use of molecular markers to determine the viral load, for the diagnosis and management of HBV infection. The qualitative detection of HBsAg has been the hallmark of HBV infection. Its presence for more than 6 mo is pathognomonic of chronic infection, and HBsAg seroclearance is now considered the goal for functional cure, except for occult hepatitis B, in which HBsAg is not detected despite the persistence of the infection. In recent years, efforts have focused on the search for accurate tools for the monitoring of antiviral treatment in CHB. Complete cure of CHB infection implies elimination of the HBV from infected hepatocytes, which is hardly achievable because of the persistence of the covalently closed circular DNA (cccDNA) and integrated HBV-DNA. Since cccDNA detection is difficult to perform in routine diagnosis, surrogate markers have been developed, being the quantitative HBsAg (qHBsAg), the hepatitis B core-related antigen (HBcrAg), and serum HBV-RNA the most promising ones. In the last years, different assays to qHBsAg levels have been developed. In most studies carried out on HBeAg-positive patients, a positive correlation among HBsAg titers, serum HBV DNA, and liver cccDNA has been observed[32]. In contrast, this relationship was not verified in HBeAg-negative CHB cases[33]. The lack of correlation could be a consequence of S gene mutations associated with HBeAg seroconversion, affecting expression or secretion of HBsAg[34-36]. Nonetheless, several studies have shown that qHBsAg is a useful diagnostic tool, together with HBV-DNA levels, to discriminate inactive carriers from HBeAg-negative chronic hepatitis[37,38]. Furthermore, it has been described to be useful in predicting sustained HBsAg clearance and liver disease progression in inactive carriers[33]. Likewise, baseline and on-treatment qHBsAg levels have been shown to be a reliable

prognostic marker of sustained virological response (SVR) in treatment with pegylated interferon alpha (PEG-IFN-α). Consequently, current guidelines recommend its use for the management of HBV therapy[39-42]. The HBcrAg, another recently developed marker, detects the HBcAg, HBeAg and the 22 kDa precore protein. Different studies indicate that HBcrAg depicts a more accurate correlation with intrahepatic cccDNA transcriptional activity than qHBsAg, regardless of HBeAg status[43,44]. Furthermore, HBcrAg has been suggested as a prognostic factor for virological remission and HBsAg clearance in patients undergoing antiviral treatment[45], as well as a predictive marker for the development of HCC[46]. Nevertheless, its clinical use remains controversial. HBV-RNA detection has also raised interest as a possible surrogate marker of HBV transcriptional activity since serum HBV RNA levels significantly correlated with intrahepatic cccDNA concentrations among untreated patients[47,48]. Likewise, it has been suggested that HBV RNA has a predictive value as a diagnostic tool of HBeAg loss in patients under therapy, being proposed as a reliable marker for treatment discontinuation[49,50]. However, routine implementation still requires standardization of the methodology. Finally, several studies have identified other promising markers to monitor the management of CHB patients such as quantitative anti-HBcAg or cccDNA determination[51-53]. Further validation for their use in clinical practice is still required.

Therapy

Over the last 2 decades, notable progress has been achieved in the treatment of CHB infection. Currently available antiviral agents include PEG-IFN-α and nucleoside/nucleotide analogues (NAs) among which entecavir, tenofovir disoproxil fumarate, and tenofovir alafenamide are the first-line oral anti-HBV drugs due to the high genetic barrier to HBV resistance. Although suppression of HBV replication reduces the progression of liver disease and improves the outcomes in most patients, the actual obstacle to cure CHB is the persistence of cccDNA and integrated HBV DNA. Thus, the term 'functional cure' has been accepted as the ultimate goal to reach with HBV therapies[41,42]. However, PEG-IFN-α treatment has an unsatisfactory SVR rate in addition to several adverse effects, being therefore limited to a selected group of patients. On the other hand, although NAs have shown high efficacy in inhibiting viral replication, the HBsAg sustained clearance rate is poor, with a substantial risk of relapse when treatment is discontinued, the need for retreatment, and the risk of select drug resistant strains[54]. Therefore, the main challenge at present is the implementation of new strategies that increase the rate of loss of HBsAg or the sustained suppression of HBV replication compared to existing therapies by developing more efficient antiviral agents and immune-modulatory therapies to restore the functionality of the immune system. Direct antiviral agents targeting different HBV proteins or steps of the viral replication cycle are being evaluated. HBV entry inhibitors are molecules that target the NTCP receptor (NTCP: Sodium taurocholate cotransporting polypeptide is the host cell receptor required for HBV entry), preventing both the novo infection and reinfection cycles, being of great value to control CHB infection[55,56]. Also, HBsAg release inhibitors are promising drugs that combined with current antiviral treatments might help to induce HBsAg clearance [57]. Additionally, core protein assembly modulators and small interfering RNA targeting HBV transcripts are under evaluation in different clinical trials[58,59]. Another appealing strategy implies targeting the HBV cccDNA. Several molecules and clustered regularly interspaced short palindromic repeats technology have shown the ability to inhibit synthesis or eliminate the already formed cccDNA[60]. However, they are still under investigation due to delivery issues and unintended off-target effects [61]. Furthermore, antiviral agents against the protein X are also being addressed, both for their role in the epigenetic regulation of cccDNA and in the modulation of several host cell signaling pathways[62].

As mentioned above, the impairment of the host's immune system is another important factor for HBV persistence. Several approaches are being investigated for the pharmacological activation of the intrahepatic innate immune response, including the induction of IFN genes with antiviral properties[63] or the stimulation of toll-like receptors (TLR)[64], targeting adaptive cell effectors. In line with the latter strategy, some attractive methodologies include the use of checkpoint inhibitors that block the co-inhibitory receptors overexpressed in HBV-specific T cells to reverse immune dysfunction[65], the adoptive transfer of either genetically engineered T lymphocytes expressing chimeric antigen receptors or reinfusion of autologous restored T cells[66,67], as well as therapeutic vaccination that might boost host immune response[68]. However, the wide range of functional deficiencies observed in patients with CHB represents an important pitfall for the success of these therapies. Its use together with

antivirals agents is expected to lead to viral elimination as well as mounting strong immunological surveillance that limits viral reactivation.

Vaccine

Vaccination is the most powerful tool to control the spread of HBV. Since the 1980s, a recombinant HBV vaccine obtained by expressing the small envelope protein (HBsAgS) in yeast has been available. It is currently being implemented for infants in more than 189 countries, and in 109 of these a dose within the first 24 h of life has been introduced in vaccination schedules[69]. Following the global introduction of large-scale vaccination, a substantial decrease in the rate of HBsAg carriers was observed [70]. After three intramuscular doses, a protective response against HBV is achieved in more than 90% of healthy adults and more than 95% of infants, children, and adolescents. However, the response rate declines with age, particularly after the age of 40, as well as in people with obesity, smokers, comorbidities, genetic factors, or particular settings[71]. Failure to mount an adequate immune response is one of the main concerns regarding the HBV vaccine; therefore, to overcome this shortcoming, several attempts to enhance the immunogenicity have been addressed. On the one hand, new and more powerful adjuvants have been developed and evaluated, including liposome-based formulations, cytidine-phosphate-guanosine oligodeoxynucleotide (a TLR9 agonist) or virosomes[72]. These reformulations have shown a considerable improvement in seroconversion rates compared to the conventional vaccine, particularly in individuals with poor or no response[73]. Interestingly, the novel adjuvants may reduce the current schedule from three to two doses, contributing to a higher adherence rate to compliance with the vaccination scheme, which is another drawback[74]. Likewise, intradermal administration has shown to provide better responses than the intramuscular route[75].

On the other hand, recombinant vaccines derived from mammalian cells containing the medium and large envelope proteins, in addition to the already used small envelope protein, have been developed. This approach has the advantage of antigens displaying the same post-translational modifications and protein folding that occurs *in vivo*. This alternative approach showed a faster seroprotection rate as compared to the conventional vaccine, making it of particular interest for people with poor or no response. Furthermore, it could protect against HBV strains carrying vaccine induced or spontaneous HBsAgS mutants[76]. In fact, the emergence of vaccine escape mutants (VEMs) and the role of the HBV genetic variability both have been considered as possible shortcomings of the vaccine.

Shortly after the massive implementation of the HBV vaccine, the selection of variants with mutations in the wild type epitope has been reported. In many countries, where early large-scale vaccination was introduced, along with a decrease in the prevalence rate of infection, over time a significant increase in the frequency of VEMs [77] has been observed. Different studies have shown that VEMs can replicate, with the implicit risk of becoming the predominant strains in the coming decades. In addition to the selection pressure exerted by the implementation of large scale vaccination, due to the overlapping of the open reading frames in the HBV genome, mutations in the *Pol* gene can affect the *S* gene[78]. Consequently, the use of antiviral agents targeting the viral polymerase indirectly promotes the selection of mutants affecting the HBsAgS recognition by vaccine-induced antibodies. Beyond these assumptions, the transmission of VEM is a very unusual event and, although its strict vigilance is recommended, it does not pose a threat to the control of HBV infection. Therefore, the introduction of mutated antigens in the vaccine formulation is not currently being considered[79].

Finally, the genetic variability of HBV may represent a more significant problem than VEMs. Based on the genetic diversity, HBV is classified into 10 genotypes (A to J) and several subgenotypes. The HBV vaccine used today was developed decades ago, when the existence of the different HBV genotypes was unknown, using HBsAgS of genotype A2 as a prototype. Case reports of vaccinated people subsequently infected, mainly with the most divergent genotypes, have been described[80-82]. Although there is a paucity of data regarding cross-genotype preventive effect, greater protection against homologous genotype/sub-type than against heterologous strains of HBV have been reported[83]. However, empirical data from regions where the most divergent genotypes are prevalent suggests that cross protection is sufficient to prevent infection. Therefore, HBV diversity would not represent a major obstacle to the prophylaxis of infection.

HCV

In 2020 the Nobel Prize in Physiology or Medicine was awarded to the Americans Harvey J Alter (United States National Institutes of Health) and Charles M Rice (Rockefeller University) and to the British Michael Houghton (University of Alberta) for the discovery of the HCV. Alter demonstrated the existence of a non-A non-B hepatitis virus-associated with post-transfusion hepatitis in 1975, Houghton cloned and identified the viral genome and renamed it as HCV in 1989, and Rice established, from an edited version of the virus genome, a robust *in vitro* replication system in cell cultures in the 1990s and thus laid the foundation for future genetic and functional analysis[84-86].

Epidemiology and treatment

HCV is an enveloped single-stranded RNA virus of the *Flaviviridae* family. Due to a lack of proofreading activity of HCV RNA-dependent RNA polymerase (NS5B) and their high replication rate, a large number of viral variants are produced during infection[87]. Eight genotypes have been described, among them genotype 1 is prevalent worldwide, while the others were each characterized in different geographic regions, that is genotype 2 in West Africa, genotype 3 mostly in South Asia, genotype 4 in Central and North Africa, genotype 5 in South Africa, genotype 6 in South-East Asia, and genotype 7, which has been isolated from central African immigrants in Canada[88,89]. Recently, the novel genotype 8 was described as endemic in India[90].

HCV is estimated to infect more than 1% of the global population, and around 80% develop a slowly evolving, asymptomatic chronic liver disease characterized by cell damage, inflammation, and fibrosis that can progress, after a few decades, to cirrhosis in 30%-40% of cases or to HCC in 1%-3% of them[91]. Thus, HCV infection is strongly related to liver transplantation. In the absence of a vaccine, HCV treatments went through different stages, starting with prolonged regimens based on interferon as an immune system modulator with cure rates of less than 50% and high adverse effects, and going through successive generations of direct-action antivirals (DAA). However, the real improvement of the DAA regimen began in 2013, when an interferon-free treatment was available; since then, several DAA schemes targeted against the protease, the NS5A protein, or the polymerase became the standard of care. Currently, treatments are oral with almost no side effects and with SVR rates higher than 97% after 8 to 12 wk. Nowadays, successful treatment leads to regression of clinical symptoms and complications of liver disease even in those patients with other comorbidities, co-infections, or advanced liver disease[92-94]. In this new scenario, as mentioned above, in 2016 the World Health Assembly approved a global strategy to achieve viral hepatitis elimination (C and B), which concerning HCV aims to reduce 90% of new HCV infections (incidence), 65% of deaths (mortality), and treat at least 80% of patients who require treatment[95,96]. However, this objective is far from being reached. On the one hand, it is critical that each country implements systematic and organized programs of silent carrier detection to overcome the suboptimal rates of HCV screening. On the other hand, it is necessary to ensure access to treatment for all infected people, which is still difficult due to the high cost of it. Finally, it is essential to carry out primary prevention tasks to avoid the generation of new cases and the reinfection of patients already cured, especially in the groups at greatest risk[95,96].

Pathogenesis and immune response

Chronic hepatitis C pathogenic mechanisms as well as the immune response participation in the generation of liver damage are still topics of interest[91,97]. It has been thoroughly described that HCV alters liver homeostasis, leading to stress and inflammation[98]. The liver microenvironment is extremely complex with numerous immune cell populations that, along with the cytokines that they produce, play a central role in the viral elimination; thus the interplay between virus and host immune response may influence infection outcome[99-101]. Remarkably, in the chronic stage, the role of the immune cells becomes more complex since their altered functionality would contribute to liver damage[102]. Cellular immune surveillance of HCV infection induces interferon production and activates innate immune response, hence controlling the infection. As part of the innate immune response, natural killer, dendritic, and Kupffer cells present viral antigens from infected hepatocytes to the T and B lymphocytes, which in turn contribute to virus control. The immune system triggering is not enough to control HCV infection and in consequence, a persistent infection is established. However, the results behind this data are controversial, and the underlying mechanism by which various cell populations are involved is still

under discussion.

The understanding of the relationship between achieving protection and the activation of both innate and adaptive immune responses is mandatory for the development of an effective prophylactic vaccine that may control infection and transmission. Moreover, the restoration of the immune response after DAA treatment is also under debate, particularly because most reports have focused on immune cells in peripheral blood, and little is known related to the intrahepatic immune environment after rapid clearance of chronic HCV. Remarkably, a double scenario arises after DAA treatment, certain immune features are reinvigorated but many immune exhaustion signs may continue after viral elimination[103]. The majority of pro-inflammatory cytokines and chemokines reached normal values after long-term monitoring albeit IFN-α and tumor necrosis factor-related apoptosis-inducing ligand maintained high levels for months after treatment[104,105]. Regarding HCV-specific T cells, a partial recovery of the functionality, mainly proliferation capacity, was described. Nevertheless, it does not apply for every patient, and the restoration level was not homogeneous for all individuals. The suppression of HCV replication led to a decrease in expression of T lymphocyte exhaustion markers and an increase in HCV-specific IFN-γ responses after treatment[94,106,107]. However, the restoration of exhausted HCV-specific CD8+ T lymphocyte surface phenotype does not result, *per se*, in a complete functional restoration. Regarding CD4+ T cells, HCV antiviral treatment leads to a shift from a T helper 1 cell to a follicular helper T cell (Tfh) environment within HCV-specific cells. Likewise, HCV-specific CD8+ T lymphocytes, Tfh cells are likely to persist in an antigen-independent manner[108]. Furthermore, in chronic hepatitis C, regulatory T cells are usually elevated and display an activated phenotype in the course of infection that persists even after DAA therapy[109]. Natural killer (NK) cells have an important role in HCV infection control; however, phenotype and function of NK cells are altered in chronic HCV patients[110]. In recent years, several groups have investigated the recovery of the altered NK cell compartment upon successful antiviral treatment, but it is still a matter of research whether an active reinvigoration *via* certain signaling pathways or the resolution of inflammation after virus elimination are responsible for a seemingly restored NK cell compartment. Hence, such a persistent challenge of the immune system might trigger irreversible damage that in turn could affect the success of any therapeutic vaccine design or even any immunotherapy approach against HCC[94,103,111].

DAA resistance-associated variants

Current DAA therapy has demonstrated high efficacy, but still in a minor group of patients (4%-5%) it does not succeed in eradicating HCV, largely due to inadequate adherence but also due to relapse or viral fitness[112]. Since HCV is a rapidly evolving RNA virus, the exposure to DAAs triggers strong drug selection favoring mutants that offer partial resistance to them. Thus, the high SVR achieved with DAA still faces the challenge of resistance-associated variants (RAVs), some arising after treatment but others naturally occurring in treatment naïve individuals[113,114]. DAA treatment failure may be attributable to advanced liver disease, suboptimal therapy adherence, and the presence or generation of NS5A mutations[112]. The three HCV non-structural proteins have different RAV prevalence, which may be related to their distinct roles in the HCV life cycle that defines the resistance genetic barriers[115]. RAVs affecting each of the DAA classes have different properties and occur most commonly in the NS5A region, less commonly in the NS3 region, and uncommonly in the NS5B region. In all first-line DAA regimens, NS5A inhibitors are a crucial component because their RAVs have direct clinical impact[114,116]. Treatment-emergent RAVs that remain at high frequency after the end of therapy often have other fitness compensating mutations and may be more difficult to treat. Nowadays, there are several options for patients who have failed to respond to first line DAA therapy, and more than 90% of these patients are able to achieve SVR following retreatment, but the selection of the appropriate therapy depends on several factors and may require genotype and resistance testing. It should be noted that there are some notable genotype-specific differences with respect to retreatment, particularly in the case of prior exposure to NS5A inhibitors in patients with genotype 1 infection. Rescue treatment options with multiple targeted therapies, such as the pangenotypic combinations, sofosbuvir/velpatasvir/voxilaprevir (Vosevi), and glecaprevir/pibrentasvir (Mavyret), were effective in the majority of cases with DAA failure[112]. Interestingly, a recent European multicentric study showed that even Vosevi can fail in genotype 3 and genotype 1a infected individuals with cirrhosis, but this failure is not associated with a specific pattern of RAV. It is important to note that rescue treatment with multiple targeted therapies was effective in the majority of patients[117].

DAA treatment in the context of solid organ transplantation

The field of solid organ transplantation (SOT) has also been benefited with DAA development[118,119]. The efficacy of DAA has created a new opportunity to improve survival in end-organ failure patients through greater access to organ transplantation, since transplanting organs from HCV-infected donors into infected or uninfected recipients is now under consideration. Altogether, this has led to a better transplant outcome due to healthier patients receiving SOT and a significant reduction of waitlist mortality and healthcare costs. In the post-liver transplantation setting, early treatment is now recommended due to the high efficacy of DAAs, in association with a low side effect profile and easily mitigated drug-drug interactions[118,119]. The optimal treatment duration for each organ is not yet clear; in the years to come there will be increasing data and hopefully standardization of treatment. Some reports proposed 8-12 wk of DAA treatment for liver transplantation, but 2-4 wk seems to be enough for other organs[118,119]. However, it should be kept in mind that although it is expected that treatment eliminates the risk of infection, this is not a certainty; since the persistence of HCV RNA in peripheral blood mononuclear cells and/or the liver has been shown to occur post-SVR in liver transplantation recipients, with unclear clinical consequences. Although early outcome data are encouraging, the overall experience is limited, and many ethical issues and scientific questions remain, such as avoidance of selection bias, the optimal timing of DAA therapy, detailed evaluation of drug-drug interactions between DAAs and immunosuppressants, and long-term graft-patient outcomes. Moreover, there is no data on possible long-term hepatic and extrahepatic adverse effects associated to HCV exposure, even among those cured of the infection. As such, transplanting livers from HCV-infected donors into uninfected recipients requires special approval from governing bodies in the United States and in nearly all countries around the world[120].

HCC as a consequence of DAA treatment

Regarding the plausibility of HCC development in the context of DAA therapy, a risk reduction would be expected as viral clearance reduces morbidity and mortality rates. It should be considered, however, that HCV has a direct carcinogenic potential since some of the HCV-encoded proteins interact with cellular regulatory factors and produce oxidative stress, DNA damage, and deregulation of host cell checkpoints, thus promoting tumorigenesis[121,122]. Because HCV is an RNA virus and its genome does not undergo reverse transcription into DNA, its carcinogenic effects cannot be attributed to its integration into the hepatocyte genome. Lately, the risk of occurrence or recurrence of HCC in HCV patients who received DAA has been debated. IFN-based therapy reports demonstrated that achieving a SVR significantly diminished the risk for HCC[123]. Furthermore, these patients recover liver functionality with a positive impact on long-term disease-free survival[121,124]. On the other hand, initial reports on DAA therapy exposed a potential high risk of HCC occurrence and recurrence after treatment[121,125-127] and hypothesized that the occurrence of HCC would be the result of the emergence and spread of a pre-treatment "orphan" tumor clone that escapes immunological surveillance. The rapid virus elimination following DAA therapy may lead to an imbalance of the immunity that may rebound on immune control of the neoplastic clone[121]. However, the initial results were not conclusive or were even opposed due to the lack of homogeneity in the study design [125]. Recent seminal reports support the notion of a reduced rate for occurrence or recurrence of HCC after SVR obtained after DAA treatment, so the impact of DAAs on HCC risk is nowadays an old tale[93,128,129]. However, the time of DAA therapy initiation in HCC HCV positive patients is still controversial since it seems to condition treatment success[2,125,130,131]. In this sense, the best advice for physicians is to follow approved international or local guidelines and to keep updated to minimize risks or therapeutic failures[2,125,130,131].

Vaccine

The development of a prophylactic HCV vaccine that can contribute to the eradication goal still remains as a pending issue. The diversity of the virus, different behaviors of the virus in animal models or cell cultures, the limited models or individuals to test the vaccines, and the insufficient understanding of protective immunity against HCV are barriers to the development of an effective vaccine. It has been described, both in chimpanzees and humans, that immune system surveillance of primary infection is not necessarily efficient in controlling a recurrent one[132,133]. Therefore, spontaneous HCV immune control does not certainly generate protective immunity, hence diminishing confidence that prophylactic vaccination is possible. Furthermore,

compared to the initial HCV infection, a lower peak and duration of viremia characterized the reinfection in the same individual[113,134,135]. A faster and more effective viral replication control at second exposures indicates an adaptive immune response that may avoid chronic infection even though it cannot prevent reinfection. Therefore, a vaccine that induces T and B cell responses against multiple HCV genotypes and impedes the selection of virus escape mutants is needed[111,113]. While attenuated vaccines by the passage of the virus in non-human primate cell lines could be produced and suppress or inactivate genetic virulence factors, HCV does not replicate at high levels in non-human primate cell lines and no virulence factors have been defined for HCV yet. Therefore, concerns related to the production and the potential risk of attenuated vaccines could limit their utility[113]. In addition, HCV culture strains have adaptive mutations that enhance their ability of *in vitro* replication with an unknown impact on replication in humans. Inactivated whole HCV vaccines were also described; however, the lack of effective processes in the later phases for the purification of HCV represents an obstacle for the development of a complete virus vaccine[136]. On the other hand, there are numerous approaches involving viral antigens as immunogens, namely DNA-vaccines, adenovirus-based strategies, virus-like particles, HCV recombinant antigens conjugates to HBsAg, and HCV peptides in different delivery platforms[113,137-143]. Most of these candidate's vaccines have triggered humoral and cellular immune responses in rodents, and a small subset of them causes immunity in macaques, and fewer candidates in chimpanzees[113,144-148]. Likewise, only a few HCV vaccine developments accomplished the goal of phase I trials in volunteers not at risk for HCV infection[113,149-153]. Given that the partial results of the clinical trials are not completely encouraging, new strategies are required to improve and/or maintain antiviral immunity, and therefore there is a long way to go until a successful HCV vaccine could be used[137].

HCV infection is an example of the success of translational research, as a result, HCV infection is the only chronic viral infection that can be cured, and the hepatic or extrahepatic manifestations are mostly reversible[154]. Many countries are making significant progress in their fight against it, but HCV surveillance is at the base of any effort to control and eliminate the disease, since early diagnosis can prevent health problems that may result from infection and prevent transmission of the virus. The road is long, but with clear objectives the goal can be achieved.

HDV

In 1977, Rizzetto *et al*[155] identified a new antigen in the liver and serum of HBV infected patients who showed more severe hepatitis than their counterparts[155]. This observation led to the discovery of the HDV (also called a satellite virus), an unusual defective virus whose genome consists of a negative single-stranded circular RNA that encodes a single nucleocapsid protein, the delta antigen. The HDV virion, of 36 nm, consists of a ribonucleoprotein core complex and a lipoprotein envelope composed of the three HBV envelope proteins: Small (S-), medium (M-), and large (L-) HBsAg. HBV presence is mandatory for HDV replication, since HBsAg is required for HDV cell entry by NTCP, virion assembly, and export; however, its RNA replication is autonomous. HDV is maintained as episomes in the nucleus of the infected hepatocytes and transcribes the viral RNAs on behalf of the host cell machinery[156].

Clinical, epidemiological, and virological features

Two different scenarios may allow HDV infection: Either HBV and HDV simultaneously infect the host (co-infection) or HDV infection occurs in CHB patients (superinfection). In general, HDV is a highly pathogenic virus associated with more severe forms of acute hepatitis, including fulminant hepatitis. The natural course and outcome of acute hepatitis D differ according to the way infection takes place, whereas only 2% of coinfections evolve to chronicity, superinfection results in chronic infection in over 90% of the cases[157]. Irrespective of the type of infection, the chronic state leads to more severe hepatitis associated with higher rates of HCC and a faster progression to cirrhosis compared with HBV monoinfection, increasing this risk three times among HDV-HBV coinfected patients[157,158]. At least 5% of individuals with chronic HBV are co-infected with HDV, raising the HDV global burden of infection to an estimate of more than 62 million people, nearly 1% of the world's population[2,159]. Despite having a global distribution, HDV has a higher prevalence in Africa (Central and West Africa), Asia (Central and Northern Asia), Pacific Islands, Middle East, Eastern Europe, South America (Amazonian basin), and Greenland[2,160,161].

In addition to host factors, HDV and HBV genotypes influence the course of chronic hepatitis[162]. HDV genome analysis disclosed at least eight distinct HDV genotypes (HDV-1 to -8), with some displaying two to four sub-genotypes. Infection with

genotype 1, the most common one, has been associated with a wide spectrum of disease severity, while other genotypes appear to be more geographically restricted and to be linked with different degrees of disease severity. Infections with either genotype 2 and 4, the most commonly genotypes found in the Far East, generally develop milder forms of liver disease, whereas genotype 3 exclusively found in the Amazon region, has been documented as one of the most aggressive types, associated with severe and fulminant hepatitis outbreaks. HDV-5 is predominant in West Africa, whereas HDV 6, 7, and 8 were isolated in patients from central Africa[157]. Furthermore, HBV genotype could influence HDV infection and replication, being HDV viral loads are lower in patients co-infected with HBV genotype A, whereas co-infection is more frequently seen in genotype F CHB patients[156].

Transmission and diagnosis

HDV and HBV routes of transmission are alike, namely intravenous drug users or exposure to infected blood products and serous body fluids, but HDV mother to infant transmission is rare[162]. HIV infection, intravenous drug users, men who have sex with men, and individuals from areas of high HDV prevalence who are HBV-infected are at risk for co-infection with HDV[2,161]. The HDV antigen is only detectable transiently, therefore the diagnosis is made by measuring anti-HDV antibodies. HDV IgM appears in blood between the first and third weeks after infection and remains positive in the chronic phase with variable levels according to disease activity. HDV IgG is also detectable during active and resolved infection, so this test is useful for the screening of chronic or past HDV infection, while HDV RNA detection is applied to confirm active chronic hepatitis and to supervise therapy response. Anti-HDV IgM and HDV RNA assessment, together with HBV infection acute markers, should be tested to distinguish between acute co-infection HBV/HDV *vs* HDV superinfection [163].

Immune response and pathogenesis

Experimental and clinical studies suggested that HBV is a weak inducer of innate response and has developed strategies to evade innate immune sensing, whereas HDV has shown to activate the IFN pathway *via* melanoma differentiation antigen 5. It has been suggested that both HBV and HDV could inhibit the janus kinase/signal transducer and activator of transcription signaling pathway and hence the response to exogenous IFN. So, the constant activation of the IFN pathway may contribute to chronic viral pathogenesis; however, the implications on liver disease of HDV and HBV and innate immunity interplay remain to be understood. HDV activation of the type-I IFN pathway may promote an increase in the NK cell number, thereby inducing the killing of HBV-specific CD8 T cells by tumor necrosis factor-related apoptosis-inducing ligand-dependent mechanisms, hence worsening HBV pathogenesis in co-infected patients. Additionally, it has been described that HDV proteins affect autophagy by promoting HDV replication, cause oxidative stress, and modulate the transforming growth factor-β and nuclear transcription factor-kappa B signaling pathways. However, most of the studies have been performed in artificial systems that naturally tend to overexpression, so most of them need to be confirmed in actual infectious systems[156,164,165].

HDV pathologic changes are limited to the liver with histopathologic features that are not specific for it, but they tend to be more severe in HDV disease. The hepatocyte injury is typically focal, except in the most severe cases when confluent necrosis occurs, leading to submassive or massive necrosis accompanied by infiltration of inflammatory cells within the collapsed lobules and in the portal areas[157]. Liver biopsy is still of election to achieve an accurate inflammation grading and fibrosis staging since fibrosis noninvasive markers are not reliable in chronic HDV infection. The higher inflammation in HDV compared to HBV monoinfection alters elastography measurement, so the accuracy of transient elastography seems to be reasonable to detect cirrhosis but remains to be validated for grading lesser degrees of fibrosis[1,166-168].

Viral tropism

Several studies have proved the ability of HDV to replicate in a variety of tissues and cells after transfection; moreover, HDV-like viruses have been isolated from other species (birds, snakes). These findings question the hypothesis of an escaped human gene HDV origin and also alludes to the cooperation with other viruses to egress. Furthermore, it has been shown that HDV ribonucleoprotein can be assembled with envelope proteins that come from non-HBV related viruses, raising the question of HDV may also be harbored by other viruses[156].

Treatment

The ability to achieve SVR in the treatment of HDV remains uncertain given the high rates of late relapse. Therefore, HDV remains difficult to treat with the current available therapies. PEG-IFN is the election therapy but the absence of HDV treatment guidelines generate uncertainty concerning protocols. Nucleoside/NAs are ineffective because they do not reduce HBsAg levels, which is required for HDV propagation. However, despite the presence of HDV typically suppressing HBV replication, nucleoside/NA (entecavir or tenofovir) is generally recommended, particularly in patients with cirrhosis, regardless of HBV replication status[1]. Nevertheless, the Hep-Net International Delta Hepatitis Intervention Trial, a large multicenter program, treated patients with PEG-IFN-a-2a and/or adefovir for 48 wk. Six months after treatment completion, 28% of patients who were treated only with interferon continued to have undetectable HDV RNA with no additional benefit compared to those who also received adefovir and showed no response in individuals treated with adefovir alone. In a consecutive study in which patients were treated with PEG-IFN-α with or without tenofovir, only 23% of patients with interferon therapy presented levels of RNA under the detection limit 24 wk after stopping treatment with no extra benefit from the additional use of tenofovir[169]. Therefore, treatment is still the greatest challenge in HDV infection. So far, several promising new therapies have been described, some of which in combination with interferon, may result in sustained clearance of HDV. In this regard, myrcludex and lonafarnib are two promising treatments that are at the most advanced development stages. Myrcludex is an entry inhibitor while lonafarnib prevents HDV secretion, preventing both *de novo* and reinfection cycles. Other therapies are under evaluation in different clinical trials, such as hepcludex which has already been partially approved owing to its safe profile[170,171].

Despite these promising advances, we are in need of treatments achieving permanent HDV RNA suppression since high rates of relapse are associated with current IFN therapies in addition to increased transaminase levels after discontinuation. Interestingly, HDV coinfection prior to liver transplantation reduces the risk of graft reinfection and is associated with better patient survival than HBV-monoinfected patients. However, reinfection with HDV following liver transplantation may still occur, but tends to be aborted if HBV recurrence is also prevented[2,172].

The current knowledge on HDV highlights that the critical points to be addressed in future research must be directed to explain the virus and the immune system interaction linked to the pathogenesis that might allow treatment improvement against chronic liver disease produced by HDV.

HEPATITIS E VIRUS

The hepatitis E virus (HEV) is a causative agent of endemic and epidemic hepatitis worldwide, producing approximately 20 million infections every year, leading to an estimated 3.3 million symptomatic cases[173]. It is a spherical, non-enveloped virus that belongs to the family *Hepeviridae*, genus *Orthohepevirus*, a genus that is divided into four species (A-D)[174]. The strains of species A (Orthohepevirus A) are responsible for hepatitis E in humans. It comprises eight genotypes (HEV-1 to 8) displaying a geographical distribution and different epidemiological patterns. Genotypes that infect humans are 1-4 and 7[174,175].

HEV represents a significant public health challenge in resource-limited settings, mainly from Asia and Africa. In industrialized countries, it has historically been incorrectly regarded as having little clinical relevance[4]. However, in the last years, it has been recognized as an emerging and often undiagnosed disease in developed countries and some places of America, based on increasing reports of non-travel associated sporadic cases and chronic clinical presentations[176].

Epidemiology and transmission: Old and new challenges

Two epidemiological patterns have been observed for HEV. The first one is related to genotypes 1 (HEV-1) and 2 (HEV-2), which infect only humans and are transmitted mainly by the fecal-oral route, through water contaminated with the virus, resulting in frequent sporadic cases and occasional large outbreaks. These genotypes circulate in areas of high endemicity, generally in developing countries (due to poor sanitation) in Asia, the Middle East, North Africa, and some parts of America[175-177]. The second pattern, observed mainly in industrialized countries and some parts of America, is related to the zoonotic transmission of HEV genotypes 3 (HEV-3) and 4 (HEV-4), in

which pigs are considered a viral reservoir, although these viruses have also been detected in other animals, such as wild boar or deer[175,177]. Humans can become infected through direct contact (with many studies showing that farmers have higher levels of HEV antibodies)[178] or by ingestion of raw-undercooked animal meat or derived products, such as sausages or pates, that contain the virus[5]. Shellfish, fruits, and vegetables have also been implicated in viral transmission, probably due to pig slurry contaminating watercourses, which are used for irrigation, or being used as fertilizer[4,5]. HEV-3 has a worldwide distribution, while HEV-4 is restricted to Asia and Europe[177]. Interestingly, genotype 7 (HEV-7) has only been described in the Middle East and Dubai, from sporadic human cases and camels[174].

Since HEV is transmitted by the fecal-oral route (involving contaminated waters) and also as a zoonosis (having animal reservoirs), many studies in the last years have focused on HEV detection in environmental and food matrices as sources of HEV infection[177]. HEV has been detected in many environmental matrices, such as sewage, recreational waters (river, creek, dam), and tap waters, showing fecal contamination of the environment[179,180]. Viral presence in sewage represents an indicator of viral excretion of a given population, so it is useful for monitoring HEV circulation [181]. Water resources that are contaminated with wastewater are the main origin for the dissemination of enteric viruses and, in consequence, they could represent a viral reservoir with a dramatic impact on the population's health[179].

HEV food contamination that is not derived from pork is another route that is currently being studied, such as shellfish, fruits, vegetables, or milk, which have been postulated to be possible sources of infections, particularly in places where sporadic cases without an epidemiological link occur[5]. Many new lines of study are focused on the research of HEV in foods, methodologies for its detection in food matrices, and food outbreaks. The knowledge of these sources of infection will allow for improvements in the prevention of HEV infection.

Additionally, vertical transmission from mother to child[182] and transmission through blood transfusion[183] have also been described, but as less frequent routes. However, the transfusion route is currently becoming more relevant since an increasing number of cases are being reported in Europe and Asia[176]. This is particularly important for immunosuppressed populations since these patients could develop chronic infections and are commonly subjected to blood transfusions. Asymptomatic carriers of HEV could play a possible role as human viral reservoirs, and the virus can be transmitted during the donation, when the volunteer donates blood prior to the onset of the acute stage of hepatitis E[176]. In response to the threat posed by HEV to transfusion safety, many European countries have implemented screening for HEV-RNA in blood products, and many others are considering to do so [183]. However, in the rest of the world, there is still a lack of knowledge about this route of transmission.

In recent years, a few cases of acute and chronic human hepatitis E attributed, for the first time, to the HEV-C (*Orthohepevirus C*) species were reported in many parts of the world. Until now, this virus had only been detected in rats and ferrets (known as rat-HEV), and belongs to the genus *Orthohepevirus*, as well as the human-infecting HEV-A, although they are very divergent[4,184]. HEV-C genotype 1 was identified in both immunocompetent and immunocompromised patients who displayed acute and chronic infection as described in a recent large prospective study in Hong Kong, positioning this virus as a new etiological agent of hepatitis E. It is important to mention, as observed in one case, that the pre-existing HEV antibodies did not protect against HEV-C genotype 1. Also, routine hepatitis E diagnostic tests may overlook HEV-C infection[184]. Therefore, this is a new challenge in the field of viral hepatitis and specifically in understanding HEV epidemiology.

Clinical features

In most cases the infection produces an acute self-limited illness with a variety of clinical manifestations, ranging from asymptomatic course to acute liver failure, resulting in fatality rates of 0.2%-4%. The most common symptoms are abdominal pain, nausea, vomiting, anorexia, fever, and jaundice[182]. The course of the disease could be more severe in pregnant women infected with HEV-1, with high maternal, fetal, and neonatal morbidity and mortality rates, as high as 25%[182]. In turn, it has been described in individuals who have chronic liver disease that the mortality rate increases when infected by HEV[174]. Chronic HEV infections have been identified among immunocompromised persons infected with HEV-3 or HEV-4, including patients receiving cancer chemotherapy, recipients of organ transplant, and HIV-infected persons. In these cases, HEV-RNA had been detected in serum and/or stool samples for at least 6 mo[175,177]. Chronic hepatitis E seems to be an increasing

problem, particularly in Europe, where areas with high chronicity rates have been identified.

Hepatitis E also shows a spectrum of serious complications and extrahepatic manifestations, which are being increasingly recognized[174]. Some of them include acute or chronic liver failure, neurological disorders, pancreatitis, renal injury, cryoglobulinemia, hematological disorders, and thyroiditis[174,175,177]. The mechanisms of HEV-associated extrahepatic injuries are not fully understood yet and represent a challenge for the study of hepatitis E and its management.

Diagnosis

Diagnosis of hepatitis E infection can be carried out using direct techniques, which allow for the detection of the viral antigens and nucleic acid, as well as by the detection of IgG and IgM HEV-specific antibodies, although it may require a combination of both, molecular and serological assays, to confirm infection and for monitoring the treatment in chronically infected patients[185]. Laboratory diagnostic techniques for HEV detection vary in their specificity and sensitivity, something important to consider when using any of them, and to make comparisons. Currently, the gold standard test is the PCR for HEV-RNA amplification[185]. In the case of acute hepatitis E, a differential diagnosis should be performed to exclude other viral hepatitis and other causes (autoimmune, toxic, *etc.*) of liver disease. HEV-RNA detection can be carried out in serum samples (although the viremic period is short) as well as in stool samples, in which virions are shed for a longer period of time[4]. HEV antigen can also be performed, using double-antibody sandwich enzyme immunoassay techniques, which can be detected in serum, feces, or urine[185], although it is not extensively used. Acute hepatitis can also be diagnosed by IgM anti-HEV detection[173]. It is worth mentioning that the time of diagnosis and sample extraction is crucial. HEV is not generally taken into account in an initial assessment of a sick individual, due to still being regarded as an "emerging" disease, and many clinicians have limited knowledge of the disease[4]. This delay in sampling could lead to false negative results for viral RNA detection. In these cases, specific IgM testing is useful. For chronic infections, diagnosis is performed by detecting the presence of HEV-RNA by RT-PCR (and/or its variants Nested-PCR and RT-PCR) in blood for more than 6 mo[174]. The titer of antibodies against HEV may be lower in these patients, as well as in those immunosuppressed, so detection of HEV IgM and IgG should be interpreted with caution[185]. Although viral genotyping is not routinely performed, its determination is important in order to understand the clinical and epidemiological pattern (especially in risk patients, as immunosuppressed individuals, pregnant women, *etc.*), as well as for viral surveillance and to monitor the introduction of new genotypes/strains in a given region.

Antiviral treatment and vaccine

Antiviral therapy is not usually required in acute HEV infection since the virus is spontaneously cleared. However, treatment with ribavirin may be considered in cases of severe acute hepatitis E or acute-on-chronic liver failure[174].

In the case of chronic infections, ribavirin monotherapy for 3 mo is recommended. Decreasing levels of immunosuppression at diagnosis of chronic HEV infection is also advisable in solid organ transplant recipients. After 3 mo, HEV-RNA should be assessed in stool and serum samples. If RNA is undetectable, European Association for the Study of the Liver suggests stopping ribavirin therapy. If RNA replication persists, therapy with ribavirin should be continued for an additional 3 mo (6 mo course of ribavirin monotherapy in total). In the case of liver transplant recipients with lack of response to ribavirin, PEG-IFN therapy for 3 mo could be considered[174,186].

Even though many HEV vaccines have been developed worldwide, only one has been licensed in China (Helicon®). This vaccine is based on a recombinant HEV peptide derived from genotype 1, corresponding to a fragment of the open reading frame 2, which encodes the capsid protein of HEV. It is recommended to be used in individuals aged > 16 years and at high risk of HEV infection (food handlers, animal husbandry, soldiers, women of childbearing age, travelers to endemic areas, *etc.*). However, very little is known about many aspects of this vaccine, which limits its use, such as the efficacy (it has only been proved to prevent symptomatic hepatitis E due to genotype 4), immunogenicity and safety, especially in specific populations, like pregnant women, transplant patients and subjects with chronic liver disease[187].

The foregoing highlights new challenges regarding hepatitis E worldwide, showing that further research about epidemiological, clinical, and virological aspects are needed to understand better the different HEV scenarios and implications around the world.

CONCLUSION

In this review, we summarized the most relevant topics that are being analyzed or that have recently arisen in the setting of viral hepatitis. Although in recent years significant progress has been made in the knowledge of viral hepatitis, there are still many aspects to be resolved. It is necessary to continue working on improving diagnosis to maintain a constant and continuous epidemiological follow-up of infected populations, expand knowledge on the mechanisms of pathogenesis of each virus, improve treatment, and develop or improve the efficiency of vaccines. It is important to understand that strategies must be both local and global, as this represents that most successful path for viral hepatitis to cease being a major public health problem.

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