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Review Article

A Review of Hepatitis B Virus and Hepatitis C Virus Immunopathogenesis

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Abstract

Despite the advances in therapy, hepatitis B virus (HBV) and hepatitis C virus (HCV) still represent a significant global health burden, both as major causes of cirrhosis, hepatocellular carcinoma, and death worldwide. HBV is capable of incorporating its covalently closed circular DNA into the host cell's hepatocyte genome, making it rather difficult to eradicate its chronic stage. Successful viral clearance depends on the complex interactions between the virus and host's innate and adaptive immune response. One encouraging fact on hepatitis B is the development and effective distribution of the HBV vaccine. This has significantly reduced the spread of this virus. HCV is a RNA virus with high mutagenic capacity, thus enabling it to evade the immune system and have a high rate of chronic progression. High levels of HCV heterogeneity and its mutagenic capacity have made it difficult to create an effective vaccine. The recent advent of direct acting antivirals has ushered in a new era in hepatitis C therapy. Sustained virologic response is achieved with DAAs in 85–99% of cases. However, this still leads to a large population of treatment failures, so further advances in therapy are still needed. This article reviews the immunopathogenesis of HBV and HCV, their properties contributing to host immune system avoidance, chronic disease progression, vaccine efficacy and limitations, as well as treatment options and common pitfalls of said therapy.

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Abbreviations: +ssRNA, positive sense single-stranded RNA; -ssRNA, negative sense single stranded RNA; AHB, acute hepatitis B; ALT, alanine aminotransferase; anti-HBc or HBcAb, hepatitis B core antibody; anti-HBs or HBsAb, hepatitis B surface antibody; cccDNA, covalently closed circular DNA; CHB, chronic hepatitis B; cLD, cytosolic lipid droplet; CLDN1, claudin 1; DAA, direct-acting antiviral; ESCRT, endosomal sorting complex required for transport; HBcAg, hepatitis B virus core antigen; HBeAb, hepatitis B virus e-antigen antibody; HBeAg, hepatitis B virus e-antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HLA, human leukocyte antigen; IFN, interferon; IFN-I, interferon 1; IFN-α, interferon-alpha; IFN-γ, interferon-gamma; IL, interleukin; LCMV, lymphocytic choriomeningitis virus; LDL, low density lipid; NAs, nucleos(t)ide analogues; NK, natural killer; NKT, natural killer T; NPC1L1, Niemann-Pick C1-like 1 cholesterol absorption receptor; NTCP, sodium taurocholate cotransport polypeptide; OCLN, occludin; PCR, polymerase chain reaction; pDC, plasmacytoid dendritic cell; rcDNA, relaxed circular DNA; SR-BI, scavenger receptor class B type I; ssRNA, single-stranded RNA; SVR, sustained virological response; TGF-β, transforming growth factor-beta; TLR3, Toll-like receptor 3; TNF-α, tumor necrosis factor alpha; Treg, regulatory T cell; VLDL, very low density lipid.

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Introduction

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections remain major global health issues. Although the two viruses directly infect the liver, they have very different courses. Worldwide, there are an estimated more than 250 million HBV carriers, of whom roughly 600,000 die annually from HBV-related liver disease.[1,2] Acute HBV infection in adults is normally self-limited and subclinical, resulting in chronic infection in about 5%, as compared to neonatal HBV infection, where the acute infection results in chronic infection in 90% of cases. As a DNA virus, it is capable of incorporating its covalently closed circular DNA (cccDNA) into the host cell's genome, making it rather difficult to eradicate once the infection progresses to the chronic stage.

HCV was first identified in 1989 as a major cause of non A or B viral hepatitis.[3,4] Approximately 100 million people around the world have serologic evidence of HCV exposure and 71 million have chronic hepatitis C infection, according to a 2015 World Health Organization study.[5] It is estimated that 60–80% of patients with acute HCV infection will develop chronic infection, with about 20% of these chronically-infected HCV patients developing cirrhosis over a 25 year period. Those with cirrhosis have a yearly incidence of HCC of about 4–5%.[6,7] HCV is an inconstant RNA virus with high mutagenic capacity, and this leads to frequent genome mutations that enable it to evade the immune system and thereby have a high rate of chronic progression.[8] Furthermore, due to this high level of HCV heterogeneity, it has been difficult to create an effective vaccine.

Understanding the immunopathogenesis of HBV and HCV is essential in determining disease progression, chronicity, and treatment. The purpose of this article was to review the factors associated with HBV and HCV immunopathogenesis, disease progression, and pitfalls of current treatment options (Table 1).

Hepatitis B

Background

HBV is an enveloped DNA virus from the Hepadnaviridae

Table 1. HBV and HCV immunopathogeneses

	Hepatitis B	Hepatitis C
Virus structure	rcDNA	ssRNA
Receptor entry	Hepatocyte-specific NTCP receptor: Bile acid uptake from portal blood	Multi-step entry mechanism: LDL-R, SR-BI, CD-81, CLDN1, OCLN, NPC1L
Chronic progression	Adult infection: Clearance: 95%, Chronic progression: 5% Neonatal vertical transmission: Clearance: 10%, Chronic progression: 90%	All patients: Clearance: 60–80%, Chronic progression: 20–40%
Mechanisms of immune evasion	CD4+ cell inhibiting factors: IL-10, TNF-B CD8+ cell inhibiting factors: PD-1, CD244, CTLA-4	CD4+ cell inhibiting factors (including Tregs): IL-10, TNF-B, Overall Reduced CD4+ response CD8+ cell escape mutations: PD-1, CTLA-4
Approved therapies	Interferon therapy: Standard INF-α, Pegylated-IFN-α NAs Treatment initiation decision: Presence of cirrhosis, ALT level, HBV DNA level	Interferon therapy: Pegylated-IFN-α + ribavirin DAAs Treatment selection varies by: Genotype, presence of cirrhosis, treatment history, human immunodeficiency virus co-infection, renal impairment
Goals of therapy	Attain disease suppression: Suppression of HBV DNA, Loss of HBeAg, Normalization of ALT, Decrease necroinflammatory activity, Decrease in fibrosis	Eradicate HCV RNA – attain SVR: Undetectable RNA level 12 wk after completion of therapy
Vaccines	Approved and effective vaccines: Plasma-derived vaccination, HBV three-series vaccine	No available effective vaccines

family. The virus particle consists of an exterior envelope surrounding a viral capsid containing relaxed circular DNA (rcDNA). When the virus infects hepatocytes, it leaves a stable cccDNA template within the cell's nucleus. This provides a stable, long-lasting template for viral replication and accounts for HBV's viral persistence. HBV hepatocyte infection can be divided into the following steps: cell entry, capsid uncoating, DNA repair and transcription, RNA packaging, reverse transcription, enveloping, and virus secretion (Fig. 1).

Viral entry into the hepatocyte is mediated by hepatocyte-binding specific envelope proteins. The HBV viral sur-

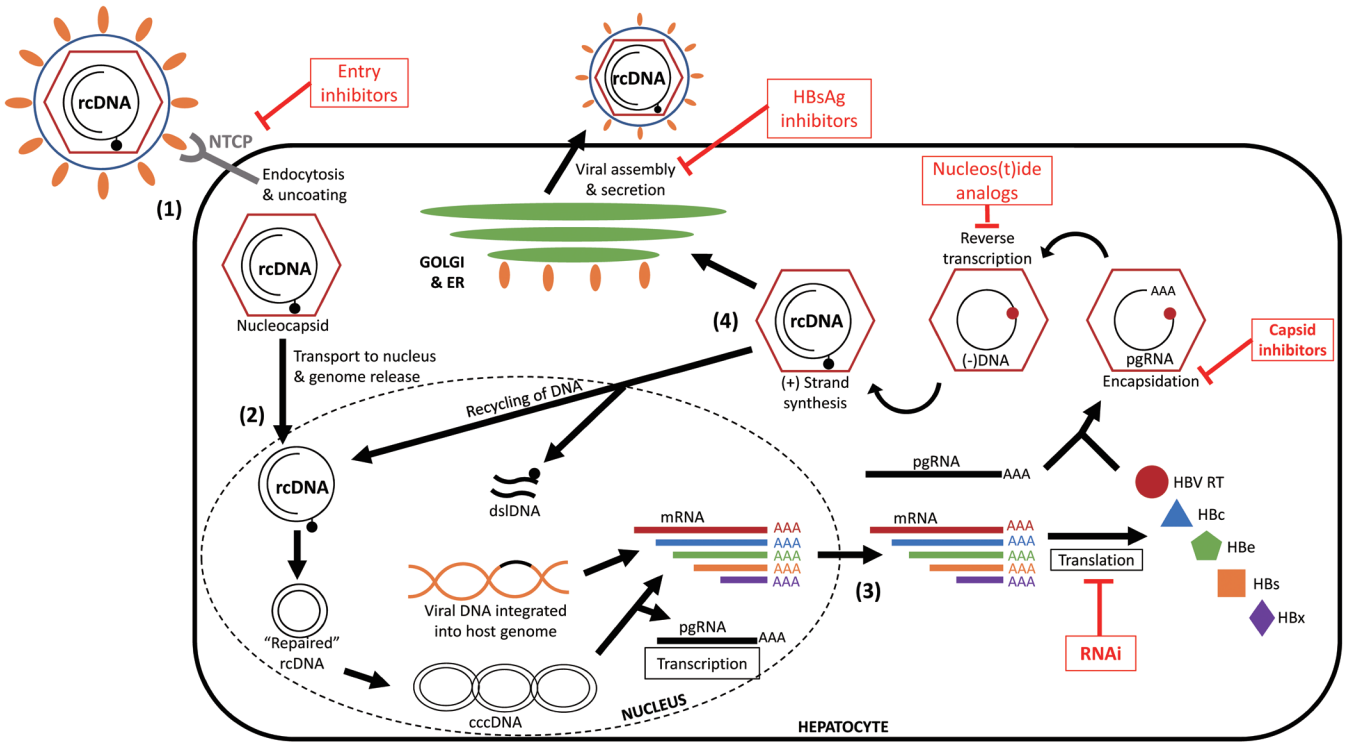

Fig. 1. Hepatitis B viral life cycle.

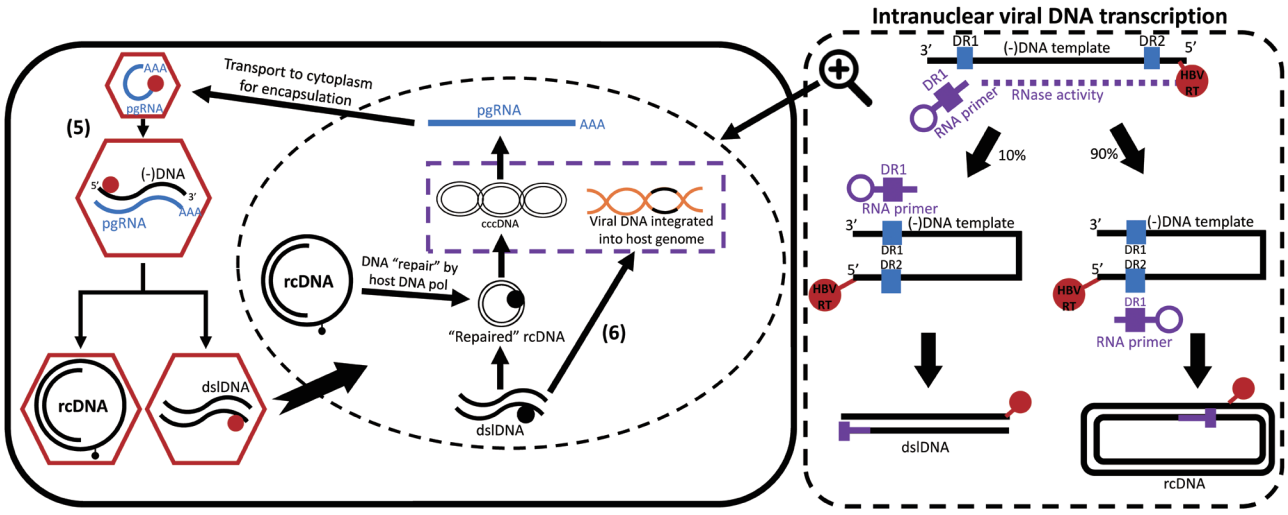

Fig. 2. Hepatitis B DNA integration. 1) HBV pre-S1 domain of envelope L protein binds to the hepatocyte-specific NTCP receptor (involved in bile acid uptake from portal blood flow), leading to cell entry.[9,10] The antigenic loop of HBV surface protein S between regions I and II interact with hepatocyte heparin sulfate proteoglycans and aid in HBV-NTCP receptor interaction.[11] 2) The viral nucleocapsid containing rcDNA is transported to the nucleus where it is "repaired" by the host cell's DNA repair mechanisms closing or "derelaxing" the rcDNA to form cccDNA. The cccDNA remains permanently in the hepatocyte's nucleus and acts as a template for viral mRNA and pgRNA.[12,13] 3) Viral mRNA and pgRNA are transported to the cytoplasm. Here, mRNA undergoes translation by host ribosomes to form viral proteins. The viral proteins and pgRNA are then assembled and encapsulated. pgRNA undergoes reverse transcription by newly transcribed HBV reverse transcriptase, producing a (−)DNA intermediate. pgRNA is then degraded and HBV reverse transcriptase completes transcription, producing an rcDNA containing nucleocapsid. 4) rcDNA containing nucleocapsids can either a) be enveloped and secreted as virions or b) cycle back to the nucleus to replenish the cccDNA pool.[117] 5) Transcription of pgRNA by HBV reverse transcriptase in a 3′ to 5′ direction resulting in a (−)DNA. pgRNA is then partially hydrolyzed by HBV reverse transcriptase, leaving an 18 nucleotide RNA primer for synthesis of (+) DNA strand. In ~90% of nucleocapsids, the RNA primer translocates to DR2 resulting in rcDNA as HBV reverse transcriptase synthesizes the (+)DNA resulting in an rcDNA containing nucleocapsid. In ~10% of pgRNA containing nucleocapsids, priming for reverse transcription occurs at the DR1 region (instead of DR2 region) of the (−)DNA template with resulting in dslDNA. 6) Viral dslDNA can then be transported to the nucleus and can incorporate into host DNA at double-strand DNA breaks.[27]

face contains three proteins: L, M, and S. The virus binds and uses the hepatocyte-specific sodium taurocholate cotransport polypeptide (NTCP) receptor, which is involved in bile acid uptake from portal blood flow for hepatocyte cell entry. In a seminal article by Yan *et al*.,[9,10] (2012), the pre-S1 domain of the HBV envelope L protein was described to bind to the hepatocyte-specific NTCP receptor, leading to cell binding and entry. The antigenic loop of HBV surface protein S between regions I and II interact with hepatocyte heparin sulfate proteoglycans and aid in HBV-NTCP receptor interaction and HBV infectivity (Fig. 2).[11]

Following binding and entry into the hepatocyte, the viral nucleocapsid containing rcDNA is released into the cytoplasm and transported to the cell's nucleus, where the rcDNA is delivered. In the nucleus, the rcDNA is "repaired" by the host cell's DNA repair mechanisms, closing or derelaxing the rcDNA to form cccDNA. This cccDNA remains permanently in the hepatocyte's nucleus and acts as a template for viral RNA transcription. The RNA transcripts are transported into the hepatocytes cytoplasm, where HBV reverse transcriptase uses the RNA as templates to produce viral rcDNA. The rcDNA is then either repackaged and enveloped for cellular export or recycled back to the nucleus, amplifying cccDNA concentration.[12,13]

Acute hepatitis b infection

Acute hepatitis B (AHB) infection occurs by the exchange of bodily fluids such as blood, semen, or vaginal fluid. About 70% of adults who are acutely infected have a subclinical and anicteric phase, while the other 30% develop clinically significant symptoms and icteric hepatitis. Studies in chimpanzees by Wieland *et al*.,[14] (2004) and transgenic mice studies by Baron *et al*.,[15] (2002) have demonstrated the immune response during HBV infection. In an acute infection, HBV has a prolonged incubation period of 45–180 days. The initial immune response is mediated by the innate immune system. Typically, the innate immune response results in about a 90% reduction in serum HBV DNA. In the chimpanzee and other model systems, it has been shown that natural killer (NK) cells and natural killer T (NKT) cells play an important role in the early viral control of acute HBV infection through interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) secretion. A surge of IFN-γ mediated by NK cells, NKT cells, and T cells has been found to coincide with a reduction in serum HBV DNA.[14] Subsequent *ex vivo* studies have shown that IFN-γ and TNF-α stimulation could destabilize cccDNA via activation of APOBEC3A and APOBEC3B.[16] These studies also suggested that the innate immune response is important for rapid viral clearance during AHB infection, although they cannot clear the virus alone.

The adaptive immune response during an acute infection function is crucial in three ways: 1) inhibition of HBV attachment and entry, 2) eradication of infected hepatocytes, and 3) conference of viral immunity. B cell response and antibody production is focused on the various HBV proteins, including core protein, surface protein, e-antigen, and polymerase. The antibodies specific for the envelope (hepatitis B surface antibody, known as anti-HBs or HBsAb) and nucleocapsid antigen (hepatitis B core antibody, known as anti-HBc or HBcAb) are both clinically important and useful to distinguish between different acute phases of HBV infection.[17] The discovery of the NTCP and pre-S1on protein L and the antigenic loop on protein S contributing to hepatocyte infection has led to the understanding of how anti-HBs leads to disease resolution and virus control. Antibodies against these entry antigens effectively block HBV infection, contributing to disease resolution and long-term immunity.[18,19]

HBV-specific T lymphocytes are responsible for viral

clearance as well as halting liver inflammation. HBV specific CD8+ T cells are found in the liver during acute HBV infection and cause lysis of HBV-infected hepatocytes. This is thought to be the mechanism of clearing cccDNA-containing hepatocytes and effectively eliminating HBV infection.[20] CD8+ T cells have also been shown to release INF-γ and TNF-α during acute HBV infection, stimulating the innate immune response, a key mechanism in serum HBV DNA clearance.[21] T cell response is modulated during an acute infection by both inhibitory and activating regulatory mechanisms, including expression of PD-1, IL-10, and CD4 T regulatory cells (Tregs).[22] Higher expression of T cell inhibitory mechanisms (i.e. PD-1, CD244, and CTLA-4) are seen in patients who develop chronic hepatitis B (CHB), indicating that inhibition of the T cell response may contribute to development of CHB.[23–25]

CHB

CHB is characterized by both persistent liver inflammation and HBV infection. Liver inflammation leading to fibrosis and cirrhosis occurs due to chronic inflammation as the immune system destroys the cccDNA-containing hepatocytes but is unable to clear the HBV infection. Clinically, this is identified by the presence of hepatitis B surface antigen (HBsAg), elevated HBV DNA, with elevated alanine aminotransferase (ALT). CHB can be further subdivided into two types: HBV e-antigen (HBeAg)-positive and HBeAg-negative. HBeAg is an immunologically distinct soluble antigen, located between the viral nucleocapsid and envelope processed from the pre-core protein. Loss of HBeAg is typically associated with remission of liver disease; however, in a subset of HBeAg-negative patients, viral reactivation can occur. The phases of HBV infection are detailed below.

The course of HBV infection can be divided into four phases that are determined by the host-virus immune response. The first phase is the immune tolerance phase, characterized by active replication of HBV without substantial hepatic inflammation but with HBeAg positivity and normal ALT level. HBeAg accumulates in the serum as an immunologically distinct soluble antigen and is used as a marker of active viral replication. The function of HBeAg is not clearly understood and is dispensable for replication, as mutant viruses without HBeAg exist and are both infectious and pathogenic. The second phase is the immune clearance phase, characterized by elevated ALT levels and decreased HBV DNA load. The third phase is the inactive carrier state, known as the immune control phase, defined by low HBV replication in which HBeAg positive patients lose HBeAg and gain antibodies to HBeAg (HBeAb). This phase is characterized by disease remission. The fourth phase is viral relapse, known as the immune escape phase, and is associated with a relatively high rate of liver inflammation, fibrosis, hepatocellular carcinoma (HCC) development, and mortality. In this phase, patients who were inactive carriers develop increased viral replication of HBeAg-negative HBV and hepatic inflammation with elevated ALT. This phase transition may occur in 20–30% of patients in the inactive carrier phase. HBeAg-negative CHB is the main form of CHB worldwide and is associated with defective T cell function.[26] It remains unclear what triggers the progression through the various phases of infection or the mechanism of defective T cell function during the immune escape.

Dysregulation of T cell immune response has been associated with progression to CHB. CD4+ helper T cells perform antiviral functions and produce a variety of cytokines crucial for viral clearance and progression to fibrosis, specifically the ratio between Tregs and Th17 T-helper cell subtypes. These two subtypes remain antagonistic to each other, where Th17 cells mediate inflammatory response leading to liver damage and fibrosis while Tregs mediate immune tolerance and contribute to the chronicity of infection. Nan *et al*.,[27] (2012) found elevated levels of both Tregs and Th17 cells in peripheral blood in patients with HBV infection; however, Th17 cells were significantly higher in patients with AHB compared to patients with CHB, who showed a higher Treg/Th17 ratio. These findings suggest Treg/Th17 imbalance is closely associated with progression of CHB. Further studies have shown higher interleukin (IL)-35, which stimulates Treg production, in CHB patients, contributing to the dysregulation of the Treg/Th17 ratio and that higher Treg/Th17 ratios are closely associated with cirrhosis.[28,29] CD4+ helper T cells play an important role in stimulating and maintaining CD8+ T cell response, and insufficient CD4+ helper T cell response is strongly associated with impairment of CD8+ T cell function and viral persistence.

Sterilizing cure is defined as the eradication of intrahepatic HBV DNA (intranuclear cccDNA or integrated HBV DNA), loss of HBsAg, and undetectable serum HBV DNA. This was the goal of interferon (IFN)-based therapies. Once viral DNA is incorporated into the host genome or cccDNA in the host nucleus, it is a challenge to remove it and may require immune modulation to destroy the infected hepatocyte due to the dysregulation of T cell immune response. Luneman *et al*.,[30] (2014) discovered that CHB patients paradoxically produce $CD56^{bright}$ NK cells that inhibit cytokine production and blunt the cytolytic activity of CD8+ T-cells. Studies have shown that interferon-alpha (IFN-α) treatment induces normal $CD56^{bright}$ NK cell activity, thereby increasing IFN-γ expression and cytotoxic function of CD8+ T cells with an associated decline viral load.[31,32] In a study by Wursthorn *et al*.,[33] (2006) IFN therapy can decrease intrahepatic HBV DNA in most patients and achieve undetectable levels on about 50% of patients based on real-time polymerase chain reaction (PCR) of post-treatment liver biopsies. Although IFN-α therapy may lead to seroconversion and viral clearance, there is a poor response rate, with about a 30% HBeAg seroconversion and 3% HBsAg seroconversion.[26] Due to the side effect profile, and the mediocre sterilizing cure rate, IFN-based therapies have fallen out of use. Functional cure, defined by seroclearance of HBsAg with persistence of HBV DNA only in the liver, is associated with improved clinical outcomes and is the current therapeutic target. Functional cure is the goal of nucleus(t)ide analogues (NAs) and is achieved by inhibiting viral replication. Due to the persistence of cccDNA in hepatocytes, NAs must be administered long term, if not indefinitely. However, some studies have shown that NA treatment improves CD8+ T cell cytotoxic function and occasionally clearance of HBV DNA-containing hepatocytes is achieved.[34,35] In a subset of patients, cure can occur after prolonged NA treatment.

HBV vaccines and limitations

HBV vaccination has been an effective measure to prevent HBV infection. Plasma-derived vaccinations were first developed then replaced by recombinant HBV vaccines in the 1980's, both with similar efficacy.[36] These vaccines stimulate anti-HBs antibodies in noninfected individuals, with a 95% effective rate of seroconversion. The reason for 5% non-response remains unclear; however, there are certain populations affected, including those with chronic disease, certain genetic mutations, and those on immunomodulatory medications at higher risk for non-response to vaccination.[37] Chronic diseases include old age (>60 years-old), human immunodeficiency virus-coinfection, and chronic kidney disease. Genetic mutations associated with non-response include homozygous mutations for human leukocyte

antigen (HLA) DRB1*0301, HLA-B8, SC01, DR-3, HLAB44, FC-31, and DR-7.[38,39] Methods that induce seroconversion include administering a 4th dose of vaccine or a repeat administration of the three-series vaccine at a higher dose. Other vaccine methods currently under investigation include intradermal administration of the vaccine, the use of adjuvants (including 3-deacylatedmonophosphoryl lipid A), use of triple S antigen recombinants, and a vaccine with the combination HBsAg and HBcAg.[37]

The implementation of a rigorous vaccination program worldwide has led to a decrease in incidence of hepatitis B infection. However, the widespread use of vaccines has led to the emergence of certain point escape mutations leading to vaccination failure. This phenomenon has been shown to cause hepatitis B infection in previously vaccinated patients. A prospective Chinese study showed 6/176 adults to be HBV DNA-positive by PCR at 1 year after receiving the HBV vaccine. Four out of these six cases had known HBV escape mutations.[40] Despite awareness of this emerging issue, there is no consensus currently on the need for vaccine redevelopment or need for therapy in individuals infected with these mutants.[41]

Experimental treatments in the pipeline

Currently, there are many classes of medications with various targets under investigation for hepatitis B therapy. One class under investigation are entry inhibitors targeting NTCP and therefore protecting against *de novo* infection of hepatocytes.[42] Myrcludex-B has been shown safe and effective in HBV/hepatitis D virus coinfection and is in phase II trials for the treatment of chronic HBV.[43] Another class undergoing active investigation includes small interfering RNAs, which target viral RNA and lead to their degradation preventing translation of viral proteins crucial for viral capsid formation and cccDNA formation.[44] Multiple small interfering RNA candidates are currently in phase I/II trials.[45] Capsid inhibitors are another class of drugs currently under investigation. The integrity of viral capsids, the protein surrounding the viral genome, are physically altered, causing both disruption of capsid integrity and preventing hepatocyte entry, thereby preventing cccDNA formation. This mechanism is distinct from entry inhibitors, as capsid inhibitors also exhibit antiviral properties.[46] Another class of agents, HBsAg inhibitors, which are currently in phase II trials, work by inhibiting the processing of HBsAg and release from the infected cell. Interestingly, they also seem to trigger an antiviral immune response, as serum HBsAg titer falls and occasionally HBsAb seroconversion occurs after monotherapy use.[47] This antiviral effect suggests that viral surface proteins in circulation may contribute to impairment of the immune system's antiviral response.[47] The role of HBsAg on antiviral suppression remains unclear and is an intriguing area of study in understanding the progression to chronic HBV and potential future immunomodulating therapies.

Investigational therapies using vaccines to stimulate cytotoxic T cell response have yielded unsatisfactory results. Fontaine *et al*.,[48] (2015) performed a phase I/II trial using NAs alongside an HBV envelope expressing DNA vaccine. The trial showed no benefit in regard to risk of relapse of HBV treated patients, rate of virologic breakthrough, or restoration of anti-HBV immune response. Lok *et al*.,[49] (2016) performed a phase II trial using T cell-based vaccine after 1 year of therapy with NAs. The vaccine did not provide significant reductions in HBsAg in virally suppressed patients with CHB. Currently, PD-1 inhibitors are being studied to explore if durable control of CHB can be achieved. Phase 1 trials have shown that in virally suppressed HBeAg-negative patients, that nivolumab (PD-1 inhibitor) led to HBsAg decline in most patients and sustained HBsAg loss in one patient.[50] Clinical trials are currently ongoing, studying various vaccines and immunomodulatory to achieve sustained CHB control.[51]

Hepatitis C

Background

HCV was isolated in 1989 and is a member of the Flaviviridae family (which includes Dengue and Zika virus). HCV has infected nearly 3% of the world's population. It is a major cause of liver disease and cancer, where about 30% of chronically infected individuals develop cirrhosis, and infected individuals are at a 17-fold increased risk of developing HCC. In the USA, HCV accounts for 50% of the cases of HCC.[52] The virus consists of a single lipid bilayer envelope surrounding a viral nucleocapsid, containing multiple viral core proteins and single-stranded RNA (ssRNA) that encodes for a single large polyprotein transcript. The single transcript is further processed into three structural proteins (core, envelope-1, envelop-2) and seven proteins involved in viral replication. In chronic HCV infection, the actions of the virus along with host immune factors cause dysregulation of the immune system, leading to failure of the immune response to clear the HCV infection. The inability of the immune system to clear the infection leads to persistent hepatic inflammation, itself leading to cirrhosis, liver failure, or the development of HCC. The virus's high mutation rate due to the lack of proofreading capability by its RNA-dependent RNA polymerase leads to escape mutations, allowing the virus to evade the immune system and contributes to treatment and vaccine failure.

This high mutation rate leads to a broad range of genetic variants categorized into seven main genotypes, each with multiple different subtypes. Each genotype has more than a 25–35% difference in their nucleotide sequence.[53] Genotype subtypes differ in nucleotide sequence from each other by 15–25%.[54] Further genetic variability can be found in each individual patient, where multiple viral quasispecies containing different mutations can co-exist. Prevalence of distribution of HCV genotypes varies geographically. Among all the genotypes, genotype 1 (i.e. HCV-1) is the most prevalent worldwide. In the USA, HCV-1a and HCV-1b subtypes account for 60–70% of all patients. HCV-2 is the most prevalent in middle and west Africa, HCV-3 in east Asia and India, HCV-4 in Egypt and sub-Saharan Africa, HCV-5 in South Africa, HCV-6 in China and southwest Asia, and HCV-7 in central Africa.[53,55]

HCV infection

HCV circulates in the blood, accessing basolateral hepatocyte surface receptors. The HCV particle has a very low density that closely resembles very low density lipoprotein (VLDL) and low density lipoprotein (LDL) and uses apolipoprotein and serum lipid uptake mechanisms to enter the hepatocyte. HCV entry is complex and uses multiple cell entry factors that are spatially arranged and bind in a temporally ordered manner. Five cell surface proteins are essential for HCV particle binding and entry, namely CD81, scavenger receptor class B type I (SR-BI), claudin 1 (CLDN1), occludin (OCLN) and Niemann-Pick C1-like 1 cholesterol absorption receptor (NPC1L1). First, HCV attaches to the hepatocyte LDL-R via viral apolipoprotein E-like protein.[56] Next, SR-BI surface protein binds to viral lipoproteins expressed by HCV surface E2 gene. SR-BI is involved in high density lipopro-

tein (HDL) and VLDL binding and is highly expressed by hepatocytes, which may explain HCV hepatotropism. SR-BI then serves to unmask virus particles and exposes the CD81 cell membrane protein which binds to HCV E2 surface protein.[57] The CD81-HCV complex moves laterally across the cell membrane to tight junctions, where CLDN1 is located, and activates CLDN1 mediated viral entry via clathrin-dependent endocytosis.[58] OCLN and NPC1L1 are tight junction proteins that are involved with HCV entry, however their exact roles are unknown. Targeting NPC1L1 with antibodies or its antagonist ezetimibe (already Food and Drug Administration-approved as a cholesterol lowering medication) has been shown to halt or delay cell entry of all seven HCV genotypes, providing an intriguing potential therapeutic target.[59]

The HCV assembly and release processes is not yet fully understood; however, it seems to be closely related to lipid packaging and release.[60] Once inside the cell, the virus particles are uncoated, the capsid is destroyed, and the viral positive sense single-stranded RNA (+ssRNA) is released into the cytoplasm.[61] The viral RNA then takes over the hepatocytes ribosome on the rough endoplasmic reticulum to synthesize a single long polyprotein that is later proteolytically processed by viral and cellular proteases into its 10 proteins, which include 3 structural proteins (core, E1, E2) and 7 nonstructural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A, NS5B).[61] Here in the cytoplasm, newly formed HCV RNA-dependent RNA polymerase transcribes the viral +ssRNA into an intermediate negative sense single stranded RNA (-ssRNA), which is then later transcribed into +ssRNA. The newly transcribed +ssRNA is then either packaged into new HCV virions or used for further HCV polyprotein translation by endoplasmic reticulum ribosomes. It is during this process, due to the high error rate and lack of proofreading ability of the HCV RNA polymerase, that many mutations are introduced and the many HCV quasispecies are formed.[61] The error rate of HCV RNA-dependent RNA polymerase is estimated to be between one mutation per every 10^3–10^6 nucleotides copied compared to one mutation per every 10^8–10^{11} nucleotides copied for DNA polymerases.[62–64] The virus proteins then arrange on the endoplasmic reticulum intracellular lipid membrane, where core proteins are shaped and bind to cytosolic lipid droplets (cLDs).[65] The cLD-bound core proteins assemble into the core nucleocapsid via microtubules and dyneins, and +ssRNA are encapsulated. The HCV nucleocapsids, containing RNA and core proteins, then bud into the endoplasmic reticulum, where surface proteins are attached.

The detailed mechanism of HCV viral release is yet unknown; however, hepatocyte exit seems to depend on the pathway for producing VLDLs and on the presence of apolipoprotein B and E. It is thought that the HCV particle complexes with apolipoprotein B and apolipoprotein E containing VLDLs in order to exit the hepatocyte. A study by Huang *et al*.,[66] (2007) showed that blocking VLDL secretion reduced HCV viral release from the hepatocytes in a growth media by 80%. Recently, another cellular pathway has been implicated in HCV release. The endosomal sorting complex required for transport (ESCRT) pathway has been implicated in HCV secretion out of the endoplasmic reticulum and cell. This pathway is normally used to bud vesicles out of the cell, and HCV may use the ESCRT system too, but little is known about this process.[67]

Innate immune response to hepatitis C infection

The innate immune response is important in HCV infection by both limiting viral dissemination through inducing infected hepatocyte apoptosis and stimulating the antigen specific adaptive immune response.[68] During an acute HCV infection, viral RNA is detected within the hepatocyte cytoplasm. The presence of intracellular HCV RNA after the uncoating of HCV virion activates TLR3, RIG-I, and MDA5 of infected hepatocytes, which in turn release interferon 1 (IFN-I, a and b) and IFN-γ. Circulating HCV RNA is also detected by plasmacytoid dendritic cells (pDCs). The activated pDCs produce IFN-α. Both IFN-I (a and b) and IFN-γ released by infected hepatocytes and circulating pDCs act to directly inhibit HCV replication and activate NK cells.[69] NK cells play a crucial role in the innate immune response to acute HCV infection by cytolytic destruction of infected hepatocytes and cytokine release, which both directly inhibit HCV replication and stimulate the adaptive immune response. Activated NK cells directly induce infected hepatocyte perforin- and granzyme B-mediated apoptosis. The cytolytic action of NK cells through the perforin/granzyme mechanism causes collateral damage to uninfected surrounding hepatocytes. NK cells also produce IFN-γ and TNF-α, which cause dendritic cell maturation, leading to IL-12 release which induces the adaptive immune response with the differentiation of CD4 and CD8 T cells.[70,71] Lastly, *in vitro* studies have shown that IFN produced by NK cells directly inhibits HCV replication.[72]

Adaptive immune response, its shortcomings, and progression to chronic hepatitis C

Generally, T cell response is the adaptive immune system's main mechanism of viremia control. In HCV, specific CD8+ T cells destroy infected hepatocytes via HLA class I antigen presenting cells as well as by inducing cytokine secretion (TNF-α, IFN-γ).[73] Helper CD4+ T cells support this function via IL-2 to stimulate CD8+ T cell and NK cell activation.[74,75]

Chronic HCV infection is defined as persistent viremia for more than 6 months.[76] In chronic HCV, a continuous yet impaired activation of the adaptive immune system occurs. HCV has multiple mechanisms to defend against immune clearance, resulting in immune system evasion and chronic infection.

The first major mechanism of chronic infection is the loss of T cell function due to chronic T cell activation. Essentially, T cell function is inhibited and their cytotoxic capacity is lost. First described in persistently-infected mice with lymphocytic choriomeningitis virus (LCMV), high levels of persistent viral antigen production results in chronic T cell activation, leading to sequential loss of T cell function.[77] During chronic infection, HCV specific CD4+ helper T cells show a reduced production of IL-2, resulting in impaired CD8+ T cells activation.[78] Additionally, HCV core antibody has been implicated in T cell suppression, as it binds to complement C1q inhibiting Lck/Akt activation of CD4+ and CD8+ T cells.[79] PD-1-mediated CD8+ T cell suppression has also been implicated in inhibiting T cells. Studies have shown that isolating CD8+ T cells from chronically infected patients and exposing the cells to PD-1 blocking drugs *in vitro* can restore T cell function.[80,81] Further studies have shown that CTLA4 (an inhibitory signal produced by Tregs) along with PD-1 may synergistically inhibit CD8+ T cell function.[82]

CD4+ T cell activity plays an important role in chronic HCV infection. It is widely known that a vigorous CD4+ T cell response during an acute HCV infection is correlated with viral clearance.[83–85] Conversely, progression of acute HCV infection to chronic infection is strongly associated with the downfall of HCV specific CD4+ T cell response.[74,83] Both the lack of robust CD4+ T cell response during acute infection and the decrease CD4+ T cell response after the acute phase of infection have both been observed and associated with the development of chronic progression.[86] The mechanism by which CD4+ T cell response dwindles during the

acute infection remains unclear. One mechanism proposed is HCV escape mutations occurring in patients with various HLA epitopes (including HLA-DRB1*15 epitope).[86,87]

Other Tregs, such as CD25+ T cells, play a role in suppressing immune system activation.[88–90] These cells work through various mechanisms to suppress the immune response during chronic HCV infection, including CD8+ T cell inhibition and inhibition of cytokine release such as IL-10 and transforming growth factor-beta (TGF-β) immunomodulating cytokines.[89,91,92] Tregs upregulated during chronic infection, decrease to the level of control individuals after spontaneous viral clearance and after treatment induced sustained virologic response (SVR).[89,93] The mechanism of Treg upregulation occurring during HCV infection remains unclear; however, it has been proposed that HCV itself can induce Treg upregulation specifically by HCV core protein.[93,94] Viral escape mutations are a major factor in the development of chronic HCV. HCV viral RNA is highly prone to mutations due to its RNA-dependent RNA polymerase and its lack of proofreading function. Multiple virus mutants can co-exist, eventually leading to the selection of CD8+ T cell escape variants. Up to 50–70% of patients with chronic HCV infections exhibit mutations at viral epitopes targeted by CD8+ T cells.[95,96] Viral escape mutations emerge during the early acute infection and remain fixed in the quasispecies for years, implicating themselves as a mechanism for immune system evasion and chronic progression.[97] However, many viral mutations have been shown to reduce fitness and viral replicative capacity.[98,99] Thus, as a consequence of the viral mutant-impaired fitness, most viral escape mutations will revert to wild-type long term and be the primary transmission agent to a new host.[100] Some HLA alleles have been shown to be protective of the viral escape mutants and are associated with a decrease in chronicity. HLA-B27 positivity is protective against chronic HCV progression; however, escape mutations can still occur. HLA-B27–binding anchor encodes a highly conserved region on viral RNA polymerase, where mutations at this epitope often lead to a non-functional RNA polymerase.[101]

IFN and DAA HCV therapy

IFN remains an important immunomodulator involved in host defense and treatment of chronic HCV. Upon viral entry into the host cell, HCV RNA is detected by TLR3 in the endosome and by RIG-I in the cytoplasm. Activation of these receptor pathways lead to interferon transcription. There are 3 major types of interferon, Type I and III IFN are produced by the infected hepatocyte and Type II IFN are produced by NK-cells and natural killer T cells (NKT cells). IFN bind to specific transmembrane JAK–STAT receptor signaling pathways to regulate gene transcription in the nucleus inducing an "antiviral state" in the cell activating both the innate and adaptive immune systems through various stimulatory pathways.[102–104]

Historically, pegylated-IFN and ribavirin have been the mainstay of treatment of chronic HCV. Today, DAAs remain the preferred treatment choice; however, IFN-based therapies remain a treatment option. With IFN use, HCV genotypes play an important role in treatment strategy and outcome. HCV genotypes 1 and 4 require longer treatment (48 weeks) and achieve a SVR of 50%. Meanwhile, HCV genotypes 2 and 3 require a shorter treatment time, with a higher SVR around 80%.[105–107] The divergent response to IFN treatment has stimulated intense research, examining the mechanisms of non-response in an attempt to identify predictors of non-response that may guide therapy. The exact mechanism of IFN therapy failure remains unclear; however, it is thought that various viral mutations and host immune factors contribute to divergent responses to IFN. Various mechanisms of IFN treatment failure via inhibition of IFN signaling pathways have been shown.[108–110] Besides HCV genotypes, multiple host factors have also been shown to affect IFN treatment response, including host sex, age, and race. Male sex, older age and African-American race are associated with inferior response to IFN therapy.[111]

The development of non-IFN-based therapy with DAAs have resulted in a much better safety profile and shorter duration of therapy, with a dramatic increase in virus cure rates, especially in difficult to treat HCV genotypes. The use of HCV screening in high risk populations (i.e. intravenous drug users) and highly effective DAA treatments have also proven cost-effective in avoiding liver-related mortality and liver transplantations.[112] There are three targets of DAA therapy, including NS3/4A protease inhibitors, NS5A polymerase inhibitors, NS5B polymerase inhibitors. DAA therapy is highly effective, with SVR rates >95% in genotype 1-infected patients without cirrhosis including those co-infected with human immunodeficiency virus, and about 78–87% effective in decompensated cirrhotics with Child–Turcotte–Pugh class C disease.[113] Despite the high rate of DAA effectiveness in achieving SVR, the sheer magnitude of individuals infected with HCV leaves a significant cohort of patients who fail their initial therapy. Clinical trials of re-treatment after failure of initial DAA therapy have shown high rates of SVR with DAA salvage therapy. Re-treatment regimens utilize strategies such as adding additional active agents (other DAA classes and/or ribavirin), using longer treatment courses, or both. Clinical trials such as POLARIS-1, POLARIS-4, MAGELLAN-3 have shown a 90–98% effective re-treatment cure rate after initial DAA failure.[114,115] The mechanism of DAA resistance is thought to be due to various mutations seen in the different HCV genotypes and the DAA interaction sites that render the drugs ineffective. After DAA initiation, wild-type DAA-susceptible HCV species are eliminated, selecting for resistant variants. Some studies have shown that some of the acquired mutations in resistant quasispecies may increase viral fitness, which may explain the rapid viral replication seen on treatment (known as breakthrough) or after treatment (known as relapse).[116] Genotype-specific DAA treatment regimens and the emergence of drug-resistant variants remain a significant concern. Genotype-specific DAA resistance is associated with polymorphisms present in the various HCV genotypes, such as the NS5A resistance-associated substitutions present in genotype 1, that lead to the decreased efficacy of ledipasvir/sofosbuvir regimens, or genotype 3 variations within the NS5A region, leading to reduced sensitivity to NAs. The emergence of genotype-specific DAA resistance has led to an increase in demand for pangenotypic therapies that have a higher barrier to drug resistance (such as sofosbuvir/velpatasvir or glecaprevir/pibrentasvir) for treatment of all HCV genotypes, especially in resource-limited areas when genotype testing is unavailable.

Conclusions

Understanding the immune response to acute HBV infection and the immune system's dysregulation that results in chronic HBV infection are important in developing preventive and therapeutic strategies. Both adaptive and innate immune responses play major roles in the eradication of HBV infection with the dysregulation of T cell immune response associated with progression to chronic HBV infection. Persistence of viral DNA in the form of cccDNA or incorporated into the host's genome, along with the failure of the immune system to clear these infected cells, contributes to the persistence of HBV infection. The implementation of

a vaccination program worldwide has led to an overall decrease in incidence of HBV infection. IFN, with its poor rate of viral clearance and significant side effects, has fallen out of favor for therapy in hepatitis B. NAs are currently the treatment of choice and are aimed at suppressing viral replication by suppressing reverse transcriptase but are rarely curative. New strategies for targeting HBV production (RNA interference, HBsAg inhibitors, capsid inhibitors) and immune response (PD-1 inhibitors, therapeutic vaccines) are currently under investigation as possible curative treatments.

Unlike HBV infection, effective curative regimens using DAAs have been developed and successfully implemented for HCV; however, no effective vaccinations exist yet. It is the sheer magnitude of infected individuals and the cost of treatment that have proven to be significant barriers to disease elimination and persistence of HCV-related morbidity and mortality. It is the combination of HCV viral escape mutations along with host immune factors that are major contributors in the development of chronic HCV. Although the exact mechanism remains unclear, persistent viral infection results in chronic suppressor T cell activation, which leads to a loss of T cell response. Ongoing research to develop a safe and effective vaccine will help decrease incidence and spread of HCV.

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Conflict of interest

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Author contributions

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