



Viral hepatitis is the most common cause of liver disease in the United States and worldwide. There are at least five different viruses known to cause hepatitis in humans. These transmission electron micrographs show particles of the viruses that cause hepatitis A (HAV), B (HBV), C (HCV), D (HDV), and E (HEV). Photos of HAV and HEV: Centers for Disease Control and Prevention. Photos of HBV and HDV: Dr. John Gerin, Georgetown University Medical Center. Photo of HCV: Drs. T. Jake Liang and Stephen Feinstone, NIDDK.

# CHAPTER 5: VIRAL HEPATITIS

## INTRODUCTION AND BACKGROUND

At least five different hepatitis viruses (hepatitis A to E virus) cause liver disease in humans. All five cause acute hepatitis, while hepatitis B, C, and D viruses can also lead to a persistent infection and chronic hepatitis. Collectively, viral hepatitis is the most common cause of acute and chronic liver disease in the United States and worldwide.

In the United States, hepatitis A virus (HAV) infection is the leading cause of acute viral hepatitis (37 percent of cases), followed by the hepatitis B virus (HBV, 45 percent), and hepatitis C virus (HCV, 18 percent); hepatitis D and E virus (HDV and HEV) infections are rare. The frequency of acute viral hepatitis has declined markedly in the United States since its incidence peaked in the 1980s. The decrease is due to many factors, including the availability of hepatitis A and B vaccines and routine screening of blood donors for HBV and HCV. Importantly, the marked decline in incidence stopped in the 1990s, and rates of acute hepatitis have remained constant for the last 10 years. Many current cases of acute viral hepatitis are preventable.

Chronic viral hepatitis can be caused by HBV, HCV, or HDV. Hepatitis B is the major cause of chronic hepatitis, cirrhosis, and liver cancer worldwide and is an important cause in the United States. Population-based surveys indicate that approximately 1.25 million adult Americans have hepatitis B surface antigen (HBsAg) in serum, which is indicative of chronic HBV infection. Most infections are silent, and many

infected individuals are unaware of having hepatitis B until they develop signs or symptoms of cirrhosis or liver cancer.

Chronic hepatitis B accounts for approximately 3 percent of liver transplants annually in the United States. A safe and effective HBV vaccine has existed for more than 20 years, but new cases of chronic hepatitis B still appear due to *de novo* infections among unvaccinated persons in this country, as well as emigration of persons from areas of the world where HBV is endemic. Currently, there are three licensed therapies for hepatitis B: interferon alfa, lamivudine, and adefovir dipivoxil. A limited or defined course of therapy (6-12 months) is associated with a low rate of sustained response (15-30 percent) to all three agents. Continuous suppressive therapy with adefovir or lamivudine is widely used, but the long-term benefits and risks of this approach have not been defined. Complete elimination of virus is rarely achieved even after lengthy courses of antiviral treatment, probably due to the stable nature of the HBV genome, which exists inside the infected hepatocyte as a covalently closed circular (ccc) molecule of viral DNA.

Hepatitis C is the major cause of chronic hepatitis, cirrhosis, and liver cancer in the United States and much of the developed world. Currently, at least half of newly diagnosed cases of chronic liver disease in the United States are due to HCV infection, and it is the main reason for liver transplantation in adults (29 percent). Population-based surveys indicate that 1.8

percent of adult Americans (approximately 4 million) have antibodies to HCV (anti-HCV) in serum and approximately 2.7 million have HCV RNA in serum, indicating chronic HCV infection. At present, there are estimated to be 8,000 to 10,000 deaths yearly in the United States attributed to cirrhosis and several thousand more deaths due to liver cancer from hepatitis C. There is presently no vaccine for hepatitis C and no specific means of prevention. Therapy for hepatitis C has been evolving. The currently recommended regimen is a 24- or 48-week course of the combination of peginterferon alfa (pegylated interferon, a long-acting form of interferon) and ribavirin that results in long-term virus eradication in 50-60 percent of cases. However, this regimen is expensive, poorly tolerated, and is contraindicated in many patients with hepatitis C for whom therapy is otherwise indicated.

Hepatitis D (delta) virus is a rare but important cause of liver disease in the United States. It affects only those who also have hepatitis B, requiring the “helper function” of HBV for its replication and spread. Chronic hepatitis D is often severe and leads to cirrhosis in up to 70 percent of cases. At present, the only means of prevention is immunization against HBV infection that is essential to sustain HDV replication. There are no therapies of proven benefit for chronic delta hepatitis.

Hepatitis E virus causes acute hepatitis only and is common in developing nations, but rare in the United States and Western Europe. HEV infections are associated with large outbreaks and usually occur after fecal contamination of the water supply. HEV can cause severe disease, particularly in pregnant women, in whom the fatality rate is at least 10 percent. An experimental vaccine for HEV has been developed and is under evaluation in humans.

In summary, the five forms of viral hepatitis exert a considerable burden on the nation’s health and pose many challenges in terms of their control and management.

## RECENT RESEARCH ADVANCES

There have been important advances recently in the understanding of viral hepatitis. All five hepatitis viruses have been identified and defined by molecular and immunological means. The molecular structure, intermediate replicative forms, viral proteins, and life cycles of each virus have been defined. Safe and highly effective vaccines are now available for hepatitis A and B and a candidate vaccine of promise has been produced for hepatitis E. Therapies have been evaluated for all three forms of chronic viral hepatitis and have been successfully applied and approved for general use in treating hepatitis B and C. Few areas of biomedical research have been as exciting and productive in the last few decades as viral hepatitis; nevertheless, many major challenges remain.

**Hepatitis A Virus (HAV):** This small, positive-stranded RNA virus is classified within the genus *Hepatovirus* of the family Picornaviridae. HAV was first isolated and identified in 1974 and grown in cell culture in 1977. Subsequently, sensitive and specific tests for HAV infection and an inactivated vaccine have been developed. Several HAV vaccines have been licensed for use, and are safe and highly effective. While the incidence of acute hepatitis A has decreased in recent years, HAV remains the most common cause of acute hepatitis in the United States. Hepatitis A can result in acute liver failure and causes at least 100 deaths annually in the United States. Nevertheless, there are no therapies for hepatitis A, and few attempts have been made to develop antiviral agents for this disease.

**Hepatitis B Virus (HBV):** This partially double-stranded DNA virus belongs to the family Hepadnaviridae. Unique among DNA viruses, HBV replicates its genome through an RNA intermediary under the control of a virally encoded reverse transcriptase/DNA polymerase. The surface antigen of HBV (HBsAg, formerly the Australia antigen) was first identified in

1963 and was shown to be associated with hepatitis B in 1968. This discovery triggered an enormous research effort, which resulted in several advances, including the:

- Characterization of the virus;
- Development of sensitive and specific tests for its detection in serum and liver; identification of animal models of the infection (e.g., ducks, woodchucks, chimpanzees);
- Full definition of the serological and clinical course of infection and disease;
- Clear association of HBV infection with cirrhosis and liver cancer;
- Identification, cloning, and sequencing of the HBV DNA;
- Molecular characterization of viral replication mechanisms;
- Description of stable cell culture systems;
- Development of an inactivated HBV vaccine (initially a plasma-derived and later a recombinant vaccine);
- Development of antiviral therapy for chronic hepatitis B (initially interferon and later nucleos(t)ide analogues);
- Partial delineation of the immunological basis of the disease; and
- Fairly complete description of the viral life cycle.

These advances were the basis for the production of a safe and highly effective vaccine that has led to a decrease in the incidence of acute and chronic hepatitis B and, in areas of high HBV endemicity, a decline in the incidence of hepatocellular carcinoma. Hepatitis B was thus one of the great success stories for biomedical research in the 20th century.

Nevertheless, further progress in HBV research is critically important. Acute hepatitis B still occurs, and, therefore, greater availability and improved efficacy of the HBV vaccine would be of benefit, particularly in

developing areas of the world. Chronic hepatitis B remains a major cause of morbidity and mortality from chronic liver disease, and current therapies are only partially effective, resulting in suppression of viral replication, but rarely virus eradication or cure.

**Hepatitis C Virus (HCV):** This positive-stranded RNA virus is classified within the genus Hepacivirus of the family Flaviviridae. This third type of viral hepatitis was first recognized in 1974, shortly after the identification of HAV. The virus itself was not identified until 1989, when molecular techniques identified viral RNA in the serum of chimpanzees and patients with acute and chronic “non-A, non-B hepatitis.” As with HBsAg, the discovery of HCV RNA led to an explosion of research and further important progress, including the:

- Development of sensitive and specific tests for detecting anti-HCV and HCV RNA;
- Introduction of tests for anti-HCV into blood banking, leading to virtual disappearance of post-transfusion hepatitis C;
- Identification of HCV as a common cause of chronic liver disease, cirrhosis, and hepatocellular carcinoma;
- Definition of the molecular structure of the virus and steps in the processing of viral antigens;
- Development of a subviral system (replicon) for study of replication of the viral RNA in cultured cells;
- Partial characterization of the immunology of acute and chronic HCV infection; and
- Introduction and improvement of antiviral therapies.

Progress in hepatitis C research has been impressive, but several critically important gaps exist, including the lack of an effective HCV vaccine. Also, existing therapies are effective in a proportion of patients, but with many limitations. Interferon was the first therapeutic agent shown to be beneficial in chronic hepatitis C, even before the identification of the virus. With



the availability of viral markers, interferon was shown to lead to suppression of viral replication and, in some cases, virus eradication and apparent cure. Later clinical trials demonstrated that the optimal regimen for therapy of chronic hepatitis C is the combination of peginterferon alfa and ribavirin given for 24 or 48 weeks (based upon HCV genotype), a regimen that leads to sustained virological clearance of virus in up to 80 percent of patients with genotype 2 or 3 HCV infection and in 40 to 50 percent of patients with genotype 1 HCV infection. Even higher rates of response have been reported in acute hepatitis C. Despite these advances, antiviral therapy of hepatitis C is problematic. Combination therapy is effective in only half of patients, is extremely expensive, and is associated with side effects that can be severe and dose-limiting. Furthermore, interferon use is contraindicated or problematic in many patients, such as those with advanced liver disease, renal failure, or a solid organ transplant. Currently, improved management of hepatitis C represents one of the most critical challenges to biomedical research.

**Hepatitis D (Delta) Virus (HDV):** This unique, unclassified RNA virus (genus *Deltavirus*) is dependent for its spread upon packaging functions provided by HBV and thus replicates efficiently only in HBV-infected persons. Delta hepatitis and HDV antigen were first described in 1977. Subsequently, several research advances were made, including the:

- Identification of the virus;
- Establishment of the chimpanzee as an accurate model of HDV infection;
- Development of serological tests for diagnosis;
- Definition of the serological and clinical course of acute and chronic infection;
- Characterization, cloning, and sequencing of the viral RNA;
- Definition of the replicative cycle of the virus; and
- Definition of the elements of the viral genome and their functions in the viral life cycle.

While the molecular structure and replication of HDV have been well defined, studies of therapy of delta hepatitis have been limited. High doses of interferon suppress HDV replication, and long-term treatment can lead to clearance of HDV RNA and HBsAg and a sustained remission of disease. However, therapy is poorly tolerated and beneficial responses are uncommon. There are no small molecule antivirals for delta hepatitis. Vaccines against HDV infection have been ineffective in animal models, and, therefore, the major focus in hepatitis D prevention has been on prevention of HBV, the helper virus without which HDV cannot replicate effectively.

**Hepatitis E Virus (HEV):** This small positive-stranded RNA virus is classified within the recently established family Hepeviridae. The existence of this entity was first recognized in 1978 based upon negative tests for HAV infection in persons acquiring hepatitis during water-borne outbreaks in India and Pakistan. The virus was isolated in 1983 from the stool of a patient with acute hepatitis E, and the RNA was characterized, cloned, and sequenced in 1990. Tests for antibody to HEV were developed later, and the epidemiology of the infection was characterized. Animal reservoirs of HEV have been identified in swine and rodents. Acute HEV infection is extremely rare in the United States. Large outbreaks of this disease have occurred in China, India, and Southeast Asia, where HEV may be the most common cause of acute hepatitis and jaundice. A vaccine against HEV has been produced and is being evaluated in Asia.

Few areas of biomedical research promise more immediate and tangible benefits to patients as does research on viral hepatitis. Advances in knowledge have proceeded rapidly, resulting in important clinical breakthroughs in prevention and control. Further advances are likely to continue to benefit patients with viral hepatitis.

## RESEARCH GOALS

The primary goals for research in this area are to develop practical, safe, and effective means of prevention, treatment, and control of the five forms of viral hepatitis.

**Basic Research:** A major goal is to gain a better understanding of the critical steps in the life cycles of the hepatitis viruses. Important areas in which to advance knowledge of these processes include the hepatocyte receptors used for virus attachment and penetration; the steps involved in virus uptake, transport, and uncoating; and the role of host-cellular and viral-encoded proteins in replication, assembly, and release of virus. Information from these studies will be important for target identification and the future development of screening assays to identify drugs that prevent, inhibit, or suppress replication, including novel antiviral therapies and vaccines. *in vitro* model systems of viral infection would facilitate these studies.

- **Research Goal:** To develop tissue culture systems that are fully permissive for HCV replication (Matrix Cell A3).

This is an important goal as it is a prerequisite for designing cell-based assays for validation of candidate antiviral drugs, analyzing viral isolates (including serotype specificity), and testing for infectivity and inhibition of viral transmission. Similarly, a small animal model of HCV replication would be helpful to analyze cellular and immunological responses to virus infection.

- **Research Goal:** To develop small animal models of HCV replication and liver disease (Matrix Cell B3).

Both *in vitro* and *in vivo* models of HCV infection would facilitate the ultimate development of a hepatitis C vaccine, as well as hepatitis C immune globulins (HCIG).

- **Research Goal:** To develop a vaccine against HCV (Matrix Cell C3).

A central question in hepatitis B research is the basis for stability of the covalently closed circular (ccc) DNA, the template for the transcription of mRNAs and the pregenomic RNA for replication. The stability of the HBV cccDNA appears to be a key factor underlying viral persistence and the inability to eradicate virus and HBsAg despite prolonged, potent antiviral suppression.

- **Research Goal:** To better characterize the HBV life cycle *in vitro* and *in vivo* and to analyze the generation and stability of HBV cccDNA in humans (Matrix Cell B3).

Better understanding of the HBV life cycle would help in developing therapies that might eradicate all virus and clear HBsAg, a goal rarely reached with current therapies using nucleoside analogues or interferon. Similarly, these models may provide insights into whether other molecularly targeted drugs or a therapeutic vaccine would be capable of inducing eradication of HBV.

**Clinical Research and Therapy:** The goals outlined above for basic research on viral hepatitis all have implications for clinical management of these diseases. Similarly, clinical research is likely to raise issues that have implications for further basic research. These factors underlie the importance of bi-directional translational research.

Clinical investigation on the pathogenesis of liver injury in chronic viral hepatitis is important as it provides the basis for therapy and in deciding who should be treated and at what stage of disease. Studies in humans directed at coordinated analyses of viral replication, viral levels, sequence diversity, immune status, immune

reactivity to viral proteins, clinical and demographic factors, and careful delineation of disease activity and stage would help greatly in defining the pathogenesis of liver disease in chronic viral hepatitis. In hepatitis B, in particular, clinical investigation of host-virus relationships is important to help explain the basis for spontaneous as well as antiviral therapy-induced remissions in disease that occur despite persistence of low levels of viral replication and HBsAg in serum (the “healthy carrier state”).

- *Research Goal:* To better characterize the relationship between HBV life cycle in the liver and clinical course of chronic hepatitis B in humans (Matrix Cell B3).

Analyses of replication of all the hepatitis viruses, including the influence of host responses, can focus on possible targets for small molecules that might inhibit replication or enhance innate cellular defenses.

- *Research Goal:* To identify new targets in the virus and host for small molecules that will inhibit HBV and HCV replication (Matrix Cell B2).

Small molecule antiviral agents are currently the mainstay of therapy of chronic hepatitis B and, ultimately, are likely to play a major role in treatment of hepatitis C. At present, however, peginterferon alfa with ribavirin is the only effective therapy against hepatitis C. Strikingly, patients vary greatly in their response to interferon and ribavirin, with HCV RNA levels falling rapidly to undetectable levels within 2-4 weeks in some patients (rapid responders), but not decreasing at all in others (nonresponders). Importantly, a rapid decline in viral levels in response to interferon is the most accurate predictor of sustained response and may also guide the duration of treatment. For reasons that are not yet understood, nonresponses are particularly frequent among African American patients.

- *Research Goal:* To elucidate the pathways of interferon action that lead to suppression of HCV and HBV replication (Matrix Cell A2).
- *Research Goal:* To define the cause of interferon resistance of HCV in humans (Matrix Cell A1).

Conducting complementary studies in hepatitis B would also be important. Analyses of human cases might best focus on host genetic, immunological, environmental, and viral factors that underlie the relative lack of antiviral action of interferon in nonresponder patients.

Delineation of the cause of interferon resistance may also uncover further antiviral targets. These studies would benefit from the integration of analyses of early events that occur with hepatitis B and C viral infection. At least 95 percent of adults with hepatitis B and 30-50 percent of adults with hepatitis C recover fully from infection, eradicating virus by normal host mechanisms. While adaptive immune responses (i.e., T and B cells) are important, cytokines (e.g., interferon) and the innate immune response appear to be particularly critical in determining outcome of infection with hepatitis viruses.

- *Research Goal:* To gain a better understanding of the early events of viral hepatitis infection and roles of both innate and acquired immune responses in leading to spontaneous recovery (Matrix Cell B1).

Knowledge of the mechanisms underlying protective CD4+ and CD8+ T cell responses and how the innate immune system contributes to clearance of virus are important in ultimately developing therapies and vaccines against both hepatitis B and C.

Current therapy for hepatitis C is also limited because the definitive trials of therapy were conducted in highly selected populations, largely adults with well-compensated liver disease and without any co-morbidities. Furthermore, there are few options for

patients who fail to respond to current therapy of hepatitis C. Finally, hepatitis C invariably occurs after liver transplantation and can lead to rapidly progressive disease in the graft and need for repeat liver transplant within 3 to 10 years.

- *Research Goal:* To better define the optimal dose and duration, rates of response, early predictors of response, and safety and tolerance of current regimens of therapy for hepatitis C in special populations, such as children, patients with solid organ transplants, renal failure, HIV-coinfected individuals, and persons with problems of substance abuse and psychiatric illness (Matrix Cell A1; see also Chapter 12, B1).
- *Research Goal:* To investigate the role of long-term maintenance interferon therapy in nonresponders to the current best regimen of therapy for hepatitis C (Matrix Cell B1).
- *Research Goal:* To develop therapeutic regimens that can effectively sustain eradication of virus in 90 percent or more of patients with hepatitis C (Matrix Cell C2).
- *Research Goal:* To develop means to prevent recurrence of hepatitis C after liver transplantation (Matrix Cell C2; see also Chapter 12, C3).

For chronic hepatitis B, several safe and effective antiviral agents have either been developed or are in the pipeline and are likely to become available in the next 1-3 years. An important research goal is to evaluate these agents, both alone and in combination, when given over a long period. The majority of industry-supported studies on antivirals for hepatitis B have been limited to one to two years of therapy, yet this is a chronic, life-long disease and, due to the persistence of viral cccDNA, HBV is not completely eradicated despite adequate suppression of viral replication. Furthermore, with prolonged therapy, antiviral resistance to individual nucleos(t)ide analogues becomes a greater issue. The clinical significance of antiviral resistance in patients with hepatitis

B is important to elucidate, not only in regard to disease progression, but also transmission.

- *Research Goal:* To define the molecular and cellular basis for antiviral resistance of HBV (Matrix Cell B2).

Combination antiviral therapy has been proposed as a means of preventing antiviral resistance in HBV-infected patients, but the long-term efficacy and safety of this approach requires prospective study with careful focus on clinical and histological outcomes.

- *Research Goal:* To evaluate the long-term benefits and risks of combination therapy of HBV (Matrix Cell C1).

New approaches to therapy of hepatitis B would improve management of this disease. Examples of new approaches include use of combinations of antiviral agents and hepatitis B immune globulin to prevent recurrence of hepatitis B after liver transplantation, combination of antivirals and hepatitis B vaccine to prevent perinatal spread of HBV in high-risk pregnancies, therapeutic use of an HBV vaccine, and use of immune modulation to improve efficacy of antiviral therapy of chronic hepatitis B.

- *Research Goal:* To develop and evaluate new therapeutic approaches to hepatitis B, including use of therapeutic vaccine (Matrix Cells C1 and C3).

Currently, no effective antiviral agents exist for hepatitis A, D, and E. Hepatitis D is a severe form of chronic liver disease. Hepatitis A and E are self-limited infections, but the illnesses can be prolonged, relapsing, and fatal.

- *Research Goal:* To evaluate the role of therapy for all five forms of viral hepatitis and to rapidly assess target-directed small molecule antiviral therapeutics in critically designed clinical trials (Matrix Cell C1; see also Chapter 12, B2).



## STEPS TO ACHIEVE RESEARCH GOALS

Continued support of investigator-initiated research on viral hepatitis is essential for advancing treatment and prevention of the five forms of viral hepatitis. Studies on prevention and treatment of hepatitis A and E are a particularly high priority, as these two diseases are not currently broadly investigated. Both the Federal Government and industry support multiple small- and large-scale trials of therapy in hepatitis B and C. These efforts would be most effective if done in a coordinated manner to avoid overlap and maximize results. Current studies of therapy of hepatitis C, such as the HALT-C, Virahep-C, Peds-C, and A2ALL “LADR” studies provide excellent opportunities for collaborations between basic and clinical investigators. Ancillary studies to these trials to help elucidate the pathogenesis of hepatitis C or investigate new means of diagnosis and staging should be encouraged. Particularly important is the availability of tissue samples to study from patients with different stages of infection and disease. As new small molecule therapeutics directed against both HBV and HCV enter clinical practice, it will be important to conduct well designed, prospective clinical studies of combination therapies that seek to demonstrate therapeutic synergy and prevention of antiviral resistance. Coordination between industry and the NIH would facilitate these goals.

Animal models of viral hepatitis have been invaluable in identifying the five agents of viral hepatitis and elucidating the life cycles of the viruses and their natural history, treatment, and prevention. For example, the chimpanzee has played an essential role in isolation and characterization of all five forms of hepatitis, including: (1) development of diagnostic tests and a vaccine for HBV, (2) cloning and sequencing of HAV, (3) determining initial transmission of non-A, non-B hepatitis and the ultimate identification of HCV, (4) elucidation of the nature of HDV, and (5) propagation and cloning of HEV. The chimpanzee remains an important model, particularly for hepatitis C research as there are no other animal models or tissue culture systems for the study of viral replication and infection. While alternative models of hepatitis C are being developed, it is important that the chimpanzee model be sustained for the most critical studies on hepatitis C aimed at developing new therapies and HCV vaccines.

## Matrix of Research Goals in Viral Hepatitis

	Short Term (0-3 years)	Intermediate Term (4-6 years)	Long Term (7-10 years)
High Risk	<p><b>A3.</b> Develop a cell culture system that is fully permissive for HCV replication.</p>	<p><b>B3.</b> Develop small animal models of HCV replication and liver disease. Better characterize the HBV life cycle, virus-host interactions, basis for generation and stability of cccDNA, and viral state of HBV in humans.</p>	<p><b>C3.</b> Develop HCV vaccine. Develop therapeutic HBV vaccine.</p>
Intermediate Risk	<p><b>A2.</b> Fully define the pathways of interferon induction and effector action against HCV and HBV <i>in vitro</i> and <i>in vivo</i>.</p>	<p><b>B2.</b> Identify new targets in viral replication and the host for development of small molecule therapeutics (HCV, HBV, HDV). Define the molecular basis for antiviral resistance of HBV.</p>	<p><b>C2.</b> Develop ways to prevent reinfection after liver transplant for HCV (e.g., HCIG, antivirals). Achieve sustained response rate of over 90 percent in chronic hepatitis C.</p>
Low Risk	<p><b>A1.</b> Define basis for interferon resistance of HCV in humans. Define efficacy of interferon and ribavirin in subgroups of HCV patients (e.g., children, liver transplant recipients, patients with renal failure, substance abusers, minorities).</p>	<p><b>B1.</b> Fully define early events during HCV and HBV infection. Define whether long-term interferon therapy is beneficial in nonresponders with HCV.</p>	<p><b>C1.</b> Evaluate new approaches to therapy in all five forms of viral hepatitis. Evaluate long-term benefits and risks of combination therapy of HBV.</p>