

DRAFT

Working Group 2. Viral Hepatitis

Introduction & 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 U.S. and worldwide.

In the U.S., hepatitis A virus (HAV) infection is the leading cause of acute viral hepatitis (~50% of cases), followed by the hepatitis B virus (HBV, ~33%) and hepatitis C virus (HCV, ~16%); hepatitis D and E virus (HDV and HEV) infections are rare. The frequency of acute viral hepatitis has declined markedly in the U.S. since its incidence peaked in the 1980's. 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 1990's, 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 U.S. Population-based surveys indicate that ~0.5% of adult Americans have hepatitis B surface antigen (HBsAg) in serum, suggesting that 1 million adult Americans have 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 ~5% of liver transplants annually in the U.S. 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%) 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 U.S. and much of the developed world. Currently, at least half of newly diagnosed cases of chronic liver disease in the U.S. are due to HCV infection, and it is the main reason for liver transplantation in adults (~37%). Population-based surveys indicate that 1.8% of adult Americans (~4 million) have antibodies to HCV (anti-HCV) in serum and 1.2%

(~2.7 million) have HCV RNA in serum, indicating chronic HCV infection. At present, there are estimated to be 10,000 to 12,000 deaths yearly in the U.S. 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 and ribavirin that results in long-term virus eradication in 50 to 60% 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 U.S. 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 at least 70% 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 U.S. 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%. An experimental vaccine for HEV has been developed and is under evaluation in humans.

Recent Research Advances

There have been important advances recently in our 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 produced for hepatitis E. Therapies have been evaluated for all 3 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.

The hepatitis A virus (HAV) is a small, positive-stranded RNA virus 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 U.S. Hepatitis A can result in acute liver failure and causes at least 100 deaths annually in the U.S. Nevertheless, there are no therapies for hepatitis A, and few attempts have been made to develop antiviral agents for this disease.

The hepatitis B virus (HBV) is a partially double-stranded DNA virus that 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 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 a 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.

The hepatitis C virus (HCV) is a positive-stranded RNA virus 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. Sensitive and specific tests for detecting anti-HCV and HCV RNA were developed; tests for anti-HCV were introduced into blood banking, leading to virtual disappearance of post-transfusion hepatitis C; HCV was shown to be a common cause of chronic liver disease, cirrhosis and hepatocellular carcinoma; the molecular structure of the virus and steps in the processing of viral antigens were defined; a subviral system (replicon) was developed for study of replication of the viral RNA in cultured cells; the immunology of acute and chronic HCV infection was partially characterized; and antiviral therapies were introduced and improved. 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 50 to 60% of patients. 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.

The hepatitis D (delta) virus (HDV) is a unique, unclassified RNA virus (genus *Deltavirus*) that 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, the virus was identified; the chimpanzee was shown to be an accurate model of HDV infection; serological tests were developed for diagnosis; the serological and clinical course of acute and chronic infection were defined; the viral RNA was characterized, cloned and sequenced; the replicative cycle of the virus was defined; and the elements of the viral genome and their functions in the viral life cycle were defined. 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.

The hepatitis E virus (HEV) is a small positive-stranded RNA virus 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 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 U.S. 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.

Research Goals

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. 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 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.

Development of tissue culture systems that are fully permissive for HCV replication is an important goal as it is a prerequisite for designing cell-based assays for validation of candidate antiviral drugs, analysis of viral isolates (including serotype specificity), and testing for infectivity and inhibition of viral transmission (Matrix Cell A3). Similarly, a small animal model of HCV replication would be helpful to analyze cellular and immunological responses to virus infection (Matrix Cell B3). Both of these tools would facilitate the ultimate development of a hepatitis C vaccine (Matrix Cell C3), as well as hepatitis C immune globulins (HCIG). Finally, a careful molecular analysis of HCV replication in infected patients will be paramount for gaining a better understanding of the dynamics of chronic HCV infection. A key question is whether HCV persists in infected hepatocytes or whether it replicates transiently and requires continuous waves of reinfection of new cells to sustain chronic infection (Matrix Cell B1).

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 for viral persistence and the inability to eradicate virus and HBsAg despite prolonged, potent antiviral suppression. Better characterization of the HBV life cycle *in vitro* and *in vivo* and analyses of the stability of HBV cccDNA (Matrix Cell B3) 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 (Matrix Cell C1). Similarly, these models may provide insights into whether other molecularly targeted drugs or a therapeutic vaccine would be capable of inducing eradication of HBV (Matrix Cells C3 and B2).

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 (Matrix Cell B2). 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, and the role of therapy deserves prospective analysis (Matrix Cell C1). Similarly, the identification and pursuit of new targets for development of novel therapeutics directed against HCV and HBV are of high priority. The rapid assessment of target-directed small molecule therapeutics in critically designed clinical trials is an important goal (Matrix Cell C1).

Improving Therapy: 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 (interferon) 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. A major goal for future research is the elucidation of the pathways of interferon action that lead to suppression of HCV (Matrix Cell A2) and how these pathways differ between responders and nonresponders. 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 (Matrix Cell A1). Delineation of the cause of interferon resistance may 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 90% of adults with hepatitis B and 30-50% 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. A better understanding of the early events of viral hepatitis infection and roles of both innate and acquired immune responses are important to elucidate the nature of spontaneous recovery and develop means to promote 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.

Other important research goals include improving understanding of optimal dose and duration, rates of response, early predictors of response, and safety and tolerance of current regimens of therapy for hepatitis B and 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). A great challenge is to develop means to prevent recurrence of hepatitis C after liver transplantation (Matrix Cell C2). Recurrence of HCV infection in the graft can cause rapidly progressive disease, resulting in the return of end-stage liver disease within 3-10 years of transplant. The role of long-term maintenance interferon therapy in nonresponders to the current best regimen of therapy for hepatitis C also deserves

investigation (Matrix Cell B1). Ultimately, a goal of research is to develop therapeutic regimens that can effectively sustain eradication of virus in 90% or more of patients with hepatitis C (Matrix Cell C2).

Several safe and effective antiviral agents have either been developed for chronic hepatitis B 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 molecular and cellular basis for antiviral resistance deserves careful analysis (Matrix Cell B2). Furthermore, the clinical significance of antiviral resistance is important to elucidate, not only in regard to disease progression, but also transmission. Combination antiviral therapy has been proposed as a means of preventing antiviral resistance, but the long-term efficacy and safety of this approach requires prospective study with careful focus on clinical and histological outcomes (Matrix Cell C1).

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.

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