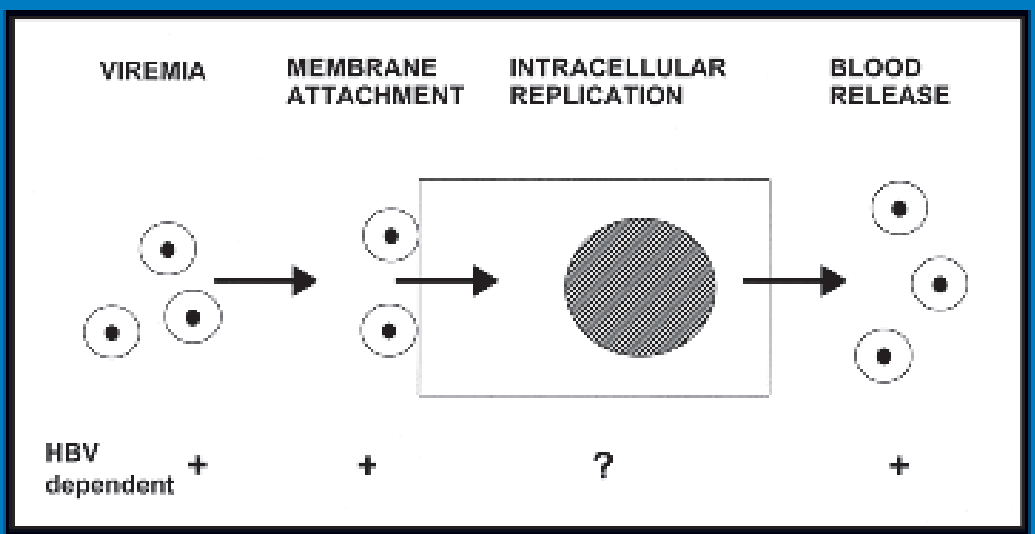
Management of Chronic Viral Hepatitis

edited by

Stuart C. Gordon



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Foreword

The expansion and transformation of hepatology over the past 40 years has been nothing short of spectacular. Scientific breakthroughs in viral hepatitis have had profound impacts on the discipline. The characterizations of the viruses that cause hepatitis A, B, C, D, and E, the development of accurate diagnostic tests, the identification of populations at risk, and the considerable expansion of understanding of the natural histories of the disorders have all occurred within a few years. In this volume, Dr. Stuart Gordon and a group of distinguished clinicians and investigators focus on the present state of knowledge in the treatment of chronic viral hepatitis. The result of these collective efforts is an excellent comprehensive text. There are full discussions of the most effective ways of evaluating and treating chronic hepatitis B, chronic hepatitis D, and chronic hepatitis C.

The development of effective vaccines to prevent hepatitis A and hepatitis B (and, therefore, also hepatitis D) and the expanding vaccination programs to prevent hepatitis B in infants born to mothers who have chronic hepatitis B gives hope

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for considerable reduction in the impact of chronic hepatitis B around the world. Universal vaccination someday may even lead to eradication of hepatitis B. The immediately realized favorable effects of screening blood for the presence of hepatitis B and subsequently hepatitis C has resulted in markedly increased blood safety. Recognition of the many routes of transmission of hepatitis viruses has refined the value of the advice given by health care providers and public health officials.

As is amply demonstrated in the text, there is much that is known and many more questions left to explore to help individuals who already have chronic viral hepatitis. To effectively manage these disorders, it is necessary to know the options available and the expectations of success.

In hepatitis B, interferon therapy and lamivudine therapy have both proven useful. There are new drugs just over the horizon. Additional antiviral agents and vaccines that may be useful in treatment are under study. Only time (and careful trials) will determine which of the new approaches that either will become a replacement for the present regimens or will become part of a multidrug viral attack in hepatitis B. Currently, the treatment algorithms presented in the text provide most useful guides. The treatment of patients who have recurrent hepatitis B after liver transplantation is fully discussed, both as to the use and expectations of antiviral and immunomodulatory agents along with hepatitis B hyperimmune globulin.

In chronic hepatitis C, the early efforts utilizing interferon therapy have been expanded to now focus on the use of pegylated interferons and ribavirin. In the past decade there has been more than a fourfold improvement in the likelihood of drug-induced control, and in many instances eradication, of the hepatitis C virus. More progress is expected as drugs that affect any of several targets along with the hepatitis C virus are developed. Candidate agents directed against the internal ribosome entry site (IRES), as well as those that have antihelicase, anti-polymerase, and anti-protease activity, are un-

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der study. Specialized antisense compounds and ribozymes may also find places as therapy.

Patient selection for therapy, viral and host modifying factors, and treatment approaches in chronic hepatitis C are all extensively covered as are the important matters of the role of treatment in patients who have normal aminotransferase levels, and those who are coinfected with HCV and HIV, as well as the management of patients who have recurrent hepatitis C after liver transplantation.

The future of further therapeutic advances in the treatment of hepatitis viruses has never been brighter. The imaginative approaches of scientists, clinical investigators, and clinicians are extraordinary and give rise to expectations of major leaps forward. The goals of therapy are expanding beyond using viral elimination as the only marker of success. It has been established that many patients have clinical and histologic improvement even if a viral eradication endpoint is not reached. The search for effective antifibrotic agents is intensifying. The day will come when there are effective vaccines to prevent hepatitis C. Until that time there is much to be done for those presently infected. Fortunately much can be done now that even 5–10 years ago was only a distant hope. This volume serves both as a primer for the present and a guide to the future.

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Preface

Worldwide, over 500 million individuals are chronically infected with viral hepatitis. Increased public awareness has focused international attention on identifying persons at risk. The U.S. Surgeon General recommends that recipients of blood transfusions before 1990 be tested for the hepatitis C virus. The National Institutes of Health convened a Consensus Development Conference to formulate guidelines for the management of chronic hepatitis C. Recognition of the dangers of chronic hepatitis B has resulted in universal vaccination against this virus, but at least 1 million Americans are chronically infected. Finally, coinfections are common: Increasing numbers of hepatitis B and C patients are also infected with the human immunodeficiency virus (HIV).

With heightened public awareness has come the interest of the pharmaceutical industry in the research and development of hepatitis antiviral agents. As antiviral therapy of chronic hepatitis enters its second decade, rapid developments have now made viral eradication a reality. Since the mid-1980s with the use of injectable alpha-interferon for the treat-

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ment of chronic hepatitis B, researchers have made slow but remarkable progress. Refinements in molecular testing have allowed for the development of precise treatment end points and a better understanding of the mechanisms of therapy. The transition from biochemical to virological measurements will mean that the era of fixed-duration therapy will evolve to treatment based on viral end points.

The treatment of chronic hepatitis B has been the unwitting benefactor of acquired immunodeficiency syndrome (AIDS) research. Many of the nucleoside/nucleotide analogs that showed remarkable effect against HIV proved to have similar efficacy against HBV DNA, and thus a new era of oral antiviral therapy against hepatitis B began. The prospect of "drug cocktails" and easily administered oral agents to control chronic hepatitis B will have an enormous effect, especially in Asia and Africa, where HBV remains a major cause of moribidity and mortality. Because many AIDS patients taking these antiviral agents are coinfected with hepatitis B virus, the interaction between nucleoside analogs, HIV RNA, and HBV DNA must be understood. Immunotherapy has entered the clinical trial phase, and the prospect of a multifaceted approach to treatment of hepatitis B is emerging.

The treatment of chronic hepatitis C is receiving increased public scrutiny, and, because of the Internet, victims of this disease are often more aware of treatment options than their physicians. There are rapid developments in the hepatitis field, and infectious disease specialists have been left largely "out of the loop." Because gastroenterologists have traditionally treated patients with hepatitis, research trials have been conducted by hepatologists, and the infectious disease community remains curiously ignorant of this infectious epidemic. Aggressive research in hepatitis continues, and much of the advice regarding treatment that was promulgated in the 1990s must now be reassessed. The concept of combination therapy has become standard care for hepatitis C, and the possibility of durable loss of virus is now a reality. Finally, intense

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interest exists regarding the prospect of direct antivirals such as the protease and polymerase inhibitors.

Management of Chronic Viral Hepatitis attempts to bring the advances of clinical and basic research into the doctor's office. Thus, each chapter begins with a brief clinical case presentation that illustrates a practical and challenging viral hepatitis dilemma. The case study format of the chapters addresses the real-life intricacies of managing patients who present with viral hepatitis, and explains how current antiviral research may benefit each patient. The authors, recognized authorities in viral hepatitis, report the latest advances in diagnostic and therapeutic modalities, and discuss how these scientific breakthroughs may benefit patients.

As research continues, clinicians need to identify patients with chronic viral hepatitis who may benefit from these exciting advances. The "algebra" of virological hepatitis markers continues to confuse the practicing physician, and it is important to understand the subtleties of this complex disease. This text is directed toward the clinician who manages patients, infectious disease specialists, hepatologists, gastroenterologists, and others who wish to better understand the developments and controversies regarding the diagnosis and treatment of chronic viral hepatitis.

Stuart C. Gordon

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1

Hepatitis B Virology

Acute and Chronic Infection—Wild-Type HBV and HBV Variants

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CASE 1

A 40-year-old healthy white male reported to his internist with symptoms of malaise and anorexia for 7 days. He also complained of diffuse joint pain. His wife noticed that his eyes had turned yellow over the last 2–3 days. He had no other medical problems and had never had jaundice or hepatitis before. He last drank alcohol about 4 weeks ago. He consumed no more than one to two beers per month. The patient denied the use of any recreational drugs, but he admitted to having extramarital sex partners. On examination, he was lethargic and obviously jaundiced. Abdominal examination revealed discomfort in the right upper quadrant. The liver span was 12 cm. There was no ascites or splenomegaly. The patient was well oriented with no evidence of encephalopathy.

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White blood cell count (K/mm^3)	4.0
Hemoglobin (gm/dL)	14
Platelets (K/mm^3)	175
Prothrombin time (INR)	1.0
Total bilirubin (mg/dL)	5.0
Unconjugated bilirubin (mg/dL)	3.8
AST (IU/L)	1240
ALT (IU/L)	1760
Alkaline phosphatase (IU/L)	105
Albumin (gm/dL)	3.4
Hepatitis A antibody IgM	Negative
Hepatitis B surface antigen (HBsAg)	Positive
Hepatitis B core antibody IgM (anti-HBc)	Positive
Hepatitis B e antigen (HBeAg)	Positive

This patient had acute icteric hepatitis B. He probably acquired hepatitis B from sexual exposure. Being an immunocompetent adult, his risk of progression to chronic hepatitis is less than 5%. Thus, antiviral therapy is not necessary. He should be retested for HBsAg in 6 months to document resolution of hepatitis B virus (HBV) infection. Once the infection resolves and with the development of hepatitis B surface antibody (anti-HBs) he should have life-long immunity to reinfection with HBV.

CASE 2

A 38-year-old Chinese male who migrated to the United States 20 years ago was referred for evaluation of hepatitis B. He was informed by the Red Cross a few years ago that he was HBsAg positive. He had no other medical problem. He was asymptomatic. During a recent preemployment check-up, he was found to have elevated liver enzymes. He was not on any medication. He was married and had two children, aged 6 and 4 years. The patient's mother was a known HBV carrier, one of his siblings had chronic hepatitis B, and an elder brother died from hepatocellular carcinoma. He consumed two to three beers per week. He had never used recreational drugs. On physical examination, he was anicteric and had no stigmata of chronic liver disease or hepatosplenomegaly.

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Total bilirubin (mg/dL)	1.2
Unconjugated bilirubin (mg/dL)	0.2
AST (IU/L)	410
ALT (IU/L)	630
Alkaline phosphatase (IU/L)	95
Albumin (g/dL)	3.9
Platelets (K/mm^3)	220
Prothrombin time (INR)	1.1
Hepatitis B surface antigen (HBsAg)	Positive
Hepatitis B core antibody IgM (anti-HBc)	Positive
Hepatitis B e antigen (HBeAg)	Positive
Hepatitis B e antibody (HBeAb)	Negative
Hepatitis B virus DNA (HBV DNA) (by hybridization assay)	300 pg/mL

Over the next two months, the patient's ALT decreased to 150 IU/L. He remained HBeAg and HBV DNA positive. A percutaneous liver biopsy revealed mononuclear infiltrate in the portal tracts with piecemeal necrosis and portal fibrosis.

This patient probably had perinatally acquired HBV infection. The presentation is consistent with an acute exacerbation in a patient with chronic hepatitis B. Other differential diagnoses include superimposed infection with other hepatitis viruses such as HAV, HCV, or HDV and exposure to hepatotoxic drugs or toxins. Once these conditions are ruled out, the most likely cause of the exacerbation is immune clearance of the virus. Because this is the first documented episode of exacerbation and the flare in ALT appears to be resolving, the best course of action would be to monitor his transaminases and hepatic function to observe for any decompensation and to repeat HBeAg, anti-HBe, and HBV DNA in a few months to document if there is spontaneous seroconversion. At this point, if the patient's transaminases are normal and he has achieved HBeAg seroconversion with undetectable HBV DNA levels, no further intervention is required. If his ALT remains elevated with positive HBeAg and detectable HBV DNA by hybridization assays, then he should be considered for therapy with either interferon or lamivudine.

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CASE 3

A 45-year-old Greek male was referred for evaluation of hepatitis B. He was first found to be HBsAg positive 3 years ago, and had mild elevation of ALT. Tests for HBeAg, anti-HBe, and HBV DNA were not performed at that time. Over the last 12 months, his ALT had increased from 50 to 60 IU/L to 150 to 200 IU/L. The patient denied any history of acute hepatitis or jaundice in the past. He was a non-smoker. He consumed alcohol on rare occasions only. He had used intravenous drugs for a few months about 20 years ago.

On physical examination, the patient was a well-built male who appeared healthy. There was no icterus and no stigmata of chronic liver disease. Abdominal examination was unremarkable.

AST (IU/L)	166
ALT (IU/L)	100
Total bilirubin (mg/dL)	1.1
Unconjugated bilirubin (mg/dL)	0.3
Alkaline phosphatase (IU/L)	75
Albumin (g/dL)	3.9
Platelets (K/mm^3)	235
Prothrombin time (INR)	1.1
Hepatitis B surface antigen (HBsAg)	Positive
Hepatitis B e antigen (HBeAg)	Negative
Hepatitis B e antibody (anti-HBe)	Positive
Hepatitis B virus DNA (HBV, DNA) (by hybridization assay)	150 pg/mL
Hepatitis C antibody (anit-HCV)	Negative
Hepatitis delta antibody (anti-HDV)	Negative

This patient had anti-HBe positive chronic hepatitis B with evidence of viral replication (HBV DNA positive) and hepatocellular injury. This picture is consistent with infection due to a HBV variant, most likely a variant with a stop codon mutation in the precore region of the HBV genome. His liver biopsy revealed evidence of chronic hepatitis with septal fibrosis. The likelihood of a sustained response to antiviral therapy is low compared to patients with wild-type HBV infection.

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INTRODUCTION

Hepatitis B virus (HBV) infection has a worldwide distribution with approximately 300 million individuals who are chronically infected. There is a wide variation in HBV carrier rates in different parts of the world. In the United States there are approximately 1 to 1.25 million carriers (1).

HEPATITIS B VIRUS STRUCTURE AND GENOME

HBV is a DNA virus which belongs to the Hepadnavirus family. There are three main hepatitis B virus particles detectable in the blood of infected patients: (a) large amounts of 20-nm spheres, (b) 20-nm filamentous structures and (c) smaller quantities of 42- to 47-nm Dane particles. All of these particles express the hepatitis B surface antigen (HBsAg). The Dane particle is the infectious virion. It consists of a lipoprotein coat that contains the viral surface glycoprotein (HBsAg) and an inner core which comprises the core protein or hepatitis B core antigen (HBcAg), the viral DNA, and DNA polymerase.

The HBV genome is a 3.2-kb, circular, partially double-stranded DNA. The genome has four open reading frames (ORF): P gene, C gene, S gene, and the X gene (Fig. 1). The P gene encodes for the viral polymerase, which is needed for viral replication. The C ORF contains the C region and a short pre-C (precore) sequence located upstream of it. The pre-C/C region encodes for two RNA transcripts; pregenomic mRNA and precore mRNA (Fig. 2). The latter translates into a precore protein which after processing at its C- and N-terminal ends is secreted as hepatitis B e antigen (HBeAg). The pregenomic RNA is translated into the core protein, HBcAg, which is present in the core of the virions and in infected liver cells but is not detectable in the serum and the polymerase protein. The pregenomic RNA also serves as a template for reverse transcription into the minus (−) strand HBV DNA. The S gene includes the pre-S1, pre-S2, and the S regions,

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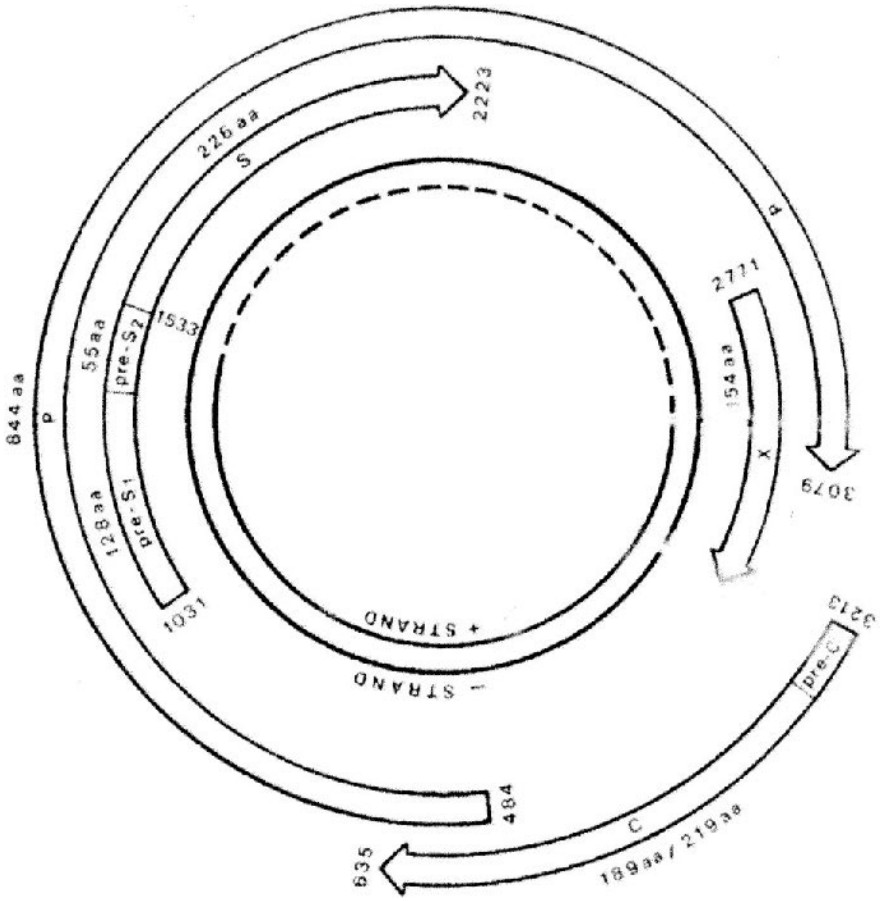

Figure 1 Structure of the HBV genome. HBV genome has four overlapping open reading frames: pre-S/S, pre-C/C, P, and X.

which code for three polypeptides: the large, middle, and small S proteins.

The most abundant is the small S protein (24 kD) coded by the S region only, which is present in the serum of HBV-infected individuals mainly as 20-nm spheres and filaments. The middle S protein (31 kD) is a product of pre-S_2 and S regions, whereas the large S protein (39 kD) is encoded by the pre-S_1, pre-S_2, and S regions (2, 3). The large S protein is pre-

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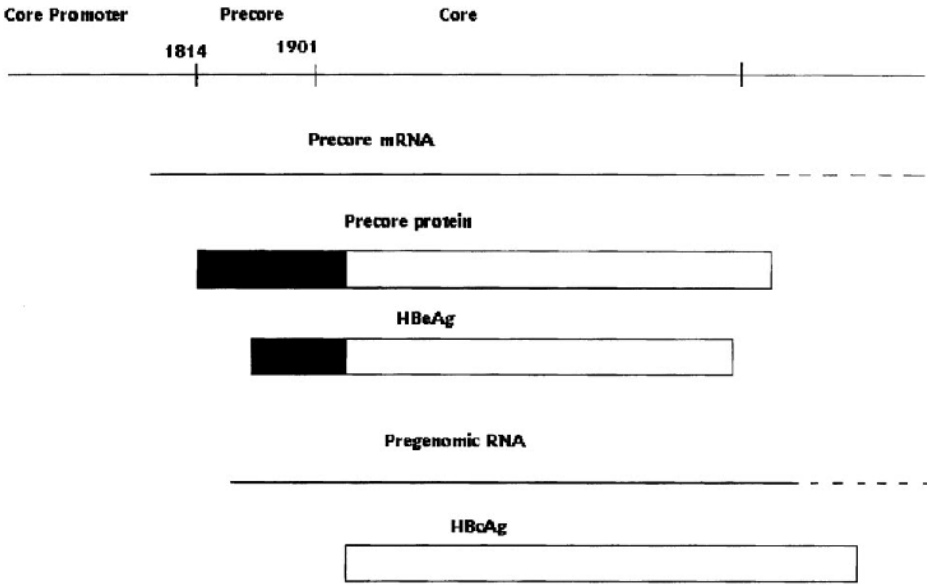

Figure 2 HBV core promotor, precore, and core regions. The precore mRNA is translated to precore protein, which becomes the secretory protein, HBeAg, after processing at the N- and C-terminal ends. The pregenomic RNA is translated into the core protein, HBcAg.

dominantly present on the surface of virions (Dane particles). Antibody response to all three S proteins can be detected after a primary HBV infection (4), but individuals vaccinated with recombinant HBsAg vaccines develop antibodies to S protein (anti-HBs) only. The significance of the X gene product is unclear. HBx protein has been shown to be a potent transactivator and has been incriminated in hepatocarcinogenesis.

CLINICAL PRESENTATIONS OF HEPATITIS B VIRUS INFECTION

Fulminant Hepatic Failure

The occurrence of fulminant hepatic failure (FHF) due to HBV infection is thought to be secondary to a vigorous immunologi-

cal response to HBV leading to massive destruction of infected hepatocytes and rapid clearance of HBV (5). Often the only marker for HBV at presentation is anti-HBc IgM. Markers of HBV replication (HBeAg and HBV DNA) are frequently absent in patients with fulminant hepatitis B. HBsAg was found to be absent from 12 to 55% of cases in some series (6). However, HBV DNA can be documented in sera and liver of these patients if assayed by polymerase chain reaction (PCR)–based methods (7). Initial reports of HBV precore HBV variant being associated with a higher likelihood of FHF (8, 9) have been questioned because of the detection of these variants in asymptomatic carriers.

The prognosis in patients presenting with FHF was universally poor until the advent of liver transplantion and aggressive ICU monitoring (10, 11). Age, factor V levels, and the presence of complications such as cerebral edema and renal failure are important predictors of outcome (12, 13). Patients who spontaneously recover from fulminant hepatitis B do not develop chronic HBV infection. Similarly, patients with fulminant hepatitis B requiring liver transplantation have very low risk of recurrent hepatitis B possibly due to the low viral load at the time of transplantation.

Acute Hepatitis B

The incubation period for HBV ranges from 1 to 4 months depending on the portal of entry and the size of the inoculum. It is important to realize that acute hepatitis B can be entirely asymptomatic and its presence can be suggested only by liver test abnormalities. In symptomatic patients, the prodromal illness resembles a serum sickness–like presentation accompanied later with a variety of constitutional symptoms including fever, malaise, fatigue, nausea, and vomiting. Abdominal pain, usually mild and located in the right upper quadrant, was reported by 32% of patients in one series (14). These symptoms are followed by an icteric phase in 30% of patients. Hence, more than two-thirds of patients with acute hepatitis

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B have no jaundice during the entire illness. The onset of jaundice usually heralds the convalescent phase. Jaundice usually resolves completely after 4 weeks but in some cases may last for 4 months.

Laboratory tests reveal markedly elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT) of up to 100 times the upper limit of normal. ALT usually is more elevated than AST. Transaminase elevations precede the development of hyperbilirubinemia. The degree of AST/ALT elevation has no bearing on the prognosis. Prothrombin time is the best prognostic marker, since it reflects the synthetic function of the remaining viable hepatocytes. Other clinically insignificant laboratory abnormalities include mild leukopenia with relative lymphocytosis.

The serological diagnosis of acute hepatitis B is discussed in a later section of this chapter. Biochemical and serological assessment remain the mainstay for diagnosis of acute hepatitis B and histological evaluation is rarely necessary.

The outcome of patients with acute hepatitis is dependent on the age at infection. Perinatally acquired HBV infection is associated with a 90% risk of chronicity (15), whereas infections between infancy and 5 years and adult-acquired infections have a 20–50% and less than 5% risk of developing chronic HBV infection, respectively (16–18). The risk of developing chronic hepatitis is also inversely proportional to viral inoculum (19). Patients with renal failure on hemodialysis and other immunocompromised patients have a higher risk of developing chronic HBV infection (20–22). Chronic HBV infection is defined as persistence of HBsAg 6 months after acute hepatitis B or after a similar duration of follow-up in a patient with no history of prior acute infection.

Chronic HBV Infection

Chronic HBV infection may manifest as an asymptomatic carrier state, chronic hepatitis, cirrhosis, or hepatocellular carcinoma.

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Hepatitis B Virus Carrier State

The carrier state is defined by the presence of HBsAg but undetectable HBeAg/HBV DNA in serum, normal serum transaminases, and the absence of symptoms. HBV carriers are usually recognized through incidental blood tests such as blood donation. HBV carriers have a low risk of development of cirrhosis and hepatocellular carcinoma (23, 24). In a 16-year follow-up study of HBsAg-positive blood donors in Montreal, only three patients died of HBV-related cirrhosis and none developed HCC (24).

Chronic Hepatitis B

Chronic hepatitis B is associated with a documented episode of acute hepatitis in 30–50% of individuals in areas of low or intermediate prevalence (25). Most patients with chronic hepatitis B, especially those in areas of high endemicity (predominantly perinatal transmission), have no prior history of acute hepatitis. Clinically, these patients are often asymptomatic. However, some may have nonspecific symptoms like fatigue and right upper quadrant discomfort. In general, physical examination is unrevealing unless patients have cirrhosis. Patients with decompensated cirrhosis may develop jaundice, ascites, hepatic encephalopathy, and portal hypertensive bleeding. Patients with chronic hepatitis B may also develop episodic exacerbation of liver disease resembling acute hepatitis. Clinically, these patients may present with jaundice, malaise, anorexia, and fatigue. However, frequently these episodes are asymptomatic. The exacerbations are often self-limited and may be followed by HBeAg seroconversion. Rarely, these exacerbations may result in hepatic decompensation.

Serum ALT is more often elevated than AST, but transaminases can be normal in patients with nonreplicative infection. ALT levels up to 50-fold upper limit of normal may be encountered during acute exacerbations, and occasionally bili-

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rubin may also be increased. The presence of cirrhosis is indicated by thrombocytopenia and leukopenia due to hypersplenism and impaired synthetic function: hypoalbuminemia, hyperbilirubinemia, and hypoprothrombinemia. Serological and molecular diagnostic tests to assess replicative versus nonreplicative infection will be discussed in a later section in this chapter. In contrast to acute hepatitis, histological evaluation is frequently needed in patients with chronic hepatitis B to assess severity of liver disease to predict prognosis, and to monitor response to therapy. Before 1995, *chronic persistent hepatitis* (CPH), *chronic active hepatitis* (CAH), and *chronic lobular hepatitis* (CLH) were commonly used terms to classify histological changes associated with chronic HBV infection. CPH was characterized by inflammation limited to the portal tracts, whereas in CAH, the inflammatory infiltrate destroys the limiting plate of the portal tract (piecemeal necrosis). CLH was usually observed in patients with exacerbations of chronic hepatitis. Knodell (26) proposed a numerical scoring system: histological activity index (HAI) for semiquantitative assessment of lobular necrosis, periportal necrosis, portal inflammation, and fibrosis. Subsequently, an international panel of experts recommended that the etiology of hepatitis, grade of inflammation, and stage of fibrosis should be the main features reported in liver histology of patients with chronic viral hepatitis (27). This has now been adopted as the nomenclature for reporting liver biopsies in patients with chronic viral hepatitis.

SEROLOGICAL MARKERS OF HEPATITIS B VIRUS INFECTION

Hepatitis B Surface Antigen and Antibody

Hepatitis B surface antigen (HBsAg) is the first viral marker to be detected in the blood of patients with acute HBV infection. Its persistence for longer than 6 months signifies transition to chronic infection. It is present in enormous quantities

in the blood of infected patients and can be detected 1–12 weeks after exposure to HBV (28, 29). HBsAg may persist after symptoms are resolved and may be detected for 4–6 months in patients who recover. Hepatitis B surface antibody (anti-HBs) may not be present until weeks or months after HBsAg has resolved (28, 30). This period in which neither HBsAg nor anti-HBs can be detected is called the window period, and the only serological marker of HBV infection is the hepatitis B core antibody (anti-HBc), IgM subclass. Hence, the serological markers of acute HBV infection are HBsAg and anti–HBc-IgM. Anti-HBs is the only protective antibody, and it confers long-term immunity against all subtypes of HBV. The coexistence of HBsAg and anti-HBs has been reported to occur in about 25% of HBsAg-positive individuals (31). In most instances, the anti-HBs antibodies are not able to neutralize the circulating virions (32). Clinically, these patients have symptoms like other patients with chronic HBV infection.

Hepatitis B Core Antigen and Antibody

Hepatitis B core antigen (HBcAg) is not detectable in blood, because it is an intracellular antigen expressed in infected hepatocytes. Hepatitis B core antibody (anti-HBc), on the other hand, can be detected approximately 3–5 weeks after HBsAg and is present throughout the course of HBV infection. Initially, the anti-HBc belongs to IgM class. IgM anti-HBc may last up to 2 years in some cases of acute hepatitis B. It is then replaced by IgG class anti-HBc. IgM anti-HBc can also be detected in patients with chronic hepatitis B during acute exacerbations.

Isolated detection of anti-HBc without HBsAg or anti-HBs has been reported with varying frequencies dependent on the prevalence of HBV infection; ranging from 0.4–1.7% in blood donors in low-prevalence areas (33, 34) to 10–20% in donors within endemic regions (35). The clinical significance of isolated anti-HBc is unclear. There is a high false-positive rate (50–70%), especially with enzyme immunoassays (EIAs)

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(36, 37). Therefore, repeat testing of anti-HBc with radioimmunoassay (RIA) should be the initial step in the work-up of these patients. The clinical scenarios compatible with a true positive isolated anti-HBc include a window period of acute hepatitis B many years after recovery from acute hepatitis B when the anti-HBs titers have fallen below detectable levels and in patients with long-standing chronic HBV infection with subdetectable HBsAg titer. HBV-DNA has been found to be present by PCR assays in up to 0–20% of individuals with isolated anti-HBc. There are also reports of transmission of HBV infection from blood and organ donors with isolated anti-HBc (35, 38–41).

Hepatitis B e Antigen and Hepatitis B e Antibody

Hepatitis B e antigen (HBeAg) is secreted from infected hepatocytes that are actively replicating virus. The presence of HBeAg is usually associated with the detection of HBV DNA in the sera of infected patients. Therefore, HBeAg is generally regarded as a marker of viral replication and infectivity. During the acute phase it appears just after the appearance of HBsAg prior to anti-HBc IgM. In resolving infection, HBeAg to anti-HBe seroconversion occurs prior to the disappearance of HBsAg. HBeAg may persist for years in patients with chronic HBV infection and is usually associated with active liver disease except in patients with perinatally acquired infection, who are in the immune tolerant phase (42–44).

The presence of anti-HBe in chronic HBV infection signifies that the virus is in a low or nonreplicative state. HBeAg seroconversion is usually accompanied by the disappearance of HBV DNA in serum by non–PCR-based assays and improvement in liver histology. In a subset of patients, however, HBV DNA remains detectable because of the persistent low levels of wild-type HBV or the presence of precore mutants which prevent production of HBeAg.

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Tests for HBV DNA in Serum

Tests for HBV DNA can be broadly categorized into PCR and non–PCR-based assays. Molecular hybridization and branched DNA (bDNA) assays represent the two commonly used non–PCR-based methods to detect HBV DNA. Hybridization assays have a sensitivity limit of 10–50 pg/mL or 10^6–10^7 viral genome equivalents/mL, whereas the bDNA assay can detect HBV DNA levels of 10^5–10^6 viral genome equivalents/mL (45). PCR-based assays are more sensitive and can detect HBV DNA levels of 10^2 viral genome equivalents/mL (46).

In acute hepatitis B, HBV DNA can be detected 1 week after the appearance of HBsAg when tested by hybridization assays (47), and it becomes undetectable prior to HBsAg clearance. Using PCR assays, HBV DNA can be detected as early as 2–3 weeks prior to the appearance of HBsAg and remains detectable for many years after HBsAg clearance (48). Similarly, in chronic hepatitis B patients with either spontaneous or treatment induced HBeAg seroconversion, HBV DNA is usually undetectable by non–PCR-based assays, but most patients remain viremic when tested by the more sensitive PCR assays. In general, non-PCR assays should be used in clinical practice to identify patients with replicative infection for treatment and to assess response to treatment. High pretreatment serum HBV DNA levels are associated with a poorer response to interferon therapy (49), but pretreatment HBV DNA levels are less reliable in predicting response to lamivudine therapy. Further studies are needed to determine the role of PCR assays in clinical practice. Some of the questions that need to be addressed include: Should patients with lower serum HBV DNA levels such as 10^4 viral genome equivalents/mL be treated? Will these patients benefit from treatment? How low should HBV DNA levels be reduced for sustained clinical benefit?

HBV DNA assays are also useful in identifying the etiology in patients with fulminant hepatitis B in whom HBsAg

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has cleared prior to presentation (50). Rarely, chronic hepatitis B patients with low levels of viral replication or patients with pre-S/S gene variants which downregulates or produces aberrant HBsAg may be mislabeled as non-B hepatitis unless tests for HBV DNA are performed.

Diagnosis of HBV Infection

The diagnosis of acute and chronic HBV infection relies on an understanding of the evolution of HBV serological markers (Figs. 3 and 4). The initial test for HBV infection should be a test for HBsAg.

If the presentation is acute, then IgM anti-HBc should also be tested. This is especially helpful if the patient is in the window period of HBsAg seroconversion (Table 1). Resolution of acute HBV infection should be documented by loss of HBsAg and detection of anti-HBs.

Patients with chronic HBV infection should additionally be tested for viral replication: HBeAg, anti-HBe, and HBV

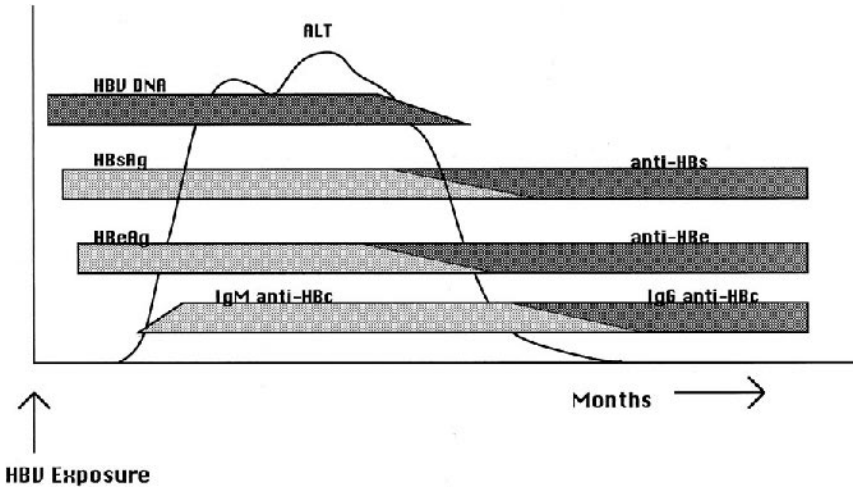

Figure 3 Serological markers in acute HBV infection.

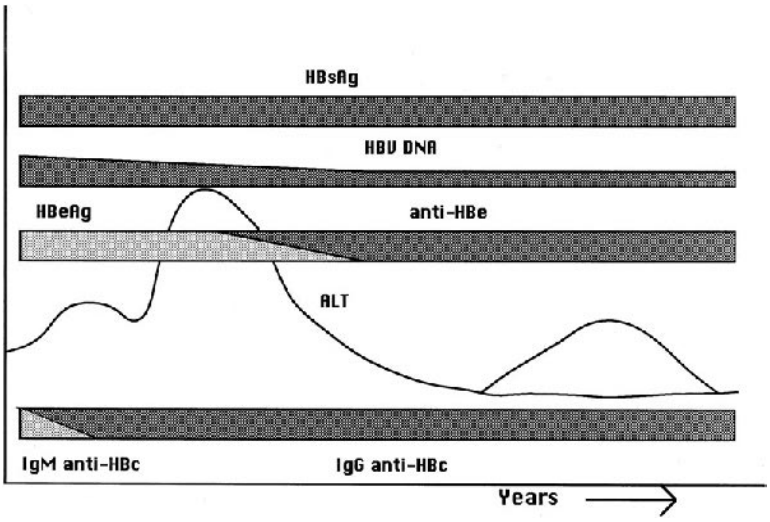

Figure 4 Serological markers in chronic HBV infections.

Table 1 Interpretation of Serological Markers Associated with HBV Infection

Acute infection	
Early phase	HBsAg, IgM anti-HBc, HBeAg, HBV DNA
Window period	IgM anti-HBc
Resolved infection	Anti-HBs, anti-HBc
Chronic infection	
Carrier state	HBsAg, IgG anti-HBc
Replicative infection	HBsAg, HBeAg, HBV DNA, IgG anti-HBc
Non- or low replicative infection	HBsAg, anti-HBe, IgG anti-HBc
Precore variants	HBsAg, anti-HBe, HBV DNA, IgG anti-HBc
Postimmunization	Anti-HBs

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DNA (see Table 1). The presence of HBeAg and HBV DNA signifies a replicative HBV infection and should prompt an evaluation for antiviral therapy. Conversely, the absence of HBeAg and HBV DNA (using non-PCR assays) and seropositivity for anti-HBe generally indicate a nonreplicative infection and antiviral therapy is not required. However, some HBeAg-negative patients may be infected with the precore HBV variant. These patients will have detectable HBV DNA in non–PCR-based assays.

NATURAL COURSE OF CHRONIC HBV INFECTION

The natural history of chronic HBV infection depends on the interplay between HBV replication and the host immune response. Patients with chronic HBV infection have a less vigorous and more restricted immune response to HBV antigens compared to patients who recovered from acute HBV infection (51). However, patients who subsequently develop spontaneous or interferon-induced HBeAg seroconversion have been reported to mount a vigorous T-cell response to HBV antigens, indicating that immune response is the key factor in viral clearance (52, 53). Other factors such as alcohol consumption, gender, and concurrent hepatitis virus infections may also affect the course of chronic HBV infection.

Chronic HBV infection is usually associated with an early replicative phase together with active liver disease followed later by a nonreplicative phase with remission in liver disease, the exception being patients with perinatally acquired infection in whom there is an additional immune tolerant phase with HBV replication but minimal liver disease.

Replicative Phase: Immune Tolerance

Immune tolerance in chronic HBV infection is a phenomenon seen in perinatally acquired infection where there is evidence

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of high levels of HBV replication in the presence of normal ALT and minimal changes on liver biopsy (42, 44, 54) (Fig. 5). The immune tolerance phase usually lasts 2–3 decades and is associated with a very low rate of spontaneous as well as treatment-induced HBeAg clearance (44, 55). This results in a high (50%) prevalence of HBeAg in young Asian adults with chronic HBV infection and hence a high frequency of maternal–infant transmission (44).

The mechanism for immune tolerance is not entirely clear, but early exposure to HBeAg may lead to a specific unresponsiveness of the helper T cells (56). Since HBeAg and HBcAg are highly cross reactive, the immune response to HBcAg is also impaired. This is supported by a lack of prolifer-

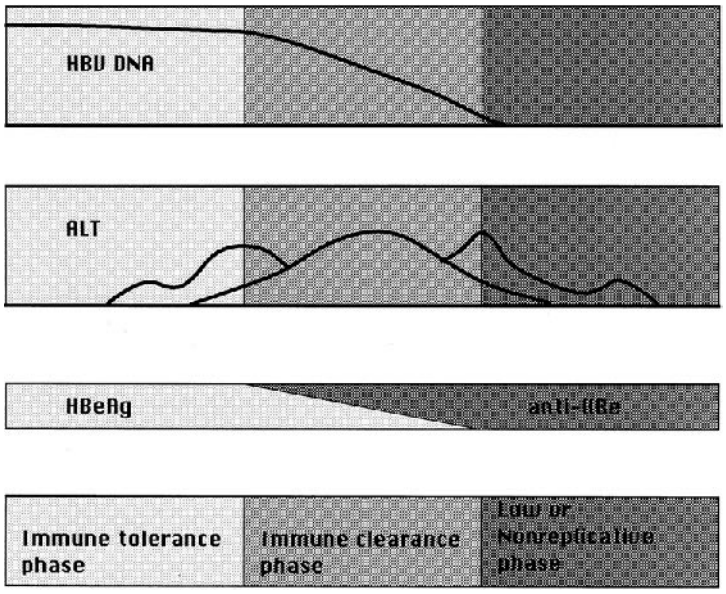

Figure 5 Natural history of perinatally acquired chronic HBV infection.

ative response to HBcAg in children who acquired HBV infection perinatally (53).

Replicative Phase: Immune Clearance

The immune tolerance phase is followed by an immune clearance phase. The annual rate of spontaneous HBeAg seroconversion is 10–20% during this period (57, 58). Exacerbations may be observed around the time of HBeAg seroconversion (59) (see Fig. 5). The exacerbations are thought to reflect immune-mediated lysis of infected hepatocytes. Not all exacerbations lead to HBeAg seroconversion. Patients with recurrent exacerbations representing repeated abortive immune clearance may sustain significant hepatocellular injury and inflammation, leading to fibrosis and increased risks of progression to cirrhosis and HCC. Exacerbations are more common in men (59). In low-prevalence regions in which HBV infection is usually acquired in adult life, the initial replicative phase is manifested by the presence of HBeAg and HBV DNA in the serum and evidence of active liver disease (Fig. 6).

Low or Nonreplicative Phase

The low or nonreplicative phase is observed in patients with perinatally acquired or adult-acquired HBV infection. Patients in the low or nonreplicative phase are HBeAg negative and anti-HBe positive but remain HBsAg positive (see Figs. 5 and 6). Liver disease is inactive in these patients as documented by normal ALT levels and resolution of inflammation on liver biopsies. There may also be regression of fibrosis in some patients who have sustained HBeAg seroconversion. HBV DNA is usually undetectable by hybridization assays but positive with PCR assays except in those individuals who lose HBsAg (60). Spontaneous clearance of HBsAg can occur in patients with chronic HBV infection but the rate is very low: 0.5–2.0% per year (61, 62). Because of the presence of small amounts of virus in the liver, institution of immunosuppres-

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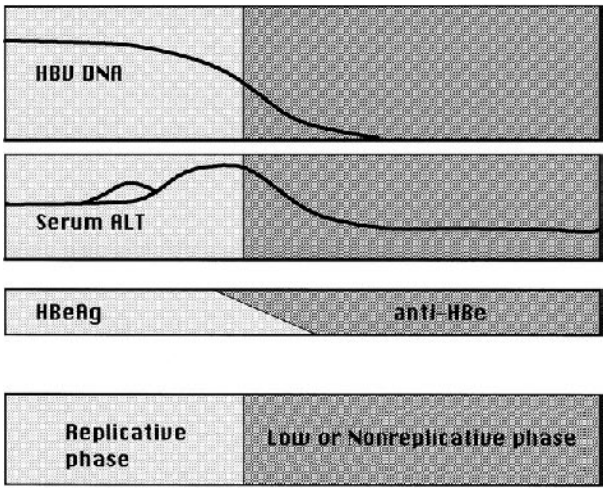

Figure 6 Natural history of adult acquired chronic HBV infection.

sive therapy or development of immunosuppressive disease may lead to reactivation of HBV replication, reversion to HBeAg, and reappearance of HBV DNA in serum (63). Reactivation of HBV replication may be asymptomatic with minimal elevation in ALT or manifest as clinical hepatitis or fulminant hepatic failure (64, 65).

HBV VARIANTS

As described in an earlier section, the HBV genome consists of four overlapping open reading frames. Although HBV is a DNA virus, it replicates through reverse transcription of an RNA intermediate and is therefore prone to mutations. Owing to the compact organization of the HBV genome, most mutations are not tolerated. HBV variants that are detected clinically may have been selected because they confer survival advantage over the wild-type virus by evading the host immune response or by enhancing viral replication. Mutations have

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been detected in all regions of the HBV genome in patients with HBV infection. However, the significance of many of these mutations is unclear.

Precore and Core Promoter Mutations

Mutation in the precore region was first reported in 1989 (66). The most common mutation is the G-A substitution at nucleotide 1896. This results in a premature stop codon at codon 28 of the precore region, thereby preventing the production of HBeAg. The exact reason why this mutation is so frequently selected is not clear. It has been suggested that the absence of HBeAg may help in evading the host immune response (67). In vitro studies showed that the precore variant may replicate more efficiently than the wild-type virus (68). Other mutations such as mutations involving the loss of the start codon in the precore region may also prevent the production of HBeAg.

The preponderance of initial reports of precore variants from Mediterranean countries and Asia is explained by the fact that the A1896 variant only occurs in certain HBV genotypes (B, C, D, and E), which are prevalent in these regions. Since the most prevalent HBV genotype in the United States and Western Europe is A, the prevalence of A1896 variant is low. The A1896 variant is replication competent and vertical, and horizontal as well as nosocomial transmissions have been reported (69).

The precore variants are usually selected around the time of HBeAg seroconversion (70). Initial conclusions that the precore variants are associated with more severe liver disease were based on reports of a high prevalence of the precore variants in patients presenting with fulminant hepatic failure or chronic active hepatitis (8, 9, 66). However, subsequent studies found that these variants were also detected in asymptomatic carriers. Thus, the pathogenic significance of the precore variants is unclear.

The presence of precore variants is suspected in patients who are HBeAg positive and anti-HBe positive with detectable

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HBV DNA in serum using non–PCR-based assays and have evidence of active liver disease. Concomitant hepatitis C or D virus (HCV, HDV) infection should be ruled out by testing for antibodies to HCV and HDV. Confirmation of the presence of precore variants requires molecular diagnostic techniques such as PCR and direct sequencing or restriction fragment length polymorphism. Patients with precore HBV variants have lower rates of sustained response to treatment with either interferon or lamivudine (71).

The core promoter region regulates the production of both precore mRNA and pregenomic mRNA. The most common core promoter mutations involve A to T change at nucleotide 1762 and G to A change at nucleotide 1764. These two changes are usually seen together and are more commonly found in HBV genotypes that preclude the development of A1896 variant (72). However, some patients may have concomitant precore and core promoter mutations. The core promoter mutations can decrease the production of HBeAg by downregulating the transcription of the precore mRNA (73). Core promoter mutations tend to appear earlier than the precore mutations and may precede HBeAg clearance by a few years (72). Initial reports suggest that the core promoter variants are associated with more severe liver disease and HCC (74), but subsequent studies found that these variants can also be found in asymptomatic carriers, and no evidence of HCC was found after up to 7 years of follow-up (72). Thus, the clinical and pathogenic significance of the core promoter mutations remain unclear.

Pre-S/S Region Mutations

HBV can be classified into four major subtypes (serotypes) (75). All subtypes share one common antigenic determinant, "a," which is a conformational epitope located in the HBsAg. There are two additional pairs of mutually exclusive subtypic determinants, d or y, and w or r, constituting four major subtypes adr, ayr, adw, and ayw.

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The most common mutation involving the S gene is the glycine to arginine substitution at codon 145 in the second loop of the "a" determinant (76–78). Other mutations in the "a" determinant have also been described. These mutations decrease the affinity of HBsAg to anti-"a" antibodies, thereby allowing the virus to escape neutralization. The G145R mutant has been described in infants born to carrier mothers who are infected with HBV despite adequate anti-HBs response to vaccination. The G145R mutant has also been described in liver transplant recipients who were reinfected with HBV despite receiving passive prophylaxis with hepatitis B immune globulin (79). The G145R mutant has been shown to be infectious and pathogenic (80). Most S mutants can be detected using currently available serological assays, especially assays that use polyclonal anti-HBs for capture or detection. To date, there is no evidence that the efficacy of HBV vaccines is declining as a result of these S mutants.

Mutations in pre-S1 and pre-S2 regions have been described in chronic HBV infections, but the prevalence and clinical significance of these mutations remain uncertain (81–83).

P Gene Mutations

The P gene codes for the viral DNA polymerase and reverse transcriptase. The most common mutations encountered in the P gene have been associated with the use of nucleoside analogues for the treatment of HBV infection.

Lamivudine is a potent inhibitor of HBV reverse transcriptase and DNA polymerase. It was recently approved by U.S. Food and Drug Administration (FDA) for use in patients with chronic hepatitis B. Unfortunately, the long-term use of lamivudine results in selection of resistant mutants. The incidence of breakthrough infections is approximately 15–25% at the end of the first year of therapy and up to 50% after 3 years of treatment (84, 85). The most important mutations involve the YMDD locus in the catalytic domain of the HBV polymer-

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ase (84, 85): substitution of the methionine at codon 552 by valine or isoleucine (M552V or M552I). In some patients, these mutations are associated with an additional mutation: leucine to methionine substitution at codon 528 (L528M) in domain B. These mutations significantly decrease the sensitivity of HBV to lamivudine by 100- to 10,000-fold. In most patients with lamivudine-resistant mutations, serum ALT and HBV DNA levels remain lower than pretreatment values if lamivudine treatment is continued. This is probably related to decreased replication fitness of the mutants. Cessation of lamivudine treatment is associated with reemergence of the wild-type virus and in some instances flare of hepatitis. Clinically, development of lamivudine-resistant mutations is suspected in patients with breakthrough infection, which is defined as the reappearance of HBV DNA using non–PCR-based assays after its initial disappearance. However, it should be recognized that breakthrough infection can also be due to noncompliance. Thus, confirmation of the presence of lamivudine-resistant mutants using molecular diagnostic assays such as PCR and direct sequencing or restriction fragment length polymorphism should be performed before contemplating addition of "rescue" therapy.

CONCLUSIONS

Case 1 presented with acute hepatitis, as evidenced by the presence of HBsAg and IgM anti-HBc. It is important to recommend prophylaxis for this patient's wife.

Case 2 had perinatally acquired HBV infection with exacerbation of chronic hepatitis B. This patient may benefit from antiviral therapy if the exacerbation does not result in sustained HBeAg seroconversion. Because of his family history of HCC, surveillance for HCC should also be considered.

Case 3 most likely was infected with a precore variant, as evidenced by the detection of HBV DNA despite the presence of anti-HBe.

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2

Hepatitis C Virology: Antigen, Antibody, and Molecular Testing

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CASE

A 54-year-old male physician with a history of acute myelogenous leukemia who received multiple transfusions in August 1989 developed abnormal liver chemistries in March 1990. Serological testing revealed that he was anti-HCV negative by the enzyme-linked immunosorbent assay (ELISA) 1 assay; anti-HAV (total) was positive but anti-HAV-IgM was negative. HBsAg and anti-HBc were negative and anti-HBs positive. Both the antinuclear antibodies (ANA) and the anti-smooth muscle antibody tests were negative.

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Date	3/90	10/91	5/93	9/94	5/95	10/95	2/96	11/96	12/96	5/97	8/97	9/97	12/97	3/98
IFN-α						▭	▭	▭	▭				▭	▭
IFN-α+ribavirin										▭	▭	▭		
Iron overload	→	→	→	→										
Hypertryiglyceridemia	→	→	→	→	→	→	→	→						
ALT	511	333	134	84	73	21	49	66	88					47
AST	222	184	76	50	47	22	45	44	66					38
Anti-HCV ELISA 1.0	Neg													
Anti-HCV RIBA 2.0		c33c												
HCV RNA RT-PCR		+	+	+		—	—	—	—	+	+	—	+	—
HCV RNA bDNA			ND[a]	1420000	497000	ND[b]	ND[b]	ND[b]	ND[b]	142600	ND[b]	ND[b]	4442000	ND[b]
HCV genotyping													2a/2c	

[a] <350,000 Eq/mL.
[b] <200,000 Eq/mL.

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This case illustrates a number of points that relate to the history of the hepatitis C virus and its associated serological assays. The first-generation test for anti-HCV was released in the United States on May 2, 1990, although it was available in Europe as early as 1989. This patient had hepatitis C in 1990, but it was not until later with the development of more refined assays that his serological profile became defined. Later, as the patient underwent treatment with antiviral agents, the development of increasingly more sophisticated molecular-based assays assisted in his clinical management.

INTRODUCTION

The hepatitis C virus (HCV) is an enveloped virus, a member of the Flaviviridae family, with a genome consisting of a single-stranded, positive sense ribonucleic acid (RNA) of approximately 9.4 kb in length and a single large open reading frame (ORF) and a highly conserved untranslated region's (UTR) at the 5′ and 3′ ends. Genotype-specific sequence difference exists in the 5′UTR; the secondary and tertiary structures are essentially preserved. The structural genes for core (C) and two envelope (E) glycoproteins, E1 and E2, are located in the N-terminal of the ORF. The nonstructural genes NS2, NS3, NS4A, NS4B, and NS5B are located in the C-terminal end (Fig. 1) (1).

HCV is a very heterogeneous virus due to random introduction of nucleotide errors by RNA polymerase during HCV replicative process (2). Isolates of the virus are distinguished based on their genetic similarity in genotypes, which can be further separated in subtypes. Isolates of the same genotype have an average sequence homology of 95% (range 88–100%) based on sequencing of relatively well-conserved regions of E1, NS4, or NS5. Subtypes within the same genotype average sequence homology of 80% (range 70–85%). Different genotypes have sequence homology of 65% (range 55–70%). Simmonds et al. (3) developed a nomenclature system for HCV genotypes that is in widespread use.

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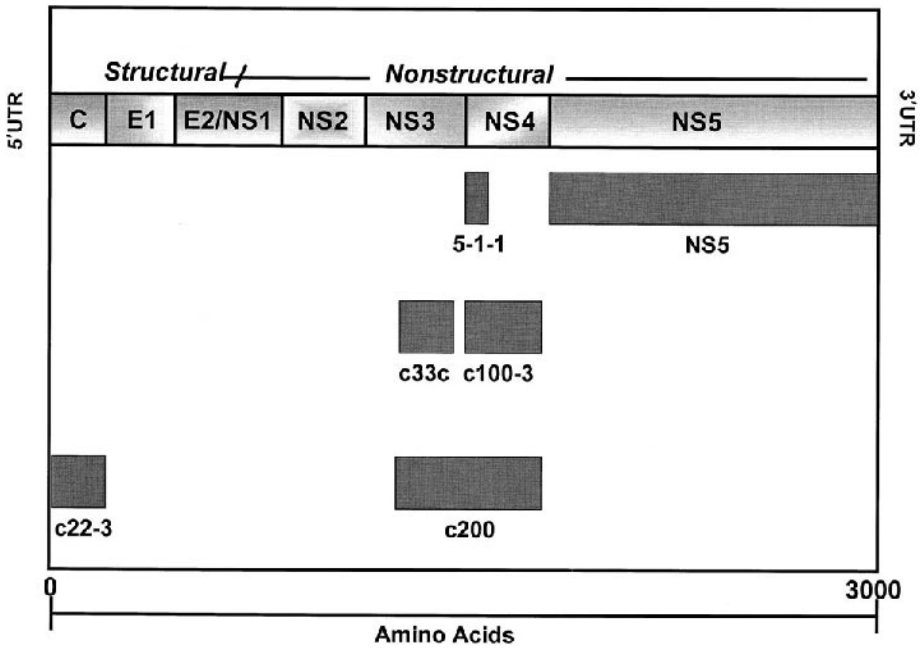

Figure 1 Hepatitis C virus genome and recombinant proteins. UTR, untranslated region. (Adapted from Ref. 20.)

Hepatitis C virus was discovered and characterized a little more than 10 years ago by Choo et al. (4), using molecular biological techniques, from a chimpanzee infected with sera from a human with chronic non-A, non-B hepatitis. Since then significant advances in molecular biology have allowed researchers to develop a wide variety of antigens and synthetic peptides that have been successfully deployed in immunoassays to detect HCV. Third-generation serological techniques based on ELISA and recombinant immunoblot assay (RIBA) have improved the sensitivity and specificity for HCV diagnosis. However, both false-positive and false-negative HCV anti-

body results can be resolved with HCV RNA determination, which directly assesses the presence or absence of virus (7). These problems arise during the acute phase of HCV infections when antibodies are not detectable among immunosuppressed subjects who do not develop antibody to HCV and among low-risk blood donors with false-positive ELISA tests (5, 6).

HCV diagnostic assays can be categorized as serological tests, which detect HCV antigen and antibodies, and molecular assays, which detect specific nucleic acid sequences of the HCV genome present in body fluids or tissues (Table 1). An algorithm summarizing the current HCV diagnostic process is shown in Fig. 2.

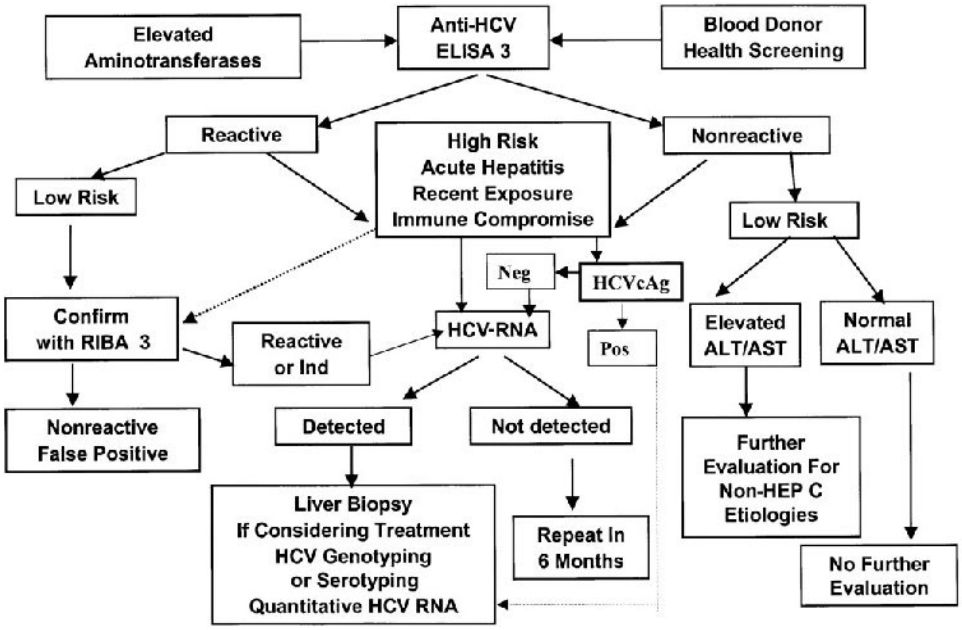

Figure 2 Algorithm for diagnosis of hepatitis C.

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Table 1 Assays in the Diagnosis of Hepatitis C

Test	Object of test	Clinical relevance
ELISA, EIA	Antibody to HCV To detect presence or absence of antibody to HCV peptides (c22-3 [core], c200 [fusion c100/c33], and NS5) in patient plasma or serum.	High-risk patients: High sensitivity and specificity: supplemental assay may or may not be necessary. Low-risk patients: Suboptimal sensitivity and specificity. Immunosuppressed patients: Decreased sensitivity.
	HCV core antigen To detect HCV core antigen in patient plasma or serum. Comparable sensitivity to RT-PCR.	Acute hepatitis C: Detection in serum prior to seroconversion to anti-HCV. Chronic hepatitis C: To confirm active HCV infection. Efficacy of antiviral therapy. Follow-up OLT patients. Valuable in areas where molecular assays cannot be implemented.
RIBA	To confirm presence or absence of antibody to HCV epitopes: c100-3, c33c, c22-3, and NS5.	Confirms or excludes ELISA-positive results: especially useful in low-risk populations such as healthy blood donors or positive patients with normal ALT values.
HCV genotyping	To determine the genetic nature of HCV. It allows genotyping of the six HCV types and their subtype.	Different types and subtypes vary in terms of treatment success, duration of therapy and prognosis.
HCV serotyping	To detect genotype-specific antibodies.	Same as genotyping but lacks subtyping.

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HCV-RNA		
Qualitative HCV-RNA:	To ascertain directly presence of HCV RNA.	1. Patients who test positive for blood HCV RNA are actively infected with HCV and at high risk for hepatic complications.
RT-PCR	The sensitivity of assays ranges from 100 to 700 viral copies/mL.	2. Can be used to assess response to antiviral interferon therapy.
TMA	Limitation: No numerical value.	3. Helps resolve indeterminate RIBA, weakly positive ELISA, or negative ELISA when clinical signs are compatible with HCV.
	Sample processing occurs in a single tube. Less susceptible to contamination.	
	Sensitivity $\leq$ 50 copies/ml ($\sim$5 IUs).	
	Specificity 99%.	
Quantitative HCV-RNA:	To measure HCV RNA levels.	
Target amplification–RT-PCR	High degree of sensitivity; lower limit of detection varies from 10 copies (RTD-PCR) and 100 copies/mL (Superquant, NGI) to 500 copies/mL (Monitor, Roche). Limitations: High variability and limited ability to measure samples with >5 million copies/mL.	
Signal amplification–bDNA	To quantify HCV RNA in blood (Quantiplex, Bayer). Reproducible and less susceptible to contamination. Limitations: Sensitivity.	
	Samples below cut-off value should be tested by PCR.	

HCV, hepatitis C virus; ELISA, enzyme-linked immunosorbent assay; EIA, enzyme immunoassay; RIBA, recombinant immunoblot assay; ALT, alanine aminotransferase; RT-PCR, reverse transcriptase polymerase chain reaction; RTD-PCR, real-time detection polymerase chain reaction; NGI, National Genetics Institute; TMA, transcription-mediated amplification; bDNA, branched DNA.

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SEROLOGICAL ASSAYS

Hepatitis C Virus Core Antigen

The hepatitis C core antigen (HCVcAg) enzyme immunoassay (Diagnostic Division, Tonen Corp., Saitama, Japan) is a one-step pretreatment method with three different types of detergents (Triton X-100, 3-[cholamidopropyl)-dimethylammnonio]-1propanesulfonate [CHAPS], and sodium dodecyl sulfate) to inactivate the interfering anticore antibody (8). The HCV core protein interacts with viral genomic RNA at a specific region to form nucleocapsids and regulates the expression of HCV by interacting with the 5′UTR (9) (Fig. 3).

HCVcAg was detected in sera from 78% (57/73) of anti-HCV-positive individuals. AMPLICOR HCV qualitative

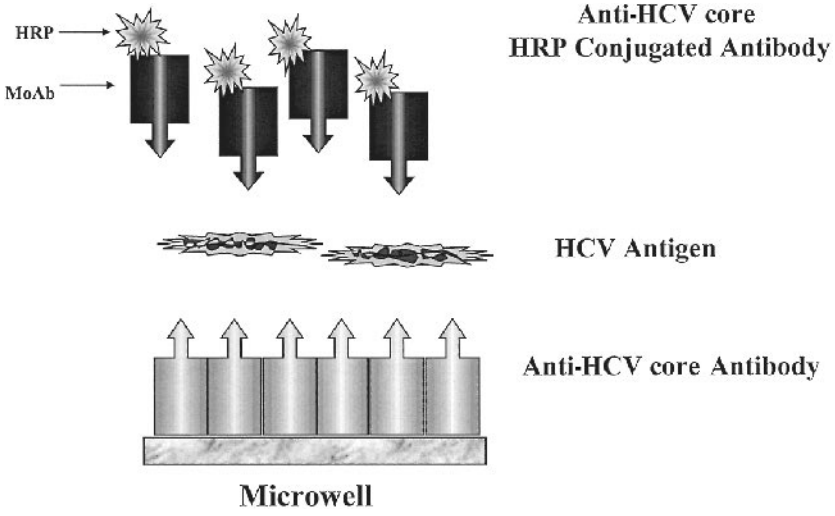

Figure 3 HCV 1.0 Antigen ELISA. The assay follows a standard ELISA protocol. Microwells coated with monoclonal antibodies recognize HCV core protein present in patient samples. Bound antigen is then detected by additional monoclonal antibodies labeled with horseradish peroxidase that recognize different epitopes on the core protein. For testing anti-HCV-positive patients, it is necessary to perform a sample pretreatment step to dissociate immune complexes.

(Roche Molecular Systems, Branchburg, N.J.) and AMPLICOR HCV Monitor quantitative HCV RNA was detected in 81 and 74%, respectively. Concentration of HCVcAg and HCV RNA measured by the AMPLICOR Monitor test significantly correlated ($r = 0.8$; $P < .001$) (8). In another study of 490 anti-HCV-positive sera, HCVcAg was detected in 90.3% of anti-HCV-positive/HCV RNA-positive samples (10). A significant correlation was also found between HCVcAg levels with alanine aminotransferase (ALT) values and quantitative HCV-RNA titers. In this study, some patients treated with interferon experienced an HCVcAg-negative conversion.

The utility of the HCVcAg test has been assessed in recurrent HCV infection among transplant patients followed for 2 years. A high serum HCVcAg level in the first 6 months was associated with histological evidence of severe recurrence of HCV; that is, bridging fibrosis, cirrhosis, severe cholestasis, and the necessity for retransplantation (11).

Peterson et al. (12) studied 24 individuals undergoing seroconversion for the presence of anti-HCV, HCV-RNA, and HCVcAg. HCVcAg was detected at the same time as HCV-RNA in 20 of 24 cases (83%). The mean time for the first detection of HCVcAg was approximately 1 day after HCV-RNA. These investigators concluded that HCVcAg could be a valuable test for early detection of HCV among anti-HCV-negative blood donors infected with HCV during the window phase of acute infection.

Tanaka et al., in a recent article (13), compared the clinical performance of HCVcAg by chemiluminescence enzyme immunoassay (EIA) with a qualitative and quantitative commercial reverse transcriptase polymerase chain reaction (RT-PCR) (AMPLICOR HCV TEST and AMPLICOR HCV Monitor Test) and an in-house nested RT-PCR. Compared with nested RT-PCR, HCVcAg showed 97% sensitivity and 100% specificity in 75 patients with chronic hepatitis C and 132 controls. HCVcAg was positive in 94% of patients with acute hepatitis C. In three subjects prospectively followed, HCVcAg was detected 1–3 weeks in the first available posttransfusion sample. In 129 anti-HCV-positive viremic patients, HCVcAg was de-

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tected in 98% compared with 100% for nested RT-PCR and 94% for commercial RT-PCR. The concentration of HCVcAg in chronic hepatitis C patients before treatment with interferon alfa was significant lower ($P < .002$) in patients with sustained response. All sustained responders remained negative for HCVcAg. However, all nonresponders remained HCVcAg positive. The concentration of HCVcAg and HCV-RNA correlated significantly (n = 48, $R = 0.627$, $P < .001$) (Fig. 4).

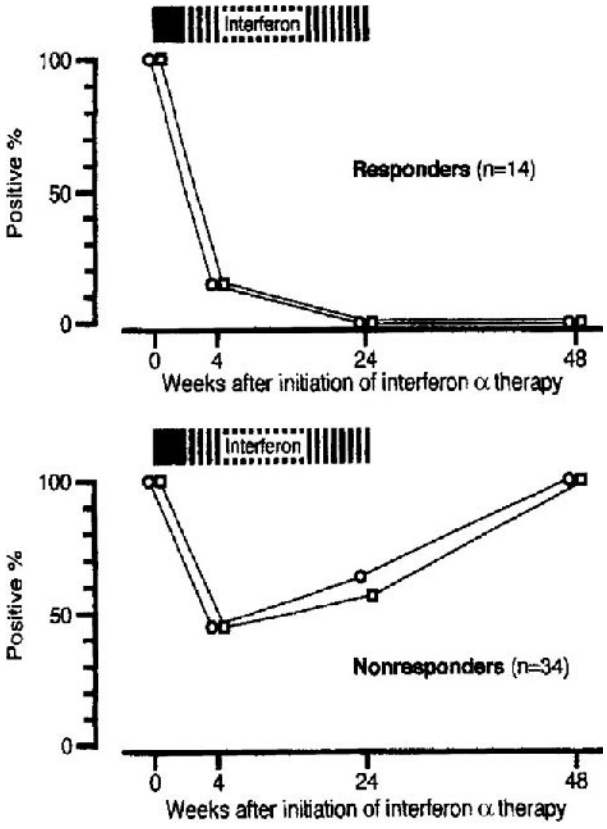

Figure 4 Comparison of clearance of HCV core antigen and HCV-RNA during and after interferon alfa therapy. Open circles indicate HCV-RNA determined by nested RT-PCR. Open squares indicate HCV core antigen. (From Ref. 13.)

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Hepatitis C Virus Antibody Testing

Since the discovery of HCV in 1989 by Choo and colleagues, highly sensitive assays for screening HCV antibodies (anti-HCV) have been developed (Fig. 5). Furthermore, supplemental assays for specificity to resolve false-positive results became available.

Three versions of anti-HCV screening tests have been developed, with each new one providing significant improvements in the sensitivity of anti-HCV (Table 2). Many of these screening tests employ the ELISA technique, in which antibodies against several different viral antigens are detected.

The third version (not deemed third-generation) assay (ELISA-3), which was approved in 1997 by the U.S. Food and Drug Administration (FDA) for blood donor screening (2), differs from the second-generation ELISA (ELISA-2) by the substitution of the NS5 protein for the 5-1-1 antigen, although this substitution does not appear to be responsible for its higher sensitivity (7). ELISA-3 is able to detect antibody against HCV earlier after infection, and it therefore offers an advantage in identifying acute infections that certainly would have been missed by previous versions of this assay. ELISA-3 is able to detect anti-HCV 7 or 8 weeks after acute infection compared with 10 weeks for ELISA-2 and 16 weeks for ELISA-1.

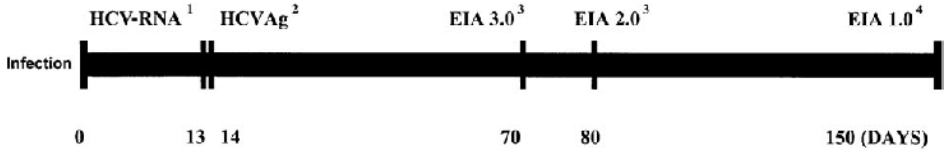

Figure 5 Declining time to detection of HCV markers during the window phase following infection. Early detection of HCVcAg approximately 1 day after HCV RNA in the window phase of acute infection. (1) Busch MP et al. Transfusion 1998; 38:7S. (2) Peterson et al. Vox Sang 2000; 78:80–85. (3) Busch MP et al. Transfusion 1995; 35:903–910. (4) Alter HJ et al. N Eng J Med 1989; 1395–1500. (From Ortho Clinical Diagnostics, Raritan, N.J.)

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Table 2 Antigenic Determinants in the Evolution of HCV Antibody Testing

Assay	ELISA	RIBA
First generation	c-100-3	c100-3 5-1-1
Sensitivity	70%–80%	
PPV low prevalence	30%–50%	
PPV high prevalence	70%–85%	
Second generation	c-200 c22-3	c100-3 5-1-1 c22-3 c33c
Sensitivity	92%–95%	
PPV low prevalence	50%–61%	
PPV high prevalence	88%–95%	
Third generation/version	c200 c22-3 NS5 recombinant Ag	c100p/5-1-1(p) c22p c33c recombinant Ag NS5 recombinant Ag
Sensitivity	97%	
PPV low prevalence	25%	
PPV high prevalence	Not done	

HCV, hepatitis C virus; ELISA, enzyme-linked immunosorbent assay; RIBA, recombinant immunoblot assay; PCR, polymerase chain reaction; sensitivity based on clinical findings and detection of HCV RNA by PCR (7); PPV, positive predictive value compared with RIBA (7).
Source: Ref. 19.

ELISAs are relatively inexpensive. ELISA is reproducible, simple to perform, and highly sensitive with low variability in high-risk populations. Major disadvantages are false-positive results in low-risk populations and poor sensitivity in the immunosuppressed patient (liver or kidney transplant, hemodialysis) (14–16).

Supplemental or Confirmatory Antibody Tests for HCV

False-positive results following ELISA testing in low-risk blood donors constitutes a significant diagnostic problem. In

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order to help resolve these false-positive results, a number of serodiagnostic tests have been developed. The strip immunoblot assay (SIA) based on Western blotting and dot-blotting techniques identifies antibodies to individual HCV antigens and therefore has a higher specificity (17).

A third-generation CHIRON RIBA HCV 3.0 SIA (RIBA-3) incorporates two recombinant antigens (c33c and NS5) and two synthetic peptides (c100p and 5-1-1p) derived from the nonstructural region and a third peptide (c22p) corresponds to the core region (Fig. 6). This assay has helped to resolve many of the indeterminate results of RIBA-2 (17–19).

The use of the RIBA test should be limited to confirmation

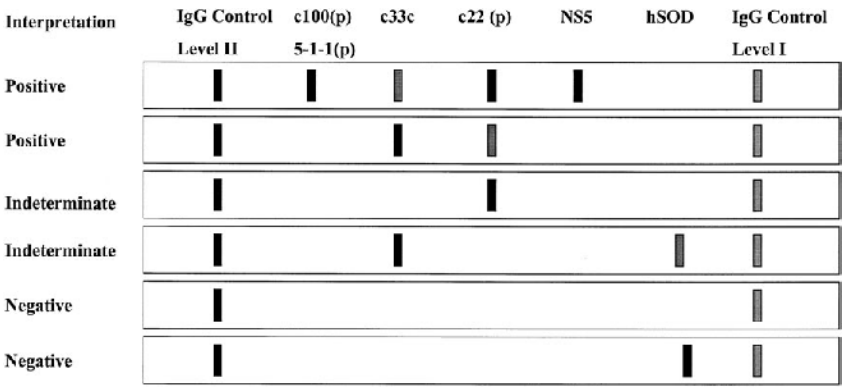

Figure 6 CHIRON RIBA HCV 3.0 SIA qualitative immunoblot assay utilizes two recombinant antigens (c33c and NS5) and two peptides (c100p and 5-1-1p) derived from the putative nonstructural region and c22p derived from the putative nucleocapsid protein (core). Since HCV recombinant antigens are produced as individual fusion proteins with hSOD, this control band enables detection of nonspecific reaction. Anti-HCV in the specimen is determined by comparing the intensity of each HCV band to the intensity of the human IgG (Level I and Level II) internal control. A positive test must have at least two HCV bands having 1+ or greater reactivity. RIBA, recombinant immunoblot assay; SIA, strip immunoblot assay; hSOD, human superoxide dismutase.

of ELISA-positive results in low-risk populations such as healthy blood donors and positive patients with normal ALT values. RIBA-3 testing is not necessary for the diagnosis of HCV infection in ELISA-positive individuals with a history of a high-risk exposure, an abnormal ALT level, or evidence of HCV-RNA positivity (18). Since a positive RIBA result is not a reliable indicator of active HCV infection (recovered patients may remain anti-HCV positive for years), many clinicians prefer confirmatory testing using HCV-RNA, because it will determine whether or not viremia is present (20).

The line probe immunoassay (LIA) is a confirmatory test that incorporates several specific HCV peptides from two nonoverlapping core regions (C1 and C2), E2, NS4 and NS5 regions, and recombinant NS3 of the HCV polyprotein (21). A recent study by Brojer et al. showed that this test could be useful for serological confirmation of HCV infection in some ELISA-negative/HCV-RNA-positive patients (22). The HCV NS5A may play a role in the development of interferon resistance through its inhibition of a single-strand RNA-dependent protein kinase (PKR), a protein activated by interferon that blocks viral protein synthesis (23). A study using the LIA and RIBA-3 (21) found an association between a primary response to interferon therapy and reactivity toward the NS5A antigen in both LIA and RIBA-3 assays.

Antibodies to HCV Envelop Proteins

A recent study has demonstrated that antibodies against E1 and E2 are predictive parameters of response to interferon therapy. Depraetere et al. assessed the role of anti-E1 and anti-E2 antibodies in 115 chronically infected with HCV genotype 1b (24). In most patients, E1 and E2 antibody levels were lower after interferon treatment, particularly in long-term responders to treatment. E1 antibody reduction was more profound than that of E2 antibody.

A sustained elevation of E1 antibody was noted even among patients who have undetectable HCV-RNA in the serum during treatment. E1 antibody titers may provide a sero-

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logical parameter that will discriminate between sustained responders and relapsers to interferon treatment.

Gane et al. described the pattern of HCV-specific antibodies and cryoglobulin in liver transplant recipients (25). Anti-E1 and anti-E2 titers, but not antibodies against other HCV antigens, increased to pretransplantation levels or higher at posttransplant month 12. These titers correlated with serum HCV-RNA levels. Cryoglobulinemia was detected in 30% of the study population and was associated with lower anti-E1 levels and more severe graft damage. The investigators concluded that anti-E1 levels may be used as a marker for monitoring patients with recurrent HCV infection after liver transplantation and suggested that the envelope-specific humoral immune response may be a reflection of HCV replication.

MOLECULAR ASSAYS

The initial diagnosis of HCV infection is often based on the presence of anti-HCV antibodies detected by the ELISA technique. However, ELISA has many limitations, including its low sensitivity in early phases of the infection and its inability to differentiate between active or resolved disease. Patients with an ongoing immunosuppressive process (e.g., HIV coinfection, hemodialysis) have lower antibody titers often resolving in a high percentage of false-negative results (26).

The detection of HCV-RNA by RT-PCR is considered the gold standard for the diagnosis of HCV infection, and furthermore is critical in the assessment of response to antiviral therapy. The detection of HCV-RNA is particularly important among patients who are viremic but have negative anti-HCV ELISA results. Both qualitative and quantitative tests are available for HCV-RNA assays.

Qualitative Tests

Qualitative HCV-RNA determination is indicated in the following clinical situations: seronegative acute hepatitis of unknown cause, chronic hepatitis of undetermined etiologies,

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immune-suppressed patients, anti-HCV-positive patients with persistently normal ALT levels, chronic hepatitis with established etiologies where coinfection with HCV is a possibility, newborns born from viremic HCV-infected mothers and, finally the monitoring of antiviral therapy (20).

The RT-PCR technique involves the amplification of components of the viral genome. In RT-PCR, the viral RNA is first converted to cDNA by a reverse transcriptase. A nucleic acid sequence is identified by a known oligonucleotide "primer." The nucleic acid is then copied multiple times using an enzymatic reaction (polymerase) (6). There are many "in-house" variations of the RT-PCR available, and the lack of standardization for HCV-RNA testing has led to confusion when comparing PCR results from different laboratories (26–28). Zaaiger et al. investigated the reliability of PCR testing in different laboratories using a standardized test (28). Only 5 of 31 laboratories scored perfectly (16%) and one-third reported either false-positive or false-negative results. In the five centers that scored perfectly, a 100-fold difference in assay sensitivity was reported for the dilution series. The investigators concluded that the observed errors were introduced by contamination. Sample contamination can occur by carryover results in the amplification of PCR products that were synthesized during previous PCR reactions and by cross contamination whereby one sample was contaminated by a positive sample at any given step of the procedure. Furthermore, before testing for HCV-RNA, the sample must be handled and stored properly to ensure accurate results (29).

Roche Amplicor HCV is based on five steps: specimen preparation; reverse transcription of target RNA to generate complementary DNA (cDNA); PCR amplification of target cDNA using specific complementary primers; hybridization of the amplified products to oligonucleotide probes specific to the target(s); and detection of probe-bound amplified products by colorimetric determination using horseradish peroxidase. Roche assays are standardized against the First International Standard for Nucleic Acid Amplification Technology (NAT)

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Assays. This standard is referred to as the World Health Organization (WHO) Standard 96/790 (27). Version 2.0 Amplicor Hepatitis C Virus Test and COBAS Amplicor Hepatitis C Virus Test (Roche Molecular Systems, Branchburg, NJ) assay has an estimated sensitivity 100 copies/mL (50 IU/mL) (Fig. 7).

Another qualitative method has been recently introduced for the diagnosis of HCV infection. The transcription-mediated amplification (TMA, Bayer-Gen-Probe, Emeryville, CA) technique has been successfully used to detect hepatitis B virus DNA and HCV-RNA in serum (Fig. 8) (30, 31). TMA is a simple, rapid, and sensitive (50 copies/mL) for all the major HCV genotypes. The TMA technique consists of three

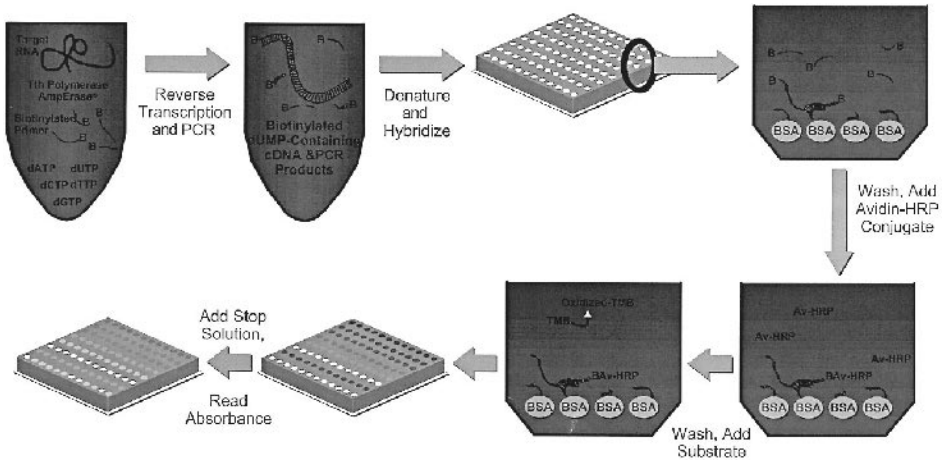

Figure 7 AMPLICOR HCV Test v 2.0 Format is based on five major processes: specimen preparation; reverse transcription of the target RNA to generate complementary DNA (cDNA); PCR amplification of target cDNA using HCV specific complementary primers; hybridization of the amplified products to oligonucleotide probes specific to the target(s); and detection of the probe-bound amplified products by colorimetric determination. (From Roche Molecular Systems, Branchburg, NJ.)

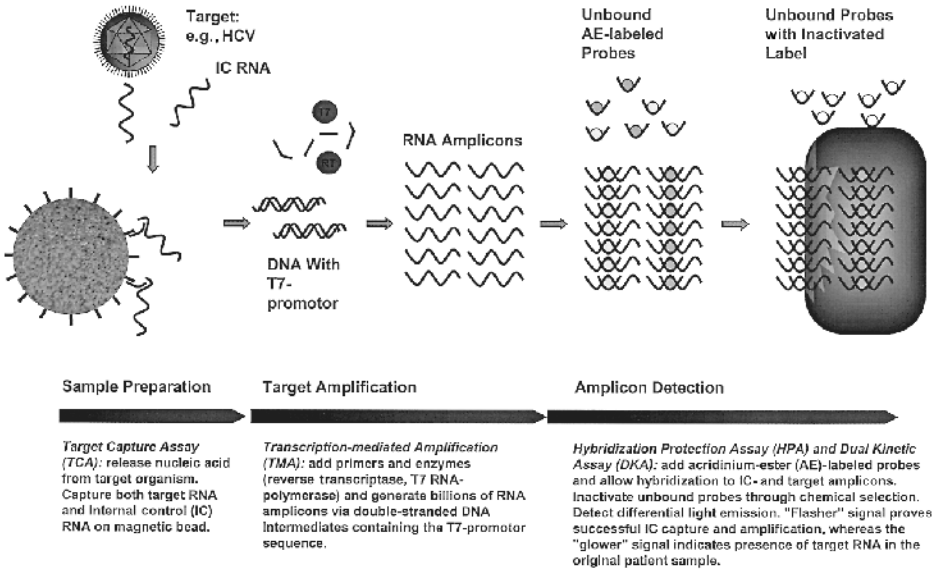

Figure 8 HCV TMA: assay overview. (From Bayer Corp., Tarrytown, N.Y.)

basic steps: sample processing using the target capture assay (TCA), target amplification using TMA, and specific amplicon detection using the hybridization protection assay (HPA). The main advantages of this methodology are (a) easier processing of the sample using a single tube with no need of centrifugation and transfers of specimens and reagents; (b) removal of inhibitors and extraneous nucleic acids, allowing a better sensitivity and specificity; and (c) a high process up to 100 samples in 5 h.

In a recent study, 47 patients who achieved a virological end of treatment response, but not a sustained response, according to HCV RNA by PCR (Amplicor HCV versions 1.0 and 2.0) were retested by TMA (31). Fifty-nine virological sustained responders and 49 nonresponders served as controls. There was a 98% concordance between PCR and TMA among virological sustained responders and nonresponders. However, residual HCV-RNA was detected by TMA at the end of

treatment in 64% (16 of 25) of relapsers who were HCV-RNA negative by Amplicor HCV version 2.0. (detection limit 100 copies/mL).

Quantitative Assays

Quantification of HCV RNA levels is a useful tool in the clinical management of patients before, during, and after specific antiviral treatment (Fig. 9). In general, there are two different technologies for quantification of HCV-RNA levels: target amplification methods using PCR technology (qPCR) and signal amplification based on a branched DNA (bDNA) technique.

The main advantage of qPCR is its high degree of sensitivity as compared to signal amplification methods of quantification (7) with reports of sensitivity as low as 1000 copies/mL (7). Three commercial assays using qPCR are available: HCV Superquant (National Genetics Institute, Culver City, CA) has a detection limit of 100 copies/mL, exhibiting linear-

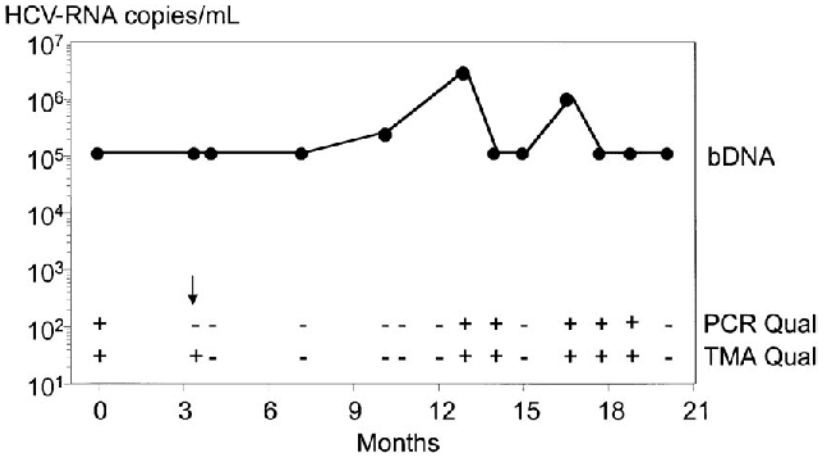

Figure 9 Monitoring of HCV-RNA reactivity using quantitative and qualitative NATs. NAT, nucleic acid testing; PCR, polymerase chain reaction; TMA, transcription-mediated amplification; bDNA, branched DNA (cut off 10^5 copies/mL).

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ity between 100 copies/mL and 5×10^6 copies/mL. Amplicor HCV Monitor 2.0 test (Roche Molecular Systems, Nutley, NJ) has a sensitivity limit of 500 copies/mL. Before the introduction of the HCV Superquant assay, the main limitation of qPCR was the high assay variability and the lack of linear range above 1 million HCV-RNA copies/mL.

Recently, a real-time detection PCR test (RTD-PCR; Perkin Elmer Corp./Applied Biosystems, Foster City, CA) based on the TaqMan chemistry system was developed (32). This method takes advantage of the reverse transcriptase activity and 5′-3′ nucleolytic activity of Taq DNA polymerase to digest a probe labeled with a fluorescent reporter and quencher dye. In this study, as few as 10 copies/mL of the HCV genome were detected, the quantification range was between 10^1 and 10^8 copies/mL, and it proved to be 10- to 100-fold more sensitive than the Amplicor HCV Monitor 2.0 test. Finally, using this methodology, HCV genome was detected in 98% of patients with chronic hepatitis, 95.8% of cirrhotic patients, and 100% of those with hepatocellular carcinoma.

The quantitative nucleic acid sequence–based amplification assay (NASBA-QT; Organon-Teknika, Boxtel, the Netherlands) was recently compared in two studies (33, 34) to Amplicor HCV Monitor 2.0 and bDNA-2 (Quantiplex HCV RNA 2.0 Assay, Bayer Corp., Emeryville, CA). NASBA-QT detection limit was 1000 copies/mL and exhibited a 10-fold better sensitivity than bDNA-2 assay with comparable sensitivity to Amplicor HCV Monitor 2.0, although the bDNA-2 and the NASBA-QT were more likely to quantify all HCV genotypes compared to Amplicor HCV Monitor 2.0.

In contrast to qPCR methods, the bDNA assay, a signal-amplification technique, is highly standardized and reproducible. The limit of detection of the bDNA-2 test is 200,000 equivalent genome/mL. This makes this test less sensitive than PCR method. Third-generation bDNA assay (Quantiplex HCV RNA 3.0) has recently become available (Fig. 10). The bDNA-3 assay has proved to have roughly a 100-fold increase in sensitivity compared to bDNA-2. Furthermore, there is equal

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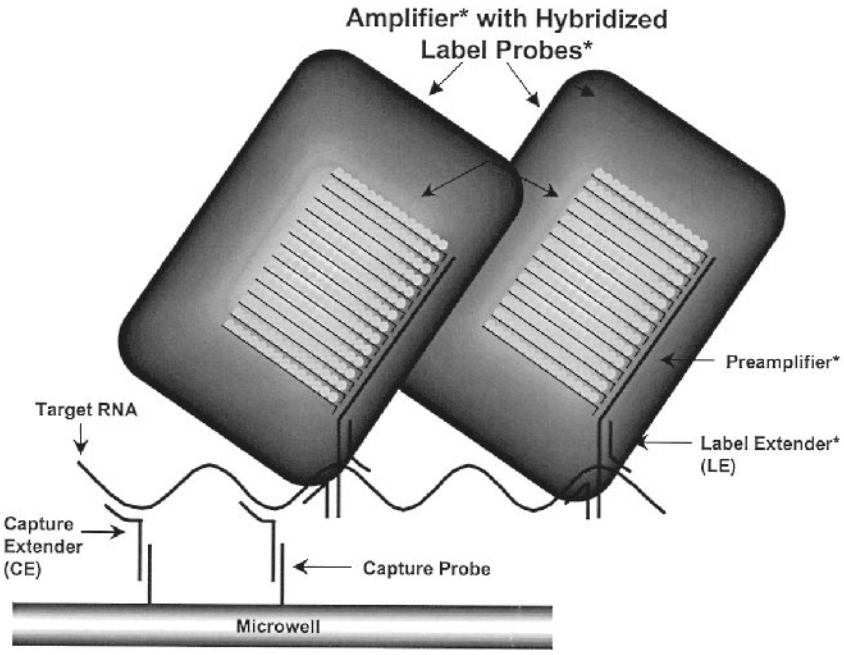

Figure 10 HCV bDNA 3.0 Assay Principle. The Bayer HCV RNA 3.0 is a sandwich nucleic acid hybridization procedure for the direct quantitation of HCV RNA in serum and plasma. After the HCV genomic RNA is released from the virions, the RNA is captured to a microwell by a set of specific synthetic oligonucleotide capture probes. A set of target probes hybridizes to both the viral RNA and the preamplifier probes. The capture probes and the target probes bind to the 5′ untranslated and core regions of the HCV genome. The amplifier probe subsequently hybridizes to the preamplifier forming a bDNA complex. Multiple copies of an alkaline phosphatase (AP) labeled probe are then hybridized to this immobilized complex. Detection is achieved by incubating the AP bound complex with a chemiluminescent substrate. Light emission is directly related to the amount of HCV RNA present in each sample. (From Bayer Corp., Tarrytown, N.Y.)

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quantification of all HCV genotypes. The assay has a reportable range of 2500 to 40 $\times$ 10^6 copies/mL.

QUASISPECIES

In patients infected with HCV, a spectrum of closely related molecular variances termed quasispecies is found. The generation of quasispecies is a potential mechanism by which HCV escapes the host immune response and continues to replicate (35). Quasispecies are more likely responsible for the failure to eradicate HCV in patients resistant to current antiviral therapy. This is due to the high error tendency of RNA-dependent polymerase of RNA viruses and its lack of proofreading capabilities. In contrast to genotypes, in which the variation on the bases are between 31 and 35% in the entire genome, quasispecies vary from each other by only 1–9% of bases (35).

Many indirect and direct methods can be used for detection and quantification of quasispecies. The single-strand conformation polymorphism analysis (SSCP) is an indirect method in which products of PCR are subjected to electrophoretic analysis under denaturing conditions, resulting in single-stranded RNA. Single-nucleotide polymorphisms result in different mobilities of the single-stranded fragments (36). Direct sequencing can also be used for quasispecies determinations (37).

GENOTYPING/SUBTYPING

Not only the HCV quasispecies express variability in different regions of the genome, but also isolates differ among themselves. All isolates separate into phylogenetically related clusters called subtypes. One or several subtypes can be classified into several major types that show similarities in 65–75% of the total genomes. The term *genotype* is generically used to refer to subtypes, types, or both. Eleven HCV genotypes are

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known to exist (37) as well as more than 90 subtypes (38). Simmonds et al. have developed a consensus system for HCV genotyping based on sequence homology in at least two regions confirmed by phylogenetic tree analysis (3). Major genotypes are assigned a number, and subtypes within each genotype are assigned a lowercase letter.

The HCV genotype can be established by either complete sequencing of the 9500 nucleotide genome with subsequent phylogenetic tree construction (39) or by subgenomic genotyping methods based on relatively well-conserved regions of the genome such as the 5′UTR, core, E1, and NS5B. The methods available are the reverse hybridization line probe assay (40) (LIPA; INNO-LIPA HCV II, Innogenetics, Zwijnaarde, Belgium) (Fig. 11), the restriction fragment-length polymorphism (RFLP) method (41), and PCR with genotype-specific primers (42). Amplification and sequencing with sequence comparison and phylogenetic tree construction for confirmation is considered to be the most reliable of these methods (39).

The 5′ untranslated region of the HCV genome is the ideal target for genotyping. Line probe assay technology is based on the reverse hybridization principle where biotinylated PCR fragments are hybridized to a selection of highly specific immobilized probes. In a second step, the biotin group in the hybridization complex is exposed by incubation with a streptavidin-alkaline phosphatase complex and the appropriate chromogen compounds (40).

In RFLP analysis, universal primers are used to amplify a single PCR fragment from a particular region of the HCV genome. Restriction fragments of varying lengths are created after cutting the PCR fragment with one or several restriction endonucleases. The estimation of the appropriate length of these fragments will allow the HCV genotype to be identified (41).

In a study of LIPA in tertiary referral centers in the United States, 72, 14, and 6% were HCV genotypes 1, 2, and 3, respectively (43). Disease activity and viremia did not differ among patients with genotypes 1, 2, and 3, but patients with

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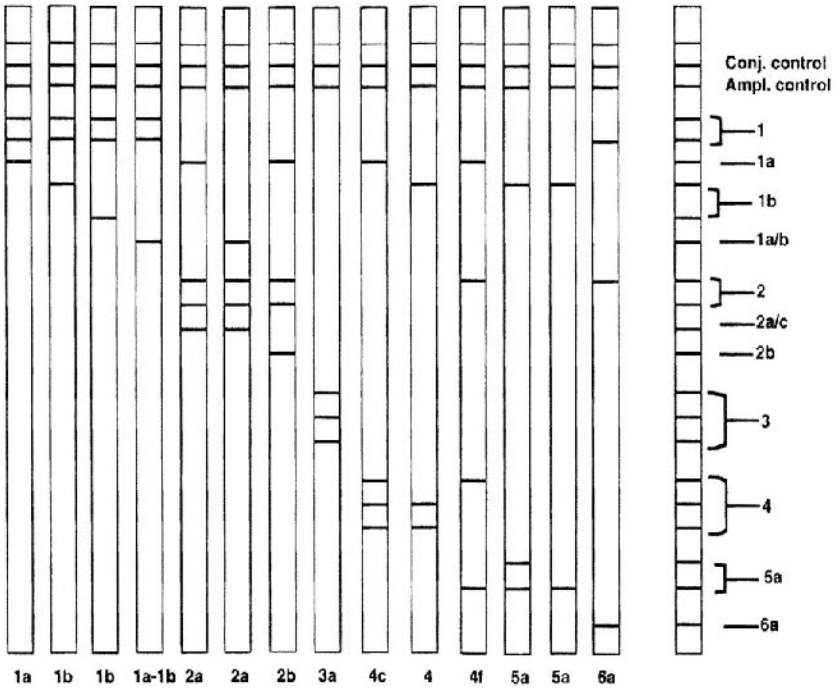

Figure 11 Reverse hybridization line probe assay (LIPA) illustrating genotype subtypes. The assay is based on variations found in the 5′ untranslated regions (5′UT) of the different HCV genotypes. Type-specific probes are tailed with a poly (T)-tail by terminal deoxynucleotidyl transferase and attached to nitrocellulose membrane. Biotin-labeled amplified products are reversely hybridized to the probes on the strip. The biotin group is incorporated by employing a 5′-biotinylated primer during amplification. The labeled product obtained from the 5′UR will only hybridize to a probe that gives a perfect sequence match, allowing stringent discrimination at the subtype level. After hybridization, streptavidin labeled with alkaline phosphatase is added and bound to any biotinylated hybrid previously formed. Incubation with BCIP/NBT chromogen results in a purple/brown precipitate. The INNO-LIPA HCV II strip contains a test control line and 20 parallel DNA probe lines. The conjugate control line is a control for the color development reaction and the amplification control line contains universal probes hybridizing to amplified products of any HCV type. (From INNO-LIPA, Innogenetics NV, Zwijnaarde, Belgium.)

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genotype 4 had lower levels of viremia than patients with genotype 1.

Genotype 2 is widely distributed but is less common than genotype 1 (10–40%). Genotype 3 is found mainly in Pakistan, Australia, and Scotland, type 4 predominantly in the Middle East and Africa, type 5 in South Africa, and type 6 in Hong Kong and Macau (44).

Variations in HCV genotype have important implications in the design of HCV vaccines and biotherapeutic agents. Antibodies elicited by one genotype may not protect patients against reinfection with other variants. Furthermore, biological differences between genotypes may relate to viral replication rates, mutation rates, histological inflammatory activity, disease severity, and response to treatment.

HCV genotype is associated with the source of the infection, not the intrinsic pathogenicity of HCV, but it is a strong predictor of sustained response to therapy. Genotype 1 patients exhibit a lower sustained virological response to combination therapy with interferon alfa-2b plus ribavirin when compared to non-genotype 1 patients (45–47). Therefore, genotype 1-positive patients should be treated with 48 weeks of combination therapy in contrast to non-genotype 1 patients, most of whom will achieve a sustained viral remission after 24 weeks of therapy (48). The clinical implication of genotype determination among liver transplant patients is unsettled. Some studies have shown an association between the presence of genotype 1b and severity of post-OLTx HCV infection (49), whereas others have failed to demonstrate such an association (50). In the posttransplantation period, recurrent HCV infection with genotype 1b or 4 has been associated with accelerated progression of chronic hepatitis in the new graft (37).

SEROTYPING

Serotyping involves an immunoassay for the detection of antibodies to serotypes of HCV (51, 52). The Murex HCV Serotyp-

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ing 1-6 Assay (SA 1-6; Murex Diagnostics Limited, Dartford, England) utilizes synthetic peptides that represent antigenic regions from HCV types 1–6. Serotyping is less labor intensive, but it is not as specific as genotyping (53). Cross reactivities between different HCV genotypes may account for the 6% rate of mistyping observed with this technology. Subtyping of 1a and 1b is still not possible with this test, but discriminating between subtypes may not be necessary in the clinical context.

Serotyping can be used as a mean of differentiating between HCV genotype 1 and non-genotype 1 patients, which is cost effective in the treatment of chronic hepatitis C patients, especially in countries where the availability and cost of genotyping is an issue. Overall, serotyping is less sensitive than genotyping based on PCR amplification of HCV RNA (53).

CONCLUSIONS

There have been major advances in the diagnosis of hepatitis C since the first marker for this virus became available in 1989. Third-generation/version assays for anti-HCV, supplemental assays for anti-HCV confirmation, sensitive qualitative, and quantitative HCV RNA methodologies and genotyping are readily available. Furthermore, an HCV core antigen enzyme immunoassay greatly facilitates the early diagnosis of acute hepatitis C as well as the assessment of antiviral response to therapy in areas where molecular testing cannot be implemented.

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3

Current Treatment of Chronic Hepatitis B

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CASE

A 25-year-old Asian-American who is a first year medical student presents for evaluation of recently discovered hepatitis B surface antigenemia. He has felt well without significant past medical history. He drinks alcohol socially. The patient has no history of recreational drug use or blood transfusion. The patient has a significant family history of liver disease with his father having had a diagnosis of hepatocellular carcinoma and chronic hepatitis B. The patient has two older siblings who have been told they have hepatitis B

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"exposure." On physical examination, the patient appears well and has stable vital signs. His abdominal examination reveals a nontender smooth liver palpable at the costal margin and no splenomegaly. The patient has no cutaneous stigmata of chronic liver disease.

Serologies		Labs	
HBsAg	Positive	ALT	125 U/L
HBsAb	Negative	AST	150 U/L
HBcAb IgM	Negative	Alkaline phosphatase	50 U/L
HBeAg	Positive	Total bilirubin	0.8 mg%
HBeAb	Negative		
HBV DNA	Positive		

Because the patient had persistent replication with HBeAg and HBV DNA present at follow-up 3 months later, treatment was advised. The role of interferon and lamivudine were discussed and he elected to receive therapy with lamivudine 100 mg po daily. The patient had rapid normalization of liver chemistries with clearance of serum HBV DNA within 12 weeks of start of the therapy. After 1 year of therapy, HBeAg had disappeared from serum and 3 months later antibody to HBeAg was detectable. Therapy was discontinued and at follow-up 6 months later, liver chemistries remained normal with markers of replication absent.

INTRODUCTION

Hepatitis B virus (HBV) is a major cause of acute and chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) affecting an estimated 300 million individuals worldwide, with approximately 250,000 deaths annually (1). The introduction of effective vaccination (2) to prevent infection and the availability of interferon alpha and more recently lamivudine to treat chronic HBV offer the potential ultimately to reduce the burden of liver disease due to HBV infection (3).

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VIROLOGY

Human HBV is a hepatotropic DNA virus, one of the Hepadnaviridae family that also includes related viruses that infect specific animal species, including the woodchuck, ground squirrel, duck, and a heron species. HBV consists of a partially double-stranded circular DNA genome of approximately 3.2 kb in length surrounded by an outer lipoprotein envelope and an inner core composed of nucleocapsid proteins with a total diameter of 42 nm (4). In vivo studies of viral kinetics have shown that the half-life of the virus is approximately 24 h with a daily turnover of 50% of the free virus population and a total viral release into the periphery of approximately 10^{11} copies (5).

EPIDEMIOLOGY

An estimated 1.0–1.25 million people in the United States are chronically infected with HBV (6). During the 1980s, the Centers for Disease Control (CDC) estimated 200,000–300,000 annual infections in the United States with a peak incidence of 70 per 100,000 persons in 1985 (7). As a result of changes in disease transmission patterns, however, the incidence dropped approximately 40% to 40 per 100,000 persons by 1991 (2). There are 1.25×10^6 chronic carriers in the United States (8). Many of these are immigrants from high- or moderate-prevalence areas such as Southeast Asia, China, and Sub-Saharan Africa (9) where the rate of HBs antigenemia is approximately 8–25% with anti-HBs prevalence of approximately 60–85%. HBV is endemic in areas containing 45% of the world's population (9). In the United States, a high prevalence is found in Alaska where the rate of HBsAg positivity in Native Americans is approximately 6.4% (10). Intermediate-prevalence areas such as Japan, Eastern Europe, Latin America, South America, and Mediterranean countries have

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a chronic infection rate of 3–5%, whereas in low-prevalence areas such as the United States, Western Europe, and Australia, the overall rate is only 0.1–2.0% (1).

NATURAL HISTORY AND RATIONALE FOR THERAPY

Identification of serum markers of HBV replication, namely, HBeAg and HBV DNA, has facilitated the development of virological indications and endpoints for therapy. A number of natural history studies have highlighted the key role of replicative status on patient survival. Thus, in a report by De Jongh et al. (11), a significantly poorer 5-year survival of 72% was observed in HBV-related cirrhosis in patients who were HBeAg positive compared to an HBeAg-negative group which had a 97% survival rate. The goals of therapy, therefore, include suppression of viral replication with an anticipated decrease in necroinflammatory activity and progression to hepatic decompensation (3).

TREATMENT

The major focus of therapy for HBV is the chronically infected patient given the high rate of spontaneous clearance of acute HBV infection: greater than 95% in the immunocompetent adult. Chronic infection is defined as the presence of HBsAg in the serum for 6 or more months. The major determinant of chronicity is the age at exposure (12). Infants born to HBV-infected mothers who are HBeAg positive have an approximate 90% probability of developing chronic infection, whereas the risk falls to 25–50% of children aged 1–5 years and to only 5% in older children and adults (13). Other factors that influence the risk of developing chronic infection include impaired immunity and old age. The ideal agent would interrupt HBV replication, abort progression of liver disease lessening the

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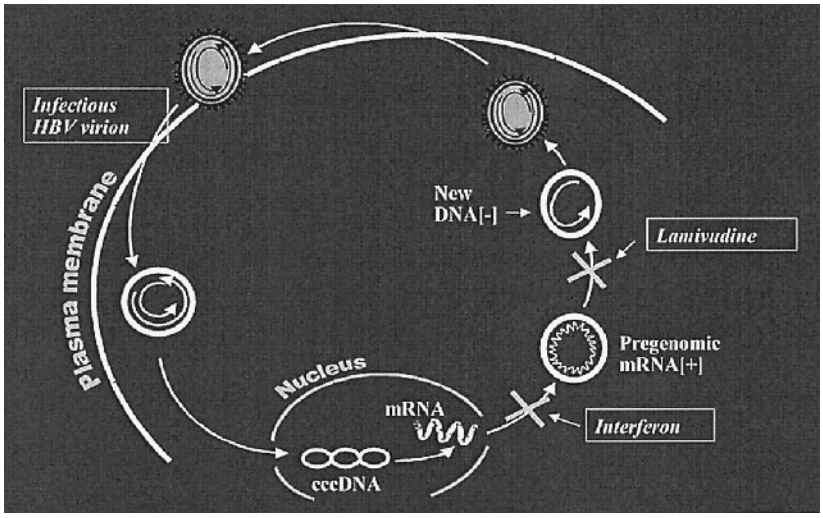

Figure 1 The ideal agent would interrupt HBV replication, abort progression of liver disease lessening the risk of cirrhosis and HCC, improve survival, and eliminate infectivity to others. (From Ref. 3.)

risk of cirrhosis and HCC, improve survival, and eliminate infectivity to others (Fig. 1). Although attempts at treating HBV date back to the 1970s, it is only in the last decade that effective therapy has been possible—initially with interferon alpha and more recently with nucleoside analogues like lamivudine (3). Treatment responses are typically evaluated on the basis of biochemical (normalization of alanine transaminase [ALT] levels), virological (sustained clearance of HBeAg and HBV DNA), and histological (decrease in necroinflammation on liver biopsy) criteria. Patients should be offered treatment if there is evidence of chronic infection (HBsAg) with necroinflammatory activity (elevated ALT) and evidence of persistent active replication (HBeAg and HBV DNA in serum) for several months.

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Interferon

The interferons (IFNs) are naturally occurring proteins secreted by many mammalian cells in response to viral infection. IFNs have antiviral, antiproliferative, and immunomodulatory effects. They increase both specific (cytotoxic T lymphocytes) and nonspecific (natural killer cell) immune response to viral proteins (14). The efficacy of interferon alpha in the treatment of chronic HBV relates to a number of mechanisms both immunoregulatory and antiviral (15). Activation of ribonucleases leads to viral mRNA degradation. Protein kinase production in response to IFN leads to peptide chain termination. IFN can also help contain viral infection by preventing viral entry and uncoating as well as mRNA translation and assembly. IFN's immunomodulatory actions enhance HLA class I expression on hepatocytes aiding in the presentation of viral encoded peptides to cytotoxic T lymphocytes (CTLs). Other immunomodulatory actions include enhanced natural killer cell activation, increased CD4/CD8 ratios, and Fc receptor expression (15, 16).

The most extensive experience with IFN therapy for chronic HBV has been with interferon alpha. It was the first agent licensed for treatment of chronic HBV in the United States and elsewhere. A seminal U.S. trial reported by Perrillo et al. (17) used interferon alpha-2b in a number of different treatment arms, including 16 weeks of interferon 5 million units daily, 1 million units daily, a tapering dose of oral prednisone for 6 weeks followed by 5 million units daily, or no treatment. Thirty-seven percent of patients in the 5 million unit arm cleared HBeAg and HBV, which was significantly greater than the 17% in the 1 million unit arm and the untreated arm (7%). Thirty-six percent of patients who had received prednisone followed by interferon cleared HBeAg and HBV DNA. Successful therapy was associated with histological improvement on posttreatment biopsy with diminished inflammation. A rise or "flare" in serum aminotransferases during therapy was a predictor of a successful response, as it was

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observed in 63% of responders but only 27% of nonresponders. The flare phenomenon is felt to be due to hepatocyte necrosis (15). The addition of prednisone to interferon therapy does not improve overall response rates but does enhance response in patients with ALT levels only modestly elevated pretreatment (15, 17).

Similar IFN response rates have been reported in other studies (18–20). A number of baseline factors have been identified to predict the likelihood of IFN response (Table 1). Notable among these are a low pretreatment HBV DNA level (less than 200 pg/mL by molecular hybridization) and ALT elevated more than three to four times the upper limit of normal in addition to other less critical factors (21).

An important observation that has been repeatedly made following successful response to IFN therapy with clearance of HBeAg is an enhanced rate of subsequent HBsAg loss with effective resolution of HBV infection (22). Typically, loss of the markers of HBV replication is associated with normalization of ALT levels and improved histology. A meta-analysis of 15 randomized control trials confirmed the benefit of IFN therapy for chronic HBV as it increased durable HBeAg loss compared

Table 1 Prediction of Outcomes to Treatment with IFN Alpha

Favorable outcome	Unfavorable outcome
HBeAg positive	HBeAg negative
Low serum HBV-DNA	High serum HBV DNA
Increased serum ALT	Normal serum ALT
Female	Male
White	Asian
Recent HBV infection	Long-standing infection
Noncirrhotic	Cirrhosis of the liver
Anti-HIV negative	Anti-HIV positive
HDV negative	HDV positive

HDV, hepatitis D virus.
Source: Ref. 21.

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to untreated controls (23). Furthermore, HBsAg loss was 6% higher in the IFN-treated group. Longer term follow-up has suggested that the ultimate rate of HBsAg loss can be greater than 70% after successful HBeAg clearance with IFN therapy (15). Although loss of HBsAg is the conventional indicator of resolution of HBV infection, HBV DNA can be recovered in some patients from serum and liver biopsy specimens for several years after this event, implying low-level replication, although this does not appear to be of major clinical importance (24). Loss of HBeAg per se also had undoubted benefit for responders to therapy and is a strong predictor of survival in treated patients as shown by the reports of Niederau and colleagues on IFN responders (25). Follow-up of IFN responders showed diminished risk of death or need for liver transplantation compared to untreated controls as well as a longer complication free survival over a mean of 50 months.

Corticosteroid "Priming." In an effort to improve IFN response rates, corticosteroids have been used, typically in a short but high-dose regimen, as an adjunct to IFN based on the clinical observation that administration of steroids to the patient with chronic HBV infection results in a decline in viral replication with increased aminotransferase levels (26, 27). A number of mechanisms have been invoked, including enhanced hepatocyte membrane expression of HBV antigens which serve as a stimulus to cytotoxic T-cell response. The HBV genome also contains a glucocorticoid-sensitive element which may increase HBV replication, leading to a more vigorous resulting immune response. Also, detectable macrophage colony-stimulating factor increases following steroid withdrawal in chronic HBV as also occurs naturally prior to HBeAg loss (15).

Analysis of the large U.S. multicenter trial reported by Perrillo et al. indicates that steroid use does not improve overall response rates when given for 6 weeks prior to IFN therapy (17). However, the subset of patients with low pretherapy ALT levels (<100 IUL) responded better to this regimen at rates comparable to patients with high pretreatment ALT levels.

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Other reports also have suggested that it is the low ALT subset of patients who benefit from steroid "priming" (28).

Use in Problem Groups: HBeAg-Negative Mutants. As discussed elsewhere in this volume, an important HBV mutant is characterized by failure to secrete HBeAg owing to an amino acid substitution in the genome at position 1896 with HBV-DNA frequently being present in serum, indicating active viral replication (29). Although biochemical and virological responses with normalization of ALT and clearance of HBV-DNA, respectively, are frequent with IFN therapy, sustained response rates have been generally low after therapy is stopped: less than 10% after long term follow-up (15). In contrast, in HBeAg-positive patients, relapse rates are generally less than 10% after successful therapy in adult-acquired HBV. More protracted IFN therapy may lessen the likelihood of relapse for the patient with a precore mutant (31). In contrast also to the HBeAg-positive treatment candidate, viral load as assessed by HBV-DNA appears to be less critical as a predictor of response (32), although the prevalence of a higher percentage of circulating precore mutants may diminish IFN responsiveness (30). Relapse generally occurs within a year of completing therapy. However, clearance of HBsAg has been reported following IFN therapy in HBeAg-negative mutants (31). In addition, histological improvement has also been observed following therapy. A report from Taiwan suggests that corticosteroid priming may aid in IFN-induced elimination of precore mutants (33).

Use in Decompensated Cirrhosis. Patients with clinically apparent cirrhosis are generally regarded as high-risk candidates for IFN therapy owing to more frequent and potentially serious side effects and less hepatic reserve to tolerate a therapy-induced flare (34). However, a number of groups have reported clinical benefit in IFN-treated cirrhotics, albeit at the cost of frequent side effects, including bacterial infection (35, 36). Hoofnagle and associates reported sustained HBV-DNA

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clearance in a third of interferon-treated subjects (35). Perrillo et al. used dose titration to minimize toxicity and enhance response in 26 cirrhotics (36). Therapy-induced flares were less frequent, but an overall response rate of 38% was still obtained. Virological responses are typically seen only in Childs-Pugh class A and B cirrhotics. The severity and frequency of side effects suggest that IFN therapy should be contemplated only if other therapeutic options have been exhausted and the patient can be closely monitored.

Use in Infancy- or Childhood-Acquired HBV. The greatest prevalence of HBV infection is in Asia and Sub-Saharan Africa (1). Early studies in Chinese children with HBV infection had resulted in low response rate to IFN, suggesting that this group was unlikely to benefit from therapy (37). Lai et al. reported that in Chinese children with chronic HBV infection, prednisone priming did not improve IFN response rates (38). However, the majority of treated children had low pretreatment aminotransferase activities, implying a quiescent immunological state. A subsequent study by Lok et al., however, in Chinese adults provided more optimistic IFN response rates. Fifteen (38.5%) of 39 patients with elevated pretreatment aminotransferases had sustained clearance of HBV-DNA from serum with loss of HBeAg (39). Other investigations have also reported successful IFN use in chronic HBV infection in adults with presumed childhood onset (40). It is also clear that IFN therapy in children with chronic HBV can be successful with high pretreatment ALT and low serum HBV-DNA levels being predictors of response (41, 42).

Lok and colleagues have also provided a long-term follow-up of Asian IFN responders; 7 of 29 (24%) responders had reactivation of viral activity with biochemical dysfunction (39). In four of these seven patients, the reactivation was sustained, lasting more than 6 months. In addition, there was a very low, only two of 29 (7%), overall loss of HBsAg (39). An American study compared the efficacy of IFN therapy for chronic HBV in Asian-Americans with a control group of Caucasians (43).

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Clearance of markers of active replication was equivalent in the two groups. No Asian patients ultimately cleared HBsAg, although two thirds of the Caucasian responders did. A higher virological relapse rate with reappearance of HBeAg in serum was also observed in the Asian responders. Thus, it appears that although IFN can result in HBeAg clearance in childhood acquired HBV, it may be temporary and is less likely to be associated with ultimate HBsAg clearance. However, a recent report from Taiwan indicated that IFN therapy in Asian patients may provide protection against subsequent development of hepatocellular carcinoma as well as improving long-term survival in IFN-treated patients (44).

Side Effects. The side effects of IFN in the treatment of chronic viral hepatitis are well characterized (3, 45). Because of the higher IFN doses necessary to treat chronic HBV, side effects however may be more intense than with the doses typically used for treatment of chronic HCV, although dose discontinuation has been necessary in less than 5% of treated patients in major studies (15). An IFN side effect unique to the treatment of chronic HBV is the therapy-induced flare, which may be poorly tolerated in the cirrhotic in whom prednisone "priming" may also be hazardous. Generic contraindications to IFN include psychiatric illness or an autoimmune diathesis (3, 45).

Lamivudine

Lamivudine is a synthetic nucleoside analogue with activity against both HBV and human immunodeficiency virus (HIV) and is the first nucleoside analogue approved for the treatment of HBV (46). It undergoes phosphorylation by intracellular kinases to form the active anabolite lamivudine 5′-triphosphate, which is a substrate for HBV polymerase. HBV replication is prevented by competitive inhibition of viral reverse transcriptase and termination of proviral DNA chain extension (4). In common with other nucleoside analogues, and

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in contrast to IFN, it is administered orally and its antiviral effect is not dependent on host immune responsiveness (46).

Preliminary dose ranging studied lamivudine in doses from 2.5 to 600 mg per day. Doses greater than 25 mg daily reduced HBV-DNA levels below the limits of detection by nonPCR testing within 2–4 weeks (46). Serum HBV-DNA levels generally returned to baseline after treatment (47, 48). Dienstag reported that 100% of patients treated for 12 weeks with 100 or 300 mg had normalization of HBV-DNA levels, with a minority having sustained DNA and serum HBeAg clearance (47). The dose of lamivudine 100 mg was selected for expanded clinical trials based on initial dose finding data.

Several expanded phase III studies of lamivudine therapy at a dose of 100 mg for 1 year have been subsequently published. In a randomized, double-blinded study conducted in 137 treatment-naïve U.S. patients, lamivudine 100 mg daily for 52 weeks was compared to placebo with a 16-week follow-up (49). Significantly greater histological response rates were noted with the lamivudine group (52 vs 23%), with a mean decrease in Knodell histological activity index of three points. Additionally, significantly fewer patients demonstrated progression of fibrosis (5 vs 20%). Patients treated with lamivudine also had statistically significant enhancement of HBeAg to anti-HBe seroconversion (17 vs 6%) and loss of HBeAg (32 vs 11%). HBV DNA suppression (44 vs 16%) and ALT normalization (41 vs 7%). Lai et al. published a similar randomized, double-blinded study in 358 Asian patients with chronic hepatitis B in which subjects were randomly assigned to receive lamivudine (25 or 100 mg) or placebo (50). Significantly more patients had improvement in hepatic necroinflammatory activity (two points or more) in the lamivudine treatment arms. Patients receiving 100 mg of lamivudine had reduced progression of fibrosis and had the highest rate of hepatitis B e antigen seroconversion (loss of HBeAg, development of antibody to HBeAg, and undetectable HBV DNA) and the greatest suppression of HBV-DNA with the highest rate of sustained normalization of ALT. This study has been extended

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to examine the efficacy and safety of continuous lamivudine therapy. HBeAg seroconversion to anti-HBe increased over time from 22% after 1 year to 40% after 3 years of continuous treatment (51). It was additionally noted that an increased baseline serum ALT level was predictive of HBeAg seroconversion. In patients treated with lamivudine with baseline ALT levels greater than twice the upper limit of normal, the HBeAg rate increased from 38% at 1 year to 65% at 3 years (52).

HBeAg seroconversion induced by lamivudine appears to be durable even after cessation of treatment. Thus, Schiff reported that up to 90% of patients treated in protocols remained HBeAg negative during a mean 6 months posttherapy (53). Thus, it is reasonable to consider withdrawing lamivudine therapy once HBeAg loss has been achieved rather than continuing it indefinitely. In common with IFN, however, Asian patients appear to have a high rate of virological relapse with return of HBeAg after therapy is discontinued (54). This observation suggests that repeated courses of lamivudine may be necessary to obtain satisfactory viral suppression in this key group of patients.

Interferon Plus Lamivudine

The potential role of combining the two agents with proven efficacy against chronic HBV, namely, interferon alpha and lamivudine, has been addressed, although not yet completely resolved in a number of studies (55, 56). In one study, patients previously treated with IFN and deemed nonresponders were randomized to receive lamivudine 100 mg with or without IFN. Histological response, HBeAg loss, and HBeAg seroconversion as well as sustained ALT normalization were more common in the lamivudine monotherapy group compared to combination treatment suggesting that combination therapy added no benefit (55). In treatment-naïve patients, no difference was noted between lamivudine monotherapy and combination therapy with interferon alfa-2b in terms of histological response, although the proportion of patients with HBeAg

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seroconversion was significantly greater among patients receiving combination treatment (56). Thus, the definitive role of combination IFN and lamivudine remains unclear (57).

Side Effects. In immune competent patients with chronic HBV enrolled in clinical trials, lamivudine has been extremely well tolerated (58). In particular, no evidence of mitochondrial toxicity has been noted even with therapy extending over several years (59). This had been a concern given the previous experience with a lethal form of mitochondrial toxicity secondary to fialuridine (FIAU) in prior HBV studies (60). Most lamivudine-related toxicity has been identified in HIV-infected patients who typically are on a number of antiretroviral agents. In non–HIV infected patients, the most clinically significant adverse event that has been recognized is a post-treatment two- to threefold ALT flare, although frank hepatic decompensation has not been observed as a result (3).

Viral Resistance. The most significant limitation to lamivudine therapy for chronic HBV has been the emergence of resistant HBV mutants related to amino acid substitutions in a key motif of the DNA polymerase, YMDD, a site of nucleoside binding. Although this event was first appreciated in liver transplant recipients receiving lamivudine monotherapy to prevent HBV (21) recurrence, it also is a frequent event in the nontransplant setting. Viral resistance increases with duration of therapy: 27% of patients by 12 months of therapy and 58% by 24 months have mutant formation in reported studies (21). Clinically, viral resistance may be associated with a rise in ALT levels, and serum HBV DNA increase or returns. This increased hepatitic activity may be asymptomatic, although patients with clinically obvious cirrhosis may experience clinical worsening of their liver disease (61). These HBV mutants are less replication efficient than so-called "wild-type" HBV (62). As discontinuation of lamivudine may lead to rapid return of this potentially more pathogenic form of HBV, the current wisdom is that treatment should be maintained. In par-

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ticular, benefit with HBeAg loss can be observed even in the presence of the YMDD mutant, although apparently less frequently than in its absence (61). As is discussed elsewhere in this volume, other agents, including adevofir, are in development, and appear to maintain efficacy even in the presence of YMDD mutants.

Lamivudine Therapy in Problem Groups: Precore Mutant. Lamivudine therapy in this subset of patients, generally reported as poor responders to IFN therapy, is associated with biochemical and histological improvement in two-thirds of patients treated for a year (63). HBV-DNA levels fall, aminotransferase levels return to normal, and hepatic necroinflammatory activity diminishes.

Use in Decompensated Cirrhosis. Lamivudine use in patients with clinically overt cirrhosis can result in clinical improvement, potentially obviating or at least delaying the need for liver transplantation. Villeneuve and colleagues (64) reported that lamivudine therapy in an uncontrolled study in patients with clinically overt cirrhosis resulted in improved hepatic synthetic function, with a resultant decrease in Child-Pugh score. As noted above, emergence of viral resistance may be poorly tolerated in the patient with advanced liver disease and prompt liver transplantation may be lifesaving (65).

Famciclovir

Although famciclovir has documented efficacy against HBV in initial clinical trials (66–68), it is overall a less potent anti-HBV agent than lamivudine (69). Viral resistance is also observed during monotherapy with famciclovir (66). Response to lamivudine may still be maintained, although the patient may subsequently develop resistance to the latter also (70). Combination nucleoside therapy is one potential application of famciclovir (71). Otherwise, its lower potency than lamivudine will continue to limit its role as monotherapy for HBV. It does

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not appear to have a major role in the treatment of YMDD mutants that result from prior lamivudine therapy (21).

CONCLUSIONS

The treatment options for chronic HBV have broadened considerably with the advent of lamivudine, the first of undoubtedly many newer agents for therapy. There is still, however, an important role for IFN therapy in well-selected patients, particularly as its successful use clearly leads to loss of HBsAg, an event which has been only rarely observed with lamivudine (Table 2). Factors to weigh in choosing an initial agent include the patient's likely tolerance of IFN side effects and predictors of successful response, notably low pretherapy HBV-DNA and elevated ALT levels (Fig. 2). The absence of severe side effects with lamivudine makes compliance easy, although by not using IFN therapy, the patient may at least theoretically be deprived of a chance of subsequent HBsAg clearance. The role of combination IFN/lamivudine remains

Table 2 Current HBV Treatment Choices

Interferon	Lamivudine
Advantages	
High rate of HBsAg clearance in responders	Available orally
Mutant formation absent	Excellent side effect profile
	Efficacy in IFN problem groups
	Does not require host immune activation
Disadvantages	
Injection necessary	Protracted therapy necessary
Difficult to use in cirrhosis	
Poor efficacy in precore mutants	Role in long-term clearance of HBsAg unclear
Requires active host immune response	Mutant formation
Frequent side effects	

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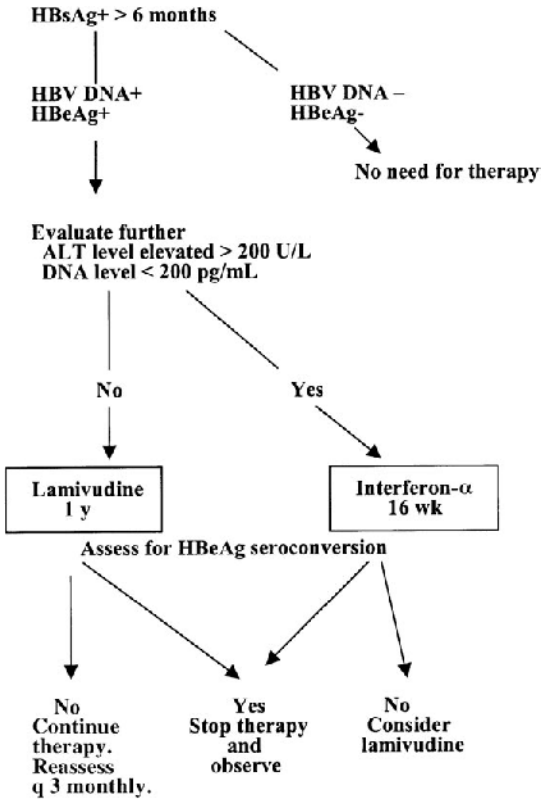

Figure 2 Proposed algorithm for treatment of chronic hepatitis B virus (HBV) infection. ALT = alanine aminotransferase; HBeAg = hepatitis B e antigen; HBsAg = hepatitis B surface antigen.

unsettled, but IFN's immunomodulating effects remain a potential benefit in HBV given that the host immune response plays such a key role in disease outcome. Improved antiviral efficacy with nucleoside therapy awaits both newer agents and, most likely, effective combination therapy, analogous with HIV (21). However, given that effective choices that are already available for the patient with chronic HBV and active replication, therapy should not be deferred. The impact of

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more recently identified viral factors such as HBV genotype will also need clarification in planning therapy (72).

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4

Treatment of Chronic Hepatitis B: Future Approaches

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CASE

A 39-year-old white man who was referred in consultation seeking a second opinion regarding his well-known history of chronic hepatitis B. The hepatitis B infection was diagnosed at age 29, when the patient complained of fatigue, weakness, and dark-colored urine. At that time, his serum alanine aminotransferases (ALT) were abnormal, and the hepatitis B virus (HBV) serology was negative for IgM anti-HBc and positive for HBsAg and HBeAg. A liver biopsy per-

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formed 6 months later was consistent with chronic hepatitis with a moderate portal inflammatory component and mild periportal fibrosis.

HBsAg	Positive
Anti-HBc (Total)	Positive
Anti-HBc (IgM)	Negative
HBeAg	Positive
ALT-90 Iu/L	Negative
Anti-HBs, anti-HBe	Negative

The patient was initially treated with interferon (IFN) alfa-2b with a 5 million unit daily dose for a 16-week period. Although a drop in serum HBV-DNA levels was observed, he did not lose the HBeAg nor normalize his ALT. One year later, a second course of IFN treatment was tried again but this time with previous administration of a short trial of prednisone. Despite developing a serum ALT flare, only a transient loss of the HBV-DNA was observed.

In 1992, the patient was enrolled in a thymosin clinical trial (1), and was randomized to the placebo arm. After 6 months of follow-up, he persisted with HBeAg positivity. For the following 5 years, he remained under clinical surveillance with no further therapy.

In 1998, a liver biopsy was repeated, showing progression of the patient's disease to a precirrhotic stage. In September of the same year, lamivudine therapy was started. Although initially his HBV-DNA became negative, later on he developed a YMDD escape mutant with detectable serum HBV-DNA and elevated ALT.

During the visit to the clinic, several issues emerged: Should the patient continue with lamivudine therapy? Should we offer him combination therapy? Are there new antivirals in the pipeline? Is there any possibility of improving the host immune response and obtaining an immunological clearance of the HBV?

INTRODUCTION

Chronic B hepatitis still represents a global public health problem for the World Health Organization (WHO). About 2

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billion people have been infected worldwide, and approximately 350 million of them are currently chronically infected. Prevalence of infection changes significantly according to different geographical areas, being more prevalent in Asia, Africa, southern Europe, and in the Amazonian basin in Latin America. According to WHO statistics, hepatitis B–induced cirrhosis or liver cancer is the ninth most common cause of death worldwide, taking more than 1 million lives each year (1a). It is estimated that chronic carriers worldwide will number 400 million in the year 2000.

Current available therapeutic regimens for chronic hepatitis B consist of IFN and lamivudine. IFN induces a response in about 38% of the treated patients, with "response" being defined by loss of HBeAg and HBV-DNA (2). Even more important is the fact that more than 85% of responders have a sustained inhibition of viral replication. Shortcomes of IFN therapy include low response rate in Asians, precore mutants, and delta (hepatitis D) coinfected patients. Furthermore, interferon is not recommended in patients with advanced cirrhosis, because the ALT flare that usually precedes HBeAg seroconversion may decompensate the patient even further.

On the other hand, lamivudine, a potent reverse transcriptase inhibitor, suppresses HBV-DNA in the vast majority of the treated patients. Unfortunately, this inhibition of HBV-DNA is not always followed by HBeAg loss, which is observed only in 17–33% of treated patients (3). Furthermore, once therapy is discontinued, serum HBV-DNA levels return to pretreatment values in a high proportion of the patients. Treatment with lamivudine for up to 2 years showed a sustained seroconversion rate of 17–27% (4). However, an important downside of this long-term therapy is the development of resistance. Lamivudine and most of the new antivirals inhibit reverse transcriptase but do not eradicate the covalently closed circular DNA (cccDNA) of the virus localized in the nucleus of the hepatocytes. The persistence of the cccDNA may yield to further transcription and synthesis of cytoplasmic HBV-RNA, predisposing to relapse and resis-

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tance. Lamivudine mutants begin to appear toward the end of the first year of treatment, reaching up to more than 50% of the cases at the end of the second year. Acute exacerbation of the hepatitis followed by clearance of HBV has also been observed in several cases. Therefore, the clinical implication of these mutations has not yet been definitively established (5).

Although treatment alternatives in hepatitis B have improved significantly when compared with what was available a decade ago, as our case describes, there is still a need for improvement in this field, and many patients are still in need of better choices. The following discussion reviews several potential therapies, both theoretical and those that are currently under study (Fig. 1).

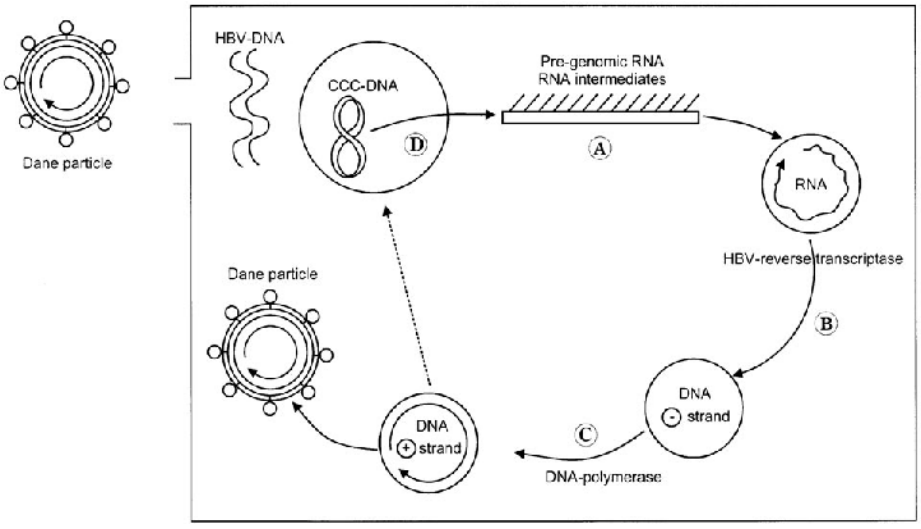

Figure 1 HBV treatment strategies according to genome replication cycle.

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ANTIVIRALS

Several antiviral drugs are currently under investigation, but among them only two are in the clinical arena: adefovir dipivoxil and entecavir (BMS-200475). Other nucleoside and/or nucleotide analogues such as DADP and L-FMAU remain at a preclinical level.

Adefovir Dipivoxil

Adefovir is an oral prodrug of an acyclic nucleotide analogue that selectively inhibits DNA polymerases and reverse transcriptases of hepadnavirus, herpesvirus, and retrovirus. This prodrug is rapidly converted to adefovir in plasma and tissues. Once within the cells, adefovir is phosphorylated by cellular kinases to adefovir diphosphate. This diphosphate is an analogue of dATP, which lacks a 3′OH, and therefore acts as a chain terminator of DNA synthesis.

Adefovir has a good oral bioavailability (30–40%), and its plasma and cellular half-life is approximately 5 and 18 h, respectively, allowing for a once-daily dosing. It is eliminated via urinary excretion, and it has no cytochrome P450 interactions (6).

In human immunodeficiency virus (HIV) studies, after prolonged therapy, about 10% of the patients developed mild to moderate nephrotoxicity reaching serum creatinine values greater than 2 mg/dL (7). Although this toxicity was usually reversible with dose reduction or discontinuation, it deserves further observation in the long-term HBV trials.

Initial clinical studies in HBV patients with adefovir include a pilot and phase II studies. Overall, after a short course of therapy, HBeAg loss was observed in 22–27% of the patients. Furthermore, between 11 and 20% of them developed anti-HBe. HBV-DNA median change from baseline was minus 4.18 and 4.84 log10 with 60 and 120 mg of adefovir, respectively (8, 9). During therapy, viral load decline followed a bi-

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phasic curve, with a median decrease in HBV-DNA at week 12 of 4.3 log10. This initial rapid phase represents the clearance of free virus from the serum and a subsequent and minor second phase, the clearance of virions from infected cells probably reflecting their death rate (10).

Other relevant features of this oral prodrug include the observation that no evidence of resistance has been detected after a short course of therapy, and that lamivudine-resistant mutants remain sensitive to adefovir as documented in several in vitro and clinical studies (10–13).

Based upon these characteristics, we should anticipate that adefovir will probably play an important role as either monotherapy or combination therapy in naive patients with HBV. Moreover, it may also benefit those patients who developed YMDD mutant strains resistant to lamivudine.

Entecavir

Entecavir, a cyclopentyl guanosine analogue (Bristol Myers Squibb 200475), is a selective and potent inhibitor of the HBV-DNA polymerase. Its efficacy against other DNA viruses such as herpes, cytomegalovirus, and varicella zoster is lower than that reported for HBV (14).

The oral bioavailability of this drug varies between 37 and 70% according to the animal model studied. Like other nucleoside analogues, entecavir is phosphorylated to monophosphate, diphosphate, and triphosphate within host cells. After phosphorylation, its half-time life is 15 h, similar to lamivudine, and is cleared mainly by the kidneys (15).

Toxicity studies in animals showed that entecavir produced similar adverse reactions than other nucleoside analogues, such as bone marrow suppression, central nervous system toxicity, and myopathy. In healthy volunteers, the most frequently observed adverse events were headaches, abdominal pain, nausea, diarrhea, dizziness, pain neck, and photophobia.

Entecavir was found to be a powerful inhibitor of HBV-

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DNA polymerase not only by inhibiting the initial priming reaction, but also at the two subsequent steps: reverse transcription and the DNA-dependent DNA synthesis (16). When compared with lamivudine, entecavir has been found to be 30 times more potent. Furthermore, liver intracellular intermediate replicatives and cccDNA levels were also reduced in the woodchuck hepatitis B model (17). If these data are confirmed in the ongoing phase II clinical trial, entecavir will probably represent an important breakthrough in the treatment of chronic hepatitis B, because it may avoid the emergence of escape mutants and decrease the high relapse rate seen with lamivudine.

Other Antivirals

Several other antivirals are being tested in experimental models. Among them, at least three have shown to be more potent than lamivudine: DAPD, L-MAU, and beta-L-5-fluorcytidine.

DAPD is a dioxolane purine nucleoside analogue that has activity against HIV reverse transcriptase and HBV-DNA polymerase in vitro. It is deaminated by adenosine deaminase, yielding dioxolane guanosine (DXG), and the corresponding triphosphate product is a potent inhibitor of the HBV polymerase. Another interesting feature of this antiviral agent is that it has shown to have synergistic effect when added to other antivirals in vitro, and that it partially preserves the antiviral activity against lamivudine-induced resistant variant (18).

L-FMAU is a novel pyrimidine nucleoside analogue that has a potent activity against HBV-DNA polymerase in vitro and in the WHV model. Like DADP, L-FMAU also has a synergistic effect when added to adefovir, and its antiviral activity is not affected by YMDD mutants. Furthermore, treatment in chronically infected woodchucks showed a twofold decrease in the cccDNA levels during the initial 2 weeks of treatment and almost undetectable DNA intermediate replicatives after 6 weeks of dosing (19).

Beta-L-5-fluorcytidine is a recently discovered L-nucleo-

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side analogue with a more potent antiviral effect than lamivudine in the woodchuck model. However, after cessation of therapy, a rebound in viremia is commonly observed and clearance of cccDNA is not achieved (20). Therefore, it seems that it will not have an important role as monotherapy in HBV treatment.

Based upon the experience achieved with the treatment of HIV infection and the emergence of resistant variants after prolonged lamivudine monotherapy, it seems reasonable to suggest that future chemotherapy will probably reside in combination drug regimens, trying to achieve the highest efficacy with the lowest escape rate possible (21).

NEW FORMS OF IMMUNE MODULATION

Owing to the mechanisms of action that current antiviral therapy have, a sustained and complete eradication of the cccDNA and the DNA intermediate replicatives from host's liver cells should not be easily expected. Instead, when elimination of the viral infection occurs because of the host immune response, chances of achieving clearance of cccDNA and therefore a sustained response are more likely. A good example of this is the high sustained response rate observed in those HBV responder patients after IFN-induced immune clearance.

The rationale for using active immune-based therapy is that in chronic hepatitis B patients the humoral and/or cellular immune responses are defective when compared against those with self-limited infection. Thus, enhancing the immune response through a more optimal antigen presentation process may indeed help to achieve viral clearance or better control of the disease (22).

From this standpoint, newer forms of immune modulation remain as an important area of research in the field of chronic hepatitis B therapy. Among these experimental approaches currently under investigation, we will briefly review those who have shown interesting rationale or results such as:

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cytokine therapy, addition of vaccine adjutants, vaccines with different antigenic components, and finally adoptive immune transfer.

Interleukin-12

Interleukin-12 (IL-12) is a glycoprotein with multiple biological effects. It is produced by monocytes and other professional antigen-presenting cells (APCs) in response to bacterial, viral, and fungal infections. After binding to a specific receptor in T lymphocytes and natural killer (NK) cells, it acts as a proinflammatory cytokine eliciting production of interferon gamma (IFN-γ) and tumor necrosis factor (TNF) (23). Among other important actions, IL-12 enhances Th1 and decreases Th2 type of response (24). This shift towards a Th1 predominant cytokine profile is critical for the patient to mount the adequate cytotoxic T lymphocyte (CTL) response necessary for the lysis of virally infected cells (25) (Fig. 2).

In HBV infection, it has been suggested that a predominance of Th2 type of response is one of the pathogenic factors involved in the evolution to chronicity (26). Furthermore, in patients with acute self-limited hepatitis B, a predominant Th1 cytokine response helped successfully to control the infection (27).

In a transgenic mouse model, IL-12 has been able to reverse this Th2 predominance to a Th1 type of response (28). In addition to its ability to promote a Th1 response, IL-12 shares other biological properties such as enhancing the lytic activity of NK and lymphokine-activated (LAK) cells. These nonspecific elements of host immune response are probably implicated in HBeAg seroconversion through a noncytolytic process after CTL-specific response takes place (29). Furthermore, IL-12 can also increase the production of IgG antibodies, particularly opsonizing subclasses of IgG. This enhanced production of virus-specific antibodies may also help to limit extracellular spread of the virus (30).

Toxicology studies in mice and primates have shown that

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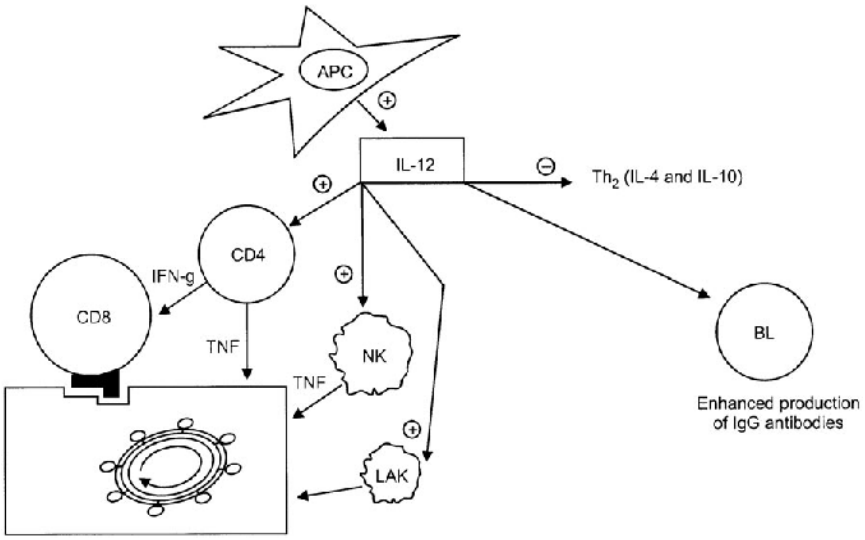

Figure 2 Biological properties of IL-12 and potential role in HBV therapy.

IL-12 can induce fever, lethargy, anemia, leukopenia, thrombocytopenia, and elevated serum aminotransferases. In mice genetically predisposed to develop diabetes, IL-12 may accelerate the onset of autoimmune diabetes mellitus (30).

Although there is consistent data suggesting that IL-12 may have a beneficial impact in the treatment of chronic hepatitis B (31), a recently published phase I/II study showed that only 25% of the patients cleared the HBV-DNA and less than those cleared the HBeAg (32). Further phase II trials are required to determine whether IL-12 treatment administered for longer periods achieves better results and is well tolerated.

Vaccine Therapy

In the past few years, several vaccine therapeutic alternatives have been explored. Already in 1994, Pol et al. conducted a pilot study in chronic hepatitis B carriers to evaluate the

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safety and efficacy of HBV vaccination in inhibiting viral replication (33). A total of 42 patients were subjected to a standard vaccination schedule of three intramuscular doses of vaccine. After completion of the vaccination, serum HBV-DNA dropped to undetectable levels in 11 patients (26%) and decreased more than 50% of the pretreatment levels in another 5 patients (12%). Overall, in approximately 38%, a conventional vaccine demonstrated some ability to decrease serum HBV-DNA levels. Even more interesting than that was the finding that an exacerbation of the hepatitis with its corresponding ALT flare preceded the HBV-DNA loss. This phenomenon suggested in some way that conventional vaccines might also have the potential to induce a CTL response. This initial data still needs to be confirmed by randomized controlled trials.

More recently, Heintges et al. published their results of another pilot nonrandomized trial of a conventional vaccine (Gen H-B-Vax) plus IFN alfa in chronic hepatitis B patients who had failed previous IFN monotherapy. Loss of HBeAg was observed in 7 of 18 (39%) of the patients, and HBV-DNA became undetectable in 9 of 18 (50%) of the patients. Furthermore, response was sustained 6 months after cessation of therapy (34). If these results are confirmed in larger and controlled clinical trials, this combination will probably represent an attractive alternative for nonresponder patients to monotherapy.

A different approach is the combination in the same vaccine formulation of several HBV antigenic components with a potent adjuvant, trying to circumvent tolerance and to elicit a stronger anti-HBV immune response. Wright et al. reported the results of their pilot phase I study in which they administered a HBV recombinant vaccine composed of HBV PreS2 and S antigens together with a potent adjuvant such as oil water emulsion MF59. A total of 13 patients with chronic hepatitis B received four injections of this vaccine at 0, 1, 2, and 6 months. Patients were then followed for 6 more months for adverse events and changes in biochemical and virological parameters. Eleven of 13 patients developed serum anti-HBs and anti-HBe

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with a wide range of titers. Although these changes were associated with a drop in serum HBV-DNA levels, HBsAg, viral DNA, and HBeAg remained detectable. During the course of the vaccination, five patients developed important and transient ALT flares which were followed by declines in HBV-DNA levels, suggesting again that in some cases non–DNA vaccines may also elicit a cellular immune response (35).

Another variant of active immune therapy studied in hepatitis B patients is the CY-1899 T-cell vaccine. This vaccine is a lipopeptide consisting of the following covalently linked components: a CTL epitope derived from HBcAg amino acids, a T helper (HTL) epitope from different residues from tetanus toxoid, and two palmitic acid molecules. These molecules when injected into normal subjects have been found to be safe and effective in inducing HBV-specific CTL (36). The rationale for using this type of vaccine is that during the course of an acute hepatitis B infection circulating CTLs to nucleocapsid are commonly detected (37). This CTL response also induces cytolysis of infected hepatocytes in chronic hepatitis B patients when they spontaneously clear the infection or when they respond to INF treatment (38). The proposed mechanism of action of this vaccine is that HTL and CTL epitopes present in it are processed by APCs or T cells, resulting in expansion of antigen-specific CTLs. This specific T-cell expansion would have the potential to clear the infected hepatocytes. In a recently published study, Heathcoate et al. described their results on the first clinical trial of the CY-1899 T-cell vaccine in patients with chronic hepatitis B infection. Ninety patients were recruited to the study; all of them had HBsAg and HBV-DNA positive in serum for at least 6 and 3 months, respectively. Patients were given CY-1899 vaccine subcutaneous in two to four doses ranging from 0.05 to 15 mg per injection 6 weeks apart. Although a CTL response was demonstrated, the peak response was well below the levels usually seen in patients with acute resolution of the infection, and therefore no significant changes in their viral serology were observed (39).

A potential explanation for the weak response observed

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to this vaccine construct is that in the expanded T cells, the cytokine profile was of a Th0Th2 HTL type rather than Th1. Based on these findings, Livingston et al. proposed to enhance the Th1 response by adding either IFN gamma or IL-12 to this vaccine, trying to overcome nonresponsiveness at the CTL level (40). Perhaps a simpler strategy could be to combine these types of vaccines to lamivudine, inasmuch as this agent has already shown to restore T-cell responsiveness in chronic hepatitis B patients by lowering the viral load (41).

Finally, if in the near future manipulation of dendritic cells becomes feasible, stimulation of this professional APC to produce IL-2 and/or IL-12 may also help to overcome nonresponsiveness to therapeutic vaccines (42).

Adoptive Immune Transfer

Initial reports on experimental models of adoptive immune transfer are from the early 1990s when Shouval et al. reported the results of their experiments in a BALB/c mouse model. They have successfully immunized female BALB/c mice with a recombinant vaccine against HBV. Later, these animals were used as bone marrow donors (BMDs). BALB/c bone marrow transplant (BMT) recipient mice were immunosuppressed with total body irradiation and then injected with bone marrow cells from immunized donors. All BMT recipient mice receiving bone marrow from an anti-HBs–positive donor seroconverted. Furthermore, a booster immunization given 1 month after transplantation resulted in an important rise in serum anti-HBs titers. These results suggested that in bone marrow recipients not only adoptive transfer of immunity to hepatitis B virus was feasible, but a secondary immune response was also possible (43).

Transfer of humoral immunity against hepatitis B virus in humans was initially reported by the same group of investigators. They retrospectively studied the outcome of 12 BMT patients who received grafts from 12 BMDs positive for anti-HBc and anti-HBs. Within several weeks after transplanta-

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tion, seroconversion was documented in all of them without developing acute hepatitis. In a second study, BMDs negative for anti-HBs were vaccinated against HBV before transplantation and after transplantation and seroconversion was assessed in their respective recipients. Although seroconversion to anti-HBs occurred in the vast majority, only a minority of the BMDs responded after several booster injections during the postransplantation period with a secondary immune response (44).

The clinical implication of this form of "passive immunization" is not only that it may help to prevent HBV infection in BMT candidates, but it can also eradicate the infection once it is already established (45–48).

Bone marrow transplantation is an expensive and limited procedure with significant morbidity and mortality. Therefore, this strategy is not a reasonable therapeutic option for conventional chronic hepatitis B patients. Nevertheless, adoptive transfer of immunity could be discussed as a strategy in those patients who already have an indication for BMT and need to prevent or treat an already established hepatitis. Other methods similar to adoptive transfer of immunity, like infusion of autologous or HLA-matched T lymphocytes after in vitro immunization and subsequent expansion, are currently under investigation and will probably soon be in the clinical trials setting.

GENE THERAPY

Gene therapy, a new branch of the already existing therapeutic modalities, is characterized by the use of genetic material or nucleic acids as drugs. The main difference with classic pharmacology is that although gene therapy can direct cells to perform new functions, the former is limited only to modifying existing cellular functions. In order to work, gene therapy requires not only the introduction of foreign DNA into host's cells, but also its adequate expression.

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Although initially considered as an appropriate modality only for the treatment of hereditary disorders, this strategy is currently being studied in a wide variety of fields such as cardiovascular diseases, cancer, autoimmune diseases, HIV infection, and chronic viral hepatitis among others (49).

A viral vector is usually used to introduce the gene into the target cell, but genes can also be directly transferred into tissues as pure recombinant plasmid DNA ("naked DNA"), incorporated into liposomes, or coated onto gold particles. Plasmid DNA gene transfer apparently offers several advantages when compared with viral vectors. Among those, and from a safety standpoint, we should emphasize the lack of immunogenicity, less risk of recombination with host's DNA, and no risk of inadvertent infection (50).

Despite progress obtained in delivery techniques, gene therapy still has several hurdles to overcome before successfully entering the clinical field. The most important technical inconveniences that need to be resolved are: difficulties in the identification of an access to the target cell, limitations in the extent of gene transfer, and magnitude and duration of its expression (51, 52).

Nevertheless, several genetic antiviral strategies are currently being explored in the treatment of chronic viral hepatitis. As is the case in classic pharmacology, gene therapy for viral hepatitis could be classified in two different approaches: those which enhance the host's immune response and those considered as being "direct antiviral." Among the first group, we will review concepts and results from experimental DNA vaccine studies, and among the latter strategies that can block viral gene expression at different levels of the viral cycle such as ribozymes, antisense oligonucleotides, and interfering peptides.

DNA Vaccines

DNA vaccines have several features that make them especially appealing. They do not require cultivation of dangerous

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infective agents, they have no risk of attenuated virus to mutate back to a virulent strain, they have lower manufacturing costs, and they have better stability at room temperature than conventional vaccines (53).

DNA-mediated immunization represents a radical change in the way antigens are delivered to the host. In this approach, a plasmid DNA vector encoding an antigenic protein is injected into the skin or muscle of the host and later expressed within cells of the organism. This leads to a strong immune response of both arms of the immune system: the humoral and cellular branches (Fig. 3) (54, 55). The introduction of the DNA can be accomplished by intramuscular or intradermal injections or by propelling DNA-coated gold particles into the skin (53).

Although DNA vaccines share similar limitations with re-

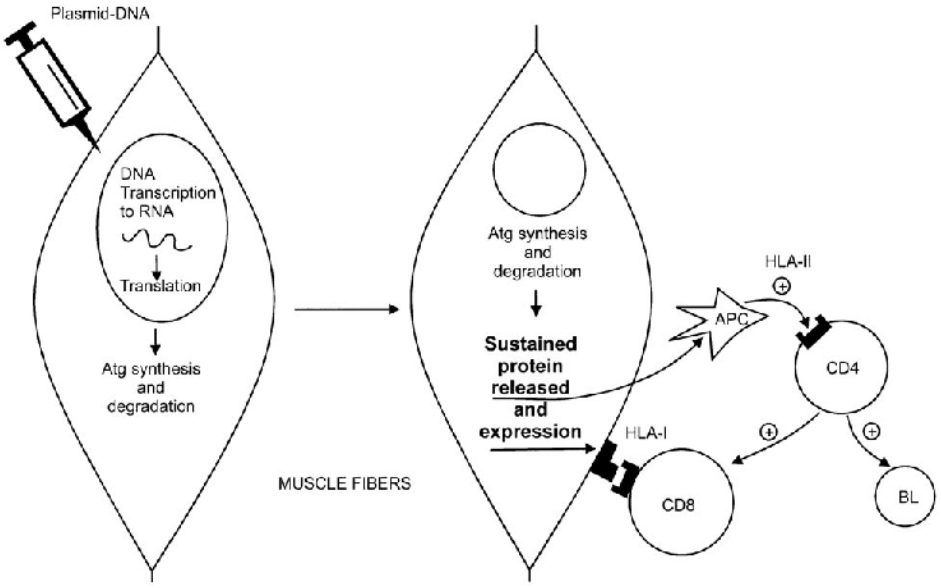

Figure 3 DNA vaccine: steps required for activation of sustained immune response.

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combinant protein vaccines, the fact that they elicit a much stronger humoral response than peptide-derived vaccines and that they indeed are able to induce a CTL response offers potential advantages for the prevention and treatment of viral infections.

The induction of this CTL response is particularly important in the prevention of the infection, because it can recognize and induce cytolysis of those cells that are already infected at the time the humoral response develops. Moreover, CTLs can cross react against different viral epitopes and therefore achieve wider protection against different strains of the same virus. Finally, owing to the central role that the CTL response plays in the immune-mediated elimination of infected hepatocytes, these vaccines also become a potentially interesting therapeutic tool in the field of chronic viral hepatitis (56,57).

DNA-mediated immunization in a transgenic mouse model of the chronic hepatitis B carrier state resulted in a complete clearance of circulating HBsAg and in a sustained suppression of transgene expression of HBV mRNA in the hepatocytes. Mice were immunized with only one intramuscular injection of a recombinant plasmid expressing S and pre-S2 domains of the gene encoding HBV envelope protein. Twelve weeks after the injection, 6 of 6 mice developed anti-HBs and had undetectable levels of circulating HBsAg. Even more striking was the finding that HBV-mRNA was also undetectable in the liver even though no cytolytic response in the liver was observed. Mancini et al. speculated that either CD4 Th1 or CD8 CTLs, via secretion of IFN γ and TNF, might have been the effector cells responsible for the observed downregulation of HBV-mRNA in the liver (58). These results suggest that DNA immunization may be able to overcome B- and T-cell tolerance and induce an adequate immune response able to prevent the spread of free virus to uninfected cells and to downregulate viral gene expression without inducing cytolysis.

Unfortunately, these results were not confirmed in a more recently reported similar hepatitis B transgenic mice

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model by Shimizu et al. In their model, CTL tolerance was broken only by dendritic cell immunization but not by plasmid DNA vaccination (59).

Therefore, until underlying reasons for these conflicting results and other issues related to the efficacy of gene delivery methods are resolved, it seems premature to speculate about the potential value of DNA immunization in the treatment of chronic HBV infection in humans.

Ribozymes

Ribozymes are ribonucleic acid enzymes that have both antisense and RNA cleavage activities. This catalytic activity is the major attraction of these enzymes, because only one ribozyme may block many target RNAs (60).

Ribozyme genes can be delivered to target cells via various viral vector delivery systems, and potentially lead to a stable expression of genetically constructed anti-HBV ribozymes. The rationale for the use of these enzymes as a treatment of hepatitis B virus infection is that HBV undergoes replication through reverse transcription of a pregenomic RNA intermediate. Thus, an enzyme that has the ability to catalyze the sequence-specific cleavage of RNA and RNA-dependent reactions becomes very attractive.

In vitro studies published during the mid-1990s have clearly shown that hammerhead ribozymes can cleave HBV-RNA (61, 62). However, it was not until recently that these promising results were also achieved in vivo. Welch et al. reported their results with the use of a hairpin ribozyme gene against hepatitis B virus in a tissue culture system. Using a retroviral vector, they have transfected ribozymes and HBV genes into hepatocellular carcinoma cell line and assessed their antiviral efficacy by monitoring viral replication. Three hairpin ribozymes targeting pregenomic HBV-RNA as well as specific mRNAs encoding HBsAg, HBV-DNA polymerase, and the X protein were designed. With the goal of increasing their

stability and catalytic efficiency, structural modifications to these ribozymes were performed generating a "tetraloop hairpin ribozyme." These modified enzymes inhibited HBV production up to 83% (63).

Ribozyme therapy will probably evolve into different directions, and the selection of new sites of activity will be one of the most relevant. An example of this is another study in which ribozymes were designed to cleave nucleotides in the HBx open reading frame (ORF). The rationale for choosing HBx-RNA as a target is that HBx protein transactivates the transcription from all HBV promoters. When these ribozymes were cotransfected with an HBx expression plasmid, they reduced the level of HBx mRNA in approximately 50% (64).

In summary, hammerhead ribozymes may become a good gene tool in the treatment of chronic hepatitis B infection, because they can bind and cleave several HBV-RNA targets without affecting cellular RNAs of the host. Furthermore, expression of more than one gene could potentially obviate the need for repeated administration and may result in sustained protection.

Antisense Therapy

Antisense nucleic acids are designed specifically to bind to RNA, resulting in the formation of RNA-DNA or RNA-RNA hybrids. Because of these hybrids, arrest of RNA replication, transcription, and/or translation may occur. Antisense effects can be enhanced by degradation of the RNA hybrids by the host's cellular RNAse. This nonantisense effect potentially associated with this strategy may end in either a benefit or a serious adverse event; therefore, it is one of the Achilles tendon of this treatment strategy (65).

Antisense nucleotides can be made in two different forms: oligonucleotides or "expressed nucleotides." Oligonucleotides are short single strands of DNA made by an automated synthesizer. Instead, expressed nucleotides are made by the

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host's cells after adenoviral or retroviral expression vectors encoding the antisense genes are administered to the patient (66).

Although in vitro and in vivo experimental antisense strategies have been successfully applied in several models, it is still unclear why results are so different, and why some nucleotides are able to inhibit HBV gene expression and others are not (67–70).

Shortcomes of antisense therapy include the destruction of oligonucleotides by the host's Dnase, difficulty in targeting oligonucleotides to a particular cell, short plasma half-life, and the need for parenteral administration. Therefore, it seems that antisense strategy in the treatment of chronic hepatitis B still has a long road to travel before entering the clinical field.

Interfering Peptides

Another form of gene therapy potentially useful in chronic hepatitis B infection is the intracellular synthesis of peptides or proteins with the ability to interfere with the assembly and function of viral proteins. This is a protein-based approach in which interfering peptides are produced within the cells and has the potential advantage over antisense strategies that their effect is independent viral sequence variations (65).

An intracellular single-chain antibody against HBV core protein has been shown to inhibit viral replication and its intermediate replicative forms in a human hepatoblastoma cell line model that produces HBV (71). The rationale for using this particular intracellular antibody approach is based upon the fact that reverse transcription of pregenomic RNA and second-strand synthesis occurs within the HBV nucleocapsid. Thus, blocking HBcAg function with an intracellular antibody may represent an innovative gene therapy approach able to inhibit viral replication.

Dominant negative (DN) mutants are those mutated proteins that suppress the activity of their normal counterparts.

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Inhibition of either RNA or DNA viral replication can be potentially achieved with this strategy. Furthermore, genetically engineered DN is also able to strongly inhibit HBV replication, apparently by interfering with packaging of the viral pregenome and reverse transcription. Their potential advantage when compared with antisense or ribozymes is that they also have a relatively low risk of developing escape mutants (65, 72).

CONCLUSIONS

Significant advances have been made in the treatment of chronic hepatitis B during the past decade. However, in a high proportion of these patients, a sustained eradication of the viral infection cannot be achieved either with IFN or lamivudine monotherapy.

For this group of patients, newer strategies are urgently needed. Combination therapy has been shown to improve the success rate in chronic hepatitis C and in HIV infection; therefore it is reasonable to speculate that it may also be helpful in the treatment of chronic hepatitis B patients. The question is whether we should combine only antiviral drugs or an antiviral plus an immunomodulatory agent. Unfortunately, the preliminary experience with combination therapy of IFN and lamivudine has been frustrating. Furthermore, clinical studies with combination of more than one antiviral agent have not been developed yet.

A wide range of novel antiviral approaches such as cytokine therapy, vaccine adjuvant, DNA vaccines, antisense therapy, and other forms of gene therapy are currently under investigation. Although in most of them their efficacy in vitro has been clearly established, similar results have not always been documented in "in vivo" models. Once they prove their efficacy and lack of toxicity, they may complement the already existing armamentarium of treatment strategies for hepatitis B infection.

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5

Treatment of Chronic Hepatitis B in Transplant Recipients

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CASE 1

A 29-year-old Asian man was emergently referred for liver transplantation with massive ascites, stage III coma, fever, and marked muscle wasting. His laboratory tests revealed: HBsAg positive (+), anti-HBc (+), anti-HBs negative (−), HBeAg (+), anti-HBe (−), HBV (hepatitis B virus) DNA (+) (2100 pg/mL), anti-HCV (hepatitis C virus) (−), anti-HDV (hepatitis delta virus) (−), alpha-fetoprotein (AFP) 11.7 ng/mL (11.7 μg/L), albumin 2 g/dL (22 g/L), bilirubin 4.1 mg/dL (70 μmol/L), INR (international normalized ratio) 2.2, aspartate aminotransferase (AST) 59 U/L, and alanine aminotransferase (ALT) 52 U/L. His ultrasound examination revealed a small liver, large spleen, massive ascites, no liver mass, patent portal vein, and extensive intra-abdominal varices. The patient was admitted to the ICU and listed as status 2A for liver transplantation due to the ris-

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ing serum creatinine in combination with the failure of his liver. A donor liver was obtained within 24 h. The consultant hepatologist and surgeon devised a plan to minimize recurrence of HBV infection in this high-risk setting.

HBsAg	Positive
Anti-HBc	Positive
Anti-HBs	Negative
HBeAg	Positive
Anti-HBe	Negative
HBV-DNA	2100 pg/mL
Anti-HCV	Negative
Anti-HDV	Negative
AFP	11.7 ng/mL
Albumin	2 g/dL
Bilirubin	4.1 mg/dL
INR	2.2
AST	59 U/L
ALT	52 U/L

BACKGROUND

Liver transplantation for patients with the diagnosis of acute and chronic HBV infection has evolved considerably over the last decade. Originally, a high rate of graft infection and early graft failure, as well as frequent patient death, led to skepticism about the use of orthotopic liver transplantation (OLT) for chronic HBV infection. Marked improvements in long-term survival have resulted from the use of low-dose immunosuppressive therapy and administration of indefinite hepatitis B immune globulin (1–6).

The goals of treatment of HBV infection include decreasing viral load, lessening infectivity, decreasing the level of hepatic inflammation, preventing or slowing the development of cirrhosis and liver failure, delaying time to liver transplantation as well as preventing liver cancer. The first step in the clearance of HBV infection is termed triple seroconversion when HBeAg becomes negative and anti-HBe becomes posi-

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tive. Concomitantly HBV DNA becomes undetectable by quantitative assay and often by the most sensitive polymerase chain reaction (PCR) test.

HEPATITIS B IMMUNE GLOBULIN

Hepatitis B immune globulin (HBIG) has been used extensively after liver transplantation to prevent graft infection for patients with HBV-induced cirrhosis or acute liver failure (7–11) (Fig. 1), although a strict requirement for compliance with injections is required for maximum efficacy. HBIG is a polyclonal antibody preparation derived from blood donors who have been exposed to and cleared HBV infection. Presently, HBIG is being derived from HBV vaccine recipients.

Samuel et al. were the first to describe the use of HBIG in a large clinical trial to prevent recurrent liver disease after liver transplantation in patients with liver failure due to HBV infection (8–10). Survival rates in this first trial were mark-

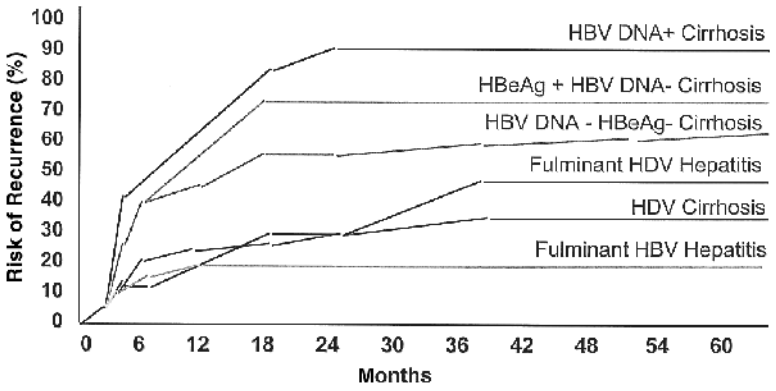

Figure 1 Risk of recurrence of hepatitis B virus after orthotopic liver transplantation depending on viral status before transplantation as reported by Samuel et al. and the European Concerted Action on Viral Hepatitis (EUROHEP) Group. HBV, hepatitis B virus; HDV, hepatitis delta virus; HBeAg, hepatitis B e antigen; (+), positive; (−), negative.

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edly improved, and other centers also demonstrated improved survival to greater than 80% after instituting indefinite HBIG therapy. HBIG is now the standard of care at most centers worldwide that provide liver transplantation (12–15) {Samuel, Bismuth, et al. 1993 ID: 16367} (4, 4, 8, 16–29). HBIG therapy is especially effective when the patient's serum is negative for HBeAg and HBV DNA before liver transplantation and if HBIG dosing is adjusted to maintain anti-HBs blood levels of at least 20 IU/mL, preferably 100 IU/mL or (according to some investigators) 500 IU/mL (30). High-dose HBIG prophylaxis has also been used with a high level of efficacy if the HBV-DNA level is significantly elevated before OLT (22, 23). One major question identified has been if and when patients can be discontinued from HBIG therapy. Preliminary findings suggest that recurrence rates of HBV infection in the liver graft are in excess of 10–20% if HBIG is stopped (28).

Attempts to find sources of high-titered HBIG have led to the innovative process of acquiring fresh frozen plasma as a possible substitute for standard HBIG (24). HBIG probably aids in managing post-OLT patients coinfected with HBV and HDV, because the binding of circulating particles that contain HBsAg includes those packages that contain delta virus. Cases have been described of fulminant hepatitis B failure in patients who received a liver transplant for end-stage liver disease due to HBV/HDV coinfection (31). If graft reinfection occurs after HBIG is discontinued or fails, a benign disease course is often seen, probably as a result of the delayed onset of disease and disease occurrence when patients underwent reduced levels of immunosuppression.

The use of a monoclonal HBIG has been described in the setting of liver transplantation (32). An initial study with a monoclonal antibody directed at the HBV surface protein was conducted in 1990 and resulted in the appearance of an aggressive mutant form of HBV in two liver transplant recipients. These escape mutants were associated with rapidly progressive liver disease (33–35). This result implies that this monoclonal antibody may have induced a genetic drift or al-

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lowed emergence of a defective virus that was extremely pathogenic. Alternatively, the presence of this highly conserved antibody allowed an existing mutant virus to escape immune surveillance. Studies with this monoclonal antibody were halted.

The use of a polyclonal HBIG preparation can also result in the emergence of HBV surface antigen viral mutations as well (36). Changes in the surface region on the HBV genome may result in "escape" from binding to HBIG and allow active and measurable viral replication. Other mutant hepatitis B viruses not associated with the use of HBIG therapy have recently been described in a number of transplantation and nontransplantation settings (37–39), including HBsAg mutants (35, 40). These newly described viruses have widely varied pathogenic potentials (41–44). The high morbidity and mortality after liver transplantation for HBV infection has been attributed to an increased rate of viral replication due to the use of immunosuppression. This immunosuppression results in the activation of viral replication resulting in aggressive hepatitis and the subsequent rapid development of liver failure (Fig. 2). This moderate to severe hepatitis evolves in some

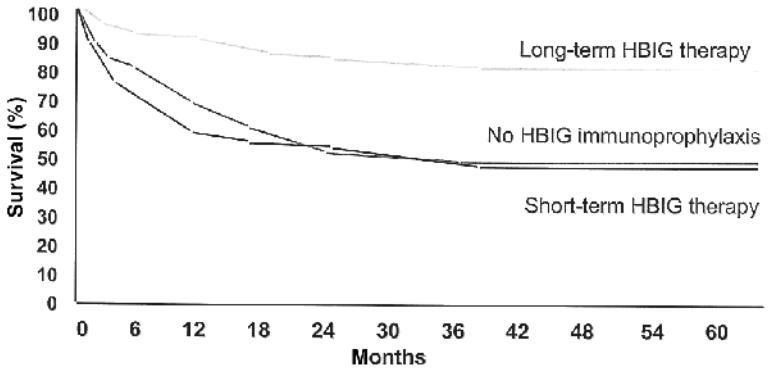

Figure 2 Survival of patients treated without and with hepatitis B immune globulin (HBIG) therapy after liver transplantation as reported by Samuel et al. and the European Concerted Action on Viral Hepatitis (EUROHEP) Group.

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patients to a rapidly progressive liver disease with cholestasis, jaundice, hepatic fibrosis, and liver failure, as well as sudden and severe multiorgan dysfunction and has been termed fibrosing cholestatic hepatitis (FCH) (33, 39, 45–49). If recurrence of hepatitis B infection is suspected after OLT, immunoperoxidase staining for the HBV core and surface antigens is useful to document disease severity and to differentiate recurrent HBV disease from rejection (15, 50). A lobular form of hepatitis has also been described in patients who have a recurrence of HBV infection (47, 51–53).

NUCLEOSIDE ANALOGUES

It now appears possible that patients who develop severe recurrent HBV infection after OLT can be managed with nucleoside analogues (46, 54–65). Lamivudine therapy was shown in a major clinical trial to decrease HBV DNA levels before liver transplantation and would be an important first step in managing the patient described in Case 1 (66). The dose of lamivudine needs to be adjusted for the level of renal insufficiency, and therefore the patient in Case 1 was started at 50 mg three times per week. HBIG therapy was initiated in the operating room and administered intravenously daily for the first week and then intramuscularly monthly thereafter. Anti-HBs titers were targeted to be approximately 100 IU/L, although this level could not always be achieved, since the HBIG therapy was often in short supply.

Lamivudine alone or in combination with HBIG has also been shown in clinical trials to be effective in the long-term management of recipients of liver transplants (Table 1) (67). The combination of lamivudine and HBIG is probably the best current therapy, since lamivudine monotherapy, especially in the setting of immunosuppression, has a very high mutation rate leading to resistance (YMDD motif mutation as well as others) and active viral replication in more than 40% of patients after 2 years of therapy post-OLT (55, 68–75). Unlike

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Table 1 Recurrence of Hepatitis B Virus at Screening Before and 24 Weeks and 52 Weeks After Orthotopic Liver Transplantation with Lamivudine Therapy

Time of testing	Number (%) of patients with positive viral examination for			Mean ALT (U/L)	Knodell score
	HBsAg	HBeAg	HBV-DNA		
Screening	52/52 (100)	44/52 (85)	45/52 (87)	116	10.0
24 weeks	51/52 (98)	36/50 (72)	2/52 (4)	71	—
52 weeks	48/51 (94)	31/49 (63)	13/52 (25)	67	6.5

HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; HBV-DNA, hepatitis B virus DNA; ALT, alanine aminotransferase.

patients who are not undergoing immunosuppression, these patients with lamivudine-resistant mutants often have more aggressive liver disease (76). If lamivudine therapy is stopped, the mutant virus may revert to the wild type (77). Nevertheless, even after the emergence of the viral mutations, lamivudine is often continued in patients because of the perception that liver disease is milder with the appearance of the YMDD mutant and continuation of lamivudine therapy as compared to discontinuation of antiviral therapy and reemergence of the wild-type virus.

The emergence of these nucleoside-resistant mutants has now also been described in patients treated with famciclovir (70, 78–80). Nucleoside-resistant mutants have provided further information on the mutation rate of the HBV virus and the similarity of the HBV reverse transcriptase (DNA polymerase) with the human immunodeficiency virus (HIV) reverse transcriptase. The most common resistant mutant is described as the YMDD mutant, with a variety of different point mutations nearby also associated with nucleoside resistance.

Multiple-point mutations have now been described in some patients with the use of one or more nucleoside analogues. Most importantly, patients who become resistant to lamivudine are resistant commonly to famciclovir and will probably be resistant to most of the new nucleoside analogues being developed (60, 63, 63, 64, 70, 76, 78–85). Adefovir (Gilead Sciences, Foster City, CA) may evolve as a "rescue" drug for patients with HBV resistant to lamivudine. Resistance to adefovir is also expected, and the ultimate management of immunosuppressed patients will evolve with the development of combination antiviral drug strategies (86–89).

Some investigators have proposed that the virus with lamivudine or nucleoside resistance is "less pathogenic," but many reports in the literature have attributed rapidly progressive liver disease to this mutant virus after liver transplantation, or for that matter in the setting of a normal immune system (55, 68, 69, 72, 77, 90–97). The natural history of such mutants has not been established and the best step may be to avoid the possibility of mutants developing by using HBIG in combination with a nucleoside analogue. To date, few patients undergoing such therapy have been reported to develop the emergence of either a nucleoside-resistant mutant or HBIG-resistant viruses. The patient in Case 1 was successfully transplanted and then maintained for 4 years with normal liver enzyme levels as well as HBsAg and HBV-DNA negative using a combination of HBIG administered intramuscularly every 4 weeks and lamivudine given at 100 mg per day.

Other nucleoside analogues have been used in liver transplant recipients with modest levels of success such as famciclovir (Famvir, SmithKline Beecham Pharmaceutical, Philadelphia, PA), which is the prodrug of penciclovir (BRL 39123; [9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine]) (58, 60, 63, 64, 70, 76, 78–85). Famciclovir appears to be a less potent nucleoside analogue than lamivudine, with only a 1- to 2-log reduction in HBV-DNA levels. Resistance is now being described

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with a number of variant mutations having been identified (33, 55, 58, 60, 64, 78, 78, 84, 85, 98–102).

INTERFERON

Application of interferon to control recurrent HBV infection after liver transplantation appears to have little effect on disease, although viral suppression can be obtained in some patients (103–106). HBsAg seroconversion has been reported in this clinical setting after the use of interferon in transplant recipients, although this is an unusual event. At this time, interferon therapy for the post-OLT patient is used but only recommended in rare circumstances.

OTHER ANTIVIRAL THERAPIES

Ganciclovir (DHPG, Cytovene, Roche, Palo Alto, CA) is a nucleoside analogue that has been shown to have efficacy for the treatment of cytomegalovirus infections in HIV-infected patients and patients who are immunosuppressed after organ transplantation. Ganciclovir has also been used to control HBV disease after liver transplantation. Unfortunately, ganciclovir must be used intravenously to achieve maximum levels of concentration in the blood and only reduces viral levels by 1–2 logs (65).

Results of two recent pilot studies of intravenous therapy with ganciclovir have demonstrated a decrease in serum levels of HBV. During intravenous infusion of ganciclovir, progressive liver disease may be slowed or controlled in most patients who have had recurrence of HBV infection with inflammation and fibrosis after organ transplantation. The high cost of intravenous infusion and the low oral bioavailability of encapsulated ganciclovir at lower levels of activity have led to only rare use of this antiviral agent. Unfortunately, both famciclovir and ganciclovir have potential toxicity, which could re-

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sult in more severe side effects when used together, and will probably never reach clinical trials as a combination therapy. Other problems that have been identified with ganciclovir include the issues of teratogenicity, carcinogenicity, and the suppression of spermatogenesis.

Management of liver transplant recipients can also be enhanced by the use of low-dose immunosuppressive therapy (5, 49, 107–112). There has been some speculation that Asian patients with chronic HBV infection may do worse than non-Asians after liver transplantation (113). Recent data from another U.S. transplant center contradicted this speculation (114). The decreased survival at some centers in this ethnic group may be based on late referral or excess immunosuppressive therapy.

A pre-OLT ultrasound did not show any evidence of liver cancer in the patient in Case 1. Liver cancer in combination with HBV infection was an ominous finding according to case series findings published in the late 1980s and early 1990s (115–121). Recent studies show little impact of liver cancer on long-term survival of the recipient when known or incidentally discovered in patients who underwent OLT for end-stage liver disease due to HBV (122). This improved survival rate is probably the result of more stringent screening for patients who have smaller tumors with less likelihood to be metastatic.

CASE 2

A 36-year-old Pakistani woman was diagnosed with hepatitis B. She presented with muscle wasting, fatigue, a sleep disorder, poor memory, and an inability to concentrate. She denied ascites, intestinal bleeding, fevers, chills, or sweats. She had some lower extremity edema over the last few weeks. Her laboratory tests included HBsAg (+), anti-HBc (+), anti-HBs (−), HBeAg (−), anti-HBe (+), HBV DNA (−), anti-HCV (−), anti-HDV (+), AFP 19 ng/mL (19 μg/L), albumin 3.2 g/L, (32 g/L) bilirubin 2.0 mg/dL (34 μmol/L), INR 1.2, AST 98 U/L (98 U/L), ALT 65 units/L (65 U/L), platelets 54 ×

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$10^3/\mu L$ ($54 \times 10^9/L$), white blood cells $2.4 \times 10^3/\mu L$ ($2.4 \times 10^9/L$) and hematocrit 34% (0.34). Her ultrasound revealed a small liver, large spleen but no ascites, no liver mass, and a patent portal vein. The patient was listed for liver transplantation and a treatment plan was discussed.

HBsAg	Positive
Anti-HBc	Positive
Anti-HBs	Negative
HBeAg	Negative
Anti-HBe	Positive
HBV-DNA	Negative
Anti-HCV	Negative
Anti-HDV	Positive
AFP	19 ng/mL
Albumin	3.2 g/L
Bilirubin	2.0 mg/dL
INR	2.2
AST	98 U/L
ALT	65 U/L

HEPATITIS B WITH SUPERIMPOSED HEPATITIS D

Interestingly, this second patient had no evidence of active viral HBV replication other than the presence of HBsAg. This lack of active replication, negative HBeAg, and negative HBV DNA occurred because of the presence of HDV infection that suppressed HBV replication. Hepatitis delta virus is an RNA virus of plant origin that requires HBV to package viral particles for secretion but does not require HBV for viral replication. HDV complicates both acute and chronic HBV infection in certain high-risk settings, including intravenous drug use, high-risk sexual activity, and travel and contact in endemic areas. Coinfection with HDV and HBV often results in more serious disease. In each patient there is a much higher chance

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of fulminant or rapid liver failure if HDV is present in combination with HBV. When new infection occurs with both viruses simultaneously, the patient will most commonly develop severe acute hepatitis. When delta infection is imposed on a chronic HBsAg carrier, new infection or "superinfection" with HDV results in accelerated liver disease with a much higher, at least 60%, chance of a patient developing cirrhosis (123–125).

Only a few effective medical therapies have been discovered that lead to a sustained decrease in the level of HBV replication or that result in clearance of HBV from the serum or liver. Treatment with interferon has now been clearly documented to a decrease the rate of progression to cirrhosis and liver failure and appears to decrease the risk of liver cancer. Clearly, the patient described in Case 2 does not fit the "ideal" subset of patients who respond to interferon and clear HBsAg, since the patient was coinfected with HDV. Interferon is only efficacious in patients who have an ongoing native immune response as shown clinically by an elevated ALT or AST (preferably greater than 200 U/L) as well as the presence of portal and interface hepatitis on liver biopsy. These ideal patients must also fit a profile in which there is only a modest level of viral replication (HBV DNA levels between 1 and 200 pg/mL). Using interferon as a single agent to manage patients with cirrhosis is complex and fraught with potential serious complications. Liver decompensation has been reported with use of interferon and the patients with cirrhosis who are undergoing interferon therapy are at increased risk of developing bacterial infections that may be life threatening (126–130).

Interferon also has a role in managing HDV infection, although treatment must take place for 1 year with the goal to reduce inflammation and slow progressive liver disease, not to clear or cure HDV/HBV infection. The patient in Case 2 received low-dose interferon at 500,000 U per day via a subcutaneous injection and the dose was increased to 1.5 million units per day over the next 3 months. Liver enzyme levels decreased to normal and liver function, including albumin levels

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and INR, returned to normal. The patient was treated for 30 months at this low dose, and nearing the end of treatment, the patient remains HDV-RNA negative and HDV antigen negative. The patient has not required liver transplantation and remains listed as a status 3 with ongoing liver cancer screening.

Currently, nucleoside and nucleotide analogues form the cornerstone of the treatment of human immunodeficiency virus (HIV). Also, nucleoside-type medications are currently used worldwide for the treatment of chronic viral infections caused by DNA viruses such as cytomegalovirus and herpes viruses. Nucleoside analogues, specifically lamivudine, are clearly able to inhibit the replication of HBV but unfortunately rarely result in clearance of HBsAg. Lamivudine (3TC, 3-thiacytadine, (−) 2′-deoxy-3′-thiacytadine 5′-triphosphate, BCH-189, Epivir [Glaxo Wellcome, Research Triangle Park, NC]) clearly decreases HBV replication in patients who were not immunosuppressed and leads to triple seroconversion in 17% of patients treated for at least 1 year (91, 131, 132). The roles and uses of current and future anti-HBV therapies are outlined in Table 2. Since the levels of HBV replication are low in concomitant HDV/HBV infection, nucleosides appear to have little role in the management of patients coinfected with these two viruses as confirmed in a prospective study by Wolters et al. (133). According to these data, the patient described in Case 2 would not be benefited by the use of lamivudine therapy before transplantation.

In Case 2, lamivudine was administered to the patient immediately before and continuously after liver transplantation to aid in the posttransplantation prevention of HBV recurrence in the graft (54, 56, 98, 134–136). One other shortfall of lamivudine and related nucleoside analogues is the risk of patients developing viral mutants that are resistant to lamivudine and subsequently to other nucleosides. To prevent such recurrence, most centers are using hepatitis B immune globulin in combination with lamivudine to prevent graft reinfection and replication (66, 137–139). Intramuscular administra-

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Table 2 Potential Roles of Antiviral Agents for Hepatitis B Virus in Managing Liver Transplantation Patients

Antiviral agent	Potential role
Lamivudine	Defined role before and after transplant
Polyclonal HBIG	Defined role after transplant
Monoclonal HBIG	No role
Famciclovir	Possible role
Ganciclovir	Possible role
FTC	Probable future role
Ribavirin	No role
Adefovir	Probable future role
Entecavir	Probable future role
Combination HBIG-nucleoside	Current best treatment
Combination nucleoside/nucleotide	Probable future role
Therapeutic hepatitis B virus vaccine	Unknown

HBIG, hepatitis B immune globulin; FTC, emtricitabine.

tion of immune globulin is rapidly replacing intravenous administration because of markedly decreased costs (111, 140).

COMBINATION THERAPIES

The use of two nucleosides to control HBV is theoretically useful and has some basis in cell and animal models. Few trials to date document the use of such agents in coinfected patients, although preliminary results are encouraging (86). No antiviral therapy is known to inhibit HDV replication except possibly interferon in humans. In patients with HBV infection alone, interferon when used after 2 months of pretreatment with lamivudine is of little benefit as well (66). Also, interferon would not be advised for the patient described in Case 2 because of the high risk of liver decompensation. Combination therapy with interferon and lamivudine would not be used, since there is no known clinical benefit before liver transplantation.

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Patients who undergo liver transplantation with HDV infection have an overall good survival rate. Suppression of HBV replication by delta hepatitis has led to better survival in the setting of coinfection compared to HBV infection alone as revealed in early reports (7, 15, 123) (see Fig. 2), probably as a result of the effect of HDV on HBV viral replication (suppression). The importance of the use of hepatitis B immune globulin (HBIG) is not well defined in patients coinfected with HDV/HBV, although most centers would use a peri- and post-OLT protocol that included the use of HBIG. The requirement for post-OLT lamivudine in Case 2 is also not clear in this patient with coinfection, although this combination (lamivudine and HBIG) is used at most centers that provide liver transplantation to any patient who is HBsAg positive.

Fortunately, the patient in Case 2 was not HCV antibody positive. Coinfection with HCV occurs in a small subset of patients and often leads, like HDV, to suppression of HBV replication (111, 141–144). The clinical course is varied when HCV and HBV coexist in the same patient, with some patients having much more rapidly progressive liver disease. Treatment, even in the setting of modest fibrosis, is complex, since such patients rarely respond to interferon therapy either in terms of HCV or HBV viral reduction or sustained response.

CASE 3

A 13-year-old male youth received a liver transplant emergently for the diagnosis of fulminant liver failure due to acetaminophen overdose. All viral markers for A, B, and C were negative before the surgery. Because of the patient's deep stage 4 coma and impending herniation, the liver transplant team accepted a liver from an anti-HBc–positive donor. The young boy did well for 8 months after transplantation and then presented with acute symptoms, including fever, jaundice, malaise, and fatigue. His laboratory tests reveal HBsAg (+), anti-HBc (+), anti-HBs (−), HBeAg (+), anti-HBe (−), HBV-DNA (+) [101 pg/mL], anti-HCV (−), anti–hepatitis delta (−), AFP [9 ng/mL (9 μg/L)], albumin [4.2 g/dL (42 g/L)], bilirubin [9.1

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mg/dL] (156 μmol/L), INR 1.2, AST 1200 U/L, ALT 900 U/L, and NH_3 280 μg/dL (165 μmol/L). His physical examination showed mild ascites, some muscle wasting, scleral icterus, and confused mental status. Imaging tests of the liver showed an enlarged liver and spleen as well as a small amount of ascites. Immediate therapy was initiated to abort a rapid progression to liver failure.

HBsAg	Positive
Anti-HBc	Positive
Anti-HBs	Negative
HBeAg	Positive
Anti-HBe	Negative
HBV-DNA	Positive
Anti-HCV	Negative
Antihepatitis delta	Negative
AFP	9 ng/mL
Albumin	4.2 gm/L
Bilirubin	9.1 mg/dL
INR	1.2
AST	1200 U/L
ALT	900 U/L
NH_3	280 μg/dL

ANTI-HBc-POSITIVE DONORS

The use of anti-HBc-positive donors presents a special problem to the transplant and infectious disease clinician (Table 3) (145–149). If a liver transplant is not immediately available, the use of a donor who is anti-HBc positive (high-risk donor) is considered at many centers. Donors who are anti-HBc positive pose a significant risk (ranging from 34 to 86%) of transmitting HBV infection to the liver transplant recipient (150–152). These donors have a high risk of transmitting HBV because of occult hepatitis infection (presence of HBV genomes in the liver) in spite of being HBsAg negative. Surgeons have even been reported to transmit HBV that can result in de novo HBV infection in liver transplant recipients (153).

Patients who receive anti-HBc-positive transplants are a challenge to manage because of the presence of a high viral

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Table 3 Risk of Hepatitis B Virus Transmission to Recipients of Orthotopic Liver Transplantation if Donor Is Positive for Antibody to Hepatitis B Core Antigen

	No. of liver organ transplant recipients				
Author (Reference)	Studied	HBsAg(+)	With (+) DNA tissue donor	Received HBIG post-OLT	Level of protection
Roque (150)	16	4	14	10	100%
Dickson (152)	23	18	NA	None	—
Wachs (163)	6	3	NA	None	—
Dodson (164)	25	18	NA	None	[a]
Prieto (165)	30	14	NA	[b]	100%

[a] Four of 18 donors positive for anti-HBs.
[b] Five recipients positive for anti-HBc before orthotopic liver transplantation.
OLT, orthotopic liver transplantation; anti-HBC, hepatitis B core antigen; HBsAg, hepatitis B surface antigen; (+), positive; HBIG, hepatitis B immune globulin (therapy); NA, not applicable; anti-HBs, antibody to HBsAg.

load in the setting of immunosuppression. Only a few clinical trials suggest that interferon has a role in managing such patients, although viral clearance is suggested by clearance of HBV-DNA and undetectable levels of HBsAg in some patients. There may be a risk of rejection if interferon is used after liver transplantation, although the risk is probably low (154). The most likely treatment choice for recipients of anti-HBc-positive transplants would be either lamivudine or famciclovir, both of which have been described in detail earlier in this chapter.

FIBROSING CHOLESTATIC HEPATITIS

Our patient in the Case 2 clinical scenario appeared to have fibrosing cholestatic viral hepatitis. In the setting of immunosuppression, HBV becomes cytopathic and fills liver cells with

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virus-derived proteins, including core and surface antigen as well as high levels of HBV-DNA in the cytoplasm as documented by in situ hybridization (155). These events often lead to severe cell dysfunction and rapid liver failure. The patient may present with ascites and may even expire of infectious complications unrelated to HBV infection. Retransplantation has been performed in patients with fibrosing cholestatic hepatitis (FCH); unfortunately patients who have rapid liver failure shortly after OLT have much poorer survival (156, 157).

Lamivudine and ganciclovir have been used to treat FCH (53, 65). Initially there is a good success rate as defined by the marked decline in concentration of bilirubin and improvement in liver enzyme levels. The INR declines and ascites, if present, often resolves. Famciclovir may also be useful for managing such patients, although no publications describe such use to date. The long-term management of these patients is more complex. The risk clearly exists of developing viral mutations and subsequent more rapidly progressive liver disease. Future management may include the use of combination nucleoside medications, including the addition of oral ganciclovir or famciclovir to lamivudine early in the course of treatment. A new form of ganciclovir, valganciclovir, is being developed by Roche Pharmaceuticals.

Patients with severe recurrent HBV infection, including FCH, can undergo liver retransplantation but costs are extremely high and long-term survival is inferior to that of patients undergoing a first liver transplantation (158, 159).

IMMUNE THERAPIES

A recent interesting proposal that has not been used often is simultaneous transplantation of liver with bone marrow from a vaccinated donor or immune donor (160). One must be careful in defining "immunity," since patients who are both anti-HBc and HBs have been reported to transmit HBV to organ transplant recipients. This method of transferring cellular im-

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munity was originally pioneered in bone marrow transplant patients (161, 162), leading to HBV clearance in the recipient of the bone marrow transplant.

The administration of standard HBV vaccine to liver transplant recipients who are infected with HBV probably has no effect on viral replication or clearance. New forms of HBV vaccine being developed by Chiron and Smith-Kline-Beecham are highly immunogenic and may, alone or in combination, allow a more vigorous T-cell response to bring about a durable seroconversion.

In our patient described in Case 2, a second transplant was obviated by the immediate initiation of the oral nucleoside agent lamivudine and rapid reduction in immunosuppression. One year after the episode of acute hepatitis, this patient's serum levels of liver enzymes were slightly elevated, all liver synthetic test results were normal, and there was no evidence of portal hypertension on physical examination, although the patient remained HBsAg positive.

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6

Alternative Therapies for Chronic Hepatitis C

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CASE

A 45-year-old man with chronic hepatitis C and hyperlipidemia was referred for antiviral treatment. He had a long history of cigarette smoking and was recently diagnosed with angina pectoris. His medications included a beta-blocker, a baby aspirin, a lipid-lowering agent, and sublingual nitroglycerin as needed. He had no known drug allergies. He complained of mild fatigue but was otherwise asymptomatic. Physical examination was unremarkable except for an S4 heart gallop. Pertinent laboratory evaluation revealed the following:

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WBC	8×10^9/L
Absolute neutrophil count	2.4×10^9/L
Hemoglobin	12.9 g/dL
Hematocrit	39%
Platelet	250×10^9/L
HCV-RNA	300,000 IU/mL
HCV genotype	1a
Serum ferritin	450 ng/mL
Liver biopsy	Grade 3; stage 3

Because of the coronary artery disease, a decision was made to avoid ribavirin. In light of the low response rate to interferon monotherapy, adjunctive therapies were discussed, including the use of thymic peptides, aspirin-like drugs, amantadine, and botanicals. The risks and benefits of iron reduction therapy were also discussed. After reviewing the alternatives, the patient opted to avoid all treatment until the availability of better antiviral options.

INTRODUCTION

The need for alternative therapies for the treatment of chronic hepatitis C is based on the realization that, despite major advances in antiviral therapy, many patients fail to respond to the best agents currently available. Up to 60% of individuals appropriately treated with interferon-based regimens will not achieve a sustained response, and in patients with genotype 1, only 25–30% will have a sustained response to combination therapy with interferon and ribavirin. Efficacy may be further limited in some patients due to dose-limiting toxicities, such as depression or anemia, or the presence of relative contraindications to therapy (cardiac disease, renal failure) that preclude the use of these agents.

THYMIC PEPTIDES

The pathogenesis of hepatitis C virus (HCV) infection is poorly understood, but the immune system is believed to play a criti-

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cal role in eradicating the virus upon initial infection and during therapy. Studies have demonstrated differences in the pattern of CD4+ T-cell responses in those individuals who have complete virological recovery after HCV infection compared to those who progress to chronic infection (1). Moreover, individuals who have a strong, polyclonal proliferative response to HCV antigens are more likely to have a low viral load (2). The cytokines produced by these CD4+ T cells are generally type I (interferon gamma, interleukin-2, and tumor necrosis factor-α (TNF-α), suggesting that cellular immune responses typically mediated by these cytokines are crucial to controlling the infection. Treatment trials of chronic hepatitis C in immunocompromised populations also lend credence to the role of the immune system in eradicating infection. Severely immunocompromised hosts are less likely to clear infection with treatment (3–5). The exact mechanism of action of interferon is unknown, but it is known that interferon has both antiviral and immunomodulatory properties. Thus, one potential method of enhancing treatment response would be to combine interferon with one or more immunomodulatory compounds.

Extracts of bovine and porcine thymus are known to have immunomodulatory properties. Both in vitro and in vivo experiments demonstrate that thymic extracts affect cellular and humoral responses. A variety of thymic peptides have been identified and subsequently synthesized. Perhaps the most immunologically active and well studied of the thymic factors is thymosin α_1. Thymosin α_1 is a 28–amino acid polypeptide initially isolated from thymosin fraction (5), a partially purified extract of bovine thymus (6). Its sequence is identical to the N-terminal sequence of prothymosin α, believed to be the precursor of thymosin α_1. Interestingly, prothymosin α shares 36% sequence homology with the C-terminal domain of interferon alfa (7). Thus, it is possible that thymosin α_1 may work in a manner similar to interferon alfa. Thymosin α_1 has been measured in the sera of normal individuals (8, 9). It has been shown to have a variety of immunoregulatory actions, including the promotion of various cytokines such as interferon alfa, interferon gamma, and interleukin-2

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(10). The peptide is also associated with increased interleukin-2 receptor expression and increased CD4+ T-lymphocyte and natural killer cell activity. One of the first studies convincingly to demonstrate the effects of thymic extract on immune function showed improvements in T-cell–mediated immune reactivity (11). Thymic extract, used to treat patients with chronic hepatitis B infection, was associated with normalization of serum aminotransferases, although the study was not designed formally to assess the efficacy of treatment. Other investigators have reported similar results using a porcine thymic extract to treat chronic hepatitis B (10). These early reports have been corroborated by other investigators using different thymus preparations (12, 13). Taken together, these pilot studies all suggested that various clinical and histological parameters could be improved with the use of thymic preparations. Despite the absence of rigorous criteria to define treatment response, the studies were sufficiently encouraging to justify further investigation.

Thymosin α_1 has been tested in animal models of hepatitis B infection. In the woodchuck model of hepadnavirus infection, thymosin α_1 was used to treat woodchucks with chronic woodchuck hepatitis virus (WHV) (14). The thymosin α_1–treated animals showed decreased levels of WHV-DNA in serum and liver tissue, although the response was not sustained upon discontinuation of treatment.

In a small pilot study, Mutchnick and colleagues demonstrated that thymosin α_1 was effective in the treatment of chronic HBV infection in humans (10, 15). All subjects were HBsAg positive and HBV-DNA positive at baseline. At 12 months, 75% of subjects who received placebo remained HBV-DNA positive compared to 25% of participants in the thymosin α_1 arm ($P < .04$). There were no significant differences in the proportion of patients with detectable HBsAg or HBeAg after 12 months of therapy. The thymosin α_1–treated subjects were less likely to have replicative HBV molecular forms in post-treatment liver specimens and tended to have improved histology compared to the group that received placebo. Treatment

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with thymosin α_1 was associated with an increase in absolute number of CD3+ and CD4+ T lymphocytes. Follow-up of these subjects revealed seroconversion (loss of HBsAg and development of HBsAb) in 42% of the 12 patients in the thymosin α_1–treated group. Unfortunately, the dramatic results seen in this small, pilot study were not reproduced in a large, randomized, placebo-controlled clinical trial where thymosin α_1 fared no better than placebo (16). Thus, thymosin is generally not accepted as being beneficial therapy for chronic hepatitis B.

The rationale for using thymosin α_1 for the treatment of chronic hepatitis C follows from several observations: (a) the pathogenesis of HCV infection involves the immunoregulatory and cytokine pathways; (b) thymosin α_1 has immunoregulatory properties; (c) thymosin α_1 stimulates interferon alfa production and may itself act in a manner similar to interferon alfa.

Thymosin α_1 Monotherapy

There are few published reports of thymosin α_1 monotherapy for the treatment of chronic HCV infection. In a preliminary report of a pilot study, thymosin α_1 was used as monotherapy to treat subjects with chronic HCV infection (17). Ten participants were treated for 6 months without evidence of benefit. The drug was well tolerated. In an anecdotal series of six patients given thymosin α_1 monotherapy, three achieved a sustained response (G. Rasi, unpublished observations). Andreone and coworkers conducted a randomized, double-blind, placebo-controlled trial to evaluate the safety and efficacy of thymosin α_1 monotherapy for the treatment of chronic HCV infection (18). Nineteen participants with compensated chronic HCV infection and no prior therapy were enrolled and nine subjects were randomized to receive thymosin α_1 for 6 months with an additional 6 months of follow-up. At the conclusion of the follow-up period, there was no difference in mean serum alanine aminotransferase levels between the placebo and thymosin-treated groups. No subjects achieved a vi-

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rological response at the end of treatment or during the follow-up period. Other than discomfort at the injection site, there were no side effects reported. Thus, multiple published reports have failed to demonstrate an advantage to therapy with thymosin α_1 when used as monotherapy.

Thymosin α_1 and Interferon Combination Therapy

In an open-label study, 15 subjects with chronic HCV infection were given lymphoblastoid interferon in combination with thymosin α_1 (19). Four participants had already failed 6 months of interferon alfa-2b therapy and 13 subjects (87%) had genotype 1b. The protocol specified a loading dose of thymosin α_1 monotherapy before the first dose of lymphoblastoid interferon. The length of therapy was 12 months and study subjects were followed for an additional 6 months posttherapy. At the end of the follow-up period, six participants (40%) demonstrated a sustained virological response. The sustained response rate for those with genotype 1b was 39%. Sustained response was associated with histological improvement.

Moscarella and colleagues reported the results of their randomized study of interferon alfa-2b and thymosin α_1 combination therapy in treatment-naive patients with chronic HCV infection (20). Per protocol, patients were excluded if they showed any clinical or histological evidence of cirrhosis. Seventeen subjects were randomized to interferon monotherapy and 17 were selected to receive thymosin α_1 and interferon combination therapy for 6 months with 12 months of post-treatment follow-up. Compared to the interferon monotherapy group, those treated with combination therapy had a significantly higher biochemical (35 vs 71%, $P < .05$) and virological end of treatment response rate (29 vs 65%, $P < .05$). However, in those individuals with genotype 1b, there was no significant difference in response between the two treatment arms. Participants with genotype 2c demonstrated a trend to having a better biochemical response at the end of treatment with com-

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bination therapy than to monotherapy (100 vs 57, $P = .051$). At 12 months after the end of therapy, the biochemical sustained response rate was not statistically different between the two treatment groups. Virological sustained response data were not available but were likely to be similar between the groups.

Perhaps the most encouraging data for thymosin α_1 comes from a large randomized, double-blind, placebo-controlled trial evaluating combination therapy with thymosin α_1 and interferon for the treatment of chronic HCV infection. Sherman et al. (21) randomized 109 subjects to one of three treatment arms: (a) interferon alfa-2b and thymosin α_1; (b) interferon alfa-2b and thymosin α_1 placebo; or (c) interferon placebo and thymosin α_1 placebo. The duration of therapy was 26 weeks. Side effects due to thymosin α_1 and interferon combination therapy were not statistically different from those experienced with interferon monotherapy.

End of treatment biochemical response was superior in the combination group compared to the interferon monotherapy (37.1 vs 16.2%, $P = .04$). Among treatment-naive subjects, the end of treatment response was significantly higher for the combination group than for the interferon monotherapy group (40.6 vs 16.6%, $P = .03$). With respect to subjects with genotype 1, the end of treatment response rate in the combination group was again superior to the interferon alone group (30.8 vs 7.4%, $P = .032$). There was no statistically significant difference in biochemical end of treatment response between the two groups for participants with non–genotype 1 viruses. The virological end of treatment response rates for combination therapy and interferon monotherapy (37.1 vs 18.9%) mirrored the biochemical end of treatment response rates. Of the three study groups, only the combination arm demonstrated a statistically significant mean reduction in HCV-RNA titers compared to baseline values. Histological improvement, defined as a decrease of more than two points in the Knodell histologic activity index (HAI) was seen in the combination group compared to the placebo group ($P < .05$). The sustained biochemi-

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cal response rates were 14.2 and 8.1% in the combination arm and interferon group, respectively. Unfortunately, data on sustained virological response rates were not provided which makes problematic assessment of the true efficacy of this regimen.

These results suggest that the combination of thymosin α_1 and interferon may be a rational approach to enhancing the response rate in patients with chronic hepatitis C. This thymic peptide is particularly appealing because of its safety profile. However, the key measure of success, sustained virological response, remains difficult to define and compare to other treatment modalities from these published studies. Additional studies are needed to fully evaluate the role of thymosin α_1 for the treatment of chronic HCV infection.

IRON REDUCTION THERAPY

Most patients with chronic HCV have normal hepatic iron despite the high prevalence of elevated serum iron, transferrin saturations, and ferritin levels (22). The roles of iron and iron-binding proteins have been studied largely in nonviral infectious diseases and to a lesser extent for viral pathogens. Bonkovsky (22) and Shedlofsky (23) have reviewed and summarized a number of important studies linking higher levels of iron in blood and liver to decreased responsiveness to interferon alfa therapy for chronic viral hepatitis. In those studies, the distribution of iron in the liver was also associated with response to interferon, with portal iron deposition portending a less favorable outcome. Because of the cross-sectional nature of most of the studies, it is not established whether the increased iron predisposes patients to more severe liver disease or whether the more advanced hepatitis leads to increased iron in serum or liver.

The issue is not merely of intellectual interest, but directly shapes development of rational therapies. If increased

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hepatic or serum iron leads to more severe forms of chronic viral hepatitis, then iron reduction therapy offers the potential to improve upon standard treatments. This rationale has spurred a number of clinical trials.

Hayashi et al. used repeated phlebotomy as iron reduction therapy for patients with chronic hepatitis C (24). Ten patients had histochemically detectable iron in the liver and underwent an initial period of weekly or monthly phlebotomy of 200 or 400 mL. Patients were bled until a serum ferritin level of 10 ng/mL or less was achieved, and maintenance phlebotomy was performed if the level rebounded. Mean serum alanine aminotransferase (ALT) activity was reduced from 152 ± 49 to 55 ± 32 IU/L ($P < .001$). In 5 of 10 patients, therapy resulted in normalization of ALT. There was no improvement in liver histology, although among the seven patients who had follow-up biopsies, there was a disappearance of iron deposits.

Studies of the effect of iron reduction on virological outcomes have generally been small and have reached differing conclusions (23). This is in part due to selection bias, small sample sizes, and the different treatment regimens employed. Van Thiel and colleagues evaluated 30 subjects with high normal hepatic iron concentrations who had failed to respond to standard interferon treatment for 6 months (25). The participants were randomized to high-dose interferon (5 million units daily) for 6 months or high-dose interferon in combination with weekly venesection to maintain hemoglobin concentrations between 10 and 11 g/dL. Patients in the phlebotomy arm were more likely to achieve a biochemical and histological response than the interferon alone arm. More impressively, sustained virological response (1 year after therapy) was achieved in 60% of the patients assigned to venesection compared to 13% in the interferon monotherapy group.

Two large clinical trials evaluating the effects of phlebotomy on response to interferon therapy have been recently published. Fontana and colleagues (26) randomized 82 previously untreated patients to treatment with interferon alone for 6

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months or to interferon therapy in conjunction with iron reduction before and during treatment. Patients treated with iron reduction had lower mean serum ALT levels during therapy and follow-up than those treated with interferon alone and were more likely to have undetectable HCV-RNA at the end of treatment ($P = .03$). However, there was no difference in sustained response rates between the two treatment groups. In another study (27) that focused on patients who had failed to respond to an initial course of interferon, 96 patients were randomized to either iron reduction alone or iron reduction by phlebotomy followed by retreatment with interferon alpha. During the initial phlebotomy period, serum ALT activity decreased to some degree in most patients, with a decrease by more than 50% from baseline in 13% and normalization of ALT in 9%. Unfortunately, no patient in either treatment group achieved a sustained virological response. Although iron reduction failed to improve sustained response rates in either study, improvement in ALT and modest changes in hepatic histology associated with iron reduction suggest that there may be some benefit in terms of reduction in necroinflammatory injury. Interestingly, ribavirin therapy results in an accumulation of hepatic iron, yet serum ALT activities routinely decrease even on ribavirin monotherapy (28). In light of these results, the role of phlebotomy as an adjunctive measure in the management of chronic hepatitis C remains to be elucidated. At present, phlebotomy cannot be recommended in the absence of documented iron overload states.

NONSTEROIDAL ANTI-INFLAMMATORY AGENTS

Nonsteroidal anti-inflammatory drugs (NSAIDs) have different mechanisms of action, but share the ability to inhibit the cyclooxygenase enzyme (Fig. 1) that catalyzes the conversion of arachidonic acid to prostaglandin E2, which is known to have immunosuppressive properties. In addition to inhibiting

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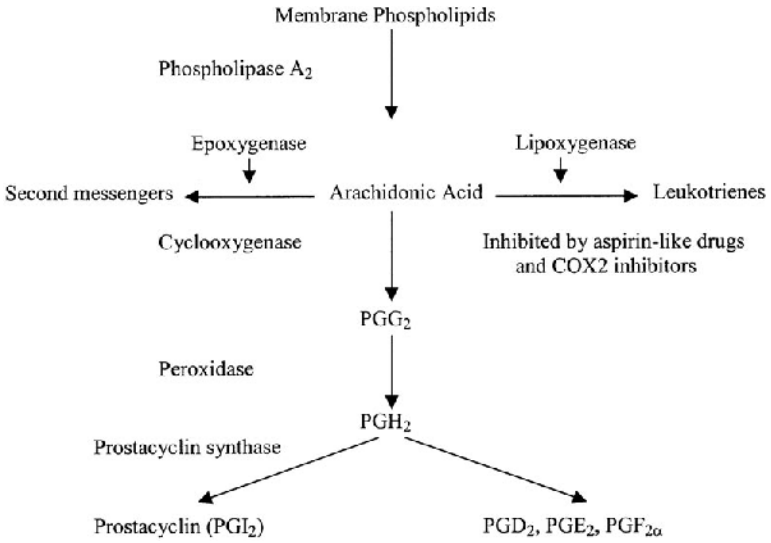

Figure 1 Metabolism of arachidonic acid to prostaglandins and site of action of aspirin-like drugs.

cyclooxygenase, NSAIDs also increase the concentration of 2′5′-oligoadenylate synthetase in patients with chronic viral hepatitis (29), although the exact mechanism by which this occurs is unknown. 2′5′-Oligoadenylate synthetase is induced by interferon α and may be important in its antimitogenic effects (30). It is possible that arachidonic acid metabolism may occur through the epoxygenase family enzymes, thereby producing the second messengers of interferon α signal (31, 32). Because interferon alfa increases prostaglandin E2 production in cultured liver tissue in subjects with chronic viral hepatitis, Andreone and colleagues postulated that aspirin-like compounds might improve the therapeutic response to interferon (33).

Zarski and coworkers randomized 149 interferon-naïve subjects with chronic HCV to receive interferon alfa-2a and placebo or interferon alfa-2a plus tenoxicam in a blinded manner (34). Biochemical end of treatment response rates were no

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different between the groups. Sustained biochemical or virological response rates were similar regardless of therapy received. The groups also showed similar improvements in the Knodell HAI. Furthermore, the same group of investigators demonstrated that interferon therapy combined with tenoxicam did not induce a significant increase of 2′5′-oligoadenylate synthetase activity in patients with chronic HCV infection (35).

More recently, a group of investigators evaluated the use of ketoprofen in combination with interferon alfa-2b to treat subjects with chronic hepatitis C (36). Seventy participants who had not previously received interferon were randomly assigned to one of three groups: (a) interferon alfa-2b alone; (b) interferon alfa-2b combined with 200 mg slow-release ketoprofen, administered three hours before interferon; (c) interferon alfa-2b in combination with 200 mg slow-release ketoprofen twice daily. Subjects assigned to receive ketoprofen were also prescribed ranitidine. The protocol specified 6 months of treatment followed by a 6-month observation period. Combination therapy with interferon alfa-2b and ketoprofen significantly decreased the incidence of flu-like symptoms. The sustained virological response rates were 5, 0, and 26% for subjects in groups 1, 2, and 3, respectively ($P = .07$ group 3 vs group 1).

The role of aspirin-like drugs as adjuncts to interferon therapy remains elusive. It is clear, however, that NSAIDs ameliorate the flu-like side effects commonly experienced by patients while on interferon. Carefully designed clinical trials with adequate assessment of confounders are needed to determine whether NSAIDs increase the response to interferon.

AMANTADINE AND RIMANTADINE

Amantadine and its structural analogue rimantadine have been used as prophylaxis and treatment of influenza A virus infection. These agents probably act by interacting with the viral membrane matrix protein (M2), interfering with the ion

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channel function of the protein. Significantly higher doses of amantadine are required to inhibit influenza B, rubella, and flaviviruses.

Multiple studies have evaluated amantadine and its analogue rimantadine for the treatment of chronic hepatitis C (Table 1). Smith reported the results of an open-label trial of 22 subjects who failed to respond to interferon monotherapy (37). Participants received 6 months of treatment with amantadine 100 mg twice daily. A positive end of treatment response was noted in 27% of study subjects and a sustained virological response was seen in 18% of treated individuals. In contrast, Fong and colleagues were unable to demonstrate

Table 1 Summary of Final Publications Using Amantadine or Rimantadine for HCV

Reference	No. subjects	Drug	SVR (%)	Conclusion
46	60 IFN non-responders	Amantadine/ IFN/ ribavirin vs IFN/ ribavirin	48 vs 5	Triple therapy superior
67	29 IFN non-responders	Amantadine/ IFN vs IFN/ ribavirin	0 vs 15	Amantadine inferior
41[a]	24	Amantadine	0	No efficacy
44	11 post–liver transplant	Rimantadine	0	No efficacy
38	17 IFN non-responders	Rimantadine	0	No efficacy
42[b]	40	Amantadine	0	No virologic response
37	22 IFN non-responders	Amantadine	18	Improved virologic response

IFN, interferon; SVR, sustained virological response.
[a] Study includes six subjects who failed prior interferon monotherapy.
[b] Study includes 9 relapsers and 12 treatment-naïve subjects.

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antiviral activity of rimantadine (38). The investigators conducted an open label-trial involving 17 interferon alfa monotherapy nonresponders. Subjects were off treatment for at least 6 months before receiving rimantadine 100 mg twice daily by mouth for 24 weeks. Compared to baseline, mean ALT levels were not significantly lower at the end of the trial and there was no change in quantitative HCV-RNA levels. Other investigators have found similar results (39–44). Thus, as a single agent, there does not appear to be any benefit to amantadine or rimantadine therapy alone.

Amantadine has also been used for the treatment of chronic hepatitis C in combination with interferon (see Table 1). Zeuzem and colleagues (45) randomized 119 previously untreated patients to 48 weeks of therapy with interferon alfa alone or interferon plus amantadine in a double-blind protocol. The sustained virological responses were actually numerically better in the monotherapy arm (22%) compared to those treated with combination interferon plus amantadine (10%, P = ns). In great contrast to this study, Brillanti et al. (46) treated 60 consecutive patients who had not responded to interferon therapy alone with either a triple combination of interferon, ribavirin, and amantadine or with combination interferon and ribavirin without amantadine. Remarkably, the sustained response rate in the triple combination therapy group was 48% compared to only 5% in those treated with the dual combination. This is the first study to demonstrate the efficacy of this unique triple combination and will need to be verified in larger clinical trials before it can be routinely considered as second-line therapy.

URSODEOXYCHOLIC ACID

Originally developed to dissolve gallstones, bile acids have been used to treat a variety of cholestatic liver diseases in recent years, including primary biliary cirrhosis and hepatic dysfunction associated with total parenteral nutrition. Urso-

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deoxycholic acid (UDCA) is a highly hydrophobic bile acid that briefly enjoyed popularity for the treatment of chronic HCV infection. It does not appear to have antiviral activity, but its use in autoimmune hepatitis and other immune-mediated hepatobiliary diseases suggests it may have modest immunomodulatory properties (22). Alternatively, UDCA may act nonspecifically by stabilizing cell membranes (39).

In a case series published by Leuschner and colleagues, UDCA was found to improve serum aminotransferase levels in individuals with non-A, non-B chronic active hepatitis undergoing treatment for cholelithiasis (47). The improvement in serum aminotransferase levels was demonstrated in subsequent studies (48–51).

Of more interest is the use of UDCA as an adjunct to interferon therapy. A table summarizing studies using UDCA has been published (39). In a recent report, treatment-naive subjects with chronic HCV infection randomly received either interferon alfa-2a (6 million units three times weekly for 6 months) monotherapy or combination therapy with the same dose of interferon plus UDCA (10 mg/kg/d for 9.5 months) (52). UDCA was initiated before the first dose of interferon and was continued for 3 months after discontinuation of interferon. Biochemical and virological end of treatment responses were similar in both groups, but combination therapy delayed the time to relapse. Combination therapy was also associated with decreased portal inflammation. Boucher and colleagues randomized treatment-naive subjects with HCV to receive either interferon alfa-2b monotherapy or combination therapy with interferon and UDCA (53). Again, there were no significant differences between the treatment groups with respect to biochemical end of treatment response rates or sustained virological response rates. Combination therapy was, however, associated with delayed time to biochemical relapse. This study did not demonstrate improvement in histology with combination treatment. Several other studies failed to show an improvement in biochemical or virological treatment endpoints when UDCA was added to interferon therapy (54–57).

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Based on these results UDCA does not appear to enhance interferon treatment for chronic HCV infection.

HERBAL PRODUCTS

There has been a great deal of interest in herbal remedies as alternatives to standard medical treatments. This trend has been reported in practically all medical fields and the popularity of herbal products will likely continue. Milk thistle contains a natural antioxidant known as silymarin, which appears to have cytoprotective properties. It has been used in a variety of liver diseases and is associated with improved biochemical markers of hepatic inflammation (58). In one study, 106 individuals were randomly allocated to receive silymarin or placebo for 4 weeks (59). Subjects were chosen based on persistently elevated liver tests despite the recommendation to abstain completely from alcohol use. Participants in the active drug group had statistically greater decreases in the serum aminotransferase levels compared to subjects who received placebo. BSP (sulfobromophthalein test) retention normalized more often in the silymarin group. Likewise, the mean percentage decrease of BSP was higher in the treated group. The study also demonstrated that histological abnormalities were more likely to resolve in the group that received silymarin. Another clinical trial attempted to ascertain the efficacy of silymarin treatment in alcoholic liver disease (60). One hundred and twenty-two participants were randomly allocated to receive 420 mg/day silymarin or placebo for 45 days. Abstinence from alcohol was encouraged in all participants. At the end of the treatment trial, only mean corpuscular volume (MCV) and gamma-glutamyltranspeptidase (GGT) levels were significantly different in the two groups. Normalization of MCV and GGT was more likely in the silymarin group. Serum aminotransferase levels and tests of liver synthetic function did not differ in the active and placebo groups.

A few studies have evaluated the effect of silymarin treat-

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ment on survival. Ferenci and colleagues (61) treated 170 subjects with cirrhosis (alcoholic and nonalcoholic) to receive 140 mg silymarin three times daily or matching placebo. Enrollment continued until all participants completed 2 years of treatment. The mean observation period was 41 months. The 4-year survival rate was 58% in the silymarin group and 39% in the placebo group ($P = .036$). Subgroup analysis revealed that the effect was more likely in subjects with alcoholic cirrhosis ($P = .01$) and in subjects who were classified as Child A at the start of the study ($P = .03$). A more recent study focused on alcoholic subjects with cirrhosis of the liver and evaluated the effects of silymarin in such a population (62). Two hundred subjects were enrolled. The 5-year survival rates were similar in both groups (75% in the silymarin group and 78% in the placebo group). No significant differences were observed between the groups with respect to clinical and biochemical parameters. A subgroup of subjects was noted to have hepatitis C infection, but the number of events in this subgroup was too small to make any meaningful comparisons.

The use of milk thistle in chronic HCV infection is largely anecdotal, and methodologically sound studies that would enable evaluation of efficacy are lacking. In fact, the efficacy of silymarin treatment for liver disease in general remains unproved.

GLYCYRRHIZIN

The botanical component glycyrrhizin, a compound extracted from the roots of *Glycyrrhiza glabra*, has been used in Japan for decades to treat chronic hepatitis (63). It is believed to be useful in the treatment of a variety of allergic conditions and is commonly administered intravenously. Van Rossum et al. summarized two early studies of glycyrrhizin for chronic hepatitis (63). One study showed improvement in serum levels of aminotransferases and the other demonstrated histological improvement.

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The exact mechanism of action for glycyrrhizin is unknown, but it appears to have antiviral and immunomodulatory properties and may also act nonspecifically. Nakamura et al. (64) performed a series of experiments using carbon tetrachloride (CCL_4) and various other hepatotoxins to induce enzyme leakage in primary cultured adult rat hepatocytes. The release of the cytosolic enzymes lactic dehydrogenase, glutamic-oxaloacetic, and glutamic-pyruvic aminotransferases were measured 24 h later. At low concentrations, CCL_4 caused dose-dependent release of soluble enzymes into medium. At higher concentrations of CCL_4, both soluble and mitochondrial glutamic-oxaloacetic aminotransferase were detected. Enzyme leakage was probably due to a change in membrane permeability. After cotreatment with glycyrrhizin, the investigators noted a dose-dependent reduction in enzyme release. They postulated that glycyrrhizin was cytoprotective by stabilizing the cell membrane (64). Other studies confirm the hepatoprotective nature of glycyrrhizin and suggest that it may have regulatory actions on the immune system as well (63).

Most clinical evaluations of glycyrrhizin have been conducted in Japan and to a lesser extent in Europe. A retrospective study of 193 patients with chronic HCV infection without cirrhosis provides evidence that long-term use of glycyrrhizin decreases the odds of developing hepatocellular carcinoma (HCC) (65). Eighty-four subjects were given intravenous glycyrrhizin as stronger neo minophagen (SNMC). This solution contains 2 mg glycyrrhizin, 1 mg cysteine, and 20 mg glycine per milliliter in physiological saline. Participants were treated daily for 2 months and then two to seven times a week for a median of 10 years. The 109 subjects chosen as controls were not treated, because logistics made it impossible for them to receive intravenous infusions several times a week. After 15 years, the cumulative rate of HCC in the treated group was 12%, compared to 25% in the control group. Normalization of alanine aminotransferase was observed in 36% of glycyrrhizin-treated patients, but only 6% of untreated subjects achieved normal enzyme levels. Indepen-

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dent of treatment, the rate of development of HCC was similar at 15 years for participants who failed to normalize their liver enzymes.

Van Rossum et al. studied 57 patients with chronic hepatitis C classified as nonresponders or unlikely to respond (genotype 1/cirrhosis) to interferon monotherapy (66). Subjects were randomized to one of four dose groups: 240, 160, or 80 mg glycyrrhizin or placebo (0 mg glycyrrhizin). Glycyrrhizin was administered intravenously three times a week for 4 weeks followed by a 4-week observation period. The mean ALT decrease at the end of active treatment was significantly higher than the placebo group (26 vs 6%, $P < .05$). A dose-response effect was not observed. Normalization of ALT at the end of treatment occurred in 10% (4 of 41), but upon discontinuation of therapy relapse occurred. There was no appreciable change in mean HCV-RNA.

The optimal dosing and length of therapy with glycyrrhizin has not been determined. In light of the results of retrospective evaluations, properly designed cohort studies and clinical trials are needed to determine whether glycyrrhizin can reduce complications of chronic hepatitis C virus infection even without clearance of viremia.

CONCLUSIONS

The mainstay of treatment for chronic HCV infection consists of combination therapy with interferon and ribavirin. However, owing to the lack of sustained virological response in the many patients and because some individuals cannot tolerate combination therapy, other treatment modalities have been actively pursued. Certain treatment alternatives have not withstood more careful scientific scrutiny beyond case reports and anecdotes, whereas others show promise and await more rigorous testing.

The role of aspirin-like compounds seems to be limited to ameliorating the side effects of interferon. UDCA has not been

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shown to be beneficial in the treatment of HCV infection, although the data do not preclude its use in special cases, such as forms of chronic HCV infection associated with severe cholestasis. More studies are needed to define its application in special circumstances, but UDCA will likely see limited use in the treatment of hepatitis C infection. Herbal products have grown in popularity as patients search for less toxic and better-tolerated treatment alternatives, but these products are not without potential risk. As a result, it is of paramount importance that such remedies be held to the same standard of evaluation before they can be recommended to patients.

The jury is still out on thymosin α_1, iron reduction therapy, amantadine/rimantidine, and glycyrrhizin. Although studies have produced mixed results, the rates of sustained biochemical and virological responses reported in some trials justify further investigation. Natural history studies of HCV infection suggest that individuals with persistently normal ALT levels are less likely to develop long-term complications of chronic HCV infection. A few retrospective studies show that individuals who achieve a sustained biochemical response to treatment are also less likely to develop hepatocellular carcinoma despite the fact that clearance of HCV viremia was not achieved. Thus, future research needs to determine whether these adjuncts to therapy hold promise of benefit in the long term.

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7

Current Treatment of Hepatitis C

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CASE 1

A 43-year-old woman was noted to have elevated aminotransferase levels on her insurance physical examination. She was seen by her primary care physician, who performed the initial evaluation and referred her to the hepatology division. She denied any complaints. She drank one to two glasses of wine per week and admitted to the use of intranasal cocaine when she was 20. Physical examination

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was unremarkable with no evidence of chronic liver disease, hepatomegaly, or splenomegaly. The presenting laboratory data were:

CBC	Normal
Chemistry panel	Normal
Total bilirubin (mg/dL)	0.7
Alkaline phosphate (IU/L)	75
Albumin (g/dL)	4.1
ALT (IU/L)	74
AST (IU/L)	36
Prothrombin Time (s)	12
Hepatitis B surface antigen	Negative
Hepatitis B core antibody	Negative
Hepatitis C antibody	Positive
ANA	Negative
Anti–smooth muscle antibody	Negative
Iron (μg/dL)	66
TIBC (μg/dL)	394
Ferritin (ng/dL)	35
Ceruloplasmin (mg/dL)	46

Impression/Outcome

With a risk factor for acquisition of the hepatitis C virus (HCV), mild elevation of aminotransferase levels, and detectable hepatitis C antibodies, she is at risk for chronic hepatitis C. Other etiologies of elevated aminotransferase levels were excluded based on the laboratory data presented. She underwent further testing for serum HCV-RNA, hepatitis C genotype, and a liver biopsy. This revealed a serum HCV-RNA viral load of 2,500,000 copies/mL, HCV genotype 1a, and mild chronic inflammation with portal fibrosis on liver biopsy. It was recommended that the patient undergo treatment with interferon and ribavirin. She experienced mild side effects of therapy, including fatigue, irritability, and myalgias. At week 24, the aminotransferase levels had normalized and the serum HCV-RNA was less than 100 copies/mL. Thus, the combination therapy was continued, and she completed another 24 weeks of therapy to complete the 48-week course. The patient returned for a follow-up visit 6 months later and her laboratory tests revealed normal aminotransferase levels with the serum HCV-RNA still being undetectable.

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CASE 2

A 47-year-old man was elevated by his primary physician for chronic fatigue and noted to have a mild elevation of alanine transaminase (ALT). After an initial evaluation, the patient was referred. Review of symptoms was unremarkable. The past medical and surgical history was significant for a motor vehicle accident that required several surgeries with blood transfusions 20 years ago. He denied the use of alcohol or medications. Physical examination revealed multiple surgical scars, but no suggestion of chronic liver disease. The presenting laboratory data were:

CBC	Normal
Chemistry panel	Normal
Total bilirubin (mg/dL)	0.3
Alkaline phosphate (IU/L)	56
Albumin (g/dL)	3.8
ALT (IU/L)	68
AST (IU/L)	56
Prothrombin Time (s)	11.3
Hepatitis B surface antigen	Negative
Hepatitis B core antibody	Negative
Hepatitis C antibody	Positive
Hepatitis C RIBA	Positive

Impression/Outcome

The clinical history and laboratory data are consistent with chronic hepatitis C. Further workup revealed a viral load of 3,600,000 copies/mL, HCV genotype 2a, with moderate chronic inflammation, portal fibrosis, and bridging fibrosis on liver biopsy. The patient was started on interferon and ribavirin. Although his hemoglobin decreased by 3 g, dose modifications were not required. At 24 weeks of therapy, the aminotransferase levels had normalized and the serum HCV-RNA was less than 100 copies/mL. Since the patient was infected with a genotype other than 1, only 24 weeks of therapy was required. He returned for follow-up 6 months later with normal aminotransferase levels and serum HCV-RNA was still undetectable. The patient had achieved a sustained virological response.

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INTRODUCTION

Hepatitis C virus (HCV) infection affects 2.7 million individuals in the United States and 150 million individuals worldwide (1,2). Although the prevalence of new HCV infections is declining in the United States, the number of patients who will potentially suffer complications of liver disease is estimated to increase significantly over the next decade (3–5). Approximately 20–30% of infected patients will develop cirrhosis and are at risk for developing complications of end-stage liver disease, including hepatocellular carcinoma (6–8), and chronic hepatitis C is now the most common indication for liver transplantation in the United States (9). Thus, there is a need for effective therapies to treat HCV infection. In this chapter, we have attempted to summarize the key issues related to antiviral therapy for patients with chronic HCV infection at this time.

WHO SHOULD BE TREATED?

Because only 20–30% of patients develop cirrhosis over 20 years, it would be useful to only select this group of patients as being potential candidates for treatment. Unfortunately, we cannot accurately predict which subgroup of patients will progress. As guidelines, the U.S. National Institutes of Health (NIH) and European Consensus Development Conferences suggest that patients with elevated aminotransferase levels, detectable serum HCV-RNA, and chronic inflammation with some degree of fibrosis should be offered therapy (Table 1) (5, 10).

There are 6 major genotypes and 30 subtypes of hepatitis C (11). Although HCV genotype 1 has lower response rates to therapy than other genotypes, this should not be considered to be a factor in selecting patients for treatment. Since symptoms in hepatitis C are nonspecific and do not correlate with disease severity, they should also not be used to select patients for therapy (12). Additionally, the degree of elevation of ALT

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Table 1 Indications for Treatment of Chronic Hepatitis C[a]

Persistent evaluations of aminotransferase levels
Detectable serum HCV-RNA
Portal fibrosis or moderate inflammation on liver biopsy

[a] Patients should meet all three criteria.
Source: Adapted from the National Institutes of Health (NIH) and the European Association for the Study of the Liver (EASL) Consensus Development Conferences on Hepatitis C (5, 10).

values does not correlate with the histological severity of the disease and thus should not be used to guide therapy. At the present time, patients with persistently normal enzymes are thought to have a relatively "benign" course that is slowly, if at all, progressive, and they should probably not be treated outside of research protocols. As future treatments potentially become more effective, treatment of these patients will probably require reevaluation.

A liver biopsy is not mandatory before therapy but is recommended (5). Patients with mild inflammation and no fibrosis have been shown to progress slowly (13, 14), whereas those with some degree of portal fibrosis are thought to have more aggressive disease (13). The biopsy allows for grading inflammation, staging fibrosis (if present), and detecting cirrhosis that may not be evident clinically or biochemically in 10–20% of patients. Treatment of patients with cirrhosis suggests that they may benefit, with reduced complication of liver disease in the future (15–17), and as such patients with compensated cirrhosis are potential candidates for therapy.

Cost-effective analyses indicate that antiviral therapy is economically beneficial (18, 19). Future cost savings observed for the treatment of hepatitis C are comparable or better than other accepted medical treatments such as hypertension screening, renal dialysis, coronary artery bypass grafting, and colorectal cancer screening. Successful therapy is associated with clearance of serum HCV-RNA, normalization of serum ALT levels, and improvement in liver histology (20, 21). Furthermore, patients who have been treated and achieve a virological sustained response have been followed for 5–15 years,

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Table 2 Contraindications to Treatment of Chronic Hepatitis C

Interferon	Ribavirin
Decompensated liver disease	Anemia (Hgb $<$ 11 g/dL)
Severe or untreated depression	Unable to tolerate anemia
Significant comorbid disease	Pregnancy
Uncontrolled diabetes	Unable to practice contraception
Uncontrolled hypertension	Coronary artery disease
Seizures	Peripheral vascular disease
Active alcohol or drug use	Renal failure
Autoimmune diseases	Gout

with 95% of the patients having no detectable HCV-RNA, normal liver tests, and histological improvement during this prolonged follow-up period (22, 23).

Patients should be carefully evaluated for any contraindications, including decompensated liver disease, significant comorbid illnesses, autoimmune diseases, depression, active alcohol or drug use, and pregnancy prior to initiating therapy (Table 2). The physician must also consider the length of therapy (usually 24–48 weeks), the cost, the frequent monitoring of patients, compliance, and the side effect profile in deciding appropriate candidates for therapy. These issues must be carefully evaluated and discussed with the patient in detail prior to initiating therapy.

DEFINITIONS OF RESPONSE TO THERAPY

There are various methods to define response to therapy, including the biochemical, virological, and histological response. The response to therapy is further subdivided into the end of treatment response or the sustained response at 24 weeks of follow-up (Table 3). Historically, *response* was defined as the propotion of patients with normal ALT values at end of treatment and follow-up (end of treatment and sustained biochemical response rates). Since the introduction of reliable and sensitive serum HCV-RNA assays, subsequent studies comparing

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Table 3 Definitions of Response to Therapy

Biochemical response	Normal alanine aminotransferase levels
Virological response	Absence of detectable HCV-RNA
Histological response	Improvements in liver biopsy results
End of treatment response	At end of therapy
Sustained response	At 24 weeks after therapy

ALT and HCV-RNA responses indicate that normalization of ALT values is a less accurate marker of viral eradication and long-term response (22, 24). For these reasons, clearance of serum HCV-RNA using a reliable sensitive assay at 24 weeks of follow-up is currently the "gold standard" of a successful response to therapy.

INITIAL INTERFERON MONOTHERAPY

Prior to the introduction of combination therapy with interferon (IFN) and ribavirin, the standard was IFN monotherapy. Type 1 IFNs are biological cytokines that have antiviral and immunomodulatory actions. There are currently four IFNs approved for the treatment of chronic hepatitis C virus infection. These include recombinant IFN-α2b (Intron-A), IFN-α2a (Roferon-A), IFN-αcon-1 (Infergen), and IFN-α1n (Wellferon). These IFNs are administered at a dose of 3 million units subcutaneously three times per week or equivalent. All four agents appear to have equivalent efficacy in achieving normalization of liver enzymes in 25–50% of patients at the end of 24 weeks of therapy (20, 25). However, only 10–15% of all patients achieve a sustained virological response 24 weeks after completing therapy (25–30). Increasing the duration of therapy to 48–72 weeks improves the sustained virological response rates marginally to 15–20% (25, 31). Higher doses of IFN and extending the duration of therapy are both associated with more frequent side effects and higher rates of discontinuation of therapy (25, 31–33).

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Predicting Response to Therapy

The cost, adverse event profile, and response rates of therapy all suggest that the ability to predict who will or will not respond to therapy may be clinically useful. Evaluation of pretreatment variables indicates that patients with low viral load, genotype 2 or 3, and the absence of fibrosis or cirrhosis on liver biopsy have a greater likelihood of responding to a 24-week course of IFN (34). These factors accurately predict response to IFN therapy in only 40–72% of the patients; thus, they are not clinically useful in predicting response for an individual patient (34). The presence or absence of HCV-RNA during therapy can be helpful in selecting patients who should continue therapy. The persistence of HCV-RNA after 12 weeks of receiving IFN monotherapy correctly identifies 98–100% of patients that will not achieve a sustained virological response (24, 35, 36). Thus, the presence of HCV-RNA during therapy is a more accurate predictor of nonresponse than the clearance of HCV-RNA as a predictor of sustained virological response.

Side Effects

Currently available type 1 IFNs have similar side effects (Table 4) (28, 29, 37, 38). The most common are flu-like symptoms (fever, chills, myalgias), fatigue, arthralgias, anorexia, and weight loss. Thyroid disturbances are also common and usually temporary. Transient hypothyroidism is the most common of these, and it usually responds well to thyroid replacement therapy. Mild bone marrow suppression (especially leukopenia and thrombocytopenia) is frequently seen, which is easily monitored and managed with dose reductions. Additionally, psychiatric disturbances are common and important to identify and treat. Depression is frequently present in untreated chronic hepatitis C patients and has previously been related to the disease itself (39). Interferon can further exacerbate depression, and thus patients should be carefully screened before therapy is contemplated. If depression is suspected, patients should be evaluated and treated by a psychiatrist. If depression can be easily controlled, treatment can sub-

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Table 4 Side Effects of Interferon Monotherapy

Type of effect	Common side effects	Rare side effects
Systemic	Fatigue, fever, malagias, arthralgias, weight loss	Vomiting, diarrhea, abdominal pain, hypersensitivity reactions
Neurological	Difficulty concentrating, sleep disturbances	Delirium, disorientation, dizziness, seizures, vertigo, decrease in vision or hearing
Psychological	Irritability, depression, anxiety	Paranoid or suicidal ideation, return of craving for alcohol, return of craving for drugs
Hematological	Decrease in platelet count, decrease in white cell count	Decrease in hemoglobin
Immunological	Susceptible to community-acquired infections: bronchitis, sinusitis, UTI	Sepsis, pneumonia, lung abscesses, spontaneous bacterial peritonitis
Autoimmune	Hyperthyroid or hypothyroid	Diabetes, thrombocytopenic purpura, lupus-like syndrome
Others		Nephritis, nephrotic syndrome, acute renal failure, proteinuria, congesive heart failure, exacerbation of liver disease

UTI, urinary tract infection.

sequently be initiated successfully. Approximately 85–90% of patients successfully complete a standard 48-week course of IFN monotherapy (20, 21).

Current Recommendations

At present, IFN monotherapy should only be considered for patients who are not candidates for treatment with IFN plus ribavirin. This includes the pediatric population, the elderly, and patients with renal disease, anemia, heart disease, or peripheral vascular disease. The currently recommended dose is 3 million units subcutaneously three times per week (usually Monday, Wednesday, Friday) or equivalent. Serum HCV-RNA should be tested at week 12, and if undetectable with a sensitive and reliable assay, patients should complete the 48 weeks of therapy. If viremia persists, termination of therapy should be considered, as the likelihood of achieving a sustained viro-

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logical response is extremely low. This approach, although acceptable, does not take into account the potential histological benefits of improvement in inflammation that has been observed in the absence of viral eradication (17, 40). However, the degree of benefit in the short and long term in this clinical situation is unknown. Prospective studies designed to address these issues are currently in progress.

INITIAL THERAPY WITH IFN PLUS RIBAVIRIN

Ribavirin

The limited efficacy of IFN monotherapy has led to the evaluation of other agents combined with IFN. The most successful combination, in terms of enhancing the sustained virological response rate, is the two-drug regimen of ribavirin and IFN. Ribavirin is an orally bioavailable synthetic nucleoside analogue resembling guanosine. It has been shown to be effective against several RNA and DNA viruses in vitro (41, 42). Ribavirin has putative antiviral and immunomodulatory actions, including the inhibition of virus-dependent polymerases, depletion of intracellular phosphate pools, macrophage inhibition, and alteration of the cytokine profile (42,43). When given alone, ribavirin monotherapy transiently decreases ALT values in patients with chronic hepatitis C, but this does not persist after stopping therapy. This normalization of ALT values is not associated with a reduction in serum HCV-RNA levels during or after therapy (44–46).

Comparing IFN with Combination Therapy

To date, five randomized, controlled trials have assessed and compared the efficacy of IFN alone or combined with ribavirin (Fig. 1). Three initial smaller studies treated patients for 24 weeks and suggested that adding ribavirin to IFN resulted in an improved sustained virological response rate of 42% (47–49). Two subsequent large, multicenter trials randomized patients to one of four treatment groups: (1) IFN plus placebo

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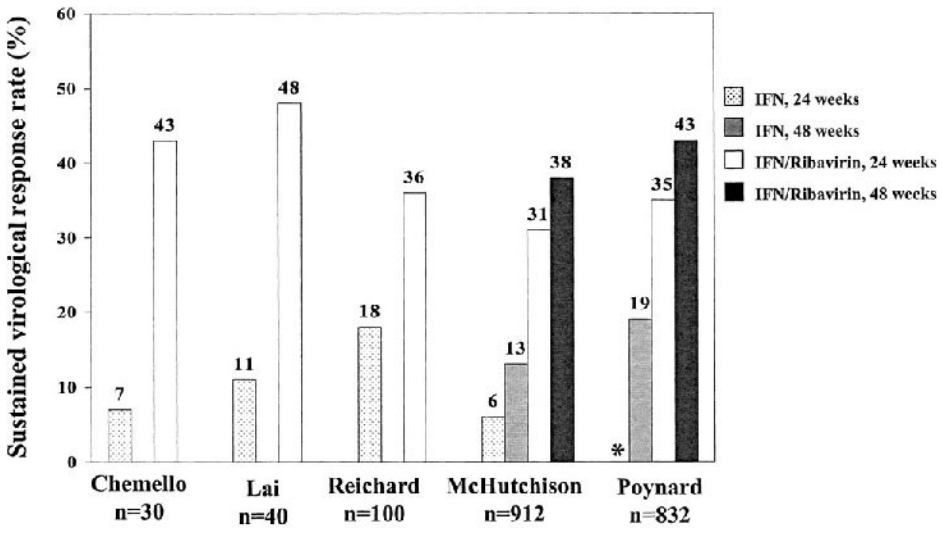

Figure 1 Sustained virological response rates in patients treated with IFN monotherapy and in patients treated with IFN plus ribavirin in the five major trials. IFN, recombinant interferon-α2b. *, none of the patients received IFN for only 24 weeks in this trial. The first three trials treated patients for only 24 weeks. Combination therapy with IFN plus ribavirin was significantly better than IFN monotherapy in all of the trials.

(24 weeks), (2) IFN plus placebo (48 weeks), (3) IFN plus ribavirin (24 weeks), and (4) IFN plus ribavirin (48 weeks) (20, 21). The dose of IFN was 3 million units three times per week subcutaneously plus 1000 mg of ribavirin (weight $<$75 kg) or 1200 mg of ribavirin (weight $\geq$75 kg). Patients underwent liver biopsy before treatment and then 24 weeks after completing therapy. The results showed a substantial benefit in terms of the biochemical, histological, and virological endpoints compared to IFN monotherapy. The combined sustained virological response rates were 33 and 41% for IFN plus ribavirin for 24 weeks and 48 weeks, respectively, compared to 6 and 16% for 24 or 48 weeks of IFN monotherapy. End of treatment response rates were also higher for IFN-plus ribavirin-treated

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patients for either duration compared to IFN monotherapy. This suggests that combination therapy enhances the end of treatment virological response rate and subsequently also decreases the relapse rate.

Predicting Response to Therapy

Analysis of current data indicates that HCV genotype and viral load are the most useful predictors of response to therapy. HCV genotype 1 is the most prevalent genotype in the United States (65–75%) and has the lowest response rates to treatment (20, 21, 32, 34, 50). With the two-drug regimen, there was a significant difference in the response rates between patients with genotype 1 or non–genotype 1 infection (Fig. 2) (20, 21, 26). Genotype 1–infected patients treated for 24 weeks had

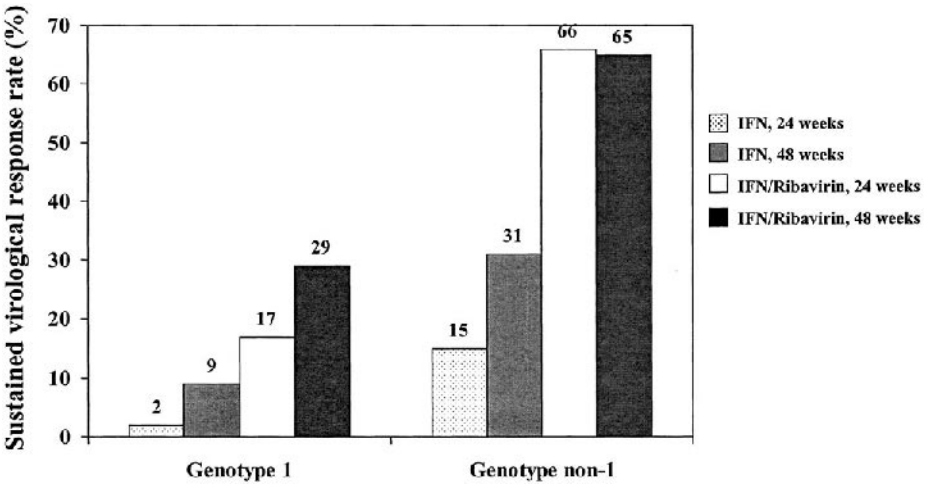

Figure 2 Sustained virological response rates in patients with genotype 1 versus other genotypes in patients treatment with IFN monotherapy and in patients treated with IFN plus ribavirin. IFN, recombinant interferon alfa-2b. Patients with genotypes other than 1 responded to all treatment arms significantly better than genotype 1 patients (20, 21).

a sutained virological response rate of 17% compared to 29% for 48 weeks of therapy. Patients with genotypes other than 1 had similar sustained virological response rates of 66% with 24 weeks of therapy and 65% with 48 weeks of therapy.

Further stratifying response based upon pretherapy viral load indicated that genotype 1–infected patients, with a viral load greater than 2 million copies/mL, only had a 10% sustained virological response when treated for 24 weeks and a higher response rate of 27% after 48 weeks of therapy (Fig. 3). In contrast, those with genotype 1 and viral load less than 2 million copies/mL had virological response rates of 32 and 33% with 24 weeks and 48 weeks of therapy, respectively.

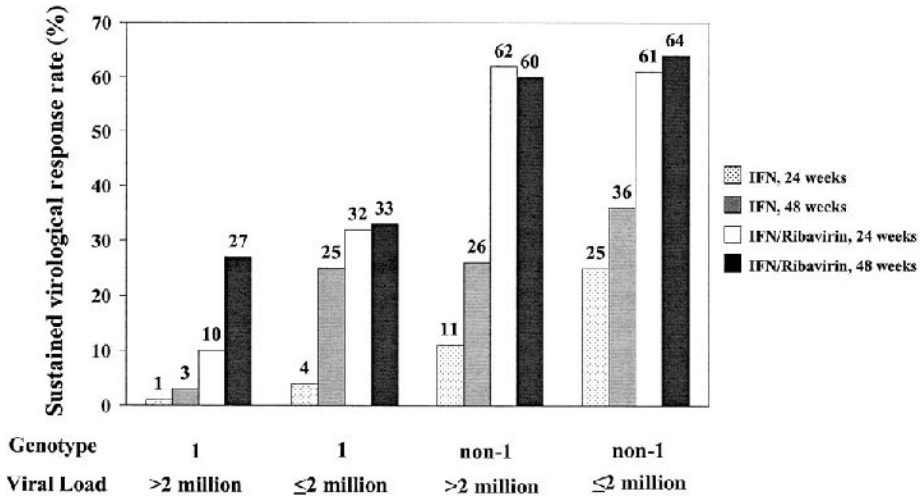

Figure 3 Sustained virological response rates stratified by genotype and viral loads in the patients treated with IFN monotherapy and in patients treated with IFN plus ribavirin. IFN, recombinant interferon alfa-2b. Viral load, million copies/mL. Based on the 1744 patients in the two clinical trials by McHutchison and Poynard. Only patients with genotype 1 and viral load above 2 million copies/mL had a greater response rate to IFN plus ribavirin therapy for 48 weeks versus 24 weeks (20, 21).

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Non–genotype 1–infected patients had a 60% sustained virological response rates regardless of viral load or duration of treatment. These data suggest that 48 weeks of combination therapy was more efficacious for patients with genotype 1 infection with high viral load and only 24 weeks of therapy is required for all others.

Poynard et al. recently reported on the clinical value of five independent characteristics that were associated with a sustained virological response: genotype 2 or 3, baseline viral load less than 3.5 million copies/mL, no portal fibrosis, female gender, and age younger than 40 years (51). The data suggests treating all patients for 24 weeks, and then using the above factors as well as the absence or presence of serum HCV-RNA measured at 24 weeks to determine if patients should receive an additional 24 weeks of treatment. Further prospective analysis of this concept is required.

Monitoring HCV-RNA During Therapy

Testing for serum HCV-RNA during therapy can help predict which individual patients will be nonresponders; thus allowing early termination of therapy in those unlikely to respond. Data from McHutchison and Poynard indicate that testing serum HCV-RNA at 24 weeks correctly identified 98% of nonresponders (20, 21, 52). Combination therapy with IFN plus ribavirin is occasionally associated with late initial viral clearance. Persistence of serum HCV-RNA at week 12 was observed in 7% of patients who were eventual sustained responders (52). Stopping therapy at week 12 in these patients would have denied them the benefits of a later sustained response.

Side Effects

Combination therapy is more likely to lead to dose reductions or to the need to discontinue therapy than IFN monotherapy (45, 46, 53). The most common cause for dose reduction is the hemolytic anemia caused by ribavirin. Approximately 10% of patients receiving ribavirin develop a significant dose-dependent and reversible intravascular hemolytic anemia. The

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mean decrease in hemoglobin is 2–3 g/dL in the first 4–8 weeks of therapy. The degree of fall in hemoglobin is similar to that seen in patients who receive ribavirin only and not exacerbated when combined with IFN (46). Patients with a hemoglobin value of less than 14 g/dL before treatment have a 20–25% chance of a 4-g or greater reduction in hemoglobin during therapy. The anemia can be managed successfully by monitoring hemoglobin before initiating therapy, at week 2, week 4, and the monthly thereafter. If the hemoglobin value falls below 10 g/dL, the dose of ribavirin should be decreased to 600 mg per day and usually does not require cessation of therapy. This dosaoge modification will lead to an increase in hemoglobin of 1.0–1.5 g. Once treatment is completed, the anemia resolves within 4–8 weeks. Patients with preexisting anemia, coronary artery disease, or other conditions that would not be able to tolerate anemia are not suitable candidates for the two-drug regimen but may be candidates for IFN monotherapy.

Ribavirin, like other nucleoside analogues, is associated with cough, dyspnea, insomnia, pruritus, rash, and anorexia in 15–30% of patients, but these symptoms do not generally correlate with the degree of anemia (Table 5). Most of these side effects are mild and usually do not require dose modification or discontinuation of treatment. Like other nucleoside an-

Table 5 Incidence of Adverse Effects with IFN Plus Ribavirin

Adverse effects of ribavirin	% of patients affected while being treated with interferon plus ribavirin
Hemolytic anemia	7–9
Cough	14–15
Dyspnea	18–19
Insomnia	39
Pruritus	19–21
Rash	20–28
Gastrointestinal intolerance	9–46
Anorexia	25–27

Source: Adapted Ref. 20.

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alogues, ribavirin is teratogenic and cannot be given to patients who are pregnant or cannot comply with adequate contraception. The most common reasons for discontinuing therapy are depression and emotional disturbances, most likely due to IFN therapy rather than ribavirin.

All patients on the combination therapy require close follow-up, initially with biweekly visits and then monthly. A complete blood count should be performed at each visit, whereas the liver tests and thyroid studies should be individualized for each patient (Table 6). A methodical approach utilizing good patient education, nursing assistance, close follow-up, and careful monitoring can help most patients successfully complete their therapy.

Current Recommendations

Suitable patients should be considered for treatment with 3 million units of IFN subcutaneously three times weekly or an equivalent along with a 1000–1200 mg oral dose of ribavirin daily depending on their body weight (5). Patients should have the HCV genotype and viral load performed prior to therapy. Patients with non–genotype 1 infection require 24 weeks of therapy, whereas patients with HCV genotype 1 infection should be tested for HCV-RNA at 24 weeks. In these patients, if serum HCV-RNA is undetectable at 24 weeks, the therapy should be continued for an additional 24 weeks. All patients who have cleared HCV-RNA during or at the end of therapy should have HCV-RNA and ALT tested at 24 weeks after completing therapy to document whether there is a sustained virological clearance.

TREATMENT OF PATIENTS WHO HAVE RELAPSED FOLLOWING IFN MONOTHERAPY

A "relapse" is defined as the loss of detectable serum HCV-RNA during treatment, which is then followed by its reappearance after cessation of therapy. This is usually accompanied

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Table 6 Recommended Laboratory Tests During Interferon/Ribavirin Therapy[a]

		Treatment				Monthly during treatment	End of treatment	Monthly for 6 months post-treatment	6 Months after completion of therapy
	Baseline	Week 1	Week 2	Week 4	Week 24				
Hemoglobin	×	×	×	×		×			
WBC	×	×	×	×		×			
Platelets	×	×	×	×		×			
HCV-RNA	×				×		×		×
Pregnancy[a]	×					×		×	
ALT/AST	×					[b]			
TSH	×					[b]			

WBC, white blood cell count; HCV-RNA, hepatitis C virus RNA; ALT, alanine transaminase; AST, aspartate transaminase; TSH, thyroid-stimulating hormone.

[a] If applicable.

[b] Should be individualized for each patient by the treating physician.

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by a return of elevated ALT values. This is a laboratory finding which usually occurs within 24 weeks of stopping therapy and is usually asymptomatic. Late relapse 24 weeks after ceasing therapy is unusual (less than 5% of patients). Retreatment of relapse patients with the same dose and duration of IFN has produced disappointing results with no increase in sustained response rates (54, 55). Heathcote et al. reported that a higher dose of IFN yielded a sustained virological response of 58% after 48 weeks of therapy and 28% after 24 weeks of therapy (56). Extending the duration of the second course beyond 24 weeks (i.e., 48 or more weeks) is probably more important than using the higher IFN dose (54–56). A recent meta-analysis indicated that following an initial relapse, 38% of patients can achieve a sustained virological remission after retreatment with a longer course of IFN monotherapy (54). Another study suggested that long-term intermittent courses of IFN may provide sustained virological respnse in 30% of relapse patients, but further prospective data is required before this can be considered a clinical viable option (57). The most important determinant of responding to the second or subsequent course of IFN appears to be clearance of serum HCV-RNA during the first course of therapy rather than normalization of ALT values without clearance of virus.

Retreatment of relapse with the combination of IFN plus ribavirin has recently been evaluated in a randomized controlled trial of 350 patients (55). A sustained virological response was reported in 49% of patients treated with 24 weeks of therapy compared to only 5% receiving retreatment with IFN monotherapy given for the same duration. The combination of IFN plus ribavirin for the treatment of relapse hepatitis C patients has now been approved in the United States. The best response was seen in patients with genotypes other than 1. Patients with unfavorable factors (genotype 1, high viral load) may theoretically benefit from retreatment with the two-drug regimen for a longer duration (48 weeks). Recent preliminary data support this concept, but further prospective data are required (58).

Thus, there are currently two options for retreatment of

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the patient who has relapsed following IFN monotherapy. They should be retreated with 24 weeks of IFN plus ribavirin or 48 weeks of higher doses of IFN (e.g., consensus IFN). Once again, the patient must be carefully evaluated prior to treatment. If patients had difficulty tolerating the initial course with significant side effects requiring dose reductions or cessation of therapy, they are likely to experience the same difficulties when they are retreated. Thus, the potential benefits as well as the potential adverse effects should be discussed with the patient before proceeding with retreatment.

TREATMENT OF IFN MONOTHERAPY NONRESPONDERS

Despite multiple studies performed in nonresponder patients, there is no universally effective therapy for this large group of IFN monotherapy nonresponders. Studies have attempted to use a higher dose regimen, extended the duration of IFN therapy, or combined agents such as amantidine, rimantidine, nonsteroidal anti-inflammatory drugs (NSAIDs), ursodeoxycholic acid, pentoxifylline, cyclosporine, steroids, and quinolones with IFN (59–61). The results have generally been disappointing, with less than 15% of patients achieving a sustained virological response (31, 50, 54, 59, 60, 62).

With the recently approved use of ribavirin with IFN, there is interest in using this combination for nonresponder patients. Currently, the efficacy of IFN plus ribavirin for these patients is unknown. Although many studies are in progress, the preliminary results show that although end of treatment rates may be as high a 37%, the sustained response rates vary between 5 and 32% (61, 63–65). Thus, further data analysis will be required to determine the effectiveness of combination therapy with ribavirin for the IFN nonresponders. These patients should probably be entered into clinical research trials until more effective therapies are available.

In this situation, "maintenance" IFN therapy may prevent disease progression. Although there is little data avail-

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able to support this in terms of virological clearance or normalization of ALT values, there may be histological benefit in terms of regression of fibrosis (17, 40). A large, multicenter trial assessing the potential long-term histological benefit of maintenance IFN is currently underway. Until further evaluation of the side effects, response rate, efficacy, and cost effectiveness has been completed, maintenance IFN therapy cannot be recommended for the IFN nonresponder outside of clinical trials.

PEGYLATED IFN

Recently, long-acting forms of pegylated IFN (the attachment of a polyethylene glycol molecule) have been evaluated. This has allowed the development of a biologically active IFN molecule with a longer half-life and much more favorable pharmacokinetics. Phase I studies have shown that the pharmacokinetics of pegylated IFN-α2a are linear with a longer half-life (>90 h) than standard IFN-α2a (66, 67). These long-acting formulations theoretically deliver a more "consistent" dose of IFN and can be administered weekly.

A Phase II study conducted in 155 patients without cirrhosis compared different doses of pegylated IFN-α2a with the standard dose of IFN-α2a. The optimal dose appeared to be 180 μg and was associated with a 36% sustained virological response rate as compared to 5% with IFN-α2a (68). A larger registration trial recently published confirms these results and indicated a similar sustained response of 39%, double that observed with 48 weeks of standard IFN-α-2a (68a). Similarly a Phase III trial involving 1219 patients compared 48 weeks of pegylated IFN-α2b with standard α2b. The sustained virologic response was 25% for pegylated IFN-α2b versus 12% for IFN-α2b (69).

A further recent trial involving 271 patients with cirrhosis showed a 29% sustained virological response with 180 μg of pegylated IFN-α2a as compared to 6% with IFN-α2a (70).

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Dose modifications were necessary in 37% of the patients as compared to 29% in patients receiving IFN-α2a. Therapy was withdrawn in 23% of patients with pegylated α2a and in 27% of patients receiving IFN-α2a (70). This compares favorably with approximately 21% of patients requiring cessation of therapy while being treated with IFN plus ribavirin. In summary, when given for a 48-week treatment course, the data suggest that pegylated IFN monotherapy doubles the sustained response observed with the regular IFNs, has a similar side-effect profile, but does not decrease relapse after therapy and is not effective in the majority of hepatitis C patients, those with genotype 1 infection and high viral load. Peginterferon-α2b is currently FDA approved for use in patients with chronic hepatitis C.

Because ribavirin combined with interferon decreases relapse rates after therapy, the combination of peginterferon plus ribavirin should increase efficacy. Two recent trials confirm these hypotheses. A randomized trial involving 1530 patients indicated that peginterferon-α2b dosed according to body weight (1.5 mg/kg/week) plus ribavirin for 48 weeks enhanced the sustained response rate to 54% compared to 47% observed with standard treatment with IFN-α2b plus ribavirin (71). Categorical analysis also indicated that weight-based dosing of both IFN and ribavirin enhanced this sustained response rate to 61% (this weight-based dosing schedule has recently been approved by European authorities for usage in the European Union). Similarly, combining pegylated IFN-α2a with ribavirin has recently been shown in a multicenter registration trial to significantly enhance the sustained response rate from 45% (with IFN plus ribavirin) to 56% with pegylated IFN-α2a (180 mg/week) plus ribavirin (72).

Both these trials also indicated that genotype 1 patients benefited most from this newer combination, patients compliant with therapy responded best, and that neutropenia and dose modifications will be more frequently required with this combination.

Thus, the combination of pegylated IFN plus ribavirin,

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once approved, will be our next best available therapy for suitable patients. This treatment will provide an incremental benefit in terms of sustained response, especially for genotype 1 infected patients, and allow convenient once weekly dosing with an acceptable safety profile.

FOLLOWING PATIENTS WITH HEPATITIS C WHO HAVE UNDERGONE THERAPY

Patients that have achieved a sustained virological response 24 weeks after completion of therapy usually remain in "remission." These patients' liver tests and HCV-RNA should be monitored at the time of their annual physical examination for possible relapse. Patients who did not respond to treatment should be evaluated for evidence of end-stage liver disease on physical examination and should be monitored with liver tests, complete blood count, and prothrombin time every 6–12 months. Repeat liver biopsy at 4- to 5-year intervals has been recommended by some authorities to assess for progression of fibrosis and cirrhosis (10). These patients should be referred for possible enrollment in ongoing clinical trials of new therapeutic agents.

Patients with established cirrhosis have an overall risk of 1–4% per year of developing hepatocellular carcinoma (73). Although, screening with the serum alpha-fetoprotein (AFP) and ultrasound examination has proven to be effective in early detection of hepatocellular carcinoma, this has not shown to reduce overall mortality (73). Screening has been recommended but should be limited to those patients in whom treatment of primary liver cancer would be a reasonable option (10, 73).

FUTURE THERAPIES

More effective and safer antiviral agents for hepatitis C are needed. The identification of the structure of the protease, helicase, and polymerase of the virus should allow more rapid

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development of new antiviral agents. Whether these will need to be given alone, in combination, or with IFN and/or ribavirin or similar drugs is unknown. Additionally, immunological approaches may be added to the therapeutic armamentarium in the future. These may involve cytokines, certain forms of immunotherapy, and molecular-based approaches, including ribozymes and antisense oligonucleotides. Because of the rapid developments in treatment, recommendations for therapy will probably change every few years. We hope that new approaches will provide effective therapy for the majority of hepatitis C patients in the future. A more complete outline of future therapies is summarized in Chapter 13.

CONCLUSIONS

With recent advances in the treatment of chronic hepatitis C, the optimal current therapy can achieve a sustained response rate in approximately 40% of the treated patients. Thus, patients with elevated aminotransferase levels, detectable HCV-RNA in the serum, and chronic inflammation with some degree of fibrosis should be screened for possible therapy. For initial treatment of patients with hepatitis C, the most effective therapy in terms of biochemical, virological, and histological response criteria is the combination of IFN and ribavirin. The duration of this therapy should be based on HCV genotype, with 48 weeks of therapy for patients with type 1 genotype and 24 weeks of therapy for other genotypes. Serum HCV-RNA should be measured at week 24 to assess response and guide further therapy in patients with genotype 1 infection. Patients who are not candidates for IFN plus ribavirin combination therapy can be considered for IFN monotherapy. However, these patients should have their serum HCV-RNA tested at week 12, and if this is not detectable, they should complete 48 weeks of therapy. Side effects and the need for dosage modification or discontinuation are more frequent with IFN plus ribavirin compared to IFN monotherapy, but these can usually be managed with close follow-up and careful monitoring.

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8

Persistently Normal Alanine Aminotransferase Levels in Individuals with Hepatitis C

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BACKGROUND

Historical Application of Alanine Aminotransferase as a Marker for Viral Hepatitis

Bloodborne infection of the liver has been recognized since the mid-1950s when elevations in liver enzymes were described following blood transfusions. After the identification of the Australia antigen in the late 1960s and the implementation of testing for hepatitis B, the incidence of posttransfusion hepatitis declined. Despite screening for hepatitis B, approximately 10% of multitransfused individuals developed alanine aminotransferase (ALT) elevations posttransfusion. For

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more than two decades, this disease, which had a clinical course similar to hepatitis B, was termed non-A, non-B hepatitis. Because no infectious agent was identified, by definition this entity was diagnosed by a history of parenteral exposure to blood products and by ALT elevations without recognized etiology.

Antiviral therapy of hepatitis B in the 1980s with interferon alpha (INF-α) showed promise of viral clearance. Patients with hepatitis B who were more likely to respond had abnormal ALT levels, and criteria for INF responsiveness included the normalization of ALT. Therefore, an early clinical trial by Hoofnagle et al. (1) using INF-α in 10 patients with a history of liver enzyme elevations following parenteral exposure to blood without hepatitis B viral markers defined response as normalization of ALT. All treated patients, regardless of the dose, showed improvement in ALT, with 7 of 10 patients demonstrating normalization. In three patients who had posttreatment liver biopsies, there was a significant improvement in histology. By the late 1980s, a large clinical trial tested INF-α for patients with non-A, non-B hepatitis, and response to treatment was defined as normalization of ALT by the end of treatment week 24 (2). Patients with a decrease of ALT of greater than 1.5 times the upper limit of normal (ULN) were defined as having a near-complete response. By definition, inclusion criteria specifically stipulated an ALT of greater than 1.5 times normal for at least 1 year. When the first-generation anti–hepatitis C virus (HCV) testing became available in 1989, 86% of these 166 patients proved to be anti-HCV positive. Every subsequent registration trial has required that patients included for treatment with HCV must have ALT above the upper limit of normal. Currently, with the wide availability of reliable viral testing by various amplification techniques, viral eradication is the "gold standard" for assessing successful antiviral treatment. Thus, increased attention is now focused on HCV-infected patients with persistently normal ALT and whether antiviral therapy should be offered to this cohort.

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Identification of Individuals with Normal ALT and Hepatitis C

The discovery of the HCV genome in 1989 established HCV as the etiological agent of non-A, non-B hepatitis in 80–90% of the cases. Before this time, donor blood products were screened using surrogate markers. That is, HBsAg donor units were excluded, but there was no reliable way to exclude non-A, non-B hepatitis. By the mid-1980s, with increased concern regarding the safety of the blood supply because of the newly recognized human immunodeficiency virus (HIV) infection, surrogate markers of hepatitis C were utilized. Donor units positive for anti-HBc and those with raised serum ALT values were excluded, because such units were more likely to transmit non-A, non-B hepatitis. The first-generation anti-HCV test was licensed in the United States in 1990, and the widespread screening of all donor blood then identified a large group of patients with normal aminotransferases and antibodies to HCV.

With the development of the second-generation serological tests and the confirmatory recombinant immunoblot assay (RIBA), many of the anti-HCV–positive patients with normal aminotransferases were actually false positives (3). A small group persisted with HCV serological positivity and risk factors for HCV. Before widespread availability of viral amplification technology, early investigators performed liver biopsies on these patients to determine if there was a group of healthy HCV-infected patients (4). In one long-term study, volunteer blood donors with antibodies to HCV underwent liver biopsies. Those donors with normal aminotransferase levels were compared to those with elevated ALT levels. Among patients with normal ALT, 65% had evidence of detectable virus in the serum compared to 95% of the patients with elevated ALT levels (5). All patients without detectable virus and normal ALT had normal liver biopsies and had probably recovered from HCV infection, whereas the other patients with normal ALT and detectable virus had mild or

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moderate hepatitis (5). Therefore, documentation of virus and/or histological activity was required to determine viral infection in the group with normal ALT. Presently, the widespread use of RNA amplification has facilitated identification of patients with persistently normal ALT and hepatitis C viremia.

Normal ALT

The concept of "normal ALT," in reality, often is based on arbitrary definitions. When comparing studies that focus on patients with normal ALT, it is evident that the upper limit of normal for ALT is variable; an observation that jeopardizes valid interpretation of results of treatment trials. The actual normal ALT depends on both gender and body mass index (BMI). Piton et al. (6) recommends an ALT of 31 U/L for women with a BMI less than or equal to 23 and up to 66 U/L for men with a BMI greater than 23. Additional investigators have also confirmed the association of ALT and BMI. Several reports have noted that women predominate in studies of patients with normal ALT (5, 7–10). This observation may merely emphasize the observation that the laboratory used for the study defined too high an upper limit of normal for ALT without gender adjustment. Other factors may contribute to the level of the ALT such as underlying diabetes, hyperlipidemia, obesity, prescription drug use, and alcohol ingestion. It is important, therefore, to be cognizant of highly variable definitions when comparing antiviral studies of hepatitis C with normal ALT values. In addition, hepatitis C can lead to chronic infection with a sawtoothed curve of ALT levels (11). The focus of this chapter, therefore, is the patient with persistently normal ALT levels. Although the definition of "persistent" as it applies to persistently normal ALT levels varies among studies, it generally refers to three consecutive normal ALT values over a 6-month period (7, 8, 12).

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HEPATITIS C AND PERSISTENTLY NORMAL ALT

Symptoms

Many patients with HCV infection and persistently normal ALT were detected incidentally during routine blood donor screening, or for other reasons (e.g., after needle stick injuries of health care workers, infected sexual partners, family members). This observation implies that such patients were asymptomatic clinically and sometimes biochemically. In fact, in one study in which all individuals were incidentally discovered to be infected with HCV and have persistently normal ALT, all had been asymptomatic (7). HCV-infected individuals with increased levels of ALT were more apt to report fatigue, headaches, dyspepsia, muscle aches, and depression, although the symptoms were mild. Shakil et al. (5) studied 60 anti-HCV–positive blood donors considered to be asymptomatic. A self-administered symptom questionnaire uncovered a large percentage of individuals with nonspecific complaints, including fatigue (61%), headaches (54%), anxiety (54%), drowsiness (53%), muscle aches (46%), itching (34%), depression (29%), excessive sweating (29%), and other less common symptoms. None of the symptoms was clearly suggestive of liver disease.

Demographics

There does not appear to be a consistent demographic feature that distinguishes patients with normal ALT from those with elevated ALT. Intravenous drug use and the level of lifetime alcohol consumption were not statistically more common in normal or abnormal ALT patients (5, 7, 13). In one study, the age of individuals with persistently normal ALT tended to be slightly lower (44.8 vs 49.3 years) (7) than those with abnormal ALT; this difference was not statistically significant. Conversely, in another study, participants with normal ALT tended to be slightly older (38.9 vs 35.7 years) (14) than those

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with abnormal ALT; again, this difference was not significant. Several investigations suggest that HCV-infected patients with normal ALT are predominantly women (5, 7–10), whereas HCV infection itself is slightly more common among males (15). Other studies, however, have not shown a gender difference (16). As noted previously, ALT should ideally be standardized for gender and BMI. Thus, the predominance of studies suggesting that women are more likely to have normal ALT must be interpreted with caution because of the lack of standardization of ALT. An analysis of a large cohort of HCV-infected patients (all with abnormal ALT values) (17) found that those individuals with near-normal, minimal ALT elevations (less than 1.3 times upper limit normal) weighed less than the cohort with higher values. When such patients then responded to antiviral therapy, their ALT values fell to less than 0.5 times upper limit normal, suggesting that the minimal pretreatment elevations were perhaps more significant than would otherwise be suspected. In summary, therefore, both weight and body mass index strongly influence the concept of normal ALT.

Genotype Predominance

Whether genotype predominance occurs in patients with normal ALT versus those with abnormal ALT has been controversial. Two studies from Italy found that genotype 2a was more prevalent in patients with persistently normal ALT than those with elevated ALT (7, 18). More recently, Mathurin (13), in a French cohort of patients with normal ALT (matched for age, sex, ethnic origin, alcohol consumption, and duration of infection with patients with increased ALT), observed no significant genotype difference between the two groups. In an American study of blood donors, Shakil (5) reported that genotype 1b was more prevalent in patients with persistently normal ALT. Clearly, interpretation of genotype data is hampered by the worldwide diversity of genotype distribution.

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Level of Viremia

Most studies have concluded that there is no difference in serum viral titers between patients with normal ALT and those with elevated ALT (5, 7, 9, 13). Jamel et al. (16) reported significantly lower HCV-RNA levels in patients with persistently normal ALT compared with patients with elevated ALT. However, there was less than a threefold difference in HCV-RNA level in patients with normal ALT compared with elevated ALT.

Histology

For the past decade, numerous investigators have studied the relationship between serum ALT and liver histology in HCV infection. There are several confounding variables, and it is difficult to compare the histology of HCV-infected patients with normal ALT and those with elevated ALT who had been matched carefully for variables that might be important in histological damage such as duration of infection, age and method of infection.

Despite the variability in scientific methods and reporting of histology, histological evidence of liver damage, as defined by the histological activity index (19), occurs in almost all patients with virus detected in the serum regardless of ALT level. Two studies performed in the early 1990s on asymptomatic patients with anti-HCV showed that significant liver disease can be present even in patients with normal ALT and hepatitis C viremia (4, 20). Various studies report more (7), less (9, 13, 16), or similar (5, 21) histological scores in patients with persistently normal ALT compared with those with abnormal ALT. In a series of patients described by Puoti et al. (7), the percentage of patients with moderate or severe liver histology activity was greater among patients with normal ALT than among patients with abnormal ALT. Overall, however, no significant diffrences in the mean histology score were found between the two groups. Shakil et al. (5) also did a comparison study between patients with normal and abnormal

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ALT. They found that histological grading was similar in three groups with normal, slightly abnormal, and abnormal ALT. Although the trend showed greater histological activity in those with higher ALT levels, it was not significant, and this study probably suffered from a beta error, since all the groups were small in size. Shindo et al. (9) compared histological findings of 19 viremic patients with persistently normal ALT to 41 biopsy-proven chronic HCV-infected patients with abnormal ALT and noted less histological activity and fibrosis score in patients with normal ALT. Similar results were seen by Mathurin et al. (13) between matched groups with normal and abnormal ALT. However, in patients with normal ALT and alcohol consumption $>$ 50g/day, significant fibrosis was seen. A recent study (16) comparing viremic patients with normal ALT with those with abnormal ALT revealed significantly lower histological activity and fibrosis in patients with normal ALT even after excluding patients with alcohol consumption $>$ 50 g/day. In an analysis of a 1700-patient database, Gordon et al. (17) found that HCV-infected patients with minimal ALT elevations (less than 1.3 times upper limit normal) had significantly lower inflammation and fibrosis than patients with higher ALT values. In summary, it would appear that HCV-infected patients with normal ALT have generally benign histology, but that liver biopsy is ultimately necessary to determine the activity of the disease in most patients.

Natural History

Very little is known of the natural history of patients with hepatitis C and normal ALT. In general, investigators conclude that there is slow or absent liver fibrosis progression in these patients. Mathurin (13) estimated the progression rate of liver fibrosis to be statistically lower in patients with normal ALT than in patients with elevated ALT by dividing the fibrosis stage by the apparent duration of infection in years. One prospective study (22) addressing natural history in 37 patients that were anti-HCV positive with persistently normal

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ALT found mild chronic hepatitis in 34 patients. The liver histology of these patients did not worsen over 5 years of follow-up. Of the three patients with normal liver histology, two were HCV-RNA negative and one was HCV-RNA positive. During follow-up, seven patients developed increased ALT levels. The increased ALT levels did not correlate with the severity of histology in these patients. Data from a controlled interferon (IFN) treatment trial for patients with normal ALT and hepatitis C viremia found the rate of fibrosis to be very slow and similar between treated and untreated patients over a 18-month period (23). Most patients with hepatitis C viremia and normal ALT levels have some degree of liver inflammation and, based on tracking the liver histology of a limited number of individuals, slowly, if ever, progress to cirrhosis.

Treatment

Early studies referred to viral hepatitis patients with persistently normal ALT as "healthy carriers." This terminology primarily applied to the hepatitis B patient, and there is now a reluctance to promulgate the use of this phrase in the patient with hepatitis C. Whether to treat the patient with hepatitis C and normal ALT remains controversial. At the time of the 1997 National Institutes of Health (NIH) Hepatitis C Consensus Conference regarding treatment recommendations, only one study treating patients with normal ALT had been published (8). Historic and current policy of the pharmaceutical companies excludes patients with normal ALT from registration trials. A summary of treatment investigations for patients with chronic hepatitis C and persistently normal ALT is listed in Table 1 (8, 10, 12, 23–29). Only three of these were randomized controlled trials (23, 26, 27). In all investigations listed, the primary outcome measured was eradication of viremia. In most studies, enrollment numbers were low, rarely exceeding 20 patients, with a follow-up of 6 months after treatment, and most investigators used standard doses (3 MU) of interferon alpha (IFN-α) for 6 months. Exceptions to this include Rossini

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Table 1 Treatment Trials for Patients with Chronic Hepatitis C and Normal Serum ALT Levels

Author (reference)	Controlled	No. of patients	Therapy	Dosing	Duration of therapy	Serum treatment HCV neg. end of treatment	Sustained response
Jacobson (24)	No	18	IFN 3 or 5, MU, and RBV	INF tiw, RBV qd	48 wks.	10/12 (83%) (at 24 wks.)	n/a
Nordoy (12)	No	23	3 MU IFN	tiw	6 mos.	9/23 (39%)	2/23 (8.7%)
Orito (25)	No	16	10 MU IFN	tiw	6 mos.	6/16 (37.5%)	6/16 (37.5%)
Rossini (26)	Yes	10	3 MU IFN	tiw	12 mos.	5/10 (50%)	2/10 (20%)
Sangiovanni (27)	Yes	16	3 MU IFN	tiw	6 mos.	1/16 (6%)	0
Serfaty (8)	No	10	3 MU IFN	tiw	6 mos.	2/10 (20%)	0
Silverman (10)	No	15	3 MU IFN	tiw	6 mos.	1/15 (6.6%)	0
Tassopoulas (28)	Yes	37	5 MU IFN	tiw	6 mos.	16/37 (43%)	8/37 (21.6%)
Tran (23)	Yes	11	3 MU IFN	tiw	12 mos.	Not reported	2/11 (18%)
Van Thiel (29)	No	21	5 MU IFN	qd	6–12 mos.	14/21 (66.6%)	Not reported

tiw, three times a week; qd, once a day.

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et al. (26), who treated for 12 months; Van Thiel et al. (29), who treated with 5 MU daily for up to 12 months; Orito et al. (25), who treated with 10 MU for 6 months; and Tassopoulos (28), who treated for 6 months using 5 MU IFN. Sustained viral eradication was similar to standard therapy in patients with elevated ALT. In patients whose viral levels fell during therapy, HCV-RNA usually returned to pretreatment levels. Only one study, using higher doses, reported a significantly higher viral clearance rate (25). Nordoy (12) hypothesized that the reason they found a higher viral clearance in their study was because of mild liver fibrosis seen in his cohort, but genotype data was not included. In contrast, two other investigators found that a lack of viral clearance was seen in patients with mild or moderate histological activity (8, 27). It has been postulated that patients with normal ALT and viremia may have a deficient immune response to infected hepatocytes that is not improved with IFN-α (27). Most of these studies were designed and implemented (a) before the importance of genotype data was realized and (b) before the advent of combination therapy (IFN and ribavirin).

Predictors of Response

A study that did explore the role of both monotherapy and combination therapy for HCV-infected patients with minimally abnormal ALT values was recently published (17). Individuals whose baseline ALT was ≤ 1.3 times the upper limit of normal responded no better to treatment than those whose baseline ALT was > 1.3 times the upper limit of normal. Hepatitis C viral characteristics may play a role in predicting outcome with IFN-α therapy. Similar to patients with abnormal ALT, genotype 1 in patients with normal ALT has been shown to respond poorly to IFN therapy alone in most studies (12). There is little data on serum viral levels and their association to IFN response in this group of patients. Sangiovanni et al. (27) suggested that the level of viremia did not seem to correlate with viral eradication in his cohort.

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Several investigators have observed ALT flares in some patients with normal ALT after starting treatment (8, 10, 26, 27). This ALT flare has also been seen in patients with viral hepatitis during IFN therapy. ALT levels usually return to normal after stopping the drug, suggesting that the rise in ALT is an effect of the IFN on the liver. Clearly, many viral and host factors may influence the response to treatment of hepatitis C and normal ALT, and the clinical significance of these so-called ALT flares has yet to be defined.

Evolving Treatment Strategies

Although the combination of IFN and ribavirin has demonstrated increased efficacy in the treatment of patients with abnormal ALT compared with IFN alone, no published studies using this agent in the normal ALT population are available except in abstract form. Jacobson et al. (24) used combination therapy in a cohort of normal ALT patients and found a superior rate of HCV-RNA clearance (61%) than has been reported in studies using IFN alone. Inasmuch as baseline ALT levels did not affect response to therapy among patients with very minimal ALT elevations (17), it now seems unlikely that patients with entirely normal ALT values will respond any differently to combination therapy than patients with higher levels. Larger controlled trials are needed.

Conclusions

HCV was isolated several decades after HBV. Because both infections targeted the liver, it was postulated that patients with hepatitis C would respond to therapy similarly to patients with HBV. Initial treatment protocols for HCV used criteria for study enrollment based on recommendations for HBV; that is, patients with elevated ALT. In addition, many patients with normal ALT were initially not even suspected to be infected with HCV. The concept of the "healthy carrier" of HCV should probably be dispelled. Still in question is why

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some patients have ALT which remains persistently normal. Although advances have been made in understanding this cohort, questions remain regarding histology, natural history, viral levels, and genotypes as well as response to therapy in patients with persistently normal ALT. To date, there is no compelling evidence that patients with persistently normal ALT are significantly different from patients with abnormal ALT. Nevertheless, the treatment of individuals with hepatitis C viremia and normal ALT has not been formally recommended. Longer treatment trials with larger numbers of patients using combination therapy and newer antivirals are necessary before definitive recommendations regarding the proper therapy of this unique cohort of hepatitis C–infected patients can be made.

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9

Retreatment of Hepatitis C: Interferon Nonresponders

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CASE

The patient is a 49-year-old man who attempted to increase the value of his life insurance in 1996 and was turned down due to discovery of elevated serum aminotransferases (alanine aminotransferase, ALT). He presented to his primary care physician for further evaluation. He denied symptoms and had always considered himself healthy. His physician repeated the ALT test, which remained elevated (Table 1), and subsequent work-up lead to discovery of chronic hepatitis C virus (HCV) infection. His sole risk factor for acquisition appeared to be multiple blood transfusions received following a motor vehicle accident 22 years previously. The patient was referred to a local hepatologist for further management of his disease. His viral

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Table 1 Treatment Course of HCV-Infected Patient

	Date	HCV-RNA (copies/mL)	ALT (ULN 39)
Diagnosis	December 1996	—	141
Begin IFN-α2b[a]	February 1997	3,546,000	80
↓ Month 3	May 1997	2,980,000	92
		Treatment stopped	
Begin IFN-α2b[a]	August 1997	3,300,000	52
↓ Month 3	November 1997	2,521,000	63
↓ Month 6	February 1998	3,000,000	41
		Treatment stopped	
Begin combination therapy[b]	December 1998	4,200,000	96
↓ Month 3	March 1999	2,734,000	55
↓ Month 6	June 1999	3,150,000	53
		Treatment stopped	

ULN, upper limit of normal.
[a] IFN-α2b 3 mU TIW.
[b] Daily ribavirin (1200 mg/day) plus IFN-α2b 3 mU TIW.

titer was 3,546,000 copies/mL and liver biopsy was consistent with chronic HCV infection with mild fibrosis. He was anxious to undergo treatment.

The patient received interferon alpha 2b (IFN-α2b) subcutaneously at 3 mU three times a week (TIW). The patient tolerated therapy well with minimal adverse effects. Viral titer, after 3 months of monotherapy, remained elevated at 2,980,000 copies/mL. He was considered a nonresponder and, given the treatment recommendations at the time, IFN-α2b was discontinued. It was decided to attempt retreatment with a longer course of therapy. He again received IFN-α2b at 3 mU TIW for 6 months. There was no change in his viral titer, and treatment was discontinued after 6 months. Following U.S. Food and Drug Administration (FDA) approval of combination therapy, a third course of treatment was attempted. The patient received IFN-α2b at 3 mU TIW plus daily ribavirin (1200 mg/day). Once again, there was no substantial change in viral titer, although slight improvement in aminotransferase levels was seen. The patient now asks you, "What next?"

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BACKGROUND

Although research was able to identify the viruses responsible for hepatitis A and hepatitis B around 1975 and 1963, respectively, it remained clear there were additional agent(s) that led to posttransfusion hepatitis. In 1989, an agent that accounted for approximately 80% of the posttransfusion hepatitis was identified (1). This agent was named hepatitis C in keeping with the existing nomenclature. Tests to detect it in the blood were developed in 1990 and 1991 and, perfected by 1992 (1, 2).

Chronic HCV infection has become a major clinical problem in the United States. It is estimated that 120,000 patients were newly infected with the HCV annually during the 1980s, decreasing to about 30,000–35,000 per annum in the 1990s. Approximately 3.9 million persons (1.8%) in the United States have antibody to HCV and about 2.9 million are chronically infected with HCV (3, 4). The majority of those infected with HCV (80–85%) develop chronic viremia and approximately 70% of those develop chronic liver disease which can progress to cirrhosis and hepatocellular carcinoma. Hepatitis C accounts for 8000–10,000 deaths per year from sequelae of chronic liver disease; a number expected to triple in the next 10–20 years. Hepatitis C–induced cirrhosis is now the most frequent indication for liver transplantation in the United States (5).

HISTORY OF TREATMENT

Interferon

The interferons (IFNs) are naturally occurring glycoproteins secreted by many mammalian cells (particularly monocytes and transformed B cells) in response to viral infections and other antigenic stimuli. There are two types of IFN. Type I IFNs (alpha, beta, and omega) are similar genetically and biologically, whereas the type II IFNs (gamma) have very little

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homology with the others (6). IFN-α is the most extensively studied regarding specific viral infections. Interferons interfere with viral replication and production. Although they are not directly virucidal or virustatic, they have effects that are antiproliferative and immunomodulatory (7). The mechanism of antiviral action remains to be completely elucidated.

Several forms of IFNα have been used in human trials: α2a (Roferon A), α2b (Intron A), αn1 (Wellferon), αn3 (Alferon N), and consensus interferon (Infergen). Four IFNs have been approved for use in the United States for the treatment of chronic hepatitis C: α2b (1991), α2a (1996), consensus IFN (1998), and αn1 (1999). In vitro studies reveal that the IFNs are similar with regard to their antiviral, antiproliferative, and gene-inducing properties (6). Clinical trials suggest that this in vitro similarity is translated into equivalent in vivo efficacy between the IFNs (8).

Definitions of Response

Either biochemical (e.g., ALT) or virological parameters (HCV-RNA) can define the response to IFN therapy. Initial clinical trials used biochemical parameters only. Normalization of serum ALT at the end of treatment with continued normal ALT levels for at least 6 months following discontinuation of therapy was considered optimal (*sustained response*) (9). *Treatment relapse* was defined as normalization of ALT at the end of treatment, with ALT becoming elevated following discontinuation of therapy. *Treatment nonresponse* was defined as failure to normalize ALT or normalization of ALT with breakthrough during IFN therapy. Virological response is now similarly defined.

Serum viral measurements were inconsistently performed in these early trials. This has created difficulty in defining true virological relapsers versus nonresponders to an initial course of IFN therapy. Patients who had an ALT relapse following discontinuation of IFN therapy may have actually been true virological nonresponders. Conversely, those

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who cleared virus at the end of treatment but did not normalize their aminotransferases may have been erroneously labeled as nonresponders when more accurately they were virological responders or relapsers. Clearly, this adversely affects categorization of prior treatment effect in subsequent clinical trials of "nonresponders" (10).

The current "gold standard" for assessing treatment outcome (*sustained response*), therefore, is clearance of HCV-RNA from the serum for at least 6 months posttherapy. Normalization of ALT does not constitute a response. Nonresponse is defined as HCV-RNA positivity by polymerase chain reaction (PCR) after 3 months of IFN monotherapy or 6 months of combination therapy. Using these criteria, well over 50% of patients treated with IFN monotherapy can be classified as nonresponders.

IFN Monotherapy

Therapy for HCV initially consisted of IFN at 3 mU TIW for a total of 6 months. Approximately 35–50% of patients treated achieved normalization of ALT at the end of treatment with about 27–35% losing circulating virus (HCV-RNA negative) (11–15). Sustained biochemical and/or virological response rates, however, remained extremely low—between 6 and 20% (9, 11, 16–22). Overall, therefore, approximately 80–90% of patients either failed to respond to IFN monotherapy or relapsed after treatment was discontinued.

Extended IFN Monotherapy

Extending IFN monotherapy to 12–18 months significantly improved sustained response rates (6 to 42%) when compared to 6-month dosing (12, 13, 15). Given the enhanced sustained response, the FDA approved the use of IFN monotherapy for up to 2 years. Nevertheless, 60–70% of those treated with extended monotherapy still remain nonresponders at the end of treatment (HCV-RNA positive) (23–29).

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Other IFN Monotherapy

There have been numerous studies evaluating differing IFN regimens and different IFNs in the treatment of hepatitis C. Differences in study design, dosing schedules, and data analysis make it impossible directly to compare most of these trials. There are some data which suggest that daily dosing may improve response rates (30, 31). Other data suggest that higher doses of IFN or higher daily IFN dosing may improve response rates as well (30, 32, 33). Higher dose induction therapy has also been associated with improvement in sustained response rates (12, 27, 34–36).

Combination Therapy

Ribavirin is a guanosine analogue that inhibits the replication of several RNA and DNA viruses (37). Its mode of action in the treatment of chronic HCV infection remains to be completely delineated. It appears to inhibit the viral RNA-dependent RNA polymerase, depleting the intracellular guanine pools. It may also interfere with the "capping" of viral RNA (38). Treatment with ribavirin monotherapy showed reduced mean serum aminotransferase levels, but the effect was short lived and there was no effect on serum viral levels (37, 39–43).

Treatment of patients who have never received therapy for their HCV (treatment-naïve patients) with IFN and ribavirin (combination therapy) is superior to either IFN or ribavirin monotherapy in producing sustained biochemical and virological responses (21, 22, 44–46). A recent meta-analysis reported that patients treated with combination therapy were more likely to achieve normalized ALT levels and negative HCV-RNA at the end of therapy (3.3-fold) and at 6-month follow-up (7.3-fold) than patients treated with IFN alone (47). This was confirmed in large randomized trials, with about 40% of treatment-naïve patients achieving a sustained response (21). And yet, over half of the patients treated with combination therapy remain unresponsive.

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There is, therefore, a large cohort of chronically infected patients who are unable successfully to eradicate virus with our current treatment therapies. These patients remain the most frustrating to treat. Available options for patients who have failed to respond to IFN monotherapy include retreatment with monotherapy at the same or higher doses, retreatment using monotherapy for extended periods, retreatment with combination therapy, retreatment using a different form of IFN or other adjunctive therapy, or watchful waiting. Options for those who have failed to achieve a response to combination therapy are even more limited.

RETREATMENT WITH IFN MONOTHERAPY

Before the introduction of combination therapy, many trials were performed using monotherapy to retreat IFN nonresponders. As mentioned above, comparing these trials remains difficult owing to differences in design and the combination of relapsers with nonresponders. Only those trials looking specifically at well-defined nonresponders and reporting virological responses based on HCV-RNA are included here.

Early retreatment trials and the "control" arms of higher dose trials have employed identical dosing to that which the patients had been previously exposed—3 mU TIW for 24 weeks. Sustained virological response rates to this regimen were poor and no sustained responses have been reported (48–50). Given these poor response rates, investigators began looking at higher doses and longer duration of IFN monotherapy. Most of these trials were small; these data are presented in Table 2.

Kawanishi et al. reported that high-dose therapy for a longer duration elicited a sustained virological response in a small percentage of patients (51). Patients were retreated with 5 mU IFN-α2b daily for 2 weeks, followed by TIW for 24 weeks, then tapered to 3 mU TIW for 8 weeks, followed by 1.5

Table 2 Retreatment of Interferon Monotherapy Nonresponders with Interferon Monotherapy

Author/date (reference)	IFN	Dose	Duration	No. of patients (No. dropped)	ETR (%)	NR (%)	R	SR (%)
Giudici-Cipriani 1993 (48)	α2b	3 mU TIW	24 weeks	21 (0)	0[a]	21 (100)	0	0
Kawanishi 1995[b] (51)	α2b	5 mU qD	×2 wk *then*	30 (2)	6 (21)	22 (73)	2	4 (14)
		5 mU TIW	×24 week *then*		6 (20)[c]			4 (13)[c]
		3 mU TIW	×8 week *then*					
		1.5 mU TIW	×8 wk					
Brillianti 1995 (49)	α2b	3 mU TIW	24 weeks	7 (0)	0	7 (100)	0	0
Rabinovitz 1996 (52)	IFN-α[d]	9 mU, 15 mU or 30 mU/week[d]	24 weeks	30 (0)	8 (27)	22 (73)	4	4 (13)
Rolachon 1997 (53)	α2b	10 mU TIW	×2 mo *then*	14 (0)	7 (50)	7 (50)	6	1 (7)
		6 mU TIW	×4 mo					
		vs	vs					
		6 mU TIW	6 mo	9 (0)	5 (56)	4 (44)	4	1 (11)
Tong 1997 (54)	α2b	3 mU TIW	48 weeks	12 (UNK)	UNK	UNK	UNK	1 (8)
Scotto 1998 (50)	Leukocyte IFN-α	3 mU TIW	24 weeks	12 (0)	3 (25)	9 (75)	3	0
			vs					
			48 weeks	12 (0)	2 (17)	10 (83)	0	2 (17)
Barbaro 1998 (55)	α2b	6 mU TIW	24 weeks	151 (5)	8 (5.3)	118 (78)	6	2 (1.3)
Bekkering 1998 (91)		10 mU qD	×7 days *then*	11 (0)	UNK	UNK	UNK	3 (27)
		10 mU TIW	×21 days *then*					
		3 mU TIW	×5 months					
Barbaro 1999 (56)	α2b	6 mU TIW	24 week	100 (5)	5 (5)	90 (90)	4	1 (1)

ETR, end-of-treatment response; NR, nonresponse; R, relapses; SR, sustained response; UNK = unknown.
[a] Measured by ALT.
[b] Abstract.
[c] Intention to treat analysis.
[d] Not specified.

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mU TIW for an additional 8 weeks. Patients who were negative for virus at 24 weeks received an additional 2 months of therapy at the 5-mU dose before being tapered. Of the 30 patients enrolled, 28 completed treatment. Twenty-two (73%) patients failed to respond at all. Six (21%) had an end of treatment response as measured by normal serum ALT and negative HCV-RNA. Four (14%) patients achieved a sustained response. Using an intention to treat calculation, these figures would be 20 and 13%, respectively.

Rabinovitz et al. treated 30 patients with IFN-α at 9 mU, 15 mU, or 30 mU a week (52). This report did not state which IFN-α was used, the dosing schedule, or how many patients were in each treatment group. Overall, eight patients (27%) achieved an end of treatment response, with four (13%) virological sustained responders. Still, two-thirds failed to achieve any response.

Rolachon et al. randomized 23 patients to receive either 10 mU TIW for 2 months followed by 6 mU TIW for 4 months (n = 14) or 6 mU TIW for 6 months (n = 9) (53). Approximately 50% in each group were nonresponders to this more aggressive regimen. Seven patients (50%) in the first group had an end of treatment response compared with five (56%) in the second group. One patient in each group (7 and 11%, respectively) sustained their response.

Tong and colleagues treated 12 patients with standard IFN-α2b therapy of 3 mU TIW for 48 weeks (54). They reported an 8% sustained response rate, but no data are given on dropout rate, end of treatment response, or relapse.

In a randomized trial by Scotto et al., patients received leukocyte IFN-α at 3 mU TIW for either 24 (n = 12) or 48 weeks (n = 12) (50). In the first group, three (25%) of the patients achieved an end of treatment response, but none sustained it. In the second group, however, two (17%) patients achieved an end of treatment response and both patients remained sustained responders 6 months after discontinuation of therapy, suggesting that longer duration was more effective in prior nonresponders.

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Barbaro et al. attempted higher dose therapy with IFN-α2b at 6 mU TIW for 24 weeks in a large cohort of patients (n = 151) (55). End of treatment response was seen in eight patients (5.3%), with only two (1.3%) maintaining the response. Barbaro and colleagues subsequently treated another 100 patients with the same dose (IFN-α2b.6 mU TIW for 24 weeks) as part of a randomized trial comparing monotherapy with combination therapy (56). Five of the 100 patients (5%) receiving monotherapy achieved end of treatment viral eradication, but only one (1%) maintained viral clearance. This finding was similar to their previous results.

Despite lengthening duration of treatment or altering the treatment regimen, retreatment of IFN monotherapy nonresponders with IFN monotherapy remains only minimally successful, with 50–100% failing to achieve any response (nonresponders). The sustained response rate is low; generally between 0 and 15%.

RETREATMENT WITH IFN–RIBAVIRIN COMBINATION THERAPY

The meta-analysis mentioned above and others have suggested that combination therapy in nonresponders might be more effective than IFN monotherapy in achieving a sustained response (47, 57–59). To this end, several investigators have looked at retreatment of IFN nonresponders with combination therapy. These data are summarized in Table 3.

Schvarz and colleagues conducted two small trials evaluating retreatment of IFN nonresponders with combination therapy at 3 mU TIW for 6 months (58, 60). In the first report, 6 patients were treated and 10 in the second. Two (33%) and four (40%) patients achieved an end of treatment response, respectively. Only one patient in the first attempt (17%) remained negative for virus at follow-up, whereas three (30%) did in the second trial. Still, 60% remained unresponsive.

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Table 3 Retreatment of Interferon Monotherapy Nonresponders with Interferon–Ribavirin Combination Therapy

Author/date (reference)	IFN	Dose	Duration	No. of patients (No. dropped)	ETR (%)	NR (%)	R	SR (%)
Schvarz 1995 (58)	α2b + RVN	3 mU TIW	24 weeks	10 (0)	4 (40)	6 (60)	1	3 (30)
Schvarz 1995 (60)	α2b + RVN	3 mU TIW	24 weeks	6 (0)	2 (33)	4 (66)	1	1 (17)
Brillanti 1995 (49)	α2b + RVN	3 mU TIW	24 weeks	7 (0)	2 (28)	5 (72)	1	1 (14)
Barbaro 1998 (55)	α2b + RVN	3 mU TIW	24 weeks	152 (17)	38 (25)	114 (75)	6	32 (21)
Sostegni 1998 (61)	α n3	RVN daily *then* 3 mU TIW	24 weeks *then* 24 weeks	31 (5)	4 (13)	29 (87)	4	0
		vs						
		RVN + 3 mU TIW	24 weeks	33 (3)	9 (27)	24 (73)	5	5 (15)
		vs						
		3 mU TIW	24 weeks	30 (2)	2 (7)	28 (93)	2	0
Barbaro 1999 (56)	α2b + RVN	3 mU TIW	24 weeks	100 (7)	21 (21)	79 (79)	7	14 (14)
Brouwer 1999 (92)	α2b + RVN	3 mU TIW	24 weeks	40 (UNK)	UNK	UNK	UNK	5 (12.5)
Preliminary Reports								
Nyberg 1999 (62)	α2b + RVN	3 mU TIW	24 weeks	5 (0)	1 (20)	3 (60)	1	Pending
Vega 1999 (63)	α2b + RVN	3 mU TIW	48 weeks	28 (1)	8/24 (33)	16/24 (67)	UNK	5/18 (27)

ETR, end of treatment response; NR, nonresponse; R, relapses; SR, sustained response; RVN, ribavirin; UNK = unknown.

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Brillanti et al. treated seven patients with combination therapy at 3 mU TIW for 24 weeks (49). These results were similar to those observed by Schvarz et al., with two patients (28%) clearing virus at the end of treatment and 1 (14%) sustaining this response at 6-month follow-up.

Barbaro et al. subsequently conducted a large prospective trial randomizing patients into two groups: (1) high-dose IFN monotherapy at 6 mU TIW for 24 weeks (n = 151) and (2) combination therapy at 3 mU TIW for 24 weeks plus daily ribavirin (n = 152) (55). Of those patients in the combination therapy group, 38 (25%) were negative for virus at the end of treatment, with six relapsing for a sustained response of 21%. This was significantly better than the sustained response rate (1.3%) seen in those on monotherapy. In a second prospective trial, these investigators randomized 200 patients into the same two groups: (1) high-dose IFN monotherapy (n = 100) and (2) combination therapy (n = 100) (56). Dosing and duration were the same as the previous trial. Twenty-one (21%) of patients had an end of treatment response, with 14 (14%) sustaining this response at follow-up. The nonresponder rate was 75–79%.

Sostegni and colleagues randomized 94 patients into one of three treatment arms: (1) ribavirin daily for 24 weeks followed by IFN-n3 at 3 mU TIW for 24 weeks (n = 31); (2) IFN-n3 at 3 mU TIW plus ribavirin daily for 24 weeks (n = 30); and (3) IFN-n3 3 mU TIW for 24 weeks (n = 30) (61). There were no sustained responders in the IFN monotherapy arm (group 3). Four (13%) patients in group 1 achieved an end of treatment response, but none sustained it. Nine (27%) patients in group 2 were end of treatment responders, with five (15%) sustained responders.

Two recent trials have noted preliminary results of retreatment with combination therapy (62, 63). Nyberg et al. reported end of treatment results on five patients treated with standard combination therapy for 24 weeks (20% response), but sustained data are pending (62). The percentage of nonresponders observed (60%) was similar to that reported by

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Scharvz et al. (58, 60). Vega and colleagues used standard combination therapy but extended the treatment period to 48 weeks (63). Currently, this is the only report in the literature using an extended treatment period of combination therapy for nonresponders. Of 28 patients enrolled, 24 have reached the end of treatment (with 8 responding) at the time of this writing. Only 18 patients have reached follow-up for a sustained response of 27% (5 of 18).

Despite the promising data on the response to combination therapy in treatment naïve and relapsed patients, the results in IFN monotherapy nonresponders remain somewhat discouraging. Sixty to 95% of patients are unresponsive to retreatment with this regimen. The sustained response rates of between 12 and 25%, however, may make retreatment with combination therapy worth attempting.

RETREATMENT WITH ALTERNATIVE FORMS OF INTERFERON

Consensus IFN

The Consensus Interferon Study Group evaluated the effect of high-dose consensus IFN (CIFN; 15 μg TIW) on those patients who had failed to respond to IFN-α2b (3 mU TIW) or lower dose CIFN (3 μg TIW or 9 μg TIW) (14, 64). Patients were randomized into two groups: (1) 15 μg TIW for 48 weeks (n = 69) or (2) 15 μg TIW for 24 weeks (n = 75). Twelve (17%) patients in group 1 and 14 (19%) in group 2 achieved an end of treatment response. This response was sustained in nine (13%) and four (5%) patients, respectively. Still, over 80% remained unresponsive to or relapsed after this therapy. Those patients in the initial 3-μg TIW group were excluded from the final analysis. Definition of response to prior therapy was based upon either virological or biochemical parameters. Also, despite the absence of discontinuation of drug in any patient, there was a high rate of dose reduction due to side effects (33%). Conclusions can be drawn from this trial only with cau-

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tion because of the exclusion of a portion of the patients and the mixed definition of response to therapy. These data are presented in Table 4.

The Consensus Interferon Study Group data was evaluated further to compare patients who had experienced an HCV-RNA response to initial monotherapy but broke through treatment with those who were HCV-RNA nonresponders throughout treatment (65). Those patients initially treated with 3 or 15 μg CIFN TIW were excluded from the analysis. Three hundred and twelve patients were nonresponders to initial monotherapy; 86 (27%) were nonresponders with breakthrough and 226 (73%) were nonresponders without breakthrough. The HCV-RNA breakthrough rate was similar between the 9-μg TIW CIFN group and the 3-mU TIW IFN-α2b group. Sixty-nine patients were retreated with 15 μg TIW for 48 weeks. Those treated for 24 weeks (n = 75) were excluded from the analysis. Those who had experienced prior breakthrough (n = 19) had a 27% sustained HCV-RNA response to retreatment with CIFN compared to 8% in those without prior breakthrough (n = 50). This difference was not statistically different. Although exciting, data from this trial should be viewed with some caution in light of the exclusion of patient groups.

IFN-β

Pilot studies using IFN-β have suggested a virological response in about 40% of treatment-naïve whites and 90% of treatment-naïve Japanese patients (66–68). Efficacy of IFN-β was maximized when it is given by intravenous infusion rather than by subcutaneous injection (69, 70). Very little information is available in regard to retreatment of IFN-α nonresponders with IFN-β.

Mazzoran treated 10 IFN-α nonresponders with IFN-β at 6 mU daily for 2 months (70). Four (40%) patients had a complete biochemical response and three (30%) became HCV-RNA negative at the end of treatment. These responders were

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Table 4 Retreatment of Interferon Monotherapy Nonresponders with Other Interferons

Author/date (reference)	IFN	Dose	Duration	No. of patients (no. dropped)	ETR (%)	NR (%)	R	SR (%)
Keeffe 1997 (14)	CIFN	15 μg TIW	48 weeks	69 (0)	12 (17)	57 (83)	3	9 (13)
			vs					
			24 weeks	75 (0)	14 (19)	61 (81)	10	4 (5)
Mazzoran 1997 (70)	IFN-β	6 mU qD IV	2 months	10 (0)	3 (30)	7 (70)	2	1 (10)
		then	*then*					
		6 mU TIW IM in responders	2 months					
Montalto 1998 (71)	IFN-β	6 mU qD	8 weeks	20 (0)	2 (10)	18 (90)	2	0
Barbaro 1999 (72)	IFN-β	6 mU qD	12 weeks	100 (12)	37 (37)	63 (63)	20	17 (17)
	vs		vs					
	IFN-α + RVN	6 mU TIW	12 weeks	100 (12)	20 (20)	80 (80)	8	12 (12)

ETR, end of treatment response; NR, nonresponse; R, relapses; SR, sustained response; RVN, ribavirin.

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treated for an additional 8 weeks with intramuscular IFN-β (6 mU TIW). Only one patient (10%) remained HCV-RNA negative at follow-up.

Montalto et al. treated 20 IFN-α2b treatment failures with intravenous IFN-β six times a week for 8 weeks (71). Two (10%) patients had an end of treatment response, but there were no sustained responders to this regimen.

In the largest IFN-β trial to date, Barbaro and colleagues randomized 200 IFN-α nonresponders into two groups: (1) IFN-β 6 mU six times a week or (2) combination therapy with IFN-α 6 mU TIW plus daily ribavirin (see Table 4) (72). Patients were treated for a total of 12 weeks. Thirty-seven (37%) patients in group 1 achieved an end of treatment response which was statistically better than the 20 (20%) in group 2 ($P = .012$). The statistical significance of this difference was not sustained, however, with 17 (17%) patients in group 1 and 12 (12%) patients in group 2 achieving a sustained response at follow-up. The number of patients who withdrew due to side effects (12 vs 9 patients) and were lost to follow-up (8 vs 6) were similar between the groups.

RETREATMENT USING ADJUNCTIVE THERAPY

Although the most promising adjunctive therapy to IFN has been the addition of ribavirin, several other alternatives have been suggested. These include iron reduction therapy, amantadine, rimantadine, ursodeoxycholic acid (UDCA), nonsteroidal anti-inflammatory medications (NSAIDs), thymosin α_1, corticosteroids, interleukin-2, zidovudine, levamisol, pentoxifylline, quinolone antibiotics, and ritonavir (73).

Iron Reduction Therapy

Iron may be an important component in the liver injury seen in chronic viral hepatitis. Removal of iron by phlebotomy improves the hepatic inflammation seen in chronic HCV infec-

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tion (74–76). Iron reduction before and during treatment with IFN increases end of treatment response rates (77–82). Further study is required to determine whether iron reduction leads to histopathological improvement or sustained long-term virological response rates.

Amantadine and Rimantadine

Smith conducted the initial trial of amantadine monotherapy in IFN-α nonresponders (83). She treated 22 nonresponders with amantadine 100 mg PO BID for 24 weeks. Six (27%) patients had both a biochemical and virological end of treatment response, and this response was sustained in four (18%) patients. Despite this promising pilot study, numerous studies (most presented in abstract form) have demonstrated little or no efficacy for either amantadine or rimantadine monotherapy (73). Little published data exist on the use of amantadine or rimantadine plus IFN-α therapy. Brillanti et al, reported preliminary results of a randomized study comparing two groups: (1) standard combination therapy and (2) standard combination therapy plus amantadine (100 mg daily) (84). Sustained data are not yet available, but end of treatment response was 10% in group 1 and 70% in group 2. It appears clear that amantadine monotherapy is ineffective in nonresponders, but combination with IFN/ribavirin suggests promise and deserves further evaluation.

Ursodeoxycholic Acid

UDCA is a hydrophilic bile acid derived from bear bile. Initially developed as therapy for dissolving cholesterol gallstones, it has been shown to be beneficial in certain forms of liver disease (e.g., primary biliary cirrhosis). UDCA improves serum aminotransferases in patients infected with chronic hepatitis C, but it has no antiviral effect (85). There have been several small pilot studies investigating UDCA alone or in combination with IFN in treatment-naïve patients (73). The results suggested little benefit. A single trial including nonre-

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sponders concluded that although there was no antiviral response, serum aminotransferases were improved, especially in cirrhotics (86). Given the sketchy data to date, there is little justification to recommend the use of UDCA in hepatitis C–infected patients who are unresponsive to IFN monotherapy. Further investigation is warranted.

Nonsteroidal Anti-inflammatory Drugs

IFN-α increases prostaglandin E production within the liver, leading to increased immunosuppressive activity (87). NSAIDS act as cyclooxygenase inhibitors, decreasing prostaglandin production, allowing increased production of antiviral proteins (87, 88). Several small pilot studies presented in abstract form suggested that NSAIDS could improve patient response to IFN in IFN monotherapy nonresponders. In a large randomized, double-blind trial of IFN-naïve patients, Zarski et al. found that the addition of tenoxicam to IFN monotherapy conferred no additional benefit (17.5% sustained response vs 17.2%) (89). In IFN nonresponders, the addition of ketoprofen resulted in no additional benefit (sustained response 0%) (90). The results of these well-designed trials have failed to demonstrate any additional benefit from NSAIDs/IFN combination therapy, and such treatment cannot currently be recommended.

Other Agents

There are small pilot studies evaluating levamisole, thymosin α-1, *N*-acetylcysteine, and α-tocopherol alone or in combination with IFN. Data are incomplete with regard to efficacy; thus their use cannot currently be recommended.

FUTURE OPTIONS

It is clear that our current therapeutic strategies for the treatment of IFN-nonresponding patients with chronic hepatitis C

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are inadequate. Despite our best efforts, approximately 50% of treatment-naïve patients receiving current therapy remain nonresponders. Given the poor response rates seen with retreatment using IFN monotherapy, there is little justification to recommend this form of therapy. It had been hoped that combination therapy with IFN and ribavirin or therapy with other types of IFN would resolve this dilemma. The present data suggest, however, that in IFN monotherapy responders we are able to achieve sustained response rates of about 15–20% with combination therapy and 5–15% with other IFNs. Although these results are better than no response, and therapy should be attempted if appropriate, future therapies directed at the virus itself are eagerly awaited. It is possible that such approaches will enhance our ability to either control or eradicate this virus. These are discussed in more detail in Chapter 13.

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10

Treatment of Chronic Hepatitis C: Transplant Recipients

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CASE

A 45-year-old female presented with ascites; subsequent evaluation revealed chronic hepatitis C with cirrhosis. Six months later, she had variceal bleeding and underwent endoscopic banding. Intermittent mental status changes, consistent with hepatic encephalopathy, developed 1 year later. At that point, she was referred for liver transplantation. Her only known risk factor was a blood transfusion in 1975 during open cholecystectomy. Prior medical history was remarkable for chronic pancreatitis diagnosed in 1995 requiring endoscopic stent placement on several occasions. There was no history of alcohol or potentially hepatotoxic drug intake and no family history of liver disease.

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Orthotopic liver transplantation (OLT) was performed in August 1999 immunosuppression with tacrolimus and prednisone was started. She developed a severe episode of acute rejection 1 month after OLT requiring OKT3 treatment. Two other episodes of acute rejection in October 1999 and November 1999 were treated with steroid boluses and mycophenolate was added to her immunosuppression scheme. A biochemically cholestatic pattern developed 6 months after OLT. Biochemical studies at that time were: aspartate aminotransferase (AST) 134 U/L alanine aminotransferase (ALT) 156 U/L, alkaline phosphatase 410 U/L, total bilirubin 9.4 mg/dL, direct bilirubin 5.8 mg/dL, albumin 3.1 g/dL. A liver biopsy (Fig. 1) revealed cholestasis, microsteatosis, spotty necrosis, ballooning degeneration, and triaditis, which are all consistent with hepatitis C virus (HCV) recurrence. Mycophenolate was discontinued, doses of prednisone were gradually reduced to 2.5 mg, and tacrolimus trough levels were maintained between 7 and 10 ng/mL. Biochemical tests showed partial improvement (AST 81 U/L, ALT 37 U/L, total bilirubin 7.2 mg/dL, direct bilirubin 4.4 mg/dL) and the patient has remained clinically stable.

INTRODUCTION

Hepatitis C has become the most frequent indication for OLT in the United States, Australia, and European countries (1, 2), and may account for up to half of the liver transplants done in many centers (3). There is also an expected increased demand for retransplantation in the next decade. The natural history of hepatitis C before transplant is generally indolent. An interval of 20–30 years between the initial infection and the onset of cirrhosis has been estimated in immunocompetent patients (4–6). In some patients, there appears to be a more aggressive course after liver transplantation. Recurrence of HCV infection leads to cirrhosis in 10% of patients 5 years after transplant (7).

Initial experiences with recurrent hepatitis B after OLT showed decreased patient and graft survival rates (8). Adequate outcomes were obtained only after effective therapies to

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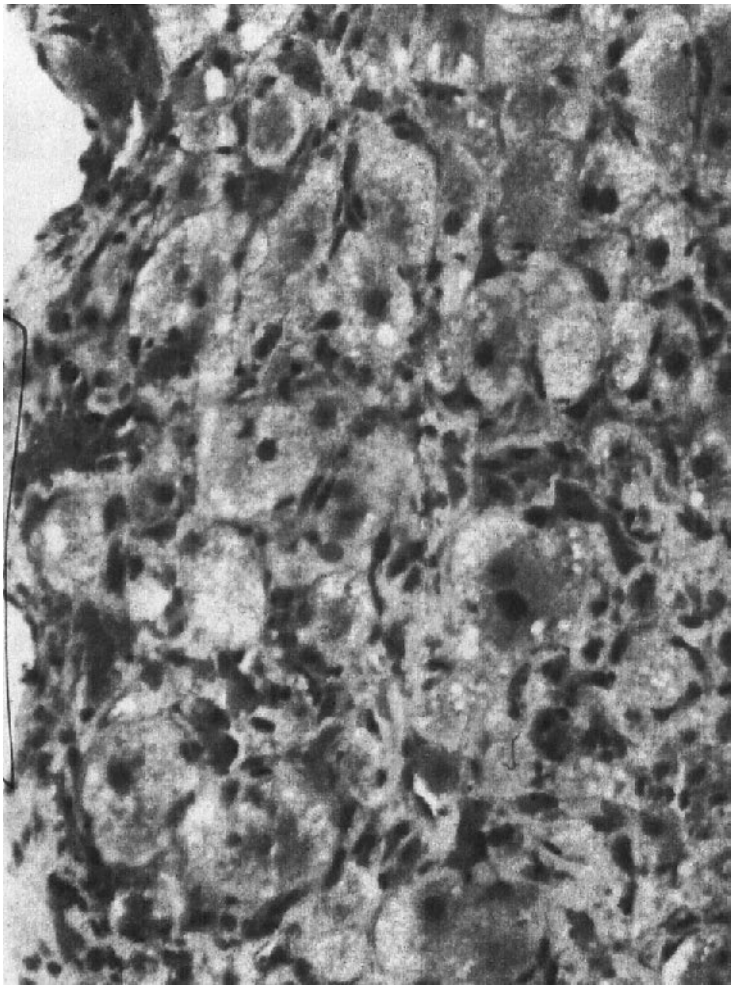

Figure 1 A liver biopsy revealing ballooning degeneration, spotty necrosis, and cholestatic features consistent with hepatitis C recurrence.

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prevent HBV infection recurrence became available. This has led to an increasing concern regarding long-term outcomes of recurrent HCV disease after transplantation and the need for adequate preventive or therapeutic measures to avoid hepatitis C–related graft damage.

VIRAL RECURRENCE

Wright et al. have demonstrated that viral recurrence, by HCV-RNA testing, occurs in 95% of patients who have viremia before liver transplant (9). Reinfection by the same genotype has been demonstrated by a high degree of nucleotide sequence homology in the hypervariable domain of the virus before and after OLT (10, 11).

Quantitative polymerase chain reaction (PCR) studies have shown the kinetics of the viral recurrence; the estimated half-life of the virus in these patients is approximately 4 h. A rapid decline in the level of HCV-RNA occurs immediately after removal of the native liver and the level continues to fall for the first 2 days. After the third day, it starts to increase, exceeding preoperative levels by the eighth day (12), and eventually reaching a level 10–20 times higher than before OLT (13–16).

NATURAL HISTORY OF HEPATITIS C AFTER TRANSPLANT

Recurrent hepatitis defined by histology has been documented in 40–80% of patients (7, 9, 17, 18). It is usually found during the first year posttransplant. Despite this, severe early dysfunction due to HCV infection is uncommon (19, 20). In a study by Ghobrial et al., deaths related to HCV occurred at a mean of 482 days, whereas deaths from other causes occurred during the first year (21).

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In most follow-up studies, graft and patient survival rates during the first decade (60 and 75–80% approximately) are similar to those of other nonmalignant indications (2, 7, 19, 21–24). However, data from the UNOS database has shown that HCV infection diminishes patient and graft survival at 2 and 5 years compared with cholestatic, autoimmune, or alcoholic liver disease (25). Insufficient number of patients in most series, methods of data collection, as well as confounding pretransplantation and posttransplantation variables may explain these discrepancies.

During the first 5 years after transplant, progression to cirrhosis has been found in 10% of patients with hepatitis C in the largest follow-up series from Europe and the United States (7, 19, 26). A 10-year follow-up study showed histological hepatitis in 88%, fibrosis in 24%, and a survival of 62% (22). Long-term outcomes after 10 years have not yet been assessed. Once cirrhosis is present, the prognosis seems poor with a mortality of up to 75% (7).

Recurrent HCV infection is also associated with a lower quality of life, lower physical functioning, depression, and a higher psychological distress (17). Singh et al. have found severe depression (i.e., Beck depression inventory score $\geq$15) in up to 45% of patients (17). Realization and awareness of disease recurrence appeared to be a greater contributor to depression associated with HCV infection than the physical impairment per se.

ACUTE AND CHRONIC HEPATITIS RECURRENCE: CLINICAL PRESENTATION

The clinical presentation of HCV, recurrence and acute graft infection after transplantation varies from asymptomatic or minimal liver damage to an influenza-like syndrome with or without jaundice. Clinical differentiation from acute rejection is necessary; acute rejection is more common during the first

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2 months posttransplant. The differential diagnosis also includes chronic rejection, protracted biliary complications, and lymphoproliferative disorders.

Patients with chronic hepatitis due to HCV recurrence may be asymptomatic or complain of nonspecific symptoms such as fatigue. ALT may be normal in up to one-half of patients with histological hepatitis (27).

A severe pattern characterized by intrahepatic cholestasis is found in 4–9% of patients with recurrent HCV infection after heart, kidney, or liver transplantation (28, 37, 59, 83). It has been suggested that high levels of HCV-RNA replication in this group of patients (29) are responsible for direct cytopathic injury, and typically they progress rapidly to graft loss within 2 years from their transplant. In a series of 135 patients with hepatitis C who required OLT, cholestatic hepatitis was found in 10 (7%), most of whom had been treated for acute rejection (28). A differential diagnosis with other cholestatic disorders such as chronic graft rejection, bile duct stricture or obstruction, hepatic steatosis, or drug toxicities must be established.

Progression to cirrhosis with associated graft failure may occur quickly occasionally (27), especially in patients with early and/or severe early recurrence (15, 30). Rosen et al. (30) studied 34 patients with histological evidence of HCV recurrence, finding that ballooning degeneration or cholestasis was more common among those who developed allograft cirrhosis ($P = .04$). Multivariate analyses identified peak total bilirubin within 1 month of onset of histological recurrence as the only independent predictor of cirrhosis.

Although initial reports showed poor results when retransplantation (re-OLT) was performed, some more recent cases have undergone successful re-OLT once the bilirubin levels begin to rise and there are other signs of clinical deterioration (ascites, weight loss, fatigue) with the goal of performing the procedure before multiorgan failure develops (59, 83).

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HISTOLOGICAL CHANGES

Hepatitis recurrence is characterized by necroinflammatory changes in the hepatic parenchyma (31, 32), initial changes are a low-grade lobular inflammation, scattered apoptotic bodies, minimal cell swelling, and mild portal lymphocytic infiltrate. Two to 4 weeks later, a greater degree of portal and lobular inflammation and hepatocytic necrosis is found; lymphoid aggregates and midzonal fatty changes are frequently observed. Ductular damage is milder than in rejection. Portal fibrosis with or without fibrous septa develops at a higher rate than in patients who have not received transplantation (6, 34).

The differential diagnosis includes biliary obstruction, preservation injury, ischemia, drug effect, and acute and chronic rejection (Table 1). Cholestatic changes are usually more severe in patients with rejection than in those with HCV recurrence except in the small group with fibrosing cholestasis (35–37). Histological findings in the subgroup of patients with intrahepatic cholestasis include ballooning degeneration of

Table 1 Role of Liver Biopsy in the Differential Diagnosis of Hepatitis C Recurrence After Liver Transplantation

Differential diagnosis	Features
Biliary obstruction	Ductal proliferation, bile stasis, neutrophils in large ducts
Preservation injury, ischemia, drug effect	Centrilobular necrosis and ballooning
Acute rejection	Ductal injury, mixed portal infiltrate, endothelialitis and cholestasis. Parenchymal centrilobular inflammation in severe cases.
Chronic rejection	Obliterative arteriopathy leading to centrilobular hepatocyte swelling, dropout, perivenular fibrosis, parenchymal foam cells, portal fibrosis and central to central bridging necrosis. Cholestatic features invariably present.

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centrilobular hepatocytes, bile ductular proliferation, canalicular cholestasis, and some can develop perisinusoidal fibrosis resembling the pattern described in fibrosing cholestatic hepatitis B (38).

DIAGNOSIS

Virological Recurrence

Detection of HCV-RNA is considered the standard diagnostic method for HCV recurrence. The most sensitive method to detect HCV-RNA is qualitative PCR. Serological diagnostic methods for hepatitis C, including second-generation assays, may be inadequate for detection of recurrent HCV after OLT (39, 40). The capacity to produce some of the antibodies to HCV antigens such as C-100-3, C-33, and NS5 may decline with immunosuppression, resulting in false-negative tests in patients with viral recurrence and in some with de novo HCV infection (41). Donegan et al. (42) compared diagnostic methods for HCV in 101 liver transplant recipients in samples collected as late as 23 months following transplantation. Forty-one percent of them were considered to be truly infected. Of these true positives, 88% were positive by bDNA signal amplification assay, 88% by second-generation electroimmunoassay (EIA) (EIA failed to detect 12% of HCV-infected recipients), and 63% by recombinant immunoblot assay (RIBA). Single reactive band patterns by RIBA-2 were frequently found in these immunosuppressed patients with HCV infection. In contrast, 15% of those who were HCV-RNA negative had persistently positive anti-HCV EIA.

Diagnosis of Recurrent Hepatitis

Approximately 50% of OLT recipients with recurrent HCV infection detected by HCV-RNA have normal or mildly elevated levels of ALT (43). Therefore, detection of recurrent hepatitis

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by aminotransferase levels underestimates the frequency of recurrent hepatitis C. Liver biopsy is helpful in diagnosis, and periodic liver biopsies may be required to determine whether post-OLT HCV hepatitis is a progressive process.

PREDICTORS OF DISEASE RECURRENCE

Viral Factors

Potential viral factors related with severity of HCV recurrence are shown in Table 2.

There are conflicting results regarding the relationship between HCV genotype and the risk of recurrent hepatitis after OLT (19, 23, 44–46). Two European studies have suggested that patients with type 1b HCV have more severe disease (7, 47), with a twofold relative risk of recurrent hepatitis by multivariate analysis (95% Cl = 1.3–2.9). These results contrast with series in the United States (46). Another recent study from Italy including 89 patients with HCV infection found no correlation between genotype 1b distribution and severity of recurrence (52). Variations in immunosuppression are also possibly involved in explaining these contrasting results. In a French study by DiMartino

Table 2 Potential Viral Factors

Factor	Comment	References
HCV genotype	Controversial; correlation found in some European studies only	7, 47
Viral load pre-OLT and early post-OLT	Related with disease progression and survival	13, 15, 23, 48
CMV infection	Possibly related to suppression of immune control of HCV	49
Hepatitis B coinfection	Milder form in coinfected patients	7, 47, 50

CMV, cytomegalovirus.

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et al., progression to chronic hepatitis in patients with genotype 1b was marked by a significant decrease of liver HCV-RNA (15). It was hypothesized that the host immune response against HCV is involved in pathogenicity and is stronger for genotype 1b than for other genotypes in the setting of liver transplantation.

Perioperative viral levels do not correlate closely with the severity of viral recurrence; however, high pretransplantation levels of HCV-RNA have been found to be associated with higher rates of disease progression (48), and lower survival rates (23) and high early posttransplantation viral levels have been associated with a worse outcome (15). Charlton et al. followed 166 patients with HCV infection after OLT for 5 years; those with pretransplantation HCV-RNA titers higher than 1×10^6 Eq/mL had a cumulative 5-year survival of 57 versus 84% in those with lower titers ($P = .001$).

It has been postulated that cytomegalovirus (CMV) infection can suppress cellular immunity, which might impair the control of HCV proliferation, allowing a more aggressive clinical course in this group of patients. In a series of 43 liver transplant recipients followed during 6 months, half of those who developed CMV viremia eventually developed allograft cirrhosis versus only 11% of CMV-negative patients ($P = .027$) (49). Further studies are required to confirm this conclusion.

A milder form of recurrence has been found in patients coinfected with HBV and HCV (7, 47). Possible explanations for this include passive immunity by antibodies to HCV administered with polyclonal HBIg infusions as suggested by a study from Villejuif (80) which showed detection of passively transmitted anti-HCV and reduced incidence of acquired HCV infection in patients receiving HBIg during the pre-HCV era (47). Direct viral interactions could also explain this phenomenon. Histopathological patterns may vary from features of HCV infection to those of hepatitis B. A series of coinfected patients from the University of California at San Francisco

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has suggested that the presence of HCV may improve the clinical outcome as compared with the expected outcome of persistent HBV infection alone (50).

Host Factors

Potential host factors that may predispose to a more severe viral recurrence are shown in Table 3.

The course of chronic hepatitis C before and after transplant may be influenced by the immunogenetic background, determining protection and susceptibility to HCV. HLA-DRB1*11 has been found to be associated with less advanced liver disease in nontransplant patients with HCV infection (51). Belli et al. (52) followed 89 HCV-positive patients for a mean of 35 months after OLT, demonstrating in a multivariate regression model that HLA-DRB1*11, HLA-B14 (Risk Ratio = 3.4), HLA-DRB1*04 (risk ratio = 2.86) had independent correlation with the risk of HCV recurrence. None of the 10 patients who developed stage 5–6 fibrosis carried the HLA-DRB1*11.

Although a previous study suggested that donor recipient match at the HLA-DQB locus was associated with more severe disease (53), two studies that are more recent did not show an association between HLA-DQ and HCV recurrence (54) or histological outcome (19).

Table 3 Host Factors

Factor	Comment	References
HLA and immunogenetic background	Viral recurrence: HLA-B14 (RR = 3.4), HLA-DRB1*04 (RR = 2.86). HLA-DRB1*11 is not found in patients with severe recurrence.	52
HLA matching	Unrelated in recent studies	19, 54
Race	Worse in nonwhite transplant recipients	23

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An increased fibrosis progression and decreased patient survival in nonwhite transplant recipients has been suggested (23). Charlton et al. found a 2.8 relative risk (0 = 0.001) of graft loss among nonwhite patients versus white patients in a cohort of 166 HCV-infected recipients followed for a mean of 5 years. In pretransplant patients, sustained viral responses to interferon (IFN) monotherapy, as well as to consensus IFN, have been lower in African-American patients than whites (55, 56). Response to combination therapy (IFN and oral ribavirin), however, appears to be less dependent on race (56a). The basis of these racial differences is still not clearly defined and needs further assessment.

Immunosuppression (Table 4)

The intensity of the immunosuppressive therapy defined by requirement of methylprednisolone boluses (57–59), OKT_3 requirement (57, 58, 60), and cumulative doses of steroids (13) correlates with the severity of HCV recurrent hepatitis. The interval to hepatitis recurrence is significantly shorter in those treated with OKT_3 for rejection than in patients with steroid-responsive rejection (60). The incidence of recurrent HCV after OLT is significantly lower in patients who did not have any episode of rejection versus those who required immunosuppressive therapy for rejection (18 vs 72%) (58).

The capacity of the immune system to mount an efficient

Table 4 Immunosuppression Factors

Factor	Comment	Reference
Management of acute rejection required	Severe recurrence in those requiring OKT3, methylprednisolone boluses, and high cumulative doses of steroids	13, 57–60
Cyclosporine vs tacrolimus	No differences found	19, 22, 68

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response to control the replication of the virus determines the outcome of the infection. T cells are important in controlling HCV replication and elimination (61); HLA class I restricted cytotoxic T lymphocytes (CTLs) and other effector type cells are recruited following CTL activation. This results in the destruction of infected cells by CD8+ CTLs and viral suppression by antiviral cytokines (62). OKT_3 produces opsonization of circulating T cells, modulates the CD_3/T-cell receptor complex, and induces T-cell apoptosis (63). OKT_3 may suppress normal mechanisms responsible for viral clearance, potentiating HCV recurrence and persistence. Rapid progression to cirrhosis due to HCV has been noted in some HIV-infected individuals (64) and in patients with hypogammaglobulinemia (65) and has been ascribed to a lack of normal immune surveillance and containment of HCV. OKT3 may also enhance production of proinflammatory cytokines tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), leading to more severe histological injury (66, 67).

No difference in rate or severity of recurrent HCV has been documented between patients receiving cyclosporine- versus tacrolimus-based immunosuppression (19, 22, 68).

In an analysis of 374 patients with HCV infection followed during 8 years, requirement for re-OLT for recurrent HCV in patients receiving tacrolimus was similar to those receiving cyclosporine (3 vs 3.6%, respectively).

LYMPHOPROLIFERATIVE DISORDERS AND HCV AFTER OLT

The incidence of posttransplant lymphoproliferative disorders (PTLDs) after liver transplantation is 2–4% in adults, and it is even higher in the pediatric population. When monomorphic, 93% are non-Hodgkin's lymphomas (69) and up to 44% of them may involve the graft (70). This disease is fatal in about 50% of cases despite aggressive management. The

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pathogenesis of PTLDs has been clearly linked to B-lymphocyte stimulation by Epstein-Barr virus (EBV) (69).

The incidence of PTLD 4 years post-OLT in those with HCV infection has been found to be significantly higher than in patients with a different etiology (12.3 vs 2.2%; $P = .015$) (71). Current evidence shows that HCV infection is associated with B-cell clonal expansion (71), which results from inhibition of B-cell apoptosis, lowering of the B-cell activation threshold, possibly through binding to tetraspanin CD81, and by a synergistic or triggering effect of HCV on EBV oncogenicity due to the B-cell proliferation inducing effect of these two viruses.

LIVER TRANSPLANTATION FROM HEPATITIS C–INFECTED DONORS

Given the prevalence of hepatitis C infection in the population, a significant number of potential donors are infected with hepatitis C as well. The estimated mean prevalence in a national collaborative study of 3078 cadaver organ donors was 2.4% by PCR (72).

HCV may be transmitted from infected donors to non–HCV-infected recipients (73); in fact, all recipients from donors with positive HCV-RNA develop the infection (74). The risk of acquisition of HCV during or after OLT before 1991 was 17–35% (9). Current advances in diagnostic tests have minimized this risk. More recently, the rate of de novo infection during the peritransplant period determined by sensitive methods such as EIA2 and PCR has been shown to be as low as 2.5% (75–77). The infection may be transmitted from the organ donor or rarely from blood transfusions, since the mean donor exposure to blood products in liver transplant recipients is very high (1).

Owing to the shortage of donors, individuals with evidence of hepatitis C infection, but no clinical evidence of liver dysfunction, have been accepted by several groups to trans-

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plant hepatitis C–positive patients with end-stage liver disease. Published results of these series have shown similar outcomes to those who received noninfected livers. Vargas et al. (78) found no significant difference in the 1- and 5-year survival of patients or graft failure between groups having positive versus negative HCV donors. Moreover, they found that liver recipients whose HCV genotype belonged to the donor were significantly less likely to develop recurrent hepatitis than those who retained their pre-OLT genotype.

Johnson et al. (79) analyzed the outcomes of transplants for patients with hepatitis C registered in the UNOS SR (scientific registry) for the period April 1994 to June 1997. A significantly improved patient survival was found in recipients of hepatitis C–positive livers versus those who received noninfected livers (90 vs 77% at 2 years; $P = .01$). Utilizing hepatitis C–positive donor livers exclusively for transplanting hepatitis C–positive recipients may be safe and effectively expands the organ donor pool.

MANAGEMENT

The best possible approach for this group of patients would be to prevent the recurrence of HCV infection. Unfortunately, current treatments have a low efficacy in eradicating the infection. In those with established viral recurrence, the goal is to prevent the development of HCV-related graft failure.

Preemptive Therapy

Prophylactic approaches to prevent severe viral recurrence include immunoglobulin administration, pretransplant IFN therapy, and early post-OLT antiviral therapy.

In a French study (80) done in the era before HCV donor screening, the incidence of recurrent HCV viremia 1 year after OLT was significantly lower in patients receiving polyclonal immunoglobulins (25 of 46 patients; 54%) compared with those who did not (162 of 172; 94%), suggesting that passive

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immunization could have prevented HCV recurrence in some cases. However, with current screening methods, polyclonal immunoglobulin preparations are not expected to have antibodies against HCV.

Several trials have used pretransplant IFN therapy with the objective of stabilizing hepatic function and suppressing viral replication to reduce the risk of post-OLT viral recurrence or aggressive recurrence. Cirrhotic patients have a lower virological sustained response than noncirrhotics to monotherapy with IFN (9 vs 16%) (1), and IFN is contraindicated in patients with decompensated cirrhosis, since it can potentially worsen the hepatic function. However, in patients with compensated cirrhosis, some trials have shown a reduction in variceal bleeding and the need for transplantation even among those without a sustained virological response (84). There has been a reduction in the incidence of hepatocellular carcinoma (81–84). Serfaty et al. followed 668 patients with compensated cirrhosis for a median of 40 months, demonstrating that the absence of IFN therapy was the only independent factor predictive for both hepatocellular carcinoma (HCC) and decompensation by multivariate analysis (84). In a meta-analysis of randomized controlled studies, Poynard et al. showed that IFN treatment in patients with compensated cirrhosis secondary to HCV infection can reduce the incidence of hepatocellular carcinoma by 13% (85).

Pretransplant IFN therapy has also been advocated as a means to decrease the viral load with the goal of minimizing the severity of posttransplant recurrence, since viral RNA levels in the perioperative period correlate with disease progression and outcome (86).

Interferon therapy started 1 week after OLT has been shown to produce a decreased viral load, with 25–41% HCV-RNA negativity at 1 year after transplant (87). In one study, the combination of IFN and ribavirin, starting 2–3 weeks after OLT, resulted in a very low rate of chronic active hepatitis (5%) 1 year after transplant despite the presence of viremia

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in 59% of patients. Side effects such as hemolytic anemia and asthenia were frequent (88). Although IFN has not prevented viremia in most patients, it may have additional effects by immune modulation of T cells and cytokines, thereby diminishing the liver damage in HCV-positive patients after liver transplant.

Antiviral Therapy for Patients with Recurrent Hepatitis C after OLT

Promising results with antiviral therapies have been obtained in some pilot studies. Taking into consideration that only a minority of patients with recurrent HCV progress to fibrosis and cirrhosis, potential therapies should ideally be aimed to patients with severe disease or evidence of progression (2).

The outcome of treatment with IFN alone once hepatitis C recurrence has been established has been disappointing; it has a low viral response, almost universal relapse after discontinuing therapy (96, 89–91), and histological improvement in hepatic damage is minor (1). IFN can increase the expression of HLA class I and II antigens and stimulate cellular immunity (92, 93). This observation led to initial concerns about IFN-induced graft rejection; however, in clinical studies, the incidence of IFN-induced rejection in liver transplantation is rare (94).

Combination therapy with IFN and ribavirin in patients with hepatitis C recurrence has been studied by Bizollon et al. in a series of 21 patients with recurrent hepatitis C after OLT treated with IFN (3 MU TIW) and ribavirin (1000 mg/day) for 6 months followed by ribavirin maintenance for another 6 months. The viral response at the end of combination treatment was 48% and 28% at the end of 12 months (95). All patients who tolerated this protocol had improved liver histology. Rejection was not associated with this treatment, but reversible hemolytic anemia was observed in three patients. The investigators suggested early treatment with combined ther-

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apy in patients with recurrent hepatitis, ideally before the development of advanced histological disease. Additional studies are required in liver recipients with recurrent hepatitis C to define prognostic factors and variables predictive of response to treatment (96).

Immunosuppression Management

Potent immunosuppression may lead to greater viral load and more rapid liver injury (97), as described previously, in patients treated for rejection. An adequate balance is required to minimize graft rejection and hepatitis C–associated liver injury. The ideal combination regimen for immunosuppression induction, maintenance, and rejection has not been defined. No difference has been observed between cyclosporine- and tacrolimus-based immunosuppression (19, 23, 68). Mild or unclear episodes of rejection are no longer treated in some centers, and therapeutic trials of immunosuppression to differentiate HCV infection from rejection may be detrimental (1) owing to enhanced viral activity. Direct conversion to FK506 has been as effective as OKT3 (and probably more cost effective) as initial treatment of steroid-resistant rejection (98, 99), and should be considered in patients with HCV infection.

Utilization of potent, new agents such as IL-2 receptor antibodies (Daclizumab), which are frequently used in renal transplant recipients, may result in a more severe outcome of HCV infection. In a series of 16 liver transplant recipients who received Daclizumab to prevent acute rejection, severe viral recurrence with a cholestatic pattern was found within the first year in three recipients (19%) (100). This represents a worse course than historical controls. Zenapax inhibits IL-2–mediated lymphocyte activation, potentially blocking immune response against HCV and enhancing viral replication. The utilization of this antibody in patients with HCV infection undergoing transplant is currently limited to clinical research

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protocols and better HCV treatment strategies are required to control potentially increased viral proliferation.

RETRANSPLANTATION

Re-OLT is indicated most frequently because of primary graft dysfunction, vascular complications, chronic rejection (101), and increasingly in patients with severe viral recurrence who develop cirrhosis (23). Although initial reports suggested poor outcome (83, 102, 103), adequate results have been found if re-OLT is performed before infectious and renal complications develop (104, 105). Outcomes analysis in a series of 14 patients with recurrent HCV infection requiring re-OLT showed a graft and patient survival at a median of 926 days in 8-of them (105). Four perioperative deaths were registered; all of them secondary to sepsis. Risk factors for these deaths were pretransplant renal failure in three and fever of unknown source in the remaining patient. One patient died after CMV infection developed at 4 months, and another one had vanishing bile duct syndrome and recurrent hepatitis and died 5 months after OLT. In this small series, a 38% survival rate at 2 years was found in critically ill patients versus 85% ($P = .06$) in noncritical patients (105).

Re-OLT in general carries a worse prognosis than primary OLT for all indications. In re-OLT, the main cause of death is sepsis (63%). Death from recurrent HCV infection occurred in only 4/76 patients undergoing re-OLT. Variables associated with poor outcome for re-OLT include UNOS status 1 and poor preoperative condition (106, 107). There appears to be no difference in the survival rate for patients undergoing re-OLT for recurrent HCV when compared with other causes. Survival at 2 years after re-OLT has been found by Ghobrial et al. (21) to be 58% for all causes of retransplantation versus 59% for recurrent HCV. In conclusion, re-OLT is an important

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option in the treatment of recurrent HCV if performed before systemic complications develop.

CONCLUSIONS

Hepatitis C has become the most frequent indication for OLT and an increased demand is expected in the next decade. Viral recurrence occurs in almost all hepatitis C–positive patients but only a proportion of them develop severe recurrence. Recurrent hepatitis C leading to cirrhosis has been demonstrated to occur in 10% of patients 5 years after transplant. Risk factors involved in a more severe recurrence are still being studied. Immunosuppressive medications result in increased viral proliferation and a worse outcome of hepatitis C recurrence. Histological features are important, but can be confusing in differentiating HCV recurrence from other post-OLT complications such as acute rejection. Combination therapy with IFN and ribavirin may lead to an improved liver histology in patients with severe recurrence, but few patients have a sustained viral response and further advances in the management of this group of patients are required. Re-OLT is an option for those with severe dysfunction secondary to HCV recurrence, with similar outcomes to those who require re-OLT for other indications.

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11

Treatment of Chronic Hepatitis B and C: HIV-Coinfected Patients

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INTRODUCTION

Liver enzyme abnormalities are very common in patients infected with the human immunodeficiency virus (HIV). Various series describe biochemical evidence of hepatocellular injury in excess of 70% during the course of HIV infection. Multiple etiologies are associated with serum alanine aminotransferase (ALT) abnormality and include viral hepatitis, drug toxicity, concurrent alcohol abuse, as well as infiltrating disease (Kaposi's sarcoma, non-Hodgkin's lymphoma), mycobacterial infection, and secondary to acquired immunodeficiency syndrome (AIDS)–related cholangiopathies. Chronic viral hepatitis B and C are among the leading etiological factors associated with hepatocellular injury. The significance of these viral

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infections was thought to be limited owing to the high early mortality associated with primary HIV infection. However, improved treatments for HIV utilizing highly active antiretroviral therapy (HAART) with multidrug regimens has resulted in increased life expectancy. This change has affected the course and natural history of hepatitis B and C and increased the importance of liver disease in HIV-infected patients.

CASE: HEPATITIS B

A 40-year-old white man with HIV and hepatitis B virus (HBV) presented to the Infectious Disease Clinic for treatment of his HIV infection. He had been doing well on an HIV antiviral regimen of azidothymidine (AZT) and lamivudine (3TC) until recently. Over the last 2 months, the patient's HIV viral load increased, and his antiretroviral regimen was changed to efavirenz 600 mg Qd, stavudine 40 mg BID, and saquinavir 600 mg TID. Over the next several weeks, the patient developed jaundice, increased fatigue, nausea, and vomiting.

Biochemical and Serologic Findings

ALT 875 U/L	HCV ELISA negative
AST 587 U/L	HCV RNA by PCR negative
Bilirubin 6.4 mg/dL	HBsAg positive
Alkaline phosphatase 234 U/L	Anti-HBc IgM positive
Albumin 3.1 mg/dL	HBV-DNA 1400 pg/mL
Prothrombin time 15 s	HIV viral load <400
H/H 13.5/34	CD4+ 323
Platelet count 101,000	

Treatment and Outcome

The patient had evidence of acute hepatic decompensation manifested by jaundice, thrombocytopenia, and coagulopathy. Etiology included acute viral hepatitis, drug toxicity, or ischemic injury. The latter was considered unlikely, as there was no reported period of

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hypotension or recent surgery. Drug toxicity was certainly a consideration with HAART agents, and with this presentation, many clinicians would withhold these medications while awaiting laboratory findings.

Hepatitis B flare in the setting of effective immune reconstitution however, is an emerging problem, and the most likely explanation of the clinical condition. In this setting, adding or substituting lamivudine 100 mg/day back into the regimen was effective in suppressing HBV replication, and the patient showed dramatic clinical improvement over the next 8 weeks, returning to baseline liver function.

HBV: NATURAL HISTORY IN HIV-INFECTED PATIENTS

Coinfection with hepatitis B is very common, with evidence of past or current HBV infection in more than 90% of patients with AIDS (1). Approximately 10% of patients with AIDS are chronic carriers of HBV. In immunocompetent hosts, the risk of developing chronic HBV is inversely related to the age the infection was acquired. In infants, chronic infection occurs in 90% of those exposed, but in adults, only 5–10% of those infected become chronic carriers (2). The HIV-positive patient has a more than fivefold increased risk of becoming a chronic HBV carrier compared to rates of chronicity at typical age of acquisition. Furthermore, HIV-infected patients with HBV infection demonstrated only 4% seroconversion rates from HBeAg to anti-HBe (3). This is in contrast to HIV-negative patients who had an annual seroconversion rate of 11%. This observation suggests that HIV infection may have the capacity to extend the period of replicative viral replication. This extended period of increased viral infectivity theoretically puts more patients at risk for further disease transmission. In most coinfected patients, the laboratory and histological features of HBV are generally milder in HIV-positive patients compared to immunocompetent HBV-infected controls. The milder histological features observed in coinfected HIV and HBV patients are present despite high titers of hepatitis Be antigen (HBeAg)

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and a greater replication of HBV as measured by HBV-DNA levels (4). HIV-infected patients with chronic HBV infection are also less likely to demonstrate elevated ALT levels. If the ALT was elevated, it was lower than in HIV-negative controls. The lower aminotransferase levels seen in HIV-positive patients with HBV infection are probably secondary to the defective cell-mediated immunity in HIV-infected patients. With HIV infection, the cytotoxic T-cell response to infected hepatocytes is decreased and there is less subsequent hepatocyte injury, which seems to result in a lower ALT. Histological findings are also compatible with less liver injury observed in HIV-infected patients and with reduced CD4+ counts. Therefore, the course of the liver injury is directly related to the host's ability to mount an immune attack against HBV-infected hepatocytes. In an immunosuppressed state, the HBV-DNA level in serum and the expression of antigens on the hepatocyte is increased. Following immune reconstitution with HAART, CD4+ counts rise dramatically. In theory, this may be associated with increased destruction of hepatocytes and expression of clinical liver disease. In fact, this phenomenon has been observed and has been associated with severe liver injury, manifested by high serum aminotransferase levels, elevated bilirubin and hepatic decompensation in some patients (5).

HBV: INTERFERON-BASED THERAPY

Interferon (IFN) therapy for treatment of hepatitis B in immunocompetent hosts was approved by the U.S. Food and Drug Administration (FDA) in 1992. The mechanism of action is unclear, but it is thought that recombinant IFNs demonstrate both immunomodulatory and antiviral effects. Interferon can induce the display of HLA class I molecules on hepatocyte membranes and so promote lysis by CD8+ cytotoxic lymphocytes. A meta-analysis of IFN treatment efficacy in immunocompetent patients demonstrated an overall response rate of 33% for treated patients compared with 12% for untreated controls (6). Prognostic factors that serve as markers of IFN

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response include high serum aminotransferase concentrations (>200 U/L), low serum HBV-DNA concentrations (<100 pg/mL), active histological changes, short duration of disease before therapy, wild-type (HBeAg-positive) virus, and the absence of immunosuppression (7). Application of these factors to HIV-infected patients with HBV infection suggests that a poor response might be expected. As noted above, HBV/HIV–coinfected patients typically have low ALT levels, very high HBV-DNA levels in serum, reduced histological injury features, and are generally immunosuppressed. Clinical studies support the concept that IFN treatment in HBV and HIV–coinfected patients is problematic. McDonald et al. reported on treatment of HBsAg-positive and HBeAg-positive patients with IFN of whom 41% were HIV positive (8). The HIV-positive patients had lower aminotransferase levels, higher mean baseline HBV-DNA, and milder histological disease, as might be expected. Thirty-three percent of HIV-negative patients responded to treatment versus none of the HIV-positive patients. The investigators suggested that the failure of IFN therapy in patients coinfected with HBV and HIV may be secondary to the effects on viral antigen display in the hepatocyte or changes in lymphoid effector cells. The known effects of HIV on helper T-cell function would prevent amplification of the lysis of infected cells by cytotoxic T cells. Zylberberg et al. (9) reported the results of a treatment trial in which 25 HIV-positive patients with HBV and "chronic active hepatitis" were given 6 months of interferon alfa-2a (IFN-α2a) for 6 months at a dose of 18 MU/week. Although 36% became HBV-DNA negative, only one patient demonstrated HBe seroconversion (9). Wong et al. described treatment of 50 HBV-infected patients. The patients were predominantly homosexual males and half were HIV infected. Response was defined as loss of serum HBV-DNA and HBeAg conversion to HBe antibody. Twenty-four percent of patients without HIV infection were classified as responders versus 4% of HIV-positive patients (10). A recent case series from France did suggest that immunocompetent HIV carriers with HBV (CD4+ 340–553 mm^3) might demonstrate improvement with IFN therapy (11). Chen

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et al. (12) reported on the long-term follow-up of HIV-infected patients who were treated with IFN between 1986 and 1991. Patients were reevaluated for HBeAg seroconversion an interval range of 4–9 years. Late seroconversion was uncommon and did not occur at a rate higher than untreated HBV-infected patients (12). These studies strongly suggest that IFN-based regimens are not generally effective for treatment of HBV in HIV-infected patients, although patients without immune dysfunction may respond better than those with low CD4+ counts. There are no published trials describing IFN use following immune reconstitution associated with HAART.

NUCLEOSIDE ANALOGUES AND HBV

Nucleoside analogues have become an important component of HIV antiviral therapy. Among FDA-approved drugs, only lamivudine (3TC) has received approval to treat patients with both chronic HBV infection and HIV.

Short-term trials showed that lamivudine was well tolerated and produced a rapid and profound decrease in serum HBV-DNA. Dienstag et al. evaluated 32 immunocompetent patients with chronic HBV (13). The patients were randomly assigned to receive 25, 100, or 300 mg of oral lamivudine daily for 12 weeks. The levels of HBV DNA became undetectable in 70% of patients who received the 25-mg dose and in 100% of those who received the 100- or 300-mg dose. In most patients, HBV-DNA reappeared after therapy was completed. Six patients (19%) had sustained suppression of HBV-DNA. A European multicenter trial by Nevens et al. confirmed Dienstag's original findings (14). Several long-term trials have also been conducted which also demonstrate good responses to lamivudine. Lamivudine treatment is associated with the selection of drug-resistant strains due to mutations in the YMDD motif. Treatment for 52 weeks resulted in 24% having the YMDD mutation. This suggests that single-agent therapy with lamivudine will have limited value for long-term viral suppression.

Among HBV/HIV-coinfected patients, the effects of lami-

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vudine on replication of HBV are similar to that observed in immunocompetent HBV singly infected patients. Forty consecutive patients coinfected with HBV/HIV and receiving lamivudine as therapies for HIV were enrolled in a trial by Benhamou (15). After 12 months of treatment with lamivudine, 96.3% of patients had HBV-DNA less than 5 pg/mL. This was not a randomized blinded study, but it does suggest that coinfected HBV/HIV patients do respond to lamivudine (15). Prior to HAART, HIV infection was associated with a higher rate of HBeAg in the blood and less clinically significant hepatitis, as described above. Recent reports suggest that the clinical significance of HBV infection in the era of HAART may be different. Bessesen et al. described five patients in whom lamivudine withdrawal or emergence of HBV resistance in HIV-positive patients was associated with severe clinical outcomes (5). Withdrawal of lamivudine led to the exacerbation of HBV infection in one coinfected patient, which led to fulminant hepatic failure and death. In several other patients, the continued administration of lamivudine as part of an antiretroviral regimen led to emergence of HBV with YMDD mutations and resistance to lamivudine. This was associated with flares of clinical hepatitis characterized by high aminotransferases and the appearance of HB anti–core IgM antibodies in several patients at the time when their CD4+ counts had risen dramatically from baseline levels. Often elevations of hepatic enzymes during HAART therapy are attributed to the use of the medication cocktail. Physicians treating HBV/HIV-coinfected patients must monitor their patients closely and consider the possibility of HBV-mediated injury. With immune reconstitution with HAART, a better understanding of the natural course of HBV and HIV-coinfected patients is needed.

COMBINATION THERAPIES AND NEW TREATMENT MODALITIES FOR HBV/HIV

New treatments for HBV and HIV-coinfected patients are on the horizon. New drugs or combinations of the drugs available

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now will be the treatments of the future. The combination of zidovudine (azidothymidine, AZT) and IFN has been described (16). Ten HIV-positive patients on AZT were treated with IFN. Serum HBV-DNA and HBeAg disappeared and serum ALT returned to normal in two patients. Patients had been receiving AZT for 2–22 months prior to starting IFN therapy. How much of a role immune reconstitution played is not known. Famciclovir and IFN have been studied in immunocompetent subjects with HBV infection who had previously failed IFN monotherapy. The combination effect appeared to be additive in terms of viral suppression. However, there are no data on this combination in HBV/HIV-coinfected patients (17).

The immunomodulatory peptide thymosin may be effective in the treatment of HBV. Ninety-seven patients were followed in a U.S. double-blind study with thymosin. A complete response was seen in 14% of patients treated with thymosin and 4% treated with placebo (18). Although there was a trend toward efficacy, this study failed to confirm treatment efficacy reported in other studies, because there was insufficient statistical power for the observed difference. Thymosin has also been used in combination with IFN or lamivudine in HIV-negative patients but not in HIV-positive patients to date.

Adefovir dipivoxil is an adenine nucleotide analogue with activity against retroviruses. Gilson et al. evaluated its antiviral activity in patients with chronic HBV of which 13 of 20 were coinfected with HIV. Patients were treated with adefovir 125 mg Po for 28 days. Hepatitis B virus DNA levels fell rapidly in all active drug recipients but returned to baseline following discontinuation of the active drug. Serum HBV-DNA rose more quickly in HIV-positive versus HIV-negative patients after completion of therapy (19). In vitro studies suggest that mutations in the YMDD motif are not associated with adefovir resistance; raising the possibility that adefovir could be an important salvage agent in patients with lamivudine resistance (20). Clinical studies with combinations of therapy including lamivudine with adefovir are anticipated.

Famciclovir is another nucleoside analogue that is ap-

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proved for treatment of herpes infections. Trepo et al. studied 333 patients randomized to three different doses for 16 weeks of treatment (21). All doses resulted in a rapid reduction of both HBV-DNA and aminotransferases. There was no difference in the observed efficacy with regard to previous IFN treatment. Famciclovir may also select mutant strains in the YMDD motif, although selection of unique downstream mutants in the polymerase has also been described (22). There are no reported clinical studies of famciclovir in HIV-infected patients.

Clinical trials with HAART have shown long-term suppression of HIV replication improves immune status. This creates a situation in which a large pool of patients with previously innocuous hepatitis B infection will be at potential risk of ongoing liver injury. This will become more clinically evident as coinfected HIV and HBV patients live longer and have the time to develop complications of late-stage liver disease. Current evidence suggests that nucleoside analogues including lamivudine, famciclovir, and adefovir can suppress viral replication with limited toxicity. However, monotherapy with these agents has failed to clear HBsAg or seroconvert the majority of patients with coinfection. Rebound of infection after therapy is stopped is the rule rather than the exception. Long-term single-agent therapy seems to lead to a relatively high rate of mutant viral emergence. These observations clearly suggest that continued research and the development of new drugs and different combinations of existing drugs needs to be pursued.

CASE: HEPATITIS C

A 35-year-old white man was recently diagnosed with HIV after having presented with esophageal thrush. He was found to have a CD4+ count of 110 mm^3, an HIV viral load of 57,000, and abnormal liver enzymes. The patient has a history of remote intravenous drug abuse. He drinks four to six beers/day. Subsequent laboratory evaluation revealed the following.

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Biochemical and Serologic Findings

ALT 104 U/L	HBsAg negative
AST 76 U/L	Anti-HBs negative
Alkaline phosphatase 101 U/L	Anti-HBc negative
Bilirubin 1.1 mg/dL	HCV ELISA repeatedly reactive
Albumin 3.7 mg/dL	HCV RNA 29,000,000 copies/mL
GGT 334 U/L	Anti-HAV total negative
Prothrombin time 11.1 s (INR 1.1)	

Treatment and Outcome

The patient was started on a HAART regimen for control of HIV. Since hepatitis C treatment outcomes appear to be influenced by CD4+ count, it is reasonable to deal with the HIV infection first and then consider therapy with IFN-based regimens. Furthermore, the patient was told that alcohol accelerates disease progress and that abstinence from alcohol was indicated. Vaccination for hepatitis A and B was performed, although response may be suboptimal in this population. On therapy, the patient demonstrated an increasing CD4+ count and a lower HIV viral load. Two months later, it was noted that his liver enzymes had risen to an ALT of 330 U/L and an AST of 180 U/L. There was concern that the worsening aminotransferase abnormality was associated with antiretroviral therapy, but in fact was most likely associated with hepatitis C–related injury following HAART initiation. A liver biopsy revealed active hepatitis consistent with a hepatitis C etiology and bridging fibrosis. The patient has multiple HAART associated side effects and does not wish to consider IFN-based HCV therapy at this time.

HCV: NATURAL HISTORY IN HIV-INFECTED PATIENTS

Hepatitis C virus coinfection is emerging as an important complication of HIV. In the United States, it is estimated that over 4 million people, or 1.8%, of the population are chronically infected. Among patients with HIV infection, the rate of infec-

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tion is directly associated with the risk for acquisition of both viruses. Prevalence studies demonstrate HCV/HIV coinfection rates of 5–15% for homosexual males to as high as 80–90% for intravenous drug abusers and hemophiliacs who received factor concentrates prior to 1987. There is a growing body of evidence that coinfection leads to an accelerated course of liver disease. This is particularly well described in hemophilic cohorts, where end-stage liver disease is emerging as a major cause of mortality in HCV/HIV-coinfected patients. The natural history of HCV among hemophilic patients has been described in several longitudinal series to date. As early as 1992, Eyster et al. warned that they were observing a new and significant impact of HCV-associated liver disease among hemophiliacs in Pennsylvania (23). An increased risk of liver disease progression in hemophiliacs with HIV coinfection was subsequently described in larger cohort studies (24, 25). The reason for this observation is not clear. Dorrucci et al. found that the rate of CD4+ loss and development of other clinical parameters of AIDS was not affected by the presence or absence of HCV among homosexual men and intravenous drug abusers (26). In contrast, Piroth et al. described more rapid clinical progression of HIV-associated disease in patients with HCV in a similar cohort of patients (27). Rockstroh et al. found that a declining CD4+ count was independently associated with the risk of progression to liver failure using a multivariate Cox regression analysis (28). The association with liver disease rate of progression and CD4+ level was also observed among coinfected parenterally acquired HCV-infected patients in Spain (29).

Hepatitis C virus is believed to be cytotoxic to hepatocytes. A patient with HIV has decreased cell-mediated immunity which allows increased HCV replication and increased histological destruction mediated by HCV, resulting in rapid progression to end-stage disease (23). Liver histology is also worsened in patients coinfected with HCV and HIV (30). Liver fibrosis progresses faster in HIV/HCV-coinfected patients (31). One study showed that after 15 years 25% of patients coinfected with HCV and HIV developed cirrhosis in contrast

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to only 6% of patients who were HIV negative (32). To date, there are few studies that describe the relationship of the virus in terms of either classification (genotype) or viral load (HCV-RNA titer) with risk of progression. Limited data from Sherman et al. first suggested that coinfection of HCV and HIV is associated with higher serum HCV-RNA levels (33). Another study confirmed those HIV-infected patients with CD4 less than 200 had higher HCV-RNA levels as compared to the HIV-infected patients (34). Several studies have described the genotype distribution among large coinfected hemophilic men in various cohorts. In England, Telfer et al. used restricted fragment length polymorphism (RFLP) methodology to classify the genotype among 189 HCV-RNA–positive subjects. Type 1 was found to be predominant, occurring in 121 or 64% of the infected men. Type 3 was the next in prevalence, accounting for 19% of their cohort. Mixed infections were identified in only 1.6% of patients (35). An Argentinean cohort was found to have slightly more type 1 and slightly less type 3 using the same assay methodology (36). In contrast, surveys of the Multicenter Hemophilia Cohort Study derived from patients in the United States and Europe demonstrates a decrease in the prevalence of genotype 1 HCV and an increase in type 3. These studies were performed using a reverse hybridization assay (Inno LiPA, Immunogenetics, Alpharetta, GA), which is the most widely used assay technique (M.E. Eyster personal communication). Recently, extensive sequence-based genotype classification in a longitudinal cohort confirmed that genotype change does occur (37). This finding is supported by a report from the Royal Free Hospital in London that demonstrated a genotype change in 3 of 11 (27%) of longitudinally studied patients (35). These findings may have relevance to treatment outcomes.

IFN-BASED THERAPIES FOR HCV

Until recently, the only approved treatment for HCV was IFN-α. In immunocompetent, singly infected patients with HCV, a

sustained virological response was observed in 5–15% of cases following 6 months to 1 year of treatment. Prognostic factors for sustained viral response include host and viral factors. Undesirable host factors include long duration of infection, increased patient age, the presence of cirrhosis, male gender, normal/near normal serum ALT levels, alcohol use, and immunosuppression. Viral factors associated with poor response includes high viral load, genotype 1, and increased quasispecies complexity. Based on these predictors, it is not surprising that patients with HCV/HIV coinfection might manifest lower response rates than is seen in other populations. As noted above, coinfected patients tend to have higher viral titers and more rapid histological progression. The majority of HIV-infected patients are male. Quasispecies variability is increased in HCV/HIV–coinfected patients as well (38) (Fig. 1).

For patients coinfected with HIV, the optimum treatment of HCV remains debated. An early report of HCV treatment described 1 of 12 (8.3%) of patients showed a sustained biochemical response to therapy (39). In one study in Spain,

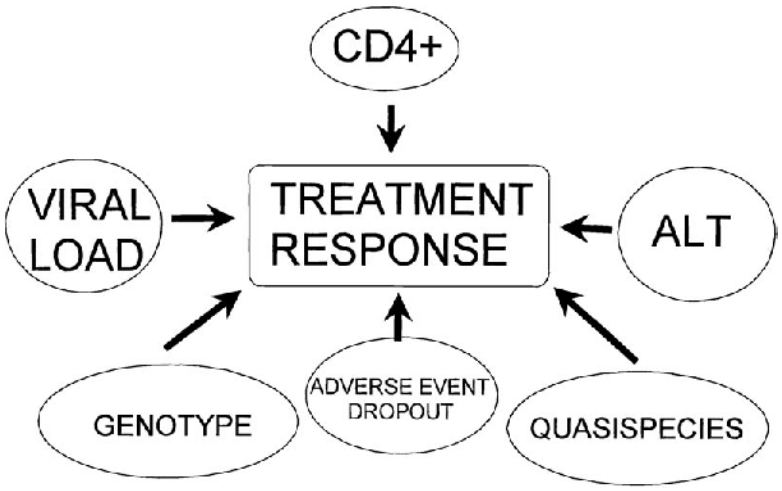

Figure 1 Major factors associated with response of HCV to interferon-based therapies in HIV-coinfected patients.

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38% of coinfected patients versus 47% of HIV-negative patients achieved a complete biochemical response 8 months after completion of therapy. Viral response was not reported (40). This study showed a positive correlation between CD4 counts and response to therapy. These data were extended by the same group to a total of 90 HIV-positive patients with HCV. The response was very similar in both groups. In the HIV-negative patients, 10 of 27 (37%) responded, and 26 of 80 (32.5%) of the HIV-positive patients had a biochemical and virological response at the end of treatment. After 12 months of follow-up, sustained response was observed in 20% of patients based on intent to treat parameters versus 24% of non–HIV-infected controls (41). A better response was seen in those patients with a higher CD4 count (>500 mm^3). A study from Germany confirmed that the efficacy of IFN in coinfected patients with HIV is influenced by their CD4+ count. A higher CD4+ count was significantly associated with a sustained response, and no patient had a CD4+ count less than 300 mm^3. Overall, 29.5% of 17 patients treated with IFN-α2a at a dose of 5 MU TIW for 52 weeks had sustained viral response (42).

Soriano et al. also assessed the utility of escalating dosage of IFN in the coinfected patient (43). They treated patients who did not respond to an initial 5 MU dosage of IFN and increased the IFN to 8 MU. An escalating dosage only induced a complete response in 1 of 12 patients who did not respond to the initial dose of 5 MU. The investigators suggest that these data did not support the hypothesis that lower rates of response to IFN in HIV-infected patients might reflect primary dependence of IFN on a preserved immune system, because a larger dose did not overcome the outcome of treatment failure. In most cases, the absence of response is not related to the dose of IFN, but to other factors.

The sum of patients described in published studies of HCV/HIV-coinfected patients is relatively low. The rates of primary response during treatment appear to be acceptable, but there are insufficient data to analyze the true sustained

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response rate at this time. It appears that CD4+ count does serve as an important surrogate marker for the likelihood of response, raising the question of whether immune reconstitution with HAART would influence outcomes.

Adverse effects to IFN are well known and include flu-like syndromes, thrombocytopenia, leukopenia, fatigue, emotional disturbance, alopecia, and thyroid dysfunction. In HCV/HIV-coinfected patients, some groups have described a sudden decrease in CD4+ counts after the administration of IFN (44). Other groups have confirmed this observation and described patients who then went on to develop AIDS-defining diagnoses. These associations are difficult to confirm, because sudden decreases in CD4+ counts can occur in HIV-infected patients without any explanation. The CD4+ count was monitored in another study which only demonstrated 5.2% of patients having a fall in the CD4+ count while on treatment (29). Controlled assessment of this phenomenon is indicated.

HCV AND RIBAVIRIN

Combination therapy with IFN and ribavirin has been found to be an important development in the treatment of HCV, with significant increases in sustained viral response rates for naïve, immunocompetent patients with HCV alone (45, 46). Treatment with combination therapy using IFN and ribavirin in HCV/HIV–coinfected patients is under study, and only limited and incomplete data are available, primarily in abstract form. Landau et al. used combination therapy with IFN and ribavirin in 20 patients with HIV and HCV coinfection. The average CD4+ count was 350 mm^3 and 85% were receiving antiretroviral therapy. Ten patients (50%) had undetectable HCV by 6 months of treatment. The responses did differ with regard to genotype, with type 3a having the best response. This is similar to results described for immunocompetent patients. The aminotransferase levels significantly improved with treatment (47).

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Another study reported the response data from 21 HCV/HIV–coinfected patients who were randomized to IFN or IFN plus ribavirin over an initial 3-month period. Then patients receiving IFN alone were shifted to combination therapy. IFN alone decreased mean the HCV-RNA load by 2.65 million copies/mL. Combination therapy was associated with a mean decrease of 450,000 copies/mL. By 6 months, patients in the combination therapy arm had a mean HCV viral load of 600 copies/mL. Dropout during this period was 27% in the combination treatment arm due to adverse effects despite the use of erythropoietin to control ribavirin-associated anemia (48). This high dropout rate is of concern, because an inability to complete treatment regimens will be associated with poor intention to treat analysis results even if improved efficacy over monotherapy is present.

There is considerable theoretical concern that ribavirin use will have a detrimental effect on HIV therapy. In vitro, there is a concentration-dependent interaction between ribavirin and zidovudine (AZT), which is thought to be secondary to competition for intracellular phosphorylation (49). There is likely to be interaction with other phosphorylation-dependent nucleoside analogues. It is not known if this will alter the efficacy of HIV therapy.

EFFECT OF HAART ON HCV

Although protease inhibitors and other therapeutic agents used to treat HIV do not appear to have intrinsic activity against HCV, it is possible that manipulation of the immune system via HIV suppression and subsequent immune reconstitution might have some effect. A study was done comparing three groups of patients with different triple therapy regimens. Plasma HCV-RNA levels, HIV load, and CD4+ and CD8+ cell counts were monitored. Regardless of the specific type of HAART, AST and ALT levels rose sharply within 1

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month of starting triple therapy. It was also shown that HCV-RNA levels increased initially, CD4+ and CD8+ cell numbers increased, and HIV-RNA levels fell. Liver biopsy performed 1–2 months after starting triple therapy demonstrated an increase in the mean Knodell histologic activity index score from 8 to 13. The biopsies showed more pronounced lobular necrosis and inflammation. A small but significant number of these patients experienced decompensation of their chronic liver disease, and so treatment was stopped (50). This study demonstrates that powerful antiretroviral therapy reduces HIV replication and increases CD4+ and CD8+ cells, but it also can increase HCV replication leading to further liver inflammation and hepatocyte damage.

Treatment of HIV with combination therapy with reverse transcriptase inhibitors modifies the prognosis of HIV. A case report describes the rapidly evolving HCV-related cirrhosis that paralleled immune restoration. A sampling phenomenon cannot be excluded, but it is suggested that triple antiretroviral therapy favored the occurrence of cirrhosis by promotion of immune-mediated mechanisms (51).

CONCLUSIONS

Coinfection of hepatitis B or hepatitis C viruses with HIV is becoming increasingly important in the clinical course of patients with multiple viral infections. Before the era of HAART, early HIV-associated mortality and significant immunosuppression were associated with minimal to mild consequences of hepatitis viral infections in these patients. Immune reconstitution of HIV-infected patients with HAART has changed the course of disease outcomes. There is now a critical need to understand the natural history and the value of treatment intervention for hepatitis viruses.

Treatments for HBV and HCV will continue to change and improve. The unique nature of HIV infection makes it im-

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perative that critically designed, well-controlled clinical trials be performed in these patients so that optimal efficacy and safety can be assured.

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12

Treatment of Hepatitis D

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CASE 1

A 40-year-old heterosexual man was found in June 1990 to be HBsAg and anti-HDV positive while screening a familial cluster of HBV/HDV carriers (mother and two younger brothers were HBsAg and anti-HDV positive). The patient had no history of acute hepatitis, intravenous drug use, or alcohol abuse and was asymptomatic. His aminotransferase levels were abnormal (aspartate aminotransferase [AST] 88 U/L ALT 150 U/L UNL <40 U/L) with other liver tests (γ-glutamyl transferase [GGT], alkaline phosphatase [ALP], bilirubin) being in the normal range. HBsAg and anti-HBe were positive, whereas HBeAg and HBV-DNA (dot-blot) were negative. Antibodies to HDV (total and IgM class) were present, as was HDV-RNA by

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polymerase chain reaction, (PCR). Antibodies to the hepatitis C virus (anti-HCV) were negative. Non–organ-specific autoantibodies (antinuclear antibody [ANA], antimitochondrial antibody [AMA], smooth muscle antibody [SMA]) were negative except for liver-kidney microsomal antibodies (LKM), which were positive at a titer of 1:160. A liver biopsy showed features of severe chronic hepatitis with bridging necrosis and early cirrhosis. Immunohistochemistry showed HBsAg in the cytoplasm of most hepatocytes, no HBcAg, and HDAg in 30% of liver cell nuclei.

HBsAg	Positive
anti-HDV (IgG, IgM)	Positive
HBeAg	Negative
anti-HBe	Positive
HBV-DNA	Negative
AST	88 U/L
ALT	150 U/L
anti-LKM	1:160

A few months later, treatment was started with recombinant interferon alpha (IFN) at a dose of 10 MU TIW for 12 months. Upon starting therapy, alanine aminotranferase (ALT) was 170 U/L, HBV-DNA was negative, and HDV-RNA and IgM anti-HDV were positive at high titer. After 6 months of IFN, ALT was almost normal and HDV-RNA undetectable, but IgM anti-HDV were still positive at low titer. At 12 months, before stopping IFN, ALT were normal and HDV-RNA, IgM anti-HDV, and HBV-DNA were negative. While on treatment the patient did not experience significant side effects besides mild fatigue. A moderate reduction of platelets (from 145,000 to 90,000/mm^3) and a twofold increase of LKM titer were the only abnormalities. Both subsided within 1 month after therapy.

ALT remained normal and HDV-RNA negative during the first 6 months posttreatment, but then relapsed to pretreatment values, with the reappearance of IgM anti-HDV and HDV-RNA. No further treatment was offered. The patient has been regularly followed with clinical, biochemical, and ultrasound controls over the following

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years. He remains asymptomatic, with ALT levels fluctuating between two and four times normal and with no evidence of portal hypertension. A second liver biopsy was performed in 1998, showing moderate chronic hepatitis with cirrhosis. Immunohistochemistry showed HBsAg in the cytoplasm of most hepatocytes and no HBcAg and HDAg in 10% of liver cell nuclei. HBsAg and HDV-RNA are always positive, and low titers of IgM anti-HDV are present. HBV-DNA is negative by dot-blot but a small number of HBV genomes can be found in serum by PCR.

CASE 2

A 27-year-old woman with a history of current intravenous drug addiction and of sexual promiscuity, but no overt alcohol abuse, was found in 1993 to be HBsAg positive, with abnormal liver enzymes, because of a history of mild fatigue in the previous months. At admission in our unit, AST was 238 U/L and ALT 450 U/L, with a sixfold increase of GGT but nearly normal alkaline phosphatase (ALP) and bilirubin. High-level HBV (HBsAg and HBeAg positive, IgM anti-HBc positive, HBV-DNA 100 pg/mL by dot-blot) and HDV replication (anti-HDV IgM and total positive, HDV-RNA positive) was present. Antibodies to HCV and to human immunodeficiency virus (anti-HIV) were negative. Non–organ-specific autoantibodies (ANA, AMA, SMA, and LKM) were negative. A liver biopsy showed severe chronic hepatitis with marked intralobular necrosis. There was marked portal fibrosis, but no bridging. Immunohistochemistry showed HBsAg on the membrane of most hepatocytes, large amounts of HBcAg (70% of liver cell nuclei), and HDAg (10% of liver cell nuclei).

HBsAg	Positive
HBeAg	Positive
IgM anti-HBc	Positive
HBV-DNA	100 pg/mL
AST	238 U/L
ALT	450 U/L

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Soon after diagnosis, the patient was started on 9 MU OD of recombinant IFN-α2a. Upon starting therapy, ALT was 370 U/L, HBV-DNA 65 pg/mL, and HDV-RNA positive. Three months after starting IFN, she experienced malaise and fatigue. A few days later, dark urine and mild jaundice were noted. ALT levels were increased (1,123 U/L) and bilirubin was up to 4.7 mg/dL. Autoantibodies were negative. HBV-DNA had become undetectable by dot-blot, but the other viral markers were unchanged. The clinical pattern was interpreted as being compatible with impending HBeAg to anti-HBe seroconversion and IFN was not stopped. Clinical symptoms disappeared in a few days, and within the following 8 weeks, ALT values went down to the normal range. HBV-DNA remained negative, and the HBeAg to anti-HBe seroconversion actually occurred. HDV-RNA was also negative, but by the ninth month of therapy, it reappeared in serum, together with a mild elevation of ALT (112 U/L), and persisted thereafter. Treatment was stopped at 12 months. HBeAg and HBV-DNA stayed negative, but a sharp rise of ALT, up to 256 U/L, was observed in the following months, together with the reappearance of IgM anti-HDV. A second liver biopsy was performed 1 year later. Moderate portal and periportal inflammation with some progression of fibrosis were seen. Immunohistochemistry showed HBsAg in the cytoplasm of most hepatocytes, no HBcAg, and HDAg in 20% of liver cell nuclei.

The patient has stopped intravenous drug use and has remained asymptomatic over the last 6 years without any antiviral treatment. Her biochemical and virological status has not changed since.

CASE 3

In September 1998, a 57 a year-old heterosexual man with a 15-year history of HBV/HDV–related liver disease was admitted to our unit because of decompensated cirrhosis. Upon admission, his AST was 288 Ul/L and ALT 345 Ul/L. There was hyperbilirubinemia (3.3 mg/dL), leukopenia (2100/mm^3), thrombocytopenia (45,000/mm^3), and hypoalbuminemia (2.6 g/dL). An ultrasound scan confirmed the presence of moderate ascites. HBsAg and anti-HBe were positive and HBeAg negative. HBV-DNA was positive by dot-blot (20 pg/mL). The patient was HDV-RNA positive, total anti-HDV positive, IgM negative, and anti-HCV was negative.

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HBsAg	Positive
Anti-HBe	Positive
HBeAg	Negative
HBV-DNA	Positive
HDV-RNA	Positive
Anti-HDV (total)	Positive
Anti-HDV (IgM)	Negative
AST	288 U/L
ALT	345 U/L

The patient had been found to be HBsAg positive at the time of a blood donation in 1983. There was no history of acute hepatitis or familial clustering of HBV or HDV infection. When first screened, he was HBeAg negative and anti-HBe positive and also total anti-HDV positive. A liver biopsy, performed in 1986, showed features of cirrhosis with severe necroinflammatory activity. Immunohistochemistry showed HBsAg in the cytoplasm of most hepatocytes, HBcAg (10% of cells, mostly cytoplasmic), and HDAg in 50% of liver cell nuclei.

The patient was enrolled in a randomized trial of IFN (1) and received 10 MU, then reduced to 5 MU of recombinant IFN α2b tiw for 12 months. No consistent effects on ALT levels were seen during therapy. At end of treatment, HCV-RNA (previously untested) and IgM anti-HDV were positive. HBV-DNA became transiently negative under IFN, but then reappeared. He remained asymptomatic under no treatment, with his ALT levels ranging between two and four times normal values. An ALT flare was, however, observed in July 1998 followed by ankle edema and abdominal distension leading to admission in September.

At the time of admission, HBV-DNA was found to be $>$100 pg/mL, with IgM anti-HDV and HCV-RNA positive. Since September 1998, the patient has received diuretic therapy and lamivudine at a dose of 100 mg daily. Upon starting therapy, ALT was 270 U/L. Within 2 months, ALT levels were normal, with a negative HBV-DNA. HDV-RNA and IgM anti-HDV remained positive. Liver function tests were clearly improved (bilirubin 1.7 mg/dL, albumin 3.1 g/dL) without any side effects of therapy. He was put an a waiting list for liver transplantation.

Treatment was continued for the 6 months. In April 1999, an ultrasound scan revealed an hypoechoic, 40-mm focal lesion in the

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fifth segment of the liver. Liver biopsy showed a trabecular hepatocellular carcinoma. Radiofrequency thermal ablation of the tumor was apparently successful. The patient underwent orthotopic liver transplantation (OLT) in August 1999, still with a negative HBV-DNA and HDV-RNA positive. Immunohistochemistry of the explanted liver showed HBsAg in the cytoplasm of most hepatocytes, no HBcAg, and HDAg in 10% of liver cell nuclei.

Lamivudine therapy was never stopped, and HBIg prophylaxis was performed. Six months post-OLT, the patient is alive and well, on steroids and FK506, with no evidence of HBV or HDV reinfection.

BACKGROUND

The hepatitis delta agent, or hepatitis D virus (HDV), is an incomplete defective RNA virus with a replicative cycle dependent on a "helper" function of another virus (HBV) resembling plant viroids and satellite viruses. Its small genome is a single-stranded circular negative RNA of 1700 nucleotides, nonhomologous with HBV-DNA except for a little area of the polymerase gene. The HDV genome contains a peculiar hairpin structure with ribozyme activity. HDV-RNA replicates through RNA-directed RNA synthesis by transcription of genomic RNA to a complementary, antigenomic (plus strand) RNA that acts as a template for further genomic RNA synthesis. Both genome and antigenome strands of HDV possess a self-cleavage site. The only protein encoded by HDV-RNA is delta antigen (HDAg), which exists in two forms: small, 195 amino acids long, with a role in HDV-RNA replication, and large, 214 amino acids long, which seems to suppress viral replication and is necessary for virion assembly. The viral nucleocapsid and genome are coated by an outer envelope of HBsAg, scarce of pre-S1 and pre-S2 peptides. HDV hence uses the same mechanisms of entry into the liver cells and of immune escape of HBV, from which presence is strictly dependent (Table 1).

HDV is the most unusual of all hepatitis viruses, being the only human satellite agent currently known (1). Its pecu-

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Table 1 Main Features of HBV and HDV

Classification	HBV Hepadnavirus	HDV Viroid
Genome	3.2-kb DNA circular, ss/ds plus-stranded	1.7-kb RNA, Circular, ss, plus-stranded
Virus particle	42 nm	35–37 nm
Morphology	Spherical, filamentous double-shelled (HBsAg coat, HBcAg core)	Enveloped hybrid (HBsAg coat, HDV core)
Transmission	Parenteral, sexual, direct contact	Parenteral, sexual, direct contact
Incubation period	30–180 days	30–180 days
Carrier state	Yes	Yes
Chronicity	Yes	Yes
Antigens	HBsAg, HBcAg, HBeAg	HDAg

liar life cycle as a defective subviral RNA particle, depending upon HBV for its assembly and replication, would seemingly make HDV a good target for antiviral treatments because of the complexity of replicative mechanisms with potential HDV- and HBV-specific points for drug action. Actual evidence of in vivo action of different antiviral drugs is however missing, and the issue of clinically effective etiological therapy for HDV hepatitis is still open.

It is estimated that HDV infection is present in 10–15 million chronic HBV carriers worldwide (2). In areas of high endemicity, HDV superinfection is typically found in HBsAg-positive individuals without detectable HBV replication (2). In this situation, HDV is likely to represent the principal cause of liver damage (3), albeit the role of inapparent but continuous HBV infection should not be underestimated, as shown by the liver transplantation model (4). These patients may have severe liver disease with the early appearance of cirrhosis, but they often have a disease course indistinguishable from that of chronic HBV infection (5). In low endemicity areas, where HDV mostly infects drug addicts with a brief duration of HBV

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infection, simultaneous replication of the two viruses probably accounts for a more severe course of disease, especially when human immunodeficiency virus (HIV) coinfection is present (6). The reappearance of HDV hepatitis after OLT for end-stage HBV/HDV cirrhosis is uncommon (7), especially when appropriate anti-HBV immunoprophylaxis is performed (8). When it occurs, it has remarkable implications in terms of graft loss and morbidity (9).

HDV infection has a worldwide distribution, but the areas of highest endemicity are the Mediterranean basin, the Middle East, and certain parts of South America. It is found infrequently in Asia, western Europe, and North America. Albeit epidemiological evidence suggests that HDV infection rates are decreasing in the Mediterranean basin (10, 11), its prevalence is still high in some areas of the Far East where HBV carriage is endemic (2). In southern Europe, most cases of HDV superinfection occur in patients with the precore mutant of HBV (11, 12), unable to secrete HBeAg. This mutant is in turn associated with specific HBV genotypes (13), and it has a lower responsiveness to IFN therapy than wild-type HBV (14). In most Western countries, hepatitis D occurs commonly among illicit drug users and multiply transfused patients, and as many as 50% of HBV carriers in these groups may have antibody to HDV. HDV infection is observed relatively infrequently among homosexual men, institutionalised mentally retarded persons, and hemodialysis patients. It is estimated that worldwide approximately 5% of 350 million HBV carriers are infected by HDV. In the lower Mediterranean basin and in the Middle East, where HDV infection is still endemic (prevalence >20% of chronic HBV carriers), the common route of transmission is person-to-person sexual and intrafamilial spread. In these areas, the most common HDV is genotype 1. Some outbreaks of severe hepatitis have been reported in the Amazonian basin and in other areas endemic for HBV as a result of transmission by skin lesions or sexual contacts. These epidemics, in which cases of fulminant hepatitis are common, are caused by HDV genotype 3.

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DIAGNOSIS AND FOLLOW-UP

Diagnosis of HDV infection is based on a limited number of specific laboratory tests (Table 2).

- Total (mostly IgG) anti-HDV antibodies: Specific for HDV infection but unable to discriminate between acute or chronic infection. May persist for some months in resolved cases.
- IgM anti-HDV: Their de novo appearance during an episode of acute hepatitis indicates acute infection. Fairly high titers can be found in chronic infection and are directly related to the expression of HDAg in the liver.

Table 2 Diagnostic Tests for HDV Infection

Test	Sample	Technique	Clinical use
Anti-HDV (total)	Blood	EIA or RIA	Antibodies to HDAg mostly of IgG class, present in ongoing or past infection
Anti-HDV (IgM)	Blood	EIA	Antibodies to HDAg, indicative of HDV-induced liver damage
HDAg	Blood	EIA or RIA	Positive at the peak of viraemia
HDAg	Blood	Immunohistology	Detects HDV infection with a sensitivity almost equal to RT-PCR
HDV-RNA	Blood or liver	Molecular hybridization in situ for liver)	Detects active HDV replication. Easier to quantify
HDV-RNA	Blood	RT-PCR	Gold standard for HDV infection, detects up to 10 genomes/mL

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- Serum HDAg: Present in blood for a few days during acute infection and hence of limited diagnostic value. HDV-RNA: Gold standard for HDV infection—present in blood whenever HDV is replicating.

It should be reminded that, owing to viral interference, HBsAg may occasionally be undetectable in blood during acute HDV hepatitis.

Even if ideally the best test to follow the course of HDV infection is reverse transcriptase–polymerase chain reaction (RT-PCR) for HDV-RNA (15, 16), its availability is restricted to research laboratories. Moreover, the test has not been standardized across different laboratories and cannot be relied on for accurate quantification of HDV viral load. A reasonable substitute in clinical practice is the determination of HDAg in liver tissue by immunohistochemical techniques using polyclonal or monoclonal anti-HDV antibodies. Albeit necessitating a liver biopsy, this test is 95% sensitive when compared to RT-PCR and much easier to perform. It also allows semiquantification of the expression of HDAg.

IgM anti-HDAg, besides being an indicator of recent infection, has been considered to indicate HDV-induced liver damage (17). Although a rough relation with fluctuations of HDV-RNA and ALT peaks can be observed, its actual usefulness is minor.

NATURAL COURSE OF HDV INFECTION

HDV infection is acquired by two different modalities (Fig. 1): as a co-infection (simultaneous exposure of an HBV nonimmune subject to HBV and HDV) and as a superinfection (exposure to HDV of a chronic HBV carrier). Chronic hepatitis D may develop through both events: as an unresolved acute HBV/HDV hepatitis by coinfection or as superinfection of a chronic HBsAg carrier with HDV. In the first case, the rate of progression to chronicity is equivalent to HBV infection alone,

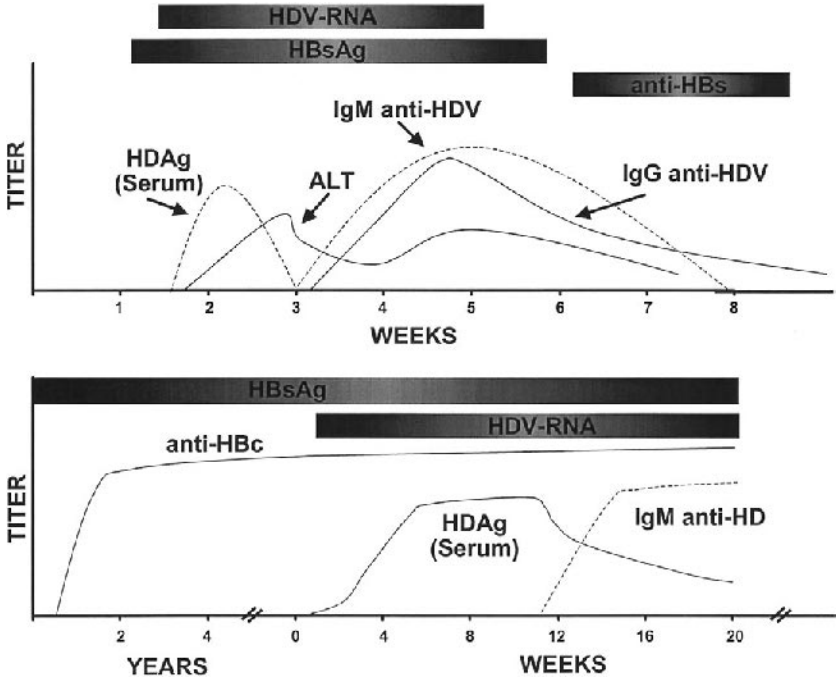

Figure 1 Acute HDV/HBV coinfection (above) and superinfection by HDV of a chronic HBV carrier (below).

but the episode of acute hepatitis may be more severe. In the case of superinfection, HDV may cause a rapid deterioration of liver disease and thus a worse long-term prognosis. Patients undergoing HBV/HDV have a low likelihood of progressing to chronicity. Clearance of HBV infection, occurring in the large majority of nonimmunosuppressed patients, necessarily terminates HDV replication. The likelihood of a more severe hepatitis is instead slightly higher than for HBV infection alone. HDV superinfection of a chronic HBV carriers is a major problem, since persistent HBV replication provides the needed long-lasting helper effect for chronic HDV infection. Chronic HDV infection causes a severe chronic hepatitis with a rapid and relentless progressive course to cirrhosis and liver failure.

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The clinical features and laboratory tests in ongoing chronic hepatitis D are like those in chronic HBV. Liver histology may show a more severe grade and stage than chronic HBV infection. A peculiar serological feature of chronic hepatitis D is the presence in serum, in 10–20% of cases, of antibodies to liver-kidney microsomes (anti-LKM3), directed to an epitope of cytochrome P450-IID6 (18) distinct from other autoantibodies found in autoimmune hepatitis (anti-LKM1) or in drug-induced hepatitis (anti-LKM2). This autoantibody is not associated with clinical evidence of autoimmunity.

We performed a prospective study (12) in order to evaluate clinical and virological factors that influence the course of HBV-related chronic liver disease. A cohort of 302 HBsAg-positive patients (mean age 34 ± 15.3 years) with chronic hepatitis (86 with cirrhosis) was assessed over a median follow-up of 94 months. Eighty-six patients (28.5%) were HBeAg positive (wild-type HBV), 80 (26.5%) were HBeAg negative, HBV-DNA positive, 76 (25.2%) had HDV superinfection, and 43 (14.2%) had dual HBV/HCV infection. Seventeen (5.6%) patients were negative for all viral markers except HBsAg. During follow-up, decompensation of disease occurred in 46 patients: 8 hepatocellular carcinoma, 36 ascites, and 2 jaun-

Table 3 Standardized Mortality Ratio (SMR) in Patients with Chronic Hepatitis B According to Baseline Virological Class as Compared to General Population

Group	No. of patients	Observed deaths	Expected deaths	SMR	95% CI
Wild-type HBV	86	8	0.47	17.0	7.33–33.49
HBeAg minus	80	6	1.15	5.20	1.91–11.34
HDV infection	76	14	0.51	27.0	14.74–45.36
HCV infection	23	0	0.37	—	—
HBV/HCV coinfection	20	7	0.14	49.0	19.65–100.94
All replicative markers negative	17	2	0.32	6.20	0.75–22.38

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dice. Five patients were transplanted. Thirty-five patients died: 33 of hepatic and 2 of nonhepatic causes. Overall mortality was 5.2-fold that of the general population (95% Cl 3.6–7.3; 35 deaths observed, 6.7 expected; $P < .0001$). A fivefold excess mortality (standardised mortality rate, SMR: 5.25; 95% Cl 3.66–7.30) in the total cohort of patients with chronic hepatitis B as compared with a matched general population was observed. Table 3 shows the SMRs of patients with chronic hepatitis B according to virological profile at enrollment. The highest risk of death was found in patients with dual HBV/HCV replicative infection, followed by HDV infection, wild-type, HBV, and HBe minus HBV. These data confirm earlier suggestions of a more severe course of HDV-related liver disease.

TREATMENT

Key Points

Treatment of chronic hepatitis D has given up to now disappointing results. Early studies and subsequent randomized trials (19, 20) found that an improvement in biochemical and viral markers of disease may occur (20) in response to high doses of IFN given for prolonged periods. The response lasts longer with the use of high doses of IFN (e.g., 9 million units TIW for at least 12 months) (20). However, an extremely high rate of relapse of HDV-RNA and of aminotransferases has been observed on termination of therapy even after prolonged courses (19). The few responders usually have recent HDV infection (7, 21). Prolonged treatment, over many years, with high doses of IFN-α should be reserved to patients who, after a few months of therapy, have a clear-cut reduction of necroinflammatory activity. Lamivudine, albeit capable of inhibiting completely HBV replication, does not affect HDV expression (22); possibly due to the relative independence of the HBV helper function from the HBV-DNA polymerase (1). Ribavirin, a nucleoside analogue, is the only other direct antiviral agent

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tested alone against HDV up to now and is also ineffective (23).

Nonantiviral Treatments

Before the age of etiological treatment for chronic viral hepatitis, immunosuppressive therapy was often given to patients with HBsAg-positive chronic hepatitis in order to reduce necroinflammatory activity. This also applied to chronic HDV hepatitis. In a small cohort of HBV/HDV–infected patients collected before 1982, Sagnelli and Manzillo (24) showed that immunosuppression with steroids and azathioprine improved, over an unstated period of follow-up, both the histology and the event-free survival rate in comparison to untreated or steroid-treated patients. This effect was more evident for HBeAg-negative patients. Albeit difficult to justify by current therapeutic standards, this experience would suggest that necroinflammation due to sustained immune response to HDV is probably the main component of the long-term unfavorable evolution of some cases of chronic HDV infection. Attempts toward immunomodulatory therapy with thymopentine, as adjunctive treatment to IFN (25) in OLT patients, have shown no consistent benefit.

Interferons

Since its first availability for clinical use, and the demonstration of its antiviral activity against HBV, IFN-α appeared to be a promising drug to treat HDV infection, since inhibition of HBV should control various steps in the HBV replicative cycle (Fig. 2). Early uncontrolled reports (26, 27) suggested, in fact, the efficacy of HDV in reducing ALT levels and controlling liver necroinflammation. This effect was clearly dose related and tended to last up to the end of therapy. Subsequently, two randomized controlled trials (19, 20), and other nonrandomized studies (28–32) confirmed that the benefit of treatment was related to the on-treatment period and tended to disappear afterward. Pooling of data obtained by meta-

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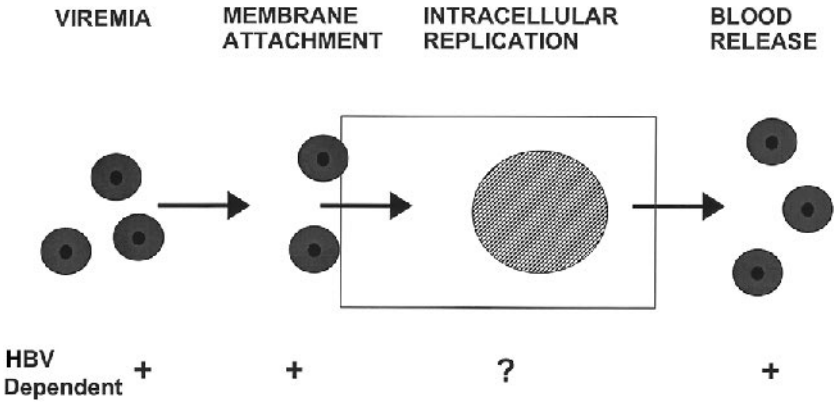

Figure 2 Steps of HDV replication.

analysis of the three evaluable studies (19, 20, 32), combining, respectively, 61, 42, and 22 patients and treating for 12 months with an intended dose of IFN ranging from 9 to 27 MU per week showed, under treatment, a pooled rate difference of ALT normalization of 25% and of HDV-RNA clearance of 40% (Fig. 3). In these three studies, the rate of spontaneous ALT normalization was 1%. However, at the end of an 18–24 months of postreatment follow-up, the success rate had dramatically diminished, since pooled rate difference for normal ALT was 5 and 1% for HDV-RNA (Fig. 4). A notable exception was found in the high-dose (27 MU/wk) arm, whose ALT normality rate was 35% at the end of follow-up even in the absence of a sustained virological response. Further follow-up data on this cohort, followed up to a mean of 118 months with some retreatments, were recently given (33). Except for one patient, none of the patients cleared HDV-RNA or HBsAg. Death or liver transplantation occurred in 13% of the patients treated with 27 MU/wk, in 45% in the 9-MU/wk group, and in 67% controls. Cumulative survival was hence significantly higher in the former group. Liver biopsies, performed after a mean of 115 months from completion of therapy, showed a reduction of the total Knodell score in the high-dose group but

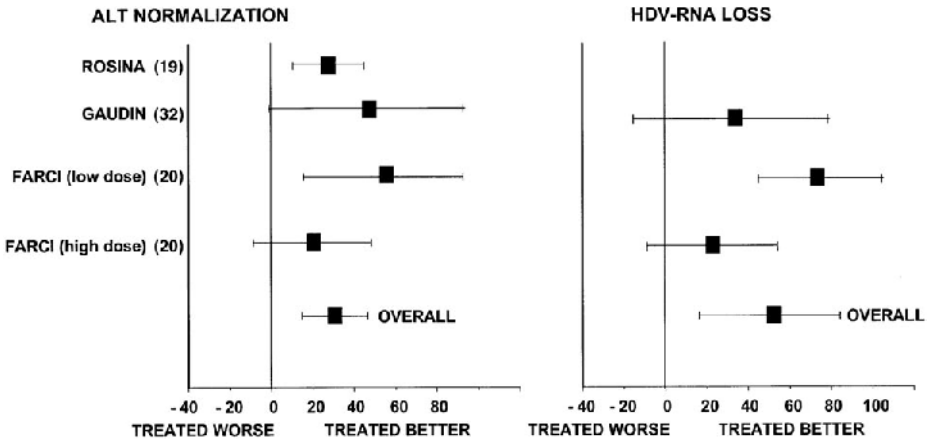

Figure 3 Meta-analysis of RCTs of IFN therapy for chronic HDV hepatitis—results at the end of therapy.

not in the 3-MU group. No significant differences in ALT values were observed among the three groups. This would suggest that, although treatment with high doses of IFN for 48 weeks did not eradicate HDV infection, it was associated with a significant improvement of the long-term clinical and histological outcome. The smallness of numbers of patients and the selective nature of this study do not allow, however, extrapolation of these results to the general population of patients with chronic HDV infection.

Experience with pediatric patients (34–36) duplicates, in terms of efficacy on HDV infection, that of adults. In these subgroup, mostly infected early in life through vertical or early horizontal transmission (37), the rate of clearance of HBV replication (loss of detectable HBeAg and HBV-DNA in serum and of HBcAg in the liver) is high during IFN therapy. Necroinflammatory activity, however, remains unchanged in these children after HBeAg to anti-HBe seroconversion. This is explained by persistence of HDV infection, expressed by the reappearance of serum HDV-RNA and of liver HDAg, sometimes after a temporary abatement under therapy. IFN thus

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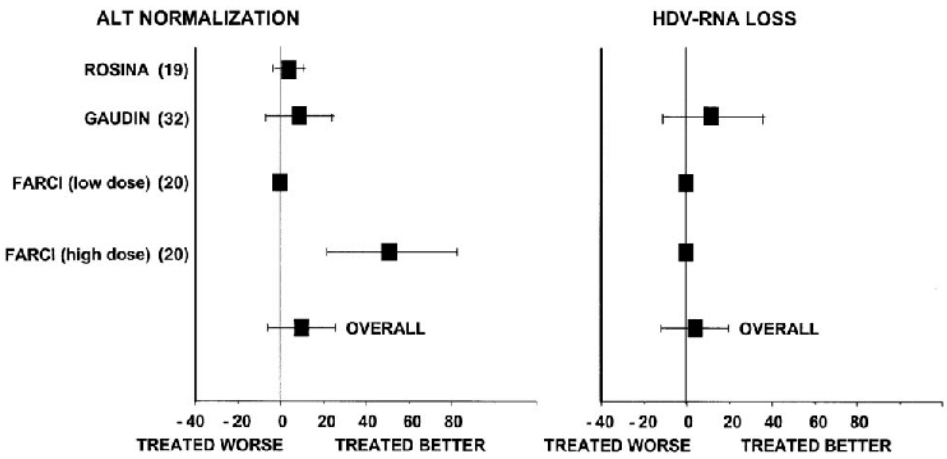

Figure 4 Meta-analysis of RCTs of IFN therapy for chronic HDV hepatitis—results at the end of follow-up.

presumably will not change the outcome of liver disease in these children.

Treatment of HIV-positive patients with HBV/HDV co-infection (26, 38) may also obtain HBeAg to anti-HBe seroconversion, but has no long-lasting effects on HDV. In this respect, no data are available up to now for HIV patients under HAART.

The only virology endpoint which can be associated with permanent clearance of HDV is HBsAg clearance (39–41) with or without the appearance of anti-HBs. In chronic HBV carriers, this event may be spontaneous, occurring at a rate of 1% per year (42), or treatment-induced (43). Although HBV-DNA genomes persist in integrated and supercoiled forms in the liver of these HBsAg-negative subjects (44), the residual HBV is unable to perform its helper function(s) for HDV (65). The incomplete defective agent thus ceases to replicate and is cleared from the liver. Such an effect may occur even after very prolonged periods of IFN treatment—like 12 years in a recent case report (12).

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The effects of IFN as an antiviral agent against HDV are rather weak (46). Anecdotal evidence (47) shows that HDV superinfection of a chronic HBV carrier with a nonmassive inoculum is possible during high-dose IFN therapy for HBV. This would suggest that the initial phases of uptake and replication of HDV are unaffected by IFN. It has instead been suggested that, in a small number of patients, treatment during the early phase of HDV superinfection could be more effective, and could also be associated with a higher likelihood of HBsAg seroconversion (21).

Current experience would hence suggest that a trial of IFN-α therapy at high doses (9–10 MU OD or 5 MU daily) should be done in patients who are IgM anti-HDV and/or HDV-RNA and/or liver HDAg positive, have abnormal ALT, and an histology of chronic hepatitis or cirrhosis. Treatment should be prolonged beyond the sixth month and over many years, with high doses of IFN-α only in those patients who have had a clear-cut reduction of ALT.

Nucleoside Analogues

Early experience (47) with the combination of IFN with a nucleoside analogue, acyclovir, in patients with chronic HDV hepatitis yielded inconsistent results; possibly due to the weakness of the effect of acyclovir on the HBV-DNA polymerase. A few years later a purine analogue, ribavirin, was tried alone in HDV-infected patients (23, 49) on account of its good antiviral action on many RNA viruses. No short-term effects on viral replication or ALT levels were consistently shown, and thus ribavirin monotherapy was abandoned in this context as for chronic hepatitis B (50). Recent evidence suggesting possible enhanced effectiveness of the IFN/ribavirin combination in chronic hepatitis B should prompt a reappraisal of this approach in chronic HDV hepatitis.

Lamivudine is an extremely effective inhibitor of HBV replication (52). The possibility that such a profound inhibition of HBV replication could actually eradicate HDV was

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evaluated by Lau (22), who treated five patients, all HDV-RNA positive, with 100 mg of lamivudine for 12 months. Four patients had low-level HBV replication (anti-HBe positive, serum HBV-DNA detectable only by PCR), whereas one was HBeAg positive. A remarkable inhibition of HBV was observed: four of five patients became HBV-DNA negative by PCR. However, all five patients remained HBsAg and HDV-RNA positive, and their ALT levels and posttreatment histology did not improve. When lamivudine was stopped, HBV replication returned to pretreatment values. We have also found similar results in four HBV-DNA–positive, HDV-RNA–positive patients with advanced cirrhosis (unpublished data). It remains to be seen whether long-term inhibition of HBV by lamivudine could obtain eradication of HDV through HBsAg clearance. The combination of lamivudine and IFN has recently been tried (53). Eight HDV-infected patients received lamivudine 150 mg QD for at least 24 weeks, thereafter combined with an initial dose of IFN-α of 10 MU daily during 4 weeks, followed by 12 weeks of 10 MU TIW. They had a follow-up of 12 weeks. During therapy, HBV-DNA was suppressed below the detection limit of the liquid hybridization assay in all, but ALT remained elevated and HDV-RNA stayed positive. The combination of lamivudine IFN induction therapy followed by prolonged IFN on therapy thus seems to be ineffective in chronic HDV infection.

Liver Transplantation

Albeit new cases of HDV infection in Europe are dwindling (11), HDV still represents one of the main causes of progressive liver disease leading to liver transplantation in chronic HBsAg carriers (54). Reinfection of the transplanted new liver is strictly linked to the reappearance of HBV in the host (55): It is thus much more frequent when the patient is HBV-DNA positive pretransplant. HDV hepatitis in the transplanted liver may lead to graft loss and to death because of liver failure and cirrhosis (56). The suppressive effect of HDV on HBV rep-

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lication (1) make this event rather less likely. Pretransplant treatment with IFN (57) or, more effectively, with lamivudine (58) dramatically reduces the chances of overt HBV reinfection of the new liver when combined to long-term immunoprophylaxis with hyperimmune anti-HBs immunoglobulins (59).

Experience with IFN in the posttransplant period is scarce and anecdotal owing to the fear of inducing graft loss and to the unsatisfactory results in chronic HDV hepatitis. No recommendations for the use of IFN in this context can be made at present.

Future Perspectives

Research on potential inhibitors of HDV is currently concentrating on the possibility of inhibiting the HDV replication mediated by the use of *trans*-ribozymes or of antisense probes (60). BZA-5B, a prenylation inhibitor, has been shown to abolish the production of HDV particles in transfected cells (61). At present, the difficulties in translating these promising in vitro results to the in vivo situation make these approaches still highly experimental.

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13

Future Hepatitis C Virus Therapy: Protease and Helicase Inhibitors and Beyond

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CASE

The patient is a 29-year-old man with chronic hepatitis C apparently acquired from a blood transfusion after a bicycle accident as a child. He has no other risk factors for hepatitis C virus (HCV) infection and no history of jaundice. The HCV infection was found in 1996 during routine blood testing for insurance qualification. He had been vaccinated against hepatitis B before the bicycle accident. At the time of initial diagnosis, his results were the following:

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AST (SGOT)	48 (Normal 10–40)
ALT (SGPT)	61 (Normal 10–40)
Alkaline phosphatase	86 (Normal 30–110)
Total bilirubin	0.6
Direct bilirubin	0.2
Albumin	3.9
Globulin	3.2
Prothrombin time	12.1/11.8
HCV Antibody	Positive
HCV RNA	Positive (by qualitative PCR)
HAV IgG Ab	Negative
HAV IgM Ab	Negative
HBsAg	Negative
Anti-HBs	Positive
Iron	72
Iron-binding content	289
Ferritin	65

The patient had no symptoms referable to the liver disease, and there was no evidence of autoimmune hepatitis, α_1-antitrypsin deficiency, or Wilson's disease. He was concerned about the long-term effect of the HCV infection and eager to undergo treatment. A percutaneous liver biopsy revealed moderate periportal inflammation (2/4) and mild hepatocellular necrosis and fibrosis (1/4). He underwent a 6-month course of therapy with interferon 3 MU TIW without significant response. He was subsequently found to have genotype 1b HCV. In 1999, the patient began a 48-week protocol treatment with PEGylated interferon (1.5 μg/kg weekly) and ribavirin (800 mg daily). He tolerated the regimen well but had no significant virological response (RNA titer fell from 1.6×10^6 to 1.1×10^6 copies/ml on therapy and rebounded to baseline after cessation). Before the course of combination therapy, a repeat liver biopsy revealed no histological change during the intervening 2.5 years. His most recent liver tests included the following:

AST (SGOT)	74 (Normal 10–40)
ALT (SGPT)	93 (Normal 10–40)
Alkaline phosphatase	90 (Normal 30–110)
Total bilirubin	0.5
Direct bilirubin	0.2
Albumin	3.6
Globulin	3.1
Prothrombin time	12.8/12.4

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Looking forward, what are the therapeutic options for this individual?

INTRODUCTION

Infection with HCV is the most common cause of chronic liver disease in the United States and the second most common cause of chronic hepatitis worldwide (1, 2). Transmitted parenterally, HCV enters the bloodstream and selectively infects hepatocytes of humans and chimpanzees, its natural hosts. Approximately 85% of infected humans develop chronic infections, some of whom exhibit progression to chronic hepatitis, cirrhosis, and hepatocellular progression. Approximately 20% of individuals chronically infected with HCV will develop cirrhosis, and between 2 and 5% will ultimately develop hepatocellular carcinoma. When progression occurs, it is generally slow, with an average time of 20 years to cirrhosis and 30 years to hepatocellular carcinoma.

Current therapies for chronic HCV infection are directed primarily at enhancing the host's immune defenses against viral infection. They include several different preparations of interferon-α, alone or more commonly in combination with ribavirin. The use and efficacy of these therapies is well described elsewhere in this volume (Chapter 7). Recent studies suggest that treatment with a combination of long-acting, PEGylated interferon and ribavirin can lead to viral clearance *and* normalization of liver test abnormalities in approximately 50% of individuals with chronic HCV infection (3,3a, 3b). These results are substantially improved from those seen with earlier combination regimens that include standard interferon and ribavirin. Even with these recent improvements, however, approximately half of treated individuals will fail to experience a sustained virological response to therapy. Moreover, in several important groups of individuals with chronic hepatitis C, including those whose infection has led to cirrhosis, the likelihood of success is even lower. Many of these treat-

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ment resistant individuals would benefit from novel therapeutic approaches to prevent further progression of their liver disease and to decrease the risk of transmission to naïve populations. This chapter focuses on the prospects for the development of new HCV antiviral agents based on more detailed understanding of the mechanisms of HCV viral replication.

Effective antiviral agents typically interfere with one or more steps in the natural process of viral infection and replication. Figure 1 depicts the major steps in the life cycle of a positive-stranded RNA virus like HCV (4). Although the precise mechanisms underlying each step have not yet been defined for HCV, this diagram provides an overview of various processes required for viral replication. Blocking any of these steps could theoretically interfere with productive viral infection. Effective antiviral agents have been developed that block many of the analogous steps in the life cycle of other viruses. (These agents are not likely to be effective against HCV, because each virus develops unique strategies to accomplish each step in its replication cycle.)

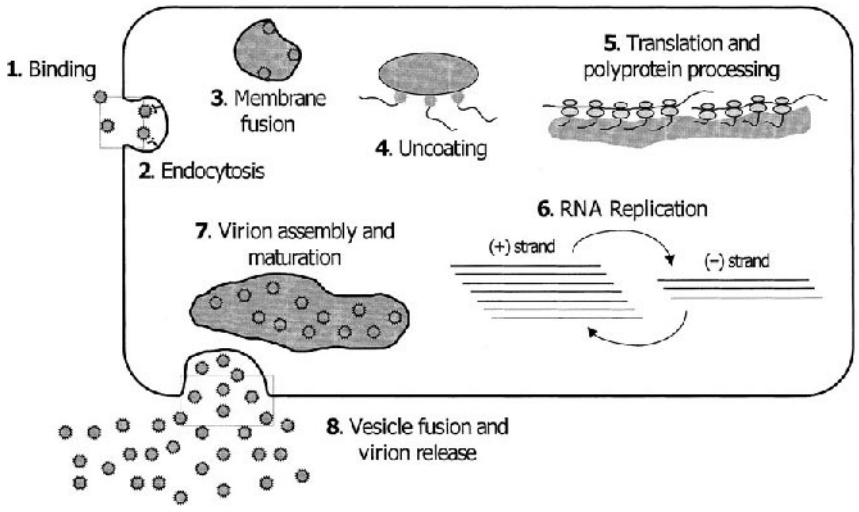

Figure 1 HCV viral life cycle.

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Unlike bacteria and parasites, viruses are unable to grow independent of host cells (primarily hepatocytes in the case of HCV). Their life cycle is intimately entwined with the biology of the infected cell, and they frequently coopt cellular processes for their own benefit. When they commandeer cellular proteins, viruses often make modifications to adapt them more closely to their purposes. In addition, viruses produce unique proteins that are encoded by their own genome that facilitates their propagation. Inhibiting the function of viral proteins and virus-specific alterations in cellular proteins are potential strategies for blocking viral replication and increasing the likelihood of virus eradication.

NEW TARGETS FOR HCV ANTIVIRAL THERAPY

Antiviral agents can be considered broadly in three categories: (a) inhibitors of viral proteins and nucleic acid (RNA in the case of HCV), (b) inhibitors of virus–host interactions necessary to support viral replication, and (c) facilitators of natural host antiviral mechanisms. Most drugs commonly considered as being specific antiviral agents (e.g., acyclovir, lamivudine, azidothymidine [AZT]) fall into the first category. To date, there have been few agents in the second category, although this is an area of intense investigation (5, 6). Interferon and ribavirin can be considered to be early members of the third category.

The effectiveness of most antiviral agents depends on their ability to block one or more steps in the viral life cycle without significantly affecting host cell physiology. Since there are important similarities between viral replication and several host cell functions, care must be taken to avoid compounds that cause toxicity by inhibiting cellular metabolism. Historically, this has been accomplished by developing compounds that directly inhibit the activity of, or even degrade, viral proteins or nucleic acids. In some cases, efforts have been

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Table 1 Potential HCV Antiviral Targets

Target	Biological function	Other properties and activities	In vitro assay	Current interest as antiviral target	Known inhibitors (candidate antiviral agents)
5′-UTR	RNA replication regulation	Conserved RNA structure; protein binding	No	Moderate	Ribozymes; antisense
IRES	Translational regulation	Conserved RNA structure; protein binding	Yes	High	Ribozymes; antisense
Translated RNA	Protein coding	Unknown	Yes	Low	Ribozymes; antisense
Core	Capsid protein	Interaction with cellular proteins	Yes	Moderate	Antisense
E1	Envelope protein	Interaction with cellular proteins	No	Low	No
E2	Envelope protein	Interaction with cellular proteins	No	Low	No

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NS2/3	Metalloprotease	Autocatalytic (cis-acting) only	Yes	Low	No
NS3	Protease	Known molecular structure	Yes	High	Peptide analogues; small molecules
NS3	RNA helicase	Known molecular structure	Yes	High	Small molecules
NS4A	NS3 protease co-factor	Known molecular structure	Yes	Moderate	No
NS4B	Unknown	Unknown	No	Low	No
NS5A	Unknown	Interaction with cellular PKR	Yes	Low	No
NS5B	RNA polymerase	Known molecular structure	Yes	High	Nucleoside analogues
3′-UTR	RNA replication regulation	Conserved RNA structure; protein binding	No	Moderate	No
Cellular PKR	Protein kinase	Interferon sensitive	Yes	Moderate	No
Cellular IMPD	Regulates cellular nucleotide pool size	Known molecular structure	Yes	High	Ribavirin, VX-497 and others

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made to develop compounds that are inert unless activated by viral enzymes. Because of the complexity of the viral life cycle and the unique characteristics of viral proteins and nucleic acids, several different molecules and processes can be considered to be targets for the development of *potent* and *selective* antiviral agents.

Therapies that interfere with specific mechanisms of the virus life cycle have been effective means of treatment for several chronic viral infections. Most notably, several nucleoside analogues ameliorate the clinical manifestations of herpesvirus infections, and combinations of protease and DNA polymerase (reverse transcriptase) inhibitors have been extremely successful at controlling the viral load and pathological manifestations of human immunodeficiency virus (HIV) infection (7).

To be effective, antiviral therapies must interfere with an essential component of the viral life cycle. For most viruses, that life cycle includes binding and uptake of the virus into the host cell, viral uncoating, synthesis of viral replicative proteins, replication of the viral genome, synthesis of viral structural proteins, viral assembly, and escape from the host cell. For HCV, which is a positive-strand RNA virus, the putative life cycle is shown in Fig. 1. In theory, interference with any step in the life cycle could block viral replication. In practice, however, some steps have been proven to be more amenable to the development of safe and effective antiviral agents. These molecular targets are the subject of this chapter. A summary is depicted in Table 1.

VIRAL ENZYMES AND OTHER PROTEINS

One of the most promising areas of HCV antiviral development is in the area of agents that interfere with virus-encoded proteins. Based on experience with herpesviruses, HIV, hepatitis B virus, and several other viruses, agents that inhibit critical steps in the viral replication cycle can be effective anti-

viral agents (7–9). By suppressing viral replication and protein synthesis, these agents can prevent or diminish the pathological effects of viral infection, including in some cases injury causes by immune responses to viral proteins. Occasionally, treatment with these replication inhibitors can lead to complete eradication of detectable virus. Although no direct inhibitors of HCV proteins are currently available for clinical use, several promising candidates have been identified through basic research efforts (5). This is an area of great excitement and activity, and we can anticipate that several agents will be available for clinical testing within the next few years.

ENZYME TARGETS

The most promising HCV enzyme targets are the viral protease, helicase, and polymerase (10). The protease and helicase are part of the same viral nonstructural protein, NS3 (Fig. 2).

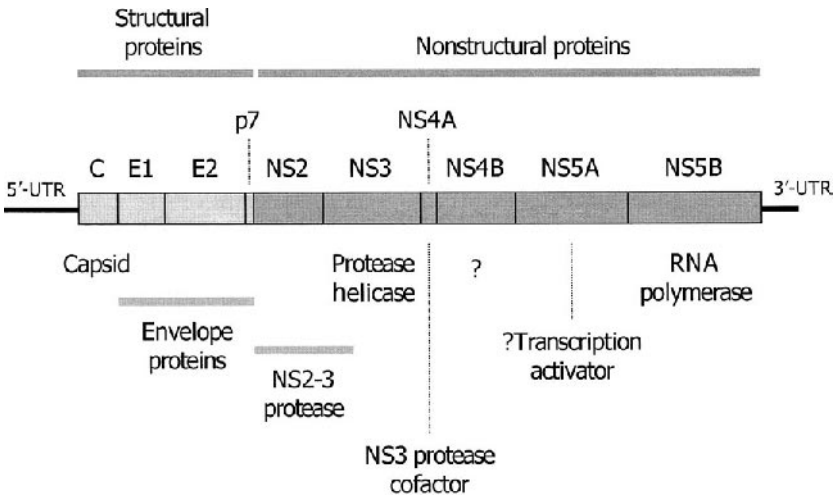

Figure 2 Proteins encoded by the HCV genome.

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Full protease activity also requires NS4, which is tightly and specifically bound to NS3. The HCV polymerase (NS5B), which is responsible for copying of the viral genome during RNA replication, is one of the most highly conserved HCV proteins, suggesting that there are severe constraints on its structure (11). Compounds that bind to and perturb this structure are likely to interfere with the biological activity of this enzyme.

Protease

There are two proteases encoded within the HCV genome (12, 13). These enzymes help to process the newly synthesized HCV polyprotein into individual proteins. This process proceeds in several steps, as shown in Fig. 3. The ribosome scans the 5′ end of the genome until it pauses on the internal ribosome entry site (IRES) and begins translation of the HCV polyprotein. A host enzyme, signal peptidase, catalyzes cleavage of the polyprotein at several sites toward the amino end.

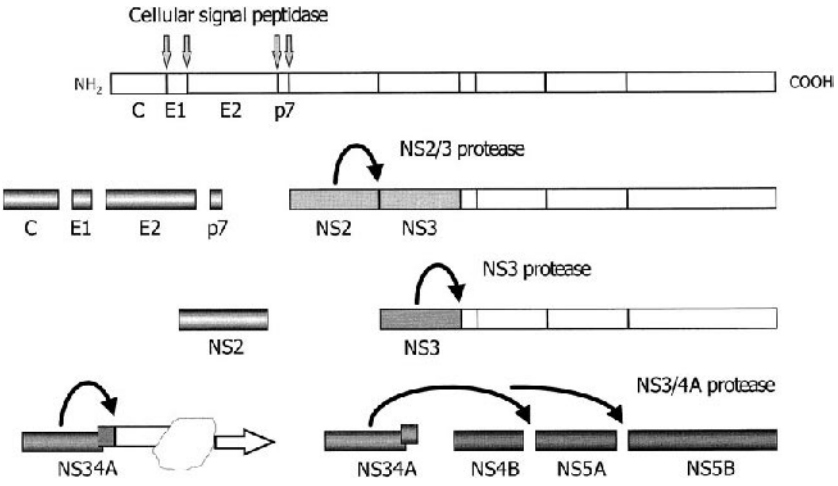

Figure 3 Scheme of HCV polyprotein processing.

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This cleavage generates the structural proteins required for RNA encapsidation, viral assembly, and budding from the infected hepatocyte (4, 14). Further processing of the nascent HCV polyprotein is catalyzed by NS2/3 protease, which cleaves NS2 from the downstream protein, and NS3 protease, which catalyzes the cleavage of NS3, NS4A, NS4B, NS5A, and NS5B from the remainder of the HCV polyprotein (14, 15). The large number of cleavage sites in the polyprotein catalyzed by NS3 suggests that this enzyme is critical for normal protein production and viral replication. NS3 protease activity is absolutely essential for the replication of several related viruses, including flaviviruses and pestiviruses. By analogy to these viruses, HCV NS3 is extremely likely to have an essential role. Definitive demonstration of this requirement, however, awaits the development of a robust system for HCV replication in vitro.

NS3 is a serine protease (serine is one of three critical amino acids in the active site of the enzyme) most similar to chymotrypsin (15) that cleaves polyproteins at specific sequences (Fig. 4). The structure of this enzyme is completely different from that of HIV protease. These differences suggest that HIV protease inhibitors would have no effect on HCV NS3 protease, a prediction that has been borne out in several studies of the HCV enzyme. As a result, development of HCV protease inhibitors has had to be started afresh. X-ray crystallography has provided detailed information about the structure of the NS3 protease (16–18). This enzyme loosely resembles an apple, with a shallow cleft at the top that contains the active site. The newly synthesized HCV polyprotein binds in this cleft, where it is cleaved at specific sites. NS3 includes a tightly bound zinc molecule that appears to stabilize its structure. This protein is also tightly bound to NS4A, which likely stabilizes the active site of the protease and increases its catalytic efficiency (17–19). Complexing with NS4A is required for NS3-catalyzed cleavage of the NS3/4A, NS4A/4B, and NS4B/5A cleavage sites, and NS4A enhances cleavage at the final, NS5A/5B, site (11).

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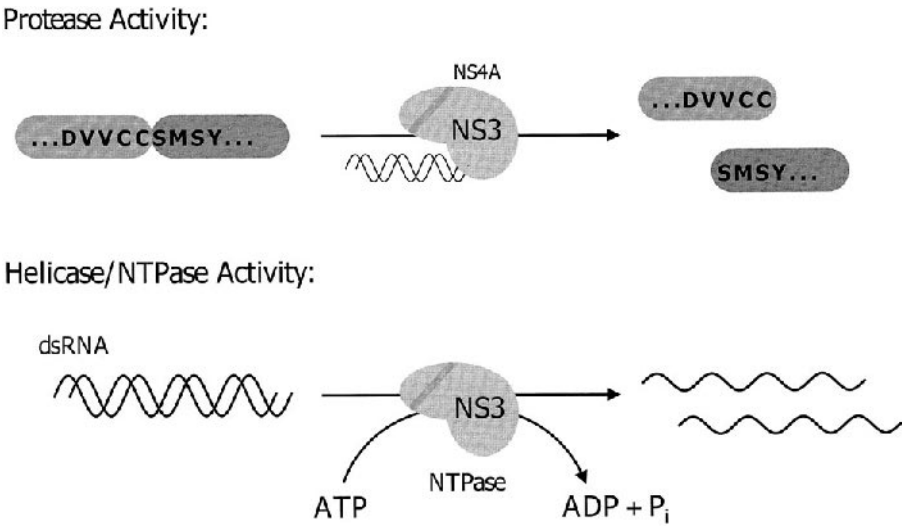

Figure 4 NS3 protease and helicase activities.

Based on these observations, several molecular targets for HCV NS3 protease inhibition have recently been identified. These include compounds that (a) physically block or inhibit activity of the substrate binding site, (b) interfere with or extrude the integral zinc atom, or (c) interfere with binding of NS4A. The most promising NS3 inhibitors identified to date belong to the first class—those that directly inhibit the active site (20–22). Several such compounds are undergoing preclinical investigation and one or two should be ready for early clinical trials within the next couple of years. Compounds have also been identified that interfere with zinc binding. They are likely to be less specific, however, with effects on other cellular zinc-containing enzymes that could generate unacceptable side effects. The tight binding of NS3 to NS4A will likely make it difficult to develop inhibitors of this interaction.

Helicase

Replication of HCV-RNA requires the unwinding of the newly synthesized strands from the template RNA (Fig. 5, panels 4–

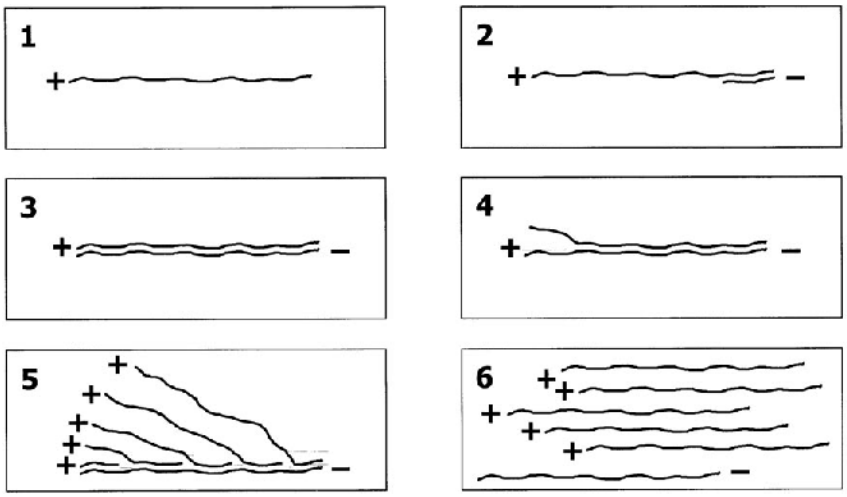

Figure 5 Scheme of HCV-RNA replication.

6). In addition, parts of the HCV genome, including the highly conserved 5′- and 3′-untranslated regions, are folded into complex structures of partial double-stranded RNA (Fig. 6). HCV helicase, a component of the NS3 protein (that also includes the protease activity described above) is an enzyme capable of

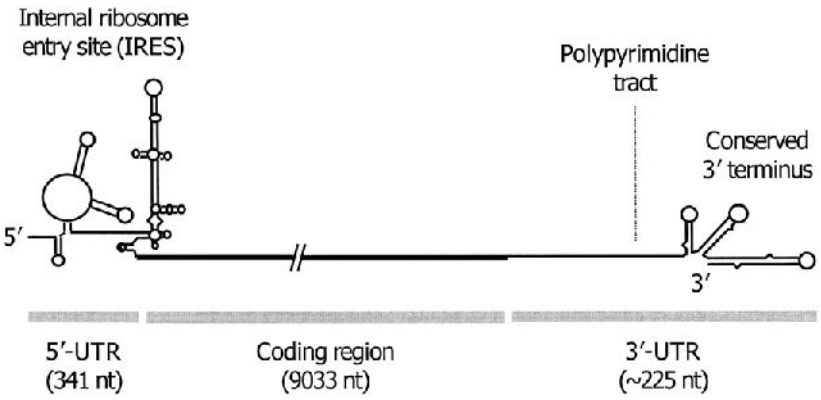

Figure 6 Structural features of the HCV RNA genome.

unwinding double-stranded RNA (see Fig. 4) (23, 24). It is likely that the NS3 helicase is essential for HCV-RNA replication. Viral helicases are essential to the replication of other RNA viruses despite the presence of several *cellular* RNA helicases. Viral helicases, including HCV NS3 helicase, must have unique qualities that make it suitable for unwinding of the viral RNA as part of the viral replication cycle. These distinguishing characteristics provide the opportunity to develop inhibitors of the viral helicase that do not block the activity of the cellular enzymes (which would likely generate unacceptable side effects).

The RNA unwinding process requires energy, and the NS3 helicase generates that energy by cleaving nucleoside triphosphates (ATP and others) into their respective diphosphates (e.g., ADP) and free phosphate. NS3 helicase thus includes a nucleoside triphosphatase (NTPase) activity, which provides an easy means of evaluating potential inhibitors of this protein (23). Thus, NS3 helicase has two important substrate binding sites, for double-stranded RNA and NTP, respectively; both of these sites are attractive targets for inhibitory compounds. X-ray crystallography has revealed the molecular structure of the NS3 helicase (25). This molecule loosely resembles a Y with the RNA binding site at the center. Its structure suggests that portions of the NS3 helicase molecule (the arms of the Y) are able to twist around each other, leading to untwisting of the two strands of the bound double-stranded RNA (18, 26). Compounds that freeze the structure of the helicase, thus preventing its unwinding action, may also be effective inhibitors of its enzymatic activity. Knowledge of the molecular structure of NS3 helicase has facilitated synthesis of several compounds that inhibit helicase and NTPase activities of this enzyme. Although these compounds are still in preclinical testing (and new ones remain to be generated), it is likely that some of them, or their structural relatives, will be suitable for clinical testing within the next few years.

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RNA Polymerase

The NS5B region of the HCV genome encodes an RNA-dependent RNA polymerase (RdRp), which catalyzes the synthesis of new strands of RNA off template HCV-RNA (see Fig. 5) (27–30). RdRp copies RNA from an RNA template, using nucleoside triphosphates (ATP, CTP, GTP, and UTP) as substrates. Normal cells do not require this activity, since cellular RNA is synthesized from a cellular DNA template by *DNA*-dependent RNA polymerases. As a result, the viral RdRp is a unique enzyme. This property makes it a logical target for antiviral development, since it should be possible to identify inhibitors of this enzyme that have no effect on normal cellular polymerases.

The molecular structure of HCV-RNA polymerase has recently been determined by x-ray crystallography (31, 32). There are several unique features of this structure, including an unusual nucleotide binding site and ringed active site, which should facilitate development of potent inhibitors. Testing of these inhibitors will be facilitated by efficient biochemical tests for polymerase activity, several of which have recently been developed (28, 33, 34). Although study of the HCV polymerase has lagged behind that of the NS3 protease and helicase, these recent developments will certainly accelerate work on this important enzyme. The effectiveness of polymerase inhibitors as antiviral agents for herpesviruses and HIV, among others, makes the NS5B RNA polymerase a particularly attractive target for HCV antiviral development.

OTHER VIRAL PROTEINS AND VIRUS-HOST PROTEIN INTERACTIONS

The HCV genome encodes several nonstructural proteins other than the protease/helicase and RNA polymerase. Protein sequences in the NS2 and NS3 regions of the viral polyprotein contribute to an NS2/3 protease that cleaves NS2

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from the downstream sequence (12, 13). The biochemical properties of this protease are distinct from those of the NS3 protease. Although it too may be a useful target for antiviral development, its limited role in viral replication and its likely action within the core of a viral protein–RNA complex may make it less accessible to potential inhibitors (35).

The roles of NS4B and NS5A in the viral life cycle are poorly understood. NS5A can bind protein kinase R (PKR), an interferon-induced, cellular protein kinase (36). Several studies have suggested that variations in the amino acid sequence of NS5A can affect HCV responsiveness to interferon therapy (37). Although the specific observations vary among investigators and studies, compounds that modify this interaction may alter viral response to interferon therapy, and thus serve indirectly as an antiviral agent. Pursuit of such therapeutic strategies remains at a very early stage.

Interference with the activities of the HCV structural proteins is also an active area of investigation. Through the work of several investigators, the HCV structural proteins have been shown to interact with several cellular proteins. The HCV core protein binds to leukotriene-β receptor and nuclear factor kappa B (NFκB), and envelope protein E2 binds PKR, the interferon-responsive protein kinase described above (38). Some antibodies to E2 appear to block viral attachment to the cell surface, suggesting that E2 has a dominant role in viral entry into the host cell (39, 40). Interference with any of these virus-host interactions may ultimately prove to be useful as antiviral therapy. At present, however, these approaches remain speculative.

VIRAL RNA AND RNA–PROTEIN INTERACTIONS

A variety of approaches are being explored to interrupt the integrity or function of the HCV genome. They include the de-

velopment of HCV-directed ribozymes, antisense oligonucleotides, and compounds to inhibit RNA–protein interactions (41–44). The 5′- and 3′-untranslated (UTR) regions of the genome are highly conserved within and among HCV genotypes, suggesting that their sequences and/or three-dimensional structures are essential to productive viral replication (4).

The 5′-UTR includes the IRES, a sequence of partially self-complementary nucleotides that assumes a complex three-dimensional structure. The IRES, which acts as a type of "landing pad" for the ribosome, is an essential regulator of the viral protein synthesis machinery. Alterations in the structure of this region have been demonstrated to block ribosome binding and/or initiation of protein synthesis (45). In other viruses that employ an IRES-dependent mechanism for protein synthesis, including poliovirus and the flaviviruses, interruption of IRES function blocks viral replication. Thus, strategies to interfere with IRES-protein interaction, or to alter the three-dimensional structure of the IRES RNA itself, are promising areas of investigation.

The 3′-UTR includes the initiation site for negative strand (replicative intermediate) RNA. This sequence is essential for viral infectivity. Recombinant HCV-RNA (so-called "infectious clones") is only infectious in chimpanzees if they contain the full 3′-UTR, including the polypyrimidine and triple stem-loop regions at the extreme 3′ end. Cellular polypyrimidine tract binding protein (PTB) binds selectively to the conserved regions of the 3′-UTR and may be involved in stabilizing the interaction between the 3′-UTR and the 5′ end of the genome (46–48). The NS5B viral RNA-dependent RNA polymerase also binds preferentially to the 3′-UTR, suggesting a possible mechanism for selective replication of HCV-RNA by this enzyme (49). Interference with specific RNA-protein interactions at either end of the viral genome may be effective in blocking viral replication. Determination of the molecular structure of these protein-RNA complexes and development of most robust systems for replication of HCV-RNA in vitro

would facilitate exploration of these intriguing antiviral targets.

Ribozymes

Ribozymes are RNA or RNA-DNA hybrid molecules with catalytic activity. Synthetic ribozymes can be designed as sequence-specific endoribonucleases, molecules that cleave other RNAs at precise sequences. Because of their sequence specificity, these ribozymes are theoretically highly selective. Moreover, a single ribozyme can cleave several target RNA molecules sequentially, enhancing the potency of this approach. Since the 5′- and 3′-UTRs of the HCV genome are highly conserved and appear critical to HCV replication, they provide attractive targets for ribozyme action. Several ribozymes have recently been developed that selectively cleave these critical regions of the HCV genome (43, 44). In preliminary experiments, when these ribozymes have been introduced into cultures of HCV-infected hepatocytes, concentrations of HCV-RNA have decreased. The long-term effectiveness of these molecules may be limited by their ability to be introduced into infected cells, their resistance to cellular nucleases, their access to viral RNA in vivo, and the ability of the viral genome to undergo repair after attack by the ribozyme. Delivery of these nucleic acids into infected cells poses a challenge not typically faced with small molecule inhibitors of viral enzymes. Unlike inhibitors of viral enzymes (e.g., proteases or polymerases), ribozymes have not yet been proven to be clinically effective inhibitors for any virus. Examination of ribozymes as potential HCV antiviral agents are at an early stage, but these offer an intriguing approach that is potentially complementary to those provided by enzyme inhibitors and immune modulators. Like other approaches, however, ribozymes must be evaluated in cell culture or animal models of viral replication that are relevant to human infection. The dearth of such models for HCV remains a serious impediment to their evaluation and development.

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Antisense Oligonucleotides

Antisense oligonucleotides are short DNA sequences that are complementary to, and therefore bind, selective regions of the HCV-RNA genome. The mechanism by which antisense oligonucleotides might block viral replication is not known. In some cases, binding of these short DNA sequences leads to degradation of the target (complementary) RNA by the action of a double-strand–specific endonuclease. In other cases, hybridization appears to block protein synthesis by inhibiting ribosome migration along the target RNA. Conceivably, well-chosen antisense oligonucleotides might even block replication of the RNA genome. Because antisense oligonucleotides act by hybridizing to the target viral RNA, their design is straightforward. The challenge lies in determining which viral sequence to bind and evaluating the ability of the oligonucleotide to interfere with viral replication. As with ribozymes, intracellular delivery of oligonucleotides and protecting them against endogenous cellular nucleases pose additional barriers. Also like ribozymes, their development as HCV antiviral agents is at a very early stage (41, 42, 50). In one provocative preliminary study, however, Shimotohno and colleagues demonstrated that antisense oligonucleotides complementary to sequences within the core protein region of the HCV genome inhibited viral replication in HCV-infected, cultured MT-2C T-cell leukemia cells (51).

MODEL SYSTEMS

Development of viral replication inhibitors requires rapid and efficient means of assessing biochemical and biological activity. Over the last several years, several biochemical (enzymatic) assays have been established for HCV NS3 protease, helicase, and RNA-dependent RNA polymerase (4, 6, 10). For most viruses, compounds that inhibit enzymatic activity are then tested in cellular and then small animal models of viral replication. Unfortunately, however, cellular models of HCV

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replication are cumbersome and exhibit limited activity. Robust animal models are currently limited to chimpanzees, which are scarce, expensive, and subject to appropriately stringent ethical controls. Several mouse models have been developed in which HCV-infected human hepatocytes are engrafted into an immunosuppressed recipient (52, 53). Although these approaches are promising, it is not yet clear how well the human hepatocyte grafts can be maintained and how effective they will be in screening potential HCV antiviral compounds. To overcome these difficulties, some investigators have begun to use a surrogate virus model. GBV-B is a virus closely related to HCV that chronically infects tamarins, which are small South American primates (54). Because of the close relationship between HCV and GBV-B, it is possible that compounds that inhibit HCV will also inhibit GBV-B replication. This model therefore allows testing of promising candidates in a small animal model before introduction into chimpanzees or humans.

Other investigators are exploring selective steps in viral replication using subgenomic HCV-RNAs, and others are developing viral chimeras, fusing parts of the HCV-RNA genome to complementary parts of the genomes of closely related pestiviruses or flaviviruses (55, 56). Unlike HCV, several flaviviruses and pestiviruses can replicate robustly in cell culture. It is hoped that some of the chimeras will also replicate in cell culture, providing a useful, cell-based assay for those biological functions of the chimeric virus contributed by the HCV-RNA. These systems are currently in early development, but work in this area has been accelerated by the development of several HCV infectious clones, recombinant HCV genomes capable of generating productive infection after in vivo injection into chimpanzees (57–59). Development of more robust, cell-based and small animal models of HCV replication are active areas of basic research that will strongly accelerate the identification and testing of new agents active against this infection.

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CHALLENGES TO THE USE OF NOVEL ANTIVIRAL THERAPIES

Development of novel therapies targeted to the HCV life cycle will undoubtedly provide important new tools in the fight against this common, chronic, and frequently debilitating disease. Nonetheless, it is likely that these specific inhibitors will pose new challenges for the clinician and clinical investigator. For many viruses, treatment with single antiviral agents leads to selection of resistant clones (9). More than 60% of patients treated with lamivudine for chronic hepatitis B infection exhibit selection of viruses with mutations in the active site of the HBV polymerase that are resistant to the drug (60). Fortunately, these mutants exhibit somewhat impaired replication and substantially reduced pathogenic effects. The situation for HIV is considerably different. Treatment with single antiviral agents (protease or reverse transcriptase inhibitors) rapidly leads to the selection of pathogenic, drug-resistant viruses (7, 9). Sequential treatment with different agents causes sequential selection of multiple resistant mutants, a process that can only be prevented by initiating treatment simultaneously with multiple agents that act at different steps in the viral replication cycle. The rapid emergence of HIV mutants during single-drug treatment likely results from the rapid spontaneous mutation rate during normal viral replication. The high degree of spontaneous mutagenesis is thought to result from multiple features of viral biology, including frequent transcription errors made by the error-prone viral reverse transcriptase, the ability of the virus to tolerate those changes without loss of replicative capacity, and the maintenance of a stable of different viral quasispecies within a single infected individual. Unfortunately, each of these characteristics of HIV appears to be equally true for HCV. As a result, it is likely that the use of new antiviral agents will rapidly select out resistant strains of HCV. It will be essential, therefore, to approach antiviral therapy for HCV much as it is done for HIV. There

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will be a need for multiple agents active against each target (with differing efficacy profiles against particular viral mutations), as well as agents active against different components of the viral replication machinery. Combination therapy will certainly be the rule, and evaluation of multiple-drug regimens will need to be done early in order to prevent sequential development of multiple-drug resistance. Since different agents will likely be produced by different companies, identification of maximally effective regimens against HCV will require more extensive collaboration among different pharmaceutical companies that has been previously evident. Overall, the rapid increases in our understanding of the mechanisms of HCV replication will provide great opportunities for new antiviral development. With careful attention to strategies that prevent or delay resistance, we should be able to avoid squandering their impact on this common disease.

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