

# Pathogenesis, Natural History, Treatment, and Prevention of Hepatitis C

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Approximately 4 million persons in the United States and probably more than 100 million persons worldwide are infected with hepatitis C virus. The virus has the unique ability to cause persistent infection in susceptible hosts after parenteral or percutaneous transmission, and its underlying mechanisms are not well understood. The immunologic correlates of protection and viral clearance and the pathogenesis of liver injury are yet to be defined, but recent studies suggest the importance of cell-mediated immune responses. Although 70% to 80% of infected persons become chronic carriers, most have relatively mild disease with slow progression. However, chronic and progressive hepatitis C carries significant morbidity and mortality and is a major cause of cirrhosis, end-stage liver disease, and liver cancer. Development of an effective hepatitis C virus vaccine is not imminent, but recent advances in technology and basic knowledge of molecular virology and immunology have engendered novel approaches to the fundamental problems encountered in vaccine development. Current therapy for hepatitis C, although effective in some patients, is problematic and still evolving. Advances in modern biology and immunology promise new therapies for this important disease.

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**D**r. T. Jake Liang (Liver Diseases Section, National Institute of Diabetes and Digestive and Kidney Diseases [NIDDK], National Institutes of Health [NIH], Bethesda, Maryland): The identification of hepatitis C virus (HCV) as the cause of non-A, non-B hepatitis represents a technical tour de force of modern molecular medicine (1). Characterization of the viral genome and the structures and functions of viral gene products has led to a better understanding of the viral life cycle and the pathogenesis of HCV-associated disease. This knowl-

edge will contribute to the development of an effective vaccine and better therapies.

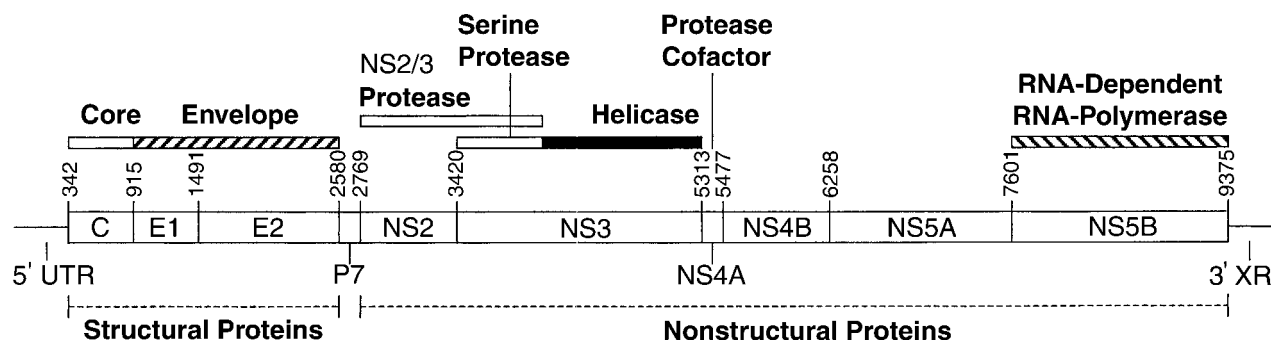
Hepatitis C virus is a member of the Flaviviridae family, which includes the flaviviruses and pestiviruses (2). There are at least 6 HCV genotypes and more than 50 subtypes. The virion contains a positive single-stranded RNA genome of 9.5 kilobases (**Figure 1**). The genome consists of 5' and 3' untranslated regions (5'UTR and 3'XR) that have little sequence variation among all genotypes and are important for translation of viral proteins and replication of the virus. The viral genome encodes a large single polyprotein of about 3000 amino acids; the N-terminal one third harbors the structural proteins, and the C-terminal two thirds contains the nonstructural proteins. The HCV structural proteins comprise the core protein and the two envelope glycoproteins E1 and E2. The nonstructural proteins, including proteases (NS2/3 and NS3), helicase (an enzyme that unwinds double-stranded nucleic acid) (NS3), and RNA-dependent RNA polymerase (NS5B), perform various functions essential for the viral life cycle (**Table 1**). Cleavage of structural proteins from the polyprotein is catalyzed by a host signal peptidase, whereas polyprotein cleavage in the nonstructural region requires HCV-encoded proteases.

Hepatitis C virus enters a susceptible host either directly, through needle inoculation or transfusion of contaminated blood products, or inadvertently, through breakage of a percutaneous barrier (as exemplified by sexual or perinatal transmission) (3). The virus then enters hepatocytes or other susceptible cells, probably through a unique surface molecule or molecules, as the viral receptor (4). After uptake, the virus uncoats and releases the genome to begin replication. The viral genome first serves as the template for translation of the polyprotein. The processed nonstructural proteins then form a complex with the genome and initiate synthesis of the negative strand, which in turn functions as the template for positive strand synthesis. The replication complex probably resides in a membranous compartment in the cytoplasm, presumably derived from the endoplasmic reticulum. The RNA replicative

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**Figure 1. Schematic diagram of the hepatitis C virus genome.** The location of HCV genes, proposed functions of gene products, and the 5' and 3' untranslated regions (5'UTR and 3'XR) are shown. Numbering refers to nucleotide positions of genes.

intermediate matures and interacts with the core and envelope proteins to assemble into a virion. Although most of the replicative processes have not been defined, some nonstructural proteins clearly play critical roles in viral replication and productive infection; these proteins are therefore the focus of antiviral development.

## Immunopathogenesis of Hepatitis C

Dr. Barbara Rehermann (Liver Diseases Section, NIDDK, NIH): Unlike other hepatitis viruses, the hepatitis C virus is more likely to cause clinically inapparent, chronic infection in persons who are otherwise considered immunocompetent. Thus, the virus is capable of circumventing an efficient immune response of the host.

### Components of Antiviral Immune Response

The mechanisms whereby HCV circumvents immune response and establishes persistent infection are currently undefined. It is well known that the specific immune response to any viral infection is primed by macrophages and dendritic cells that present viral proteins to B cells, helper T cells, and cytotoxic T cells (Figure 2). In many viral infections, B cells produce antibodies that can clear circulating virus and protect from reinfection. For example, antibodies against the hepatitis B virus surface antigen are critical for viral clearance. Through specific T-cell receptors on the cell surface, helper T cells recognize viral peptides that are derived from phagocytosed and proteolytically cleaved HCV proteins and are presented in the context of class II MHC molecules.

On activation of their specific T-cell receptors, HCV-specific helper T cells assist with activation and differentiation of B cells as well as induction (5) and stimulation of virus-specific cytotoxic T cells. Most of these effects are mediated by different sets of immunoregulatory Th1 (interferon- $\gamma$  and interleukin-2) or Th2 (interleukin-4, interleukin-5, and

interleukin-10) cytokines. In the context of class I MHC molecules, CD8-positive cytotoxic T cells recognize HCV peptides that are synthesized and processed in infected cells (Figure 2). This encounter can lead to lysis of virus-infected cells. Together with helper T cells, cytotoxic T lymphocytes may also secrete cytokines, such as interferon- $\gamma$  and tumor necrosis factor- $\alpha$ , that inhibit replication and gene expression of several viruses, such as hepatitis B virus, cytomegalovirus, and rotavirus (6–8).

### Humoral Immune Response

Hepatitis C virus can establish persistent infection despite an active humoral and cellular immune response that is generally targeted against all viral proteins. The virus may escape from the humoral immune response if the kinetics of infection and viral replication do not allow complete neutralization of the virus by HCV-specific antibodies after primary infection. Although virus-specific antibodies may interfere with viral entry into host cells and opsonize the virus for elimination by macrophages, they cannot eliminate HCV from infected cells. In

**Table 1. Functions of Genetic Elements of Hepatitis C Virus\***

Genetic Element	Function
Regulatory sequences	
5'UTR	Internal ribosomal entry site for translation; replication
3'XR	Translation and replication
Viral proteins	
Core	Nucleocapsid; assembly
E1 and E2	Envelope proteins; assembly and entry
p7	Assembly†
NS2	NS2-3 protease
NS3	Serine protease, nucleotide triphosphatase, and RNA helicase
NS4A	Cofactor for NS3 protease activity; replication†
NS4B	Replication†
NS5A	Phosphoprotein; replication† and interferon sensitivity sequence‡
NS5B	RNA-dependent RNA polymerase

\* 3'XR = 3' untranslated region; 5'UTR = 5' untranslated region.

† A proposed function.

‡ This idea is controversial.

addition, HCV has a high mutation rate, especially in the hypervariable region of the envelope proteins that can be recognized by neutralizing antibodies (antibodies that can bind and eliminate virus) (9, 10). Several studies have demonstrated that the humoral immune response can select HCV variants with sequence changes that allow escape from antibody recognition (11–14). However, recent studies in chimpanzees have suggested that HCV can cause persistent infection in the absence of mutations in the hypervariable region (15, 16). Thus, progression to persistent HCV infection is most likely a multifactorial process that depends on multiple aspects of virus–host interaction.

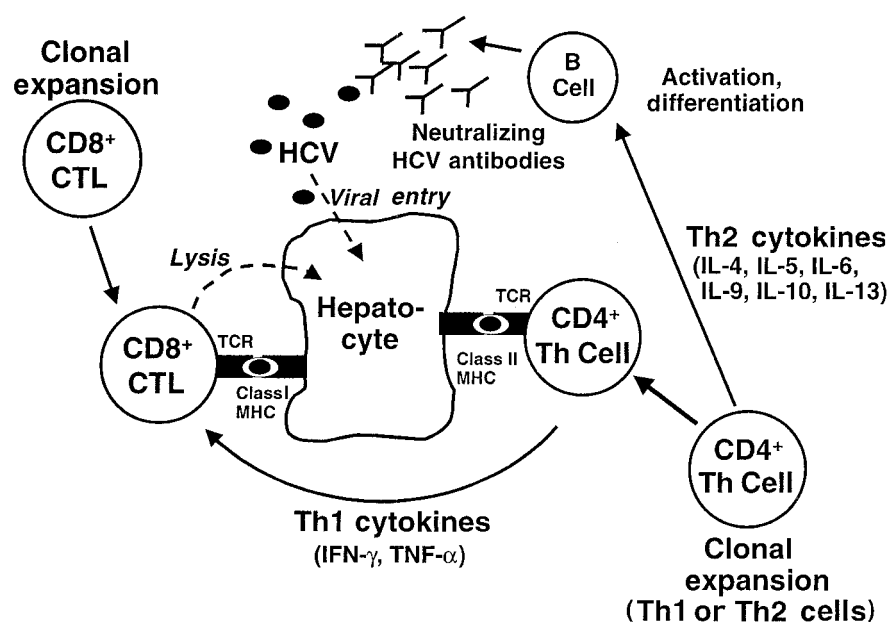
### Cellular Immune Response

The cellular immune response probably plays an important role in the outcome of HCV infection because of its ability to recognize and eliminate virus from infected cells. Most studies have concentrated on the antigen-specific immune response that is mediated by CD4-positive helper T cells and CD8-positive cytotoxic T cells.

Because chronic rather than acute infection is diagnosed in most patients, immunologic studies have been performed on patients with persistent infection who could not clear HCV. Only a few studies have analyzed the cellular immune response during the acute phase of infection. These studies suggest that the strength and quality of both helper T-cell (17, 18) and cytotoxic T-cell responses (19) differ between patients who recover and those who develop chronic infection. More important, the viral

sequences that are recognized most frequently and vigorously by HCV-specific T cells vary little among all the HCV genotypes. Furthermore, several of these frequently recognized viral peptides bind with high affinity to many different class II MHC molecules, suggesting that they can be efficiently presented and recognized by patients with different MHC haplotypes (17, 20). Thus, these viral sequences could be explored for development of preventive or therapeutic vaccines against HCV.

The cellular response against HCV could be interfered with in several ways. First, HCV elicits only a weak T-cell response in patients who develop chronic infection (17, 21). In the blood of patients with chronic hepatitis C, the frequency of cytotoxic T-cell precursors that are specific for individual HCV peptides is much lower than the frequency of T cells that recognize an influenza virus peptide as a recall antigen (22) or peptides of other viruses that can be cleared, such as cytomegalovirus (23). The reasons for this relative weakness of the cellular immune response are not known. Certainly, general immune tolerance or immunosuppression is not the cause of persistent HCV infection, because most chronically infected patients display normal immune responses against other viral agents (22). The emergence of viral mutants or quasi-species with sequence variations in T-cell epitopes may contribute to the apparent ineffectiveness of cell-mediated immune response (24–26). There is also increasing evidence that several HCV proteins, such as core (27), E2 (28), and NS5A (29), interfere with the immune response. Furthermore, infected hepato-



**Figure 2. Components of the antiviral immune response.** Although the hepatocyte is depicted as the target cell of hepatitis C virus (HCV)-specific immune response here, other cells, including dendritic cells and macrophages, are also important in antigen presentation to the immune system. CTL = cytotoxic T cell; IL = interleukin; MHC = major histocompatibility complex; TCR = T-cell receptor; Th = T helper; Th1 = helper T cells with a type 1 cytokine profile; Th2 = helper T cells with a type 2 cytokine profile; TNF = tumor necrosis factor.

cytes, which lack co-stimulatory molecules, may be relatively inefficient in priming the immune system, and the liver has been proposed as the major site where activated T cells are destroyed (30). Finally, the cellular immune response is a double-edged sword. An immune response that is ineffective in clearing HCV infection may be more harmful to the liver, causing chronic inflammation, hepatocellular injury, and, over several decades, liver fibrosis and cirrhosis.

Progression to persistent infection and the immunologic mechanisms of liver injury are the consequence of complicated interactions between the virus and host. Identification of immunologic correlates of viral clearance may contribute to the development of an effective vaccine and better therapy for HCV infection.

### **Natural History of Hepatitis C**

Dr. Leonard B. Seeff (Division of Digestive Diseases and Nutrition, NIDDK, NIH): The natural history of hepatitis C continues to be a controversial issue because of the lack of clarification of long-term outcome. Although it is widely accepted that approximately 80% of persons who become infected fail to clear the virus and progress to chronic infection, the uncertain extended outcome has prompted divergent views. Clearly, some infected persons recover completely; some remain HCV viremic without biochemical evidence of liver damage; some seem to have a static form of chronic hepatitis characterized by persistently elevated aminotransferase levels without overt symptoms or disease advancement; some progress over a difficult-to-define period to histologic fibrosis and cirrhosis; some have long-term stable cirrhosis identified only through liver biopsy; some have progressive cirrhosis that culminates in liver failure; and, finally, some develop hepatocellular carcinoma. The uncertainties lie in the relative frequencies and rates of development of these various sequelae. Indeed, the major questions are whether progression is linear and whether advancement through these increasingly severe manifestations is inevitable (31).

The only means of accurately defining outcome is to conduct well-designed, long-term, prospective studies that begin with onset of acute HCV infection and follow participants over a sufficiently extended period. Such studies must use careful clinical, biochemical, serologic, and histologic assessment. However, there are numerous impediments to accomplishing these aims. Onset of acute hepatitis C is rarely recognized owing to lack of symptoms; chronic HCV infection similarly is generally a silent condition; and the course of the disease is often

markedly protracted, spanning 20 to 40 years before the final outcome is reached. Moreover, the circumstance of exposure (for example, transfusions, parenteral drug use, hemophilia, or hemodialysis) may itself be associated with reduced life expectancy, thus competing with HCV for morbidity and mortality.

Strategies used to examine this issue have included prospective transfusion-related studies that begin with acute hepatitis C; retrospective studies that prospectively track persons with established chronic hepatitis C; and a combination of the two—retrospective-prospective (nonconcurrent prospective) studies—that requires identification of a definitive acute hepatitis outbreak in the past, with subsequent patient recall followed by long-term prospective evaluation. The prospective study approach describes the natural history of acute hepatitis C, whereas the retrospective study delineates the natural history of chronic hepatitis C. Obstacles to the first approach include the difficulty in identifying a large study cohort with acute hepatitis C and the time required to complete follow-up. The second approach introduces the problem of selection bias—namely, the focus on patients with already established chronic liver disease and the omission from analysis of persons who were infected earlier but who spontaneously recovered or were not ill enough to consult a physician.

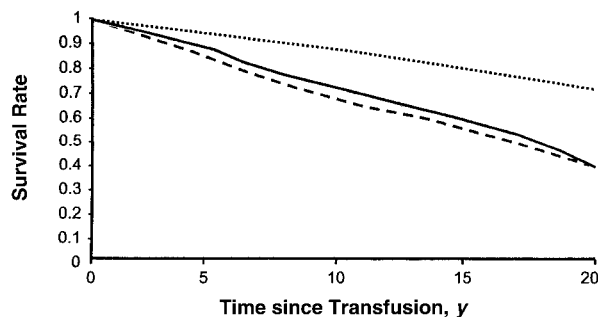
### **Prospective Studies of Acute Hepatitis C**

Early studies focused on persons with transfusion-associated hepatitis C (32–36). None of the studies had more than 14 years of follow-up, and none included a noninfected control group. Composite analysis of these studies reveals that clinical symptoms were identified in approximately 10% of patients, cirrhosis was found in about 20% (range, 8% to 24%), and hepatocellular carcinoma was rare. Liver disease appeared to be responsible for death in approximately 3% of patients (range, 1.6% to 6.0%). These studies clearly identified liver-related morbidity and mortality but in generally modest frequencies. Their limitations, however, were the relatively small numbers of patients included in each study and the short follow-up.

### **Retrospective Studies of Chronic Hepatitis**

In three important studies (37–39), a far bleaker picture emerged. Despite the relatively short follow-up (4 to 11 years), these studies reported symptoms in far greater frequency, cirrhosis in a higher proportion of patients (30% to 46%), a remarkably high frequency of hepatocellular carcinoma (11% to 19% of patients), and a significantly high rate of liver-related death. Of note, a considerable number of the patients in the U.S. study already had end-





**Figure 3.** Survival curves comparing patients with non-A, non-B transfusion-associated, predominantly type C hepatitis (solid line); matched transfusion recipients without hepatitis (controls) (dashed line); and the general U.S. population (dotted line). Survival of 100% is shown as 1 on the y axis.

stage cirrhosis or hepatocellular carcinoma when they were first seen (39). The impact of these results must, however, be tempered by the fact that they represent the worst-case scenario by focusing only on persons with already well-established chronic liver disease. Despite this “referral bias,” the accrued data nevertheless underscore how serious the condition is once cirrhosis develops.

#### Retrospective–Prospective Studies

Three studies can be classified as retrospective–prospective studies. The first, an ongoing study from Ireland, involved an outbreak of acute hepatitis C in more than 50 000 young women who had received HCV-contaminated anti-D immunoglobulin (40). In a follow-up report 17 years later, three quarters of the women were symptomatic, mainly with fatigue; serum enzyme values were normal in more than 40% of patients; and, most important, liver biopsies revealed fibrosis, which was mostly mild in 51% but represented cirrhosis in 2%. This surprising result has been attributed to the fact that these were young, healthy, nondrinking women, who seem to be at less risk than older, alcohol-imbibing men (31).

The second study followed persons from several early transfusion-related studies in whom acute hepatitis C had developed (41). Patients with acute hepatitis in these studies were combined and compared with a matched group of controls who did not undergo transfusion and did not have cirrhosis so that morbidity and mortality could be studied long-term. Analysis at 18 years (41) and 23 years (42) revealed no difference between the two cohorts with respect to overall mortality but showed a slight and slowly increasing difference in liver-related mortality (Figure 3). Follow-up for morbidity in living patients revealed that one fourth seemed to have had spontaneous resolution; in the remainder, viremia persisted, and only half of these patients had accompanying aminotransferase elevations. Liver biopsies revealed cirrhosis in 15% of patients; clinical symptoms were confined almost exclusively to pa-

tients with cirrhosis. These data are in accord with a recent report from Germany showing that mortality is increased in persons with chronic hepatitis C only if they have cirrhosis (43).

The final study is an almost 50-year follow-up of young military recruits on a U.S. Air Force base in Wyoming, from whom blood samples were obtained between 1948 and 1954 as part of a study of a streptococcal infection outbreak (44). Among the few persons found to be infected with HCV, less than 20% have died or have liver disease.

Taken together, these data suggest that approximately 15% to 20% of persons who acquire HCV infection progress to potentially serious end-stage liver disease, the critical sequela being cirrhosis. The remainder are likely to die of causes other than liver disease.

### Therapy for Hepatitis C

Dr. Jay H. Hoofnagle (Division of Digestive Diseases and Nutrition and Liver Diseases Section, NIDDK, NIH): The current recommendations for treatment of hepatitis C are based mainly on the NIH Consensus Development Conference Panel on Management of Hepatitis C that was formulated in March 1997 (45). Although the recommendations for therapy remain valid, the optimal regimen now requires modification.

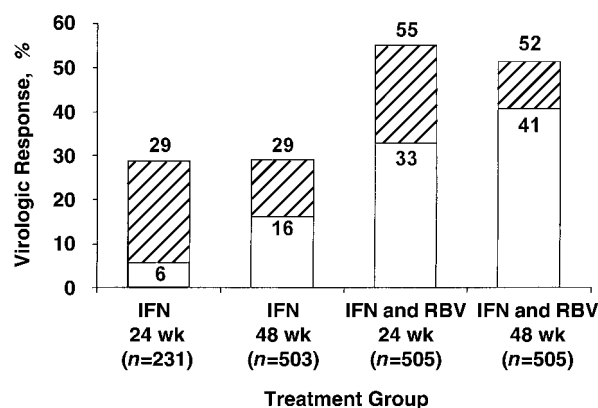
#### Indications for Therapy

Therapy for hepatitis C is clearly indicated in patients 18 to 60 years of age who have persistently abnormal alanine aminotransferase levels, HCV RNA in serum, and evidence on liver biopsy of chronic hepatitis with either fibrosis or moderate degrees of inflammatory activity (45). Patients with decompensated cirrhosis and those with persistently normal aminotransferase levels and mild hepatitis should not be treated outside of clinical trials.

#### Optimal Therapeutic Regimen

The NIH Consensus Panel stated that the optimal therapeutic regimen for hepatitis C was interferon- $\alpha$  given subcutaneously in a dose of 3 million U three times weekly for 12 months with assessment of aminotransferase levels and HCV RNA at 3 months to allow early discontinuation in patients who do not respond (45). Two years later, these recommendations clearly require modification in response to results of studies on combination therapy with interferon- $\alpha$  and ribavirin.

Ribavirin is an oral nucleoside analogue with a broad spectrum of activity against both RNA and DNA viruses. When used alone as therapy for hepatitis C, ribavirin decreases aminotransferase levels



**Figure 4.** Rates of virologic response in patients with chronic hepatitis C treated with interferon- $\alpha$  for 24 weeks (IFN 24 wk) or 48 weeks (IFN 48 wk) or interferon- $\alpha$  plus ribavirin for 24 weeks (IFN and RBV 24 wk) or 48 weeks (IFN and RBV 48 wk). The total height of the bars represents the end-of-treatment response rate; the white portion represents the sustained response rate. Data are shown for 1744 patients (the number in each treatment group is given in parentheses) from two large prospective clinical trials (49, 51). Numbers at the top of the bars represent exact percentages.

and improves hepatic histologic findings in 30% to 50% of patients (46–48). However, HCV RNA levels do not decrease, and relapses occur in almost all patients soon after treatment is stopped.

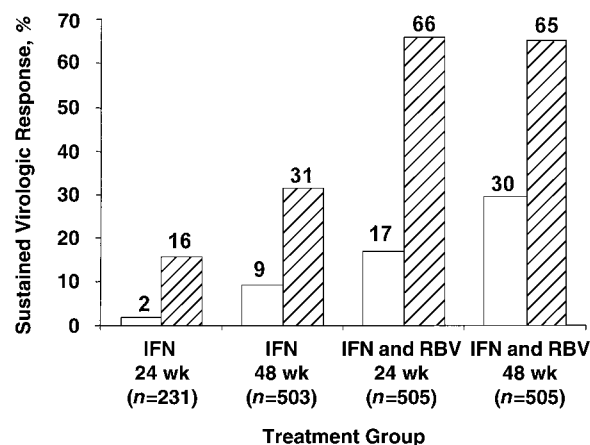
Results of three multicenter randomized, controlled trials comparing combination therapy with interferon and ribavirin with interferon- $\alpha$  alone were recently published (49–51). The similarity of design, monitoring, end points, and analysis of these trials allows presentation of combined results. A total of 1744 previously untreated patients were enrolled in two large trials (49, 51). Patients were randomly assigned to receive interferon- $\alpha$  alone or combination therapy for 24 or 48 weeks. The primary end point was lack of detectable HCV RNA in serum 6 months after stopping therapy (sustained virologic response). Biochemical (normal aminotransferase levels) and histologic responses (improvements in liver histologic findings) were also analyzed.

Combined results of these two studies are shown in **Figure 4**. In patients given interferon alone, the end-of-treatment response rates were the same after both 24 and 48 weeks (29%), but rates of sustained response were higher with the longer course of therapy. This supports the previous recommendation that interferon as monotherapy should be given for 12 rather than 6 months (45, 52). Both the end-of-treatment and sustained response rates were higher with combination therapy than with interferon alone. Most important, rates of sustained response were higher with combination therapy, and 48 weeks of treatment (41%) was superior to 24 weeks (33%). Similar differences were reported for biochemical responses and histologic improvement 6 months after stopping therapy.

Despite the impressive results obtained with combination therapy, the real issue is whether patients in whom sustained virologic response is achieved are likely to have relapse months or years later. Insufficient time has elapsed to evaluate the durability of virologic responses after combination therapy, but long-term follow-up after treatment with interferon alone indicates that most patients who fulfill the criteria for a sustained virologic response remain negative for HCV RNA, have normal serum aminotransferase levels, and have no symptoms of liver disease 5 to 12 years after treatment (53, 54).

### Factors That Predict Response to Therapy

Retrospective analyses of the two studies for features that produced a response have identified several factors important for recommending therapy (49, 51). The host factors of young age, female sex, and lesser degrees of fibrosis on liver biopsy correlated with a greater likelihood of a sustained response. Even more significant were the viral features of genotype and HCV RNA level. The sustained response rate among patients with genotypes 2 and 3 was twice as high as that among patients with genotype 1 (the number of patients with genotypes 4, 5, and 6 were too few to analyze separately) (**Figure 5**). In patients with genotype 2 or 3, a 24-week course was as effective as a 48-week course of combination therapy; two thirds of these patients had a sustained response. In contrast, the response rate among patients with genotype 1 was significantly higher with 48 weeks of therapy than with 24 weeks (30% compared with 17%).



**Figure 5.** Rates of sustained virologic response in patients with chronic hepatitis C treated with interferon- $\alpha$  for 24 weeks (IFN 24 wk) or 48 weeks (IFN 48 wk) or interferon- $\alpha$  plus ribavirin for 24 weeks (IFN and RBV 24 wk) or 48 weeks (IFN and RBV 48 wk), by viral genotype. The white bars represent patients with genotype 1 and the few patients with genotypes 4, 5, and 6; the striped bars represent patients with genotypes 2 and 3. Data are shown for 1744 patients (the number in each treatment group is given in parentheses) from two large prospective clinical trials (49, 51). Numbers at the top of the bars represent exact percentages.

**Table 2. Major Side Effects of Combination Therapy with Interferon- $\alpha$  and Ribavirin**

Common side effects usually caused by interferon- $\alpha$   
Fatigue, malaise, myalgias, headache, poor appetite  
Depression, irritability, anxiety, emotional lability  
Difficulty concentrating, forgetfulness, sleeplessness  
Bone marrow suppression, thrombocytopenia, neutropenia

Common side effects usually caused by ribavirin  
Hemolysis, hemoglobin decrease of 20 to 40 g/L  
Gastrointestinal upset  
Nasal congestion, sore throat, cough, dyspnea  
Pruritus, skin rash

Uncommon, serious side effects  
Bacterial infections  
Induction of autoantibodies and autoimmune disease  
Severe depression, psychosis, disorientation, suicide  
Relapse in alcohol or substance abuse  
Seizures  
Vision or hearing loss, tinnitus  
Acute renal or heart failure  
Fetal loss or fetal abnormalities

In both studies, patients with higher initial HCV RNA levels ( $\geq 2$  million copies/mL) had a lower response rate than those with lower levels, independent of genotype. Among patients with genotype 1 and low levels of HCV RNA ( $< 2$  million copies/mL), the response rate for a 24-week course of combination therapy was the same as that for a 48-week course (32% and 33%). Nevertheless, the variability of quantitative assays for HCV RNA and spontaneous fluctuations in levels over time make it difficult to use this factor to recommend therapy or its duration.

In both studies, all patients with a sustained response to combination therapy became negative for HCV RNA before 24 weeks. These findings suggest that patients who remain positive for HCV RNA after 24 weeks of treatment are unlikely to benefit from further therapy and that the “3-month stop-rule” of interferon monotherapy should be replaced by a “6-month stop-rule” when combination therapy is being used.

### Side Effects

The side effects of combination therapy include those of both interferon and ribavirin (Table 2). Ribavirin causes dose-related hemolysis and anemia, and prolonged treatment can cause pruritus, nasal congestion, and cough. Most side effects are mild to moderate, transient, and reversible; they can be managed with counseling, dosage adjustment, or specific treatment.

Severe side effects are more frequent with combination therapy. In two recent trials, dose reductions were required in 13% of patients receiving interferon alone compared with 17% of those receiving combination therapy for 48 weeks (49, 51). Similarly, early discontinuation of 48-week therapy was required in 8% of interferon recipients com-

pared with 20% of patients receiving combination therapy. Furthermore, ribavirin is known to cause fetal abnormalities and should not be used in patients (both men and women) who cannot practice adequate birth control during therapy and for at least 6 months thereafter.

Contraindications to combination therapy include hemolysis, anemia, bone marrow suppression, coronary and cerebrovascular disease, neuropsychiatric conditions, active alcohol or substance abuse, renal insufficiency or transplantation, and autoimmune disease. In some of these situations, interferon monotherapy can be used instead.

### Special Patients and Populations

Most therapeutic trials have focused on typical patients with chronic hepatitis C. Scant information is available for patient groups who do not fit the standard profile, including children; elderly persons; patients with acute hepatitis C who have normal aminotransferase levels, severe extrahepatic manifestations, HIV co-infection, active alcohol or substance abuse, or renal disease; patients who have had solid organ transplantation; patients receiving cancer chemotherapy or immunosuppressive agents; and patients in prisons or public institutions.

The role of combination therapy for patients previously treated with interferon- $\alpha$  is also not resolved. In a study of 24 weeks of re-treatment in 345 patients who had had relapse, the sustained response rate was 49% for combination therapy compared with only 5% for interferon re-treatment (50). These findings support use of combination therapy in patients who have had relapse, but they did not address whether a 48-week course might be preferable to a 24-week course, especially among patients with genotype 1 or high levels of HCV RNA.

Recommendations for patients who do not respond to interferon are also difficult. True non-responders have a low rate of response to combination therapy, but some sustained responses have been reported (55).

The major challenge is how to increase the rate of sustained response to antiviral therapy. Approaches being evaluated include use of different types of interferon (56), daily interferon dosing (57), higher doses and induction regimens (57), and long-acting pegylated interferons (58). Long-term, continuous interferon or ribavirin therapy is an option, particularly in patients with extrahepatic manifestations, those with marked fibrosis on liver biopsy, or those who are at high risk for hepatocellular carcinoma (59–63).

The current recommendations for treatment are summarized in Table 3. The greatest need in hepatitis C therapy is better and safer antiviral agents.

**Table 3. Current Recommendations for Treatment of Hepatitis C**

Course Dosage	24 to 48 weeks of interferon- $\alpha$ and ribavirin. Interferon, 3 million U three times weekly, and ribavirin, 1000 mg/d, for patients who weigh less than 75 kg (165 lb) or ribavirin, 1200 mg/d, for those who weigh 75 kg or more.
Monitoring	Regular visits to assess symptoms, blood counts, and serum aminotransferase levels; after 24 weeks, patients should be tested for HCV RNA by using a sensitive method.
Decision point	Therapy should be discontinued at 24 weeks for patients with genotype 2 or 3. In patients with genotype 1, therapy should be stopped if HCV RNA test results are positive and should be continued for a full 48 weeks if HCV RNA test results are negative at 24 weeks.
Follow-up	Serum aminotransferase levels should be monitored and testing for HCV RNA should be done at least 6 months after stopping therapy.

Advances in knowledge of the genomic structure, structural biology, viral life cycle, and replicative strategy of HCV will aid in the development of potent antiviral agents. Particularly attractive targets for therapy are the enzymatic activities of the non-structural polypeptides, including the helicase, protease, and RNA polymerase.

### Vaccine Development

Dr. T. Jake Liang (Liver Diseases Section, NIDDK, NIH): Because HCV infection has major public health implications, the development of an effective vaccine is of paramount importance. However, such an effort is not without daunting challenges. First, the virus exists as a quasi-species because of a high rate of mutation in the hypervariable region of the envelope proteins (9, 10). Second, the hypervariable region is a major site of anti-envelope antibody response and contains a principal neutralization epitope (11, 13, 64). Third, antibody responses to the envelope proteins develop slowly and achieve only modest titers during primary infection (65). Consequently, neutralizing antibodies may emerge too late to prevent chronic infection. In addition, anti-envelope antibodies tend to be short-lived, disappearing gradually after viral clearance (65). Fourth, immunologic correlates of protection and disease progression have not been clearly defined. These problems are further complicated by a lack of a convenient infectious tissue culture system for testing neutralizing antibodies or passage of attenuated viral strains. In addition, the only infectious animal model is the chimpanzee, an endangered species that is difficult to study; in addition, the course of HCV infection in the chimpanzee is not necessarily representative of that in humans (66). Experiments have demonstrated that challenge of apparently recovered chimpanzees with a homolo-

gous or heterologous strain of HCV resulted in reinfection, suggesting an absence of protective immunity after natural infection (14, 67).

On the more optimistic side, infected persons can develop neutralizing antibodies (12), and 15% to 25% of infected humans and 60% to 70% of infected chimpanzees ultimately recover from HCV infection (66, 68). In addition, recent studies show that a vigorous multispecific cellular immune response is implicated in viral clearance (17, 18, 69). This response involves both helper and cytotoxic T lymphocytes, particularly of the Th1 type.

The ideal HCV vaccine should elicit high-titer, long-lasting, and broadly directed anti-envelope antibodies that recognize conserved epitopes and neutralize against all HCV isolates. The vaccine should also be capable of inducing a vigorous, multispecific cellular immune response that includes both helper and cytotoxic T lymphocytes. In particular, conserved T-cell epitopes in the core, NS3, and NS4 regions should be targeted. Finally, because the Th1 response is important in viral clearance, a vaccine candidate should direct a predominantly Th1 response.

Several approaches have been used to develop an HCV vaccine. The classic approach of developing live attenuated viral strain is hindered by the lack of convenient experimental systems. The initial effort by Choo and coworkers (70) was directed toward generating recombinant HCV envelope proteins as a subunit-based vaccine, but success was limited (71). Immunization of chimpanzees with the subunit vaccine resulted in partial and transient protection against low-dose challenge of a homologous, but not heterologous, strain. Genetic vaccination has engendered enthusiasm and holds great promise for induction of broadly directed humoral and cellular immune response (72); however, preliminary experiments in chimpanzees demonstrated that DNA immunization with HCV gene constructs may not be particularly immunogenic. Furthermore, the development of chimeric viruses expressing HCV gene products is attractive, but safety and regulatory issues may surface with implementation (73). An alternative approach relies on the synthesis and production of viruslike particles (74). In contrast to the recombinant subunit-based vaccine, the structural proteins of HCV-like particles are presented in a native, virion-like conformation and may therefore be superior in eliciting a protective immune response. In addition, HCV-like particles, as a particular antigen, may elicit a cytotoxic T-cell response (75–77), which plays a critical role in viral clearance.

Given the complexity of immune responses against HCV infection and the lack of convenient experimental model systems, completely elucidating the variables and correlates of protective immunity,



viral clearance, and disease progression will be challenging. Nevertheless, development of an effective HCV vaccine requires a thorough understanding of these issues. The final product may have to incorporate multiple components that target various aspects of protective immunity. Finally, attainment of sterilizing immunity may not be necessary as long as the vaccine-induced immunity is effective in preventing chronic infection.

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