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Including Expanded Liver Transplant Section

SCHIFF'S DISEASES OF THE LIVER

TENTH EDITION

VOLUME ONE

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CHAPTER 30

Hepatitis C

GARY L. DAVIS

KEY CONCEPTS

- Hepatitis C virus (HCV) is a single-stranded ribonucleic acid (RNA) virus that replicates at a rapid rate but lacks proofreading ability. As a consequence, it has a high degree of genetic diversity that has led to evolution into several distinct viral genotypes. This genetic diversity affects the biology of the virus, in particular its susceptibility to interferon-based therapy. It may also be an important factor, along with signaling interference and effector modulation, in allowing the virus to evade elimination by the host immune response.
- The serologic test (anti-HCV) is sensitive for diagnosing HCV infection. Molecular tests that measure HCV RNA levels are extremely sensitive and are helpful in confirming infection and managing treatment.
- The incidence of acute HCV infection has fallen dramatically in the United States. The major risk factor for infection remains intravenous drug use.
- Chronic infection develops in 50% to 90% of acutely infected persons; it occurs less commonly in the young. Despite the declining incidence of HCV, the prevalence remains high (3 to 4 million persons) because of this high chronicity rate.
- Both acute and chronic HCV infections are usually asymptomatic. Chronic hepatitis C is a slowly progressive disease but results in significant disease morbidity in only a minority of infected persons. However, because HCV infection is so highly prevalent, chronic hepatitis C is among the most common causes of chronic liver disease in the United States and the leading indication for liver transplantation.
- There is no effective pre- or postexposure prophylaxis for HCV infection. Interferon-based regimens are the only effective treatment for patients with acute or chronic hepatitis, although many patients with acute infection recover spontaneously. Sustained loss of virus is now achievable in more than 50% of patients with chronic hepatitis C who are treated with the combination of long-acting (pegylated) interferons and ribavirin. Patients who have a sustained virologic response to treatment also have significant and persistent histologic improvement.

History

Although our awareness and understanding of viral hepatitis has risen dramatically over the last 4 decades, this is not a new problem. Descriptions of jaundice exist in the literature as far back as several centuries BC and are referenced in the Babylonian Talmud and the writings of Hippocrates (1). The infectious nature of the disease was first recognized in the 8th century BC

by Pope Zacharias (2). However, most of the reports of large population epidemics over the last several centuries were probably due to enteral transmission of what is now known as *hepatitis A*. It was not until the introduction of the practice of inoculation for smallpox vaccination in the 1880s that the percutaneous route of transmission of the disease was recognized (3). Numerous reports of jaundice occurring in patients receiving vaccines or injections for diabetes or syphilis followed during the early 20th century (4–6). The first association of blood transfusion with the development of hepatitis was reported in 1943 (7). The landmark studies of Krugman et al. at the Willowbrook State School in New York documented the transmissibility of hepatitis by human plasma (8) and confirmed the long-standing clinical observations that both parenteral ("serum hepatitis") and enteric ("infectious hepatitis") transmission could occur (9). Frustrating and largely unsuccessful efforts to identify the specific agents responsible for hepatitis continued over several decades (10). A serologic marker for hepatitis B was first identified by Blumberg in 1965 (11), although its association with the parenterally transmitted entity then known as *serum hepatitis was* not recognized until 2 years later (12). The recognition of the specific viral agents responsible for hepatitis B and A was made over the next few years (13,14). These discoveries were obviously major breakthroughs, but it quickly became apparent that most cases of hepatitis could not be explained by either the hepatitis A virus or the hepatitis B virus (HBV).

The entity of non-A, non-B hepatitis was formally christened by Prince in 1974 (15). An infectious agent was suspected on the basis of the observations that it was parenterally transmissible to chimpanzees and humans by blood transfusion (16). A series of experiments by Bradley et al. at the Centers for Disease Control and Prevention (CDC) characterized the nature of the infectious agent (17,18). Filtration studies suggested that the agent was between 30 and 50 nm in size. Its infectivity was abolished by chloroform, suggesting the presence of a lipid envelope (17), as well as by formalin, heat (100°C for 5 minutes or 60°C for 10 hours), and β-propiolactone ultraviolet light (18). However, the conventional virologic and immunologic techniques of the time failed to isolate the responsible agent. Scientists at Chiron Corporation, Emeryville, CA, and in Japan used a different tactic based on recently described molecular biologic techniques (19,20). This was based on Bradley's work, which suggested that the non-A, non-B agent was a virus. However, because the genomic nature of this putative virus was not known (although a flavi-like ribonucleic acid [RNA] viral agent was suspected), both deoxyribonucleic acid (DNA) and RNA were extracted for cloning from a large volume of infected serum (19). After extensive ultracentrifugation, which was sufficient to pellet down the smallest of known infectious agents, total nucleic acid was extracted and both RNA and DNA were converted to complementary DNA (cDNA). Restriction fragments were cloned into a recombinant bacteriophage vector to form a cDNA library. These phages were then inserted in *Escherichia coli* capable of transcribing and expressing the encoded peptide, and the resulting products were screened against sera from patients with non-A, non-B hepatitis under the assumption that sera from infected patients should contain antibody against the agent. After more than 1 million clones were screened, 5 clones were found to be reactive. Of these, one clone (5-1-1) was shown to bind not only antibodies present in the serum of patients with non-A, non-B hepatitis but also those in experimentally infected chimpanzees who appeared to seroconvert several weeks after exposure (21). Identification of other clones with overlapping regions of the viral complimentary DNA allowed these investigators to establish the entire viral genome.

This breakthrough led to an explosion of research on this viral agent, now designated as *hepatitis C virus (HCV)*, and its disease, now called *hepatitis C*. With the development of antibody-based detection systems (see later), HCV was found to be the major cause of non-A, non-B hepatitis (22–24). An estimated 170 million persons worldwide are infected by HCV, and it is perhaps the most common cause of chronic liver disease in the United States.

Virology

HCV is the only member of the Hepacivirus genus of the Flaviviridae family. The viral genome is contained within a nucleocapsid that is encased in an envelope derived from host membranes into which viral-encoded glycoproteins are inserted (25). Spherical 50-nm viral particles have been identified by electron microscopy (26,27). Two populations of virus appear to exist in serum on the basis of density-gradient analysis (26). The high-density fraction is thought to represent free or immunoglobulin-bound virus, whereas a lower-density fraction appears to be bound to low-density lipoproteins (LDL) (28,29). The pathogenic significance of the latter is discussed later.

GENOMIC ORGANIZATION, VIRAL PROTEINS, AND REPLICATION

HCV contains a 9.6-kb positive-sense, single-stranded RNA genome that consists of a highly conserved 341-base 5′ noncoding region, a single long open reading frame (ORF) of 9,033 to 9,099 bases, and a 3′ noncoding region. The ORF encodes a polyprotein precursor of approximately 3,000 amino acids (25). This polyprotein is cleaved co- and post-translationally by both

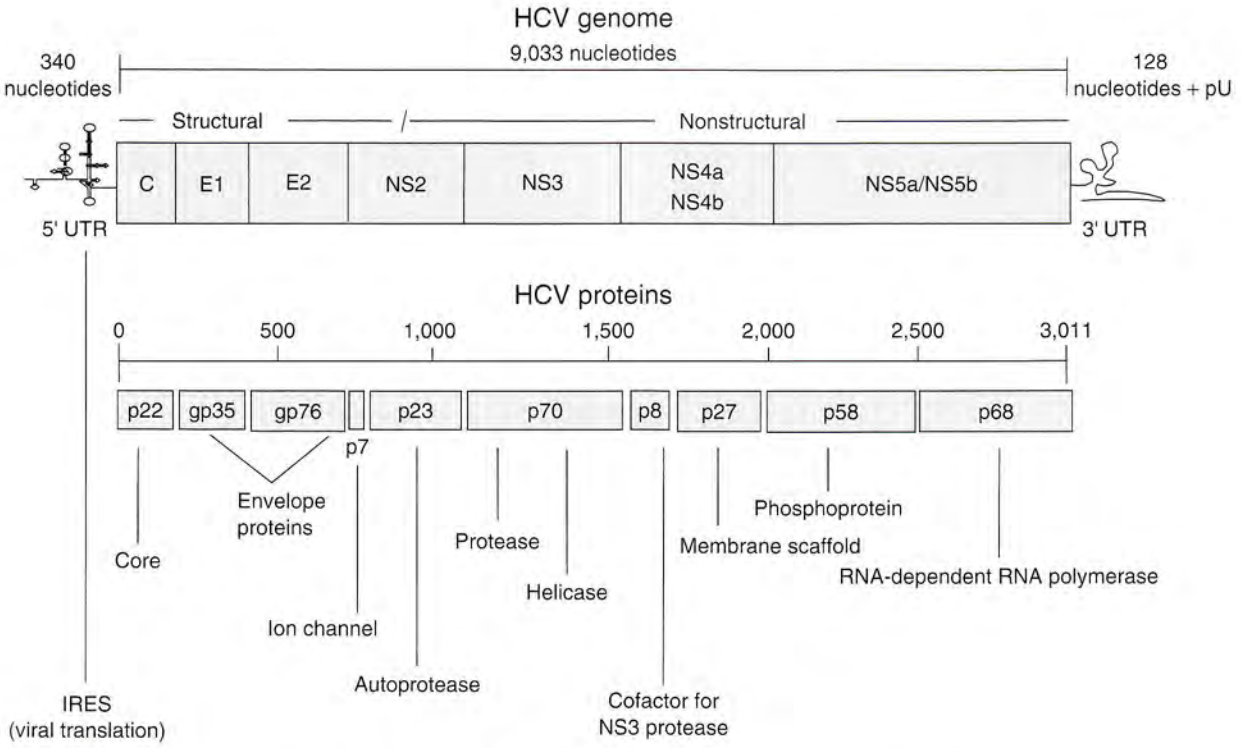
cellular and viral proteases to produce at least ten polypeptides with various functions in replication and virus assembly (30,31) (Fig. 30.1).

On the basis of phylogenetic tree analysis, genetic organization, and hydrophobicity patterns, HCV is related to flaviviruses and pestiviruses and distantly to plant viruses (32). However, HCV has sufficient diversity to justify classification into a separate genus and therefore it has been assigned as the single member of the Hepacivirus genus of the family Flaviviridae (33). The nomenclature of the respective viral proteins is also in accordance with that of the Flaviviridae family (34). Given the factors that influence viral diversity, it is difficult to estimate the age of the HCV by phylogenetic analysis. Nonetheless, ancestors of the oldest genotypes of HCV probably originated in western and sub-Saharan Africa (genotypes 1, 2, and 4) and Southeast Asia (genotypes 3 and 6) (33). Some genotypes and common subtypes evolved later. For example, the subtypes of genotype 2 probably evolved within the last 90 to 150 years. Subtypes 1b likely evolved 60 to 70 years ago, and its global distribution suggests that it disseminated over a short period (33). The recent evolution of genotype 3a (about 40 years) and its high prevalence among intravenous drug users suggest that it may have evolved and spread through the practice of needle sharing in the 1960s (33).

VIRUS REPLICATION

Identification of HCV in the cytoplasm of hepatocytes by immunohistochemistry and in situ hybridization suggests that the liver is the site of viral replication (35,36). This is further supported by the detection of negative-strand HCV RNA, the replication template, in hepatocytes. Although there is some evidence to support extrahepatic reservoirs of virus, including lymphocytes, gut epithelial cells, and the central nervous system, it remains unclear whether these sites simply harbor virus or are actually sites of replication (37–39).

Our early understanding of the replication of the HCV was based on the assumption that it must resemble the replication of genetically related flaviviruses. An important breakthrough was the creation of functional subgenomic replicons by Lohmann et al. in 1999 (40). These replicons consisted of subgenomic (incomplete) HCV RNA engineered to express a selectable marker (e.g., neomycin resistance) instead of a virus-related gene, in this case the structural gene region. A heterologous internal ribosomal entry site (IRES) was inserted to facilitate the expression of nonstructural proteins. This replicon was then inserted into Huh-7 human hepatocellular carcinoma cells and transfected cells were selected. Subsequently, replicons derived from other genotypes (Lohmann's original replicon was genotype 1b) and nonhuman and nonliver cell lines have been



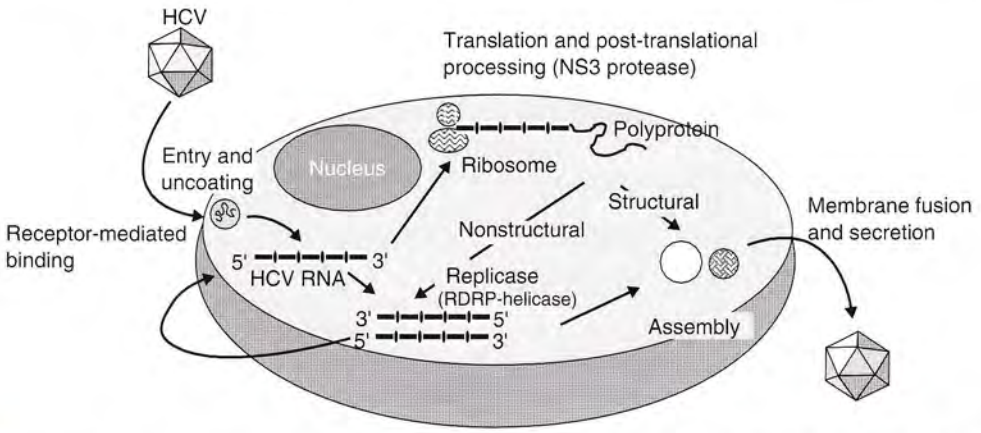
■ **Figure 30.1** Hepatitis C virus (HCV) genome and encoded polyprotein showing regions and functions. C, core; E, envelope; NS, nonstructural; IRES, internal ribosomal entry site; UTR, untranslated region; RNA, ribonucleic acid; pU, poly-uradine.

used. It became apparent that replicon RNA developed adaptive mutations that allowed it to replicate efficiently in cell culture and these increased replication manyfold (41). One persistent problem with replicon systems was that they did not release viral particles into the media despite containing the full-length HCV genome. The reasons for this were not entirely clear, but it appeared that the adaptive mutation that allowed it to replicate in culture prevented virus assembly. Recently, a full-length replicon derived from a genotype 2a isolate from a Japanese patient with fulminant hepatitis was found to replicate in culture without adaptive mutations and produce infectious particles (42). Despite the engineered and artificial nature of these recombinant constructs, these cell-based replicon systems have provided incredible information on HCV replicative mechanisms and they have also proved useful in screening potential therapeutic agents.

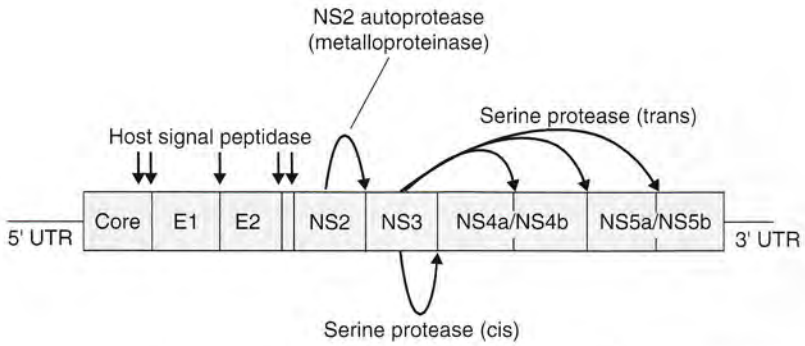
Figure 30.2 illustrates a simplified view of the infection and replication of the HCV in the hepatocyte (43). The mechanisms of HCV attachment and cell entry are still poorly understood. The envelope proteins E1 and E2 contain several *N*-linked glycosylation sites that are conserved and critical for cell entry and protein folding (44). E2 on the HCV particle surface binds to the extracellular loop of the human tetraspanin CD81 with high affinity (45). Although CD81 may serve as an essential attachment receptor for HCV, this binding is not sufficient for cell entry and, in fact, it internalizes ligand poorly (46). Other putative receptors that may participate in HCV entry include the LDL receptor (28,47), scavenger receptor class-B type 1 (SR-B1), liver/lymph node–specific intercellular adhesion molecule 3–grabbing integrin (L-SIGN), and dendritic cell–specific intercellular adhesion molecule 3–grabbing nonintegrin (DC-SIGN) (48). Once bound, the viral and cell membranes fuse, the virus is transported into the cell in an endocytotic vesicle whose acid pH uncoats the virus, releasing the positive strand of the HCV RNA into the cytosol.

The 5′ end of the HCV genome contains a highly conserved 341-nucleotide region with a complex secondary structure containing four stem loops or hairpins (49). This untranslated region (UTR) contains critical features necessary for replication and initiation of protein synthesis. First, the last stem loop of the 5′ UTR and the initial part of the core gene functions as an IRES, similar to that first described in poliovirus (50,51). Because HCV lacks a 5′ cap, and therefore does not replicate within the nucleus, it requires an IRES to direct and bind it to ribosomal subunits in the cytoplasm to form the translation complex where HCV replication ensues (51–53). This causes conformational changes in the ribosome and binding to eucaryotic initiation factor 3 (eIF3) that serves to position the AUG start codon of the viral structural gene at the decoding site of the endoplasmic reticulum membrane. In this way the positive-strand RNA serves as the messenger for translation of viral proteins.

The ORF of HCV encodes an uninterrupted stretch of 3,011 amino acids, which contains the viral proteins that are necessary for viral replication. This large polyprotein is processed co- and post-translationally by cellular and viral proteases into numerous polypeptides (Fig. 30.3). The proteins encoded by the gene are, in order from the N terminus, the structural proteins, C, E1, E2, and p7, followed by the nonstructural proteins, NS2, NS3, NS4a, NS4b, NS5a, and NS5b. The structural proteins are encoded by nucleotides in the 5′ third of the genome. Three proteolytic activities mediate the cleavages separating structural from nonstructural proteins (Fig. 30.3). Internal leader sequences within the structural precursor direct precise cleavage of the proteins by host signal peptidases within the rough endoplasmic reticulum (54). The structural proteins are produced in



■ **Figure 30.2** Schematic of the intracellular replication cycle of the hepatitis C virus (HCV). Replication takes place within the cytoplasm and is associated with the endoplasmic reticulum membrane. Assembly occurs in the Golgi. RNA, ribonucleic acid; RDRP, RNA-dependent RNA polymerase.



■ **Figure 30.3** Hepatitis C virus genome showing protease cleavage sites for corresponding polyprotein. E, envelope; NS, nonstructural; UTR, untranslated region.

more than 100-fold excess compared to positive- and negative-strand RNA (55).

The nucleocapsid or core is an unglycosylated basic protein, with RNA-binding capacity controlled by its N terminus and an essential signal for translocation to the endoplasmic reticulum located in the C terminus. The core protein modulates cell-signaling pathways, transcription, and transformation through its interactions with host proteins. Its sequence is highly conserved and contains several B-cell epitopes (40).

The envelope proteins are highly glycosylated, and some of these glycans play an essential role in protein folding and cell entry (44,56). The proteins are type I transmembrane glycoproteins with tails that serve as endoplasmic reticulum retention signals. They form noncovalently linked heterodimers and disulfide-linked aggregates, the latter being misfolded forms (56). Immune electron microscopic studies have confirmed that these proteins do, indeed, form the structural envelope of the virus (26). Both envelope proteins contain several B-cell epitopes including neutralizing epitopes. The N-terminal residues of E2 exhibit significant amino acid variation between viral genotypes and even within the same host (quasispecies) (57). These variable regions, termed *hypervariable regions* (*HVR1* and *HVR2*), are highly unstructured and are therefore able to tolerate considerable sequence variation (57). Much of this variability may be the result of immune pressure from neutralizing antibodies. Indeed, HVR1 is expressed on the viral surface, and its variability probably contributes in part to the ability of the virus to escape neutralizing antibodies (58). Studies in chimpanzees appear to confirm this because antibody against E2 confers only transient protection against HCV infection (59).

p7 is a small protein that is required for replication and may function as an ion channel (60).

In sequence, the nonstructural proteins are then cleaved from the polyprotein. First, the viral autoprotease, a zinc-dependent proteinase encoded by NS2, cleaves the NS2/NS3 junction (61,62). Second, the serine protease, a chymotrypsin-like enzyme encoded by N-terminal third of the NS3 region, cleaves the remaining nonstructural viral proteins at NS3/NS4a, NS4a/NS4b, NS4b/NS5a, and NS5a/5b junctions (Fig. 30.3) (63–65). The enzyme is obviously essential to viral replication and contains three highly conserved sites that are thought to represent the catalytic triad of the enzyme (66). The reversible binding of NS4a acts as a cofactor for the protease, allowing the enzyme to assume a more traditional trypsin-like configuration and increasing activity (67). Binding of NS4a to the protease also increases the stability of the enzyme and directs it to the endoplasmic reticulum (68). Interestingly, NS4a from heterotypic isolates can also activate the NS3 protease, although genotype 2 NS4a is a less efficient cofactor (69). In vitro studies have shown that the NS5a/NS5b junction is the most efficiently cleaved site. Protease activity is susceptible to inhibition by its cleavage products (70). The carboxy end of NS3 encodes a nucleotide triphosphatase energy source and another NS3 enzyme, the nucleoside triphosphate-binding RNA helicase, which is thought to facilitate unwinding of the RNA strands during replication (71). The unwinding activities of HCV helicase operate at the 3′ UTR for the generation of the negative-strand RNA replication template and at the 5′ UTR for the production of positive-sense RNA for assembly into infectious viral particles. The NS4 region is cleaved next, producing the NS4a and NS4b proteins. The C terminus of NS4a has been shown to be a cofactor of the NS3 serine protease as described in the preceding text (65,72). It is not essential for the function of the protease but stabilizes it and significantly improves its efficiency (66,67).

The function of NS4b has recently been clarified. It produces vesicular structures in hepatocytes that serve as a membranous web or scaffolding for the replication complex (see subsequent text) (73). NS5a is a phosphorylated protein that has been implicated in RNA binding and interferon (IFN) resistance, at least in some

isolates (74). The region has also been found to contain mutations that allow efficient replication of HCV replicons in Huh7 hepatocellular carcinoma cells (41).

The NS5b region contains the viral RNA–dependent RNA polymerase that elongates HCV RNA strands during replication (75). The enzyme is produced in excess because only approximately 1% of the total polymerase content appears to participate in replication (76). The nonstructural components assemble with the polymerase to form the membrane-associated replication initiation complex. Host cellular factors are involved with this complex as well. This multisubunit, membrane-associated replication complex is called *replicase*. The stem loop structures at the 3′ end of the coding region, but not the entire 3′ UTR, are critical for specific binding of NS5b and probably explain the specificity of the HCV polymerase (77). After binding of the 3′ end of the positive strand to the replication complex, the nascent negative–stranded RNA is elongated and the viral helicase helps separate the RNA strands (71). The negative strand of genomic length can then serve as the template for the production of nascent-positive RNA strands, which can be incorporated into the nucleocapsid that is then encoated with the viral envelope to form the mature HCV virion progeny (78–81). The HCV particles then bud from the hepatocyte.

The in vivo dynamics of HCV replication have been investigated in humans. On the basis of these studies, it is estimated that HCV replication results in more than 10^{12} viral copies per day (82). Because the half-life of these particles is short (about 2.7 hours), there is a turnover of more than 99% of viral particles daily (82). Turnover is slower (<1% to 33% per day) in infected cells, and the longevity of these hepatocytes is estimated to be 1.7 to 70 days (82).

GENETIC HETEROGENEITY

The HCV RNA polymerase is error prone because it lacks a proofreading exonuclease. As a result of this low-fidelity system of replication, random uncorrectable nucleotide errors are inevitably introduced, resulting in a heterogeneous population of viral genomes. The spontaneous nucleotide substitution rate of HCV is very high, with a frequency of between 10^{-2} and 10^{-3} substitutions per nucleotide site per year (57,83). Many of these substitutions are lethal and are not reproduced, whereas others do not result in amino acid changes and therefore remain silent. Some amino acid changes may result in increased susceptibility to elimination by the immune system and disappear quickly. Still other substitutions, especially in aggregate over time, can result in amino acid changes that have the potential to alter the biology of the virus. Regardless of the outcome of substitutions at individual sites, the virus as a whole is continually undergoing genetic evolution. As a result, HCV is a heterogeneous virus, with only approximately 70% homology among all known isolates, a level of variability similar to that of other flaviviruses (84). Obviously, the sequence heterogeneity is not evenly distributed throughout the genome. Sequences essential to replication and function are highly conserved because of constraints imposed on nucleotide substitution by secondary structures (85). For example, the 5′ UTR is 95% to 99% conserved, as is the catalytic triad site in the NS3 protease. Less well-conserved regions include core (81% to 91%), envelope (E1, 55% to 75% and E2, 65% to 72%), NS2 (57% to 71%), NS3 (70% to 80%), NS4 (65% to 79%), and NS5 (66% to 79%) (86).

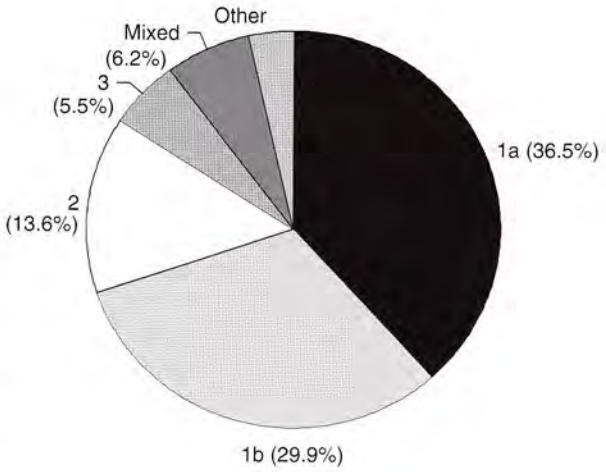
Genotypes

Isolates of a virus are usually distinguished by their genetic relatedness (genotype), much as bacteria, and sometimes viruses, are often separated by antibody reactivity (serotypes). The striking genetic heterogeneity of HCV suggested that the virus might have different genotypes. The validity of this reasoning was supported by a combination of molecular biology and statistical techniques including pair-wise distance determination and phylogenetic tree construction. Different isolates could indeed be classified by their nucleotide variability into genotypes or subtypes (33,86,87). Isolates of the same genotype have an average sequence homology of 95%, with a range of 88% to 100% on the basis of sequencing of relatively well-conserved regions of E1, NS4, or NS5. Subtypes within the same genotype have an average sequence homology of about 80% (range, 70% to 85%). By contrast, different genotypes have sequence similarity of only about 65% (range, 55% to 70%). The distribution of divergence is discontinuous, making the genotypes distinctive relatives rather than a spectrum of variants resulting from random genetic drift (86). A consensus system for HCV nomenclature based on sequence homology in at least two regions confirmed by phylogenetic tree analysis has been established by Simmonds et al. (88). In this system, major genotypes are assigned a number, and subtypes within each genotype are assigned a small letter. The numbering and lettering are assigned in the order in which the type or subtype was originally identified (88). There are six HCV genotypes and more than 100 subtypes. Reports of other genotypes have not withstood scrutiny. HCV genotypes 7, 8, and 9 were proposed several years ago on the basis of unweighted pair-group method with the arithmetic mean analysis of isolates from Southeast Asia (89). However, reanalysis by conventional phylogenetic analysis found these isolates to be subtypes of genotype 6 (88,90). Similarly, reanalysis of isolates reported to represent genotypes 10 and 11 showed that

they were most appropriately classified within types 3b and 6, respectively (91).

There are many methods for determining viral genotype. Although the most accurate is undoubtedly the sequencing of the complete 9,500 nucleotide genome with subsequent phylogenetic tree construction, this is prohibitively expensive and time consuming (92). Furthermore, multiple clones would need to be examined to exclude mixed genotype infections. Therefore, subgenomic genotyping methods have evolved, which are usually based on relatively well-conserved regions of the genome such as the 5′ UTR, core, E1, and NS5b. The nucleotide sequences within these relatively conserved regions are genotype specific, and therefore isolates can be accurately typed regardless of which of these regions is used for analysis (86,87,93). Subgenomic genotypic methods include amplification and region sequencing (94), polymerase chain reaction (PCR) with genotype-specific primers (95), restriction fragment length polymorphism of PCR amplicons (96), differential hybridization including the reverse hybridization line probe assay (LiPA, Bayer Diagnostics, Emeryville, CA) (97), and serologic genotyping (98). The line probe assay is the most popular commercial assay for genotyping. In general, these methods produce equivalent results when confirmed by amplification, sequence comparison, and phylogenetic tree construction (93). However, the method using PCR based on type-specific primers derived from the core region has been shown to be unreliable because its 1b primers frequently react with non-1b isolates, falsely suggesting a mixed genotype infection (95). This technique was one of the first genotyping methods described and was based on the very limited sequence data available at the time. Subsequent modification of the method has apparently resolved these drawbacks (99).

HCV genotype 1 is the most common genotype (40% to 80%) and has a worldwide distribution. Subtypes 1a and 1b are the most prevalent in the United States, each accounting for about a third of cases (Fig. 30.4) (100). Subtype 1b is the most prevalent genotype in Europe, Turkey, Japan, and Taiwan. Genotype 2 is also widely distributed but is less common than genotype 1 (10% to 40%). Type 3 is more common in India, Pakistan, Australia, and Scotland. Type 4 is found predominantly in the Middle East and Africa, type 5 in South Africa, and type 6 in Hong Kong and Macau (86). The geographic differences in genotype distribution appear to result from several factors. The geographic segregation of genotypes 4 and 6, their phylogenetic distances from more common genotypes, and the diversity within these genotypes all suggest that the major genotypes diverged and evolved in isolation, beginning 500 to 2,000 years ago. By contrast, the common subtypes of genotypes 1 and 2 are more widely distributed and less genetically diverse, suggesting that they evolved more recently, perhaps within the last 50 to 300 years, and were spread by population migration (33,101,102).



■ **Figure 30.4** Hepatitis C virus genotype distribution in the United States. (See reference 93.)

Quasispecies

Nucleotide substitution over time results in the evolution from a single isolate of HCV to a highly related but heterogeneous population of isolates known as *quasispecies* (103). Sequences begin to diverge about 8 weeks after infection, possibly related to the development of an HCV-specific host immune response (104). Thereafter, the same isolate may evolve into different populations of quasispecies in different patients (105). This probably reflects both some degree of randomness in nucleotide substitution and selective immune pressure. Furthermore, the diversity of the quasispecies probably reflects the duration of infection and the level of replication (103–105). Taken together, these factors result in a level of divergence after 11 to 15 years that is just as great among patients infected by the same isolate as it is in unlinked subjects (104). Nonetheless, overall sequence diversity is usually less than 5% in most infected individuals with chronic infection, even after decades of infection. The pathobiologic implications of HCV quasispecies are not well understood but they might play some role in recovery and persistence (106). The implications of quasispecies to the pathogenesis, natural history, and therapy of liver disease are discussed later.

Host Responses and Pathogenesis of Liver Disease

Multiple factors influence the interaction between the HCV virus and the infected host, thereby resulting

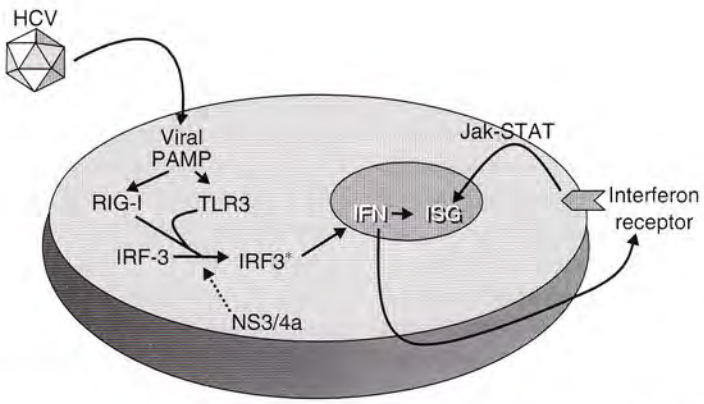
in an extremely variable presentation of the infection and liver disease. Viral factors include replication efficiency, expression of viral proteins, virus genotype and diversity, immunoreactivity of viral peptides, and, perhaps, direct liver cell injury. Host factors may include the competence of the innate immune response, local and systemic cytokine production, humoral response, and cellular immune responses. Finally, environmental factors such as alcohol intake and exogenous immune suppression may affect the course of disease. The lack of a small animal or efficient cell culture model has hampered efforts to better define the immunopathogenesis of HCV infection. As a result, most studies have been observational in infected patients. Not surprisingly, the results of these studies are often quite disparate and difficult to interpret because individual variation in the host immune response and subtle methodologic differences in experimental design may influence the findings.

It has become increasingly apparent that persistence of the virus and resistance to IFN-based therapy are related to the host's inability to overcome the viruses' defense mechanisms. The critical factor for virus clearance is the ability of the host to mount and sustain an endogenous IFN response. As described in the subsequent text, the virus works to ameliorate this response and in so doing lessens the chance for early viral clearance and subsequent immune-mediated responses. Viral heterogeneity may also contribute to persistence, but is likely not sufficient in and of itself.

INNATE HOST ANTIVIRAL DEFENSES

Proteins and nucleic acids of viruses trigger receptors that initiate antiviral mechanisms within the cells (107). The triggers for these mechanisms, termed *pathogen-associated molecular patterns* (*PAMPs*), may include viral envelope proteins or double-stranded RNA segments within the HCV genome (e.g., stems and loops in the UTRs) (108). In the case of HCV, two major pathways exist to initiate host defenses, namely the retinoic acid inducible gene-I (RIG-I) and the toll-like receptors (Fig. 30.5) (109,110). Activation of these pathways by PAMP awakens latent cellular transcription factors that trigger the expression of early response genes in the host cell. IFN regulatory factor-3 (IRF-3) and nuclear factor κB (NF-κB) are the major factors that trigger this response, although other IFN regulatory factors may also be involved (111). This process leads to the secretion of type 1 IFNs in and from the infected cell (112). NF-κB also induces other chemokines and proinflammatory cytokines that modulate the inflammatory response to infection. The secreted IFN then engages its cell surface receptor on both the infected and adjacent cells and activates the Jak-STAT pathway through which dozens of IFN-stimulated genes that produce protein kinases, oligoadenylate synthetase, and other host-protective factors are activated (113). IFN primes the further production of both IFN and the IFN-sensitive genes, thereby amplifying the antiviral responses even further. Finally, IFN modulates the host immune response by enhancing cell surface antigen expression and promotion of immune effector cells.

The effectiveness of the host response to infection relates in large part to the ability of the infected host to activate and sustain these innate cellular defenses. Some of these responses may be genetically regulated, but it also appears that the levels of IFN signaling, IFN response gene induction, and HCV-induced adaptive immune responses are controlled by the virus itself (Table 30.1) (107). Perhaps most importantly, the NS3/4a protease complex disrupts activation of IRF-3 and NF-κB by the RIG-I and toll-like receptor signaling paths. This reduces the ability of the host to effectively express antiviral defense genes and interrupts



■ **Figure 30.5** Schematic of the host innate antiviral responses to hepatitis C virus (HCV) infection. Pathogen-associated molecular pattern (PAMP) bind to retinoic acid inducible gene-I (RIG-I) or toll-like receptor (TLR3) resulting in the phosphorylation (*) and activation of IFN regulatory factor (IRF-3). IRF-3 translocates to the nucleus where it results in interferon (IFN) production. Interferon leads to activation of the Jak-STAT pathway and induction of interferon-sensitive genes (ISGs). NS, nonstructural. (See references 107 and 114.)

TABLE 30.1. INFLUENCE OF HEPATITIS C VIRAL COMPONENTS ON HOST ANTIVIRAL RESPONSES

HCV viral component	Effect on host antiviral response
AUGMENT HOST RESPONSE	
HCV viral particle	Binding to dendritic cell activates host IFN production and immune induction
dsDNA	Triggers IFN and activates PAMPs
Core	Activates PKR
NS5a	Activates STAT3
INHIBIT HOST RESPONSE	
E2	Inhibits PKR
Core	Increased suppressor of cytokine signaling blocks Jak-STAT signaling
NS3/4a protease	Disrupts RIG-I and TLR3 signaling for IRF-3 activation
NS5a	IFN antagonist
	Induces IL-8 that interferes with IFN actions
	Inhibits PKR signaling and IRF-1 action
HCV genomic sequences	RNase L recognition sites reduced in genotype 1

HCV, hepatitis C virus; IFN, interferon; dsDNA, double-stranded deoxyribonucleic acid; PAMP, pathogen-associated molecular patterns; PKR, protein kinase R; NS, nonstructural; E2, envelope protein 2; RIG-I, retinoic acid–inducible gene-I; TLR, toll-like receptor; IRF, interferon regulatory factor; IL, interleukin; RNase, ribonucleic acid proteinase.

the IFN amplification loop that attempts to suppress HCV replication (114).

ACQUIRED IMMUNITY

The host immune response to HCV infection is composed of both a nonspecific immune response, including endogenous cytokine production (see preceding text) and natural killer (NK) cell activity, and a virus-specific immune response, including both humoral and cellular components (Table 30.2). Antibodies to HCV structural and nonstructural proteins develop late during acute infection and form the basis for the detection assay of the host's exposure to the virus (See "Diagnostic Tests"). A variable cellular immune response also occurs early during acute infection and results in the emergence of $CD4^+$ and $CD8^+$ cells that recognize and respond to processed HCV antigens.

Humoral immune response

Evidence for activation of the host humoral response in HCV infection includes the presence of hepatic lymphoid aggregates containing activated B cells (115), elevated levels of the B-cell–activating interleukin-4 (IL-4) (116), and a B-cell–mediated response with the production of antibodies to several structural and nonstructural polypeptides (117). Antibodies to HCV peptides form the basis of current diagnostic assays (See "Diagnostic Tests"). However, the role of this humoral immune response in the control of infection and pathogenesis of liver disease is still largely unknown.

Antibodies to HCV antigens develop in most acutely infected patients within 4 to 8 weeks of the onset of infection. However, their relatively late onset, low level during acute infection (117,118), decrease and occasional disappearance when infection resolves (119,120), and persistence at higher levels when infection persists raises questions about their relevance in control of infection. Nonetheless, the humoral response may influence liver disease associated with HCV infection in other ways such as extracellular viral neutralization. Antibodies against envelope proteins often have neutralizing capability that could prevent viral entry into uninfected cells. Antibodies against conserved epitopes of the HCV envelope proteins (E1, E2) are found in more than 90% of patients with chronic HCV infection (117). However, the persistence of infection in most patients with anti-E1/anti-E2 suggests that either the antibodies do not have sufficient neutralization ability or the target is not relevant to viral persistence. There is no doubt that neutralizing antibodies can be raised, and these can even neutralize infectious virus ex vivo, thereby preventing infection (121,122). However, the in vivo neutralizing antibody response is usually weak and short lived. Farci et al. demonstrated that the infectivity of plasma can be neutralized by serum obtained from the same chimpanzee 2 years after infection but not by serum obtained 11 years later, presumably because of the evolution of quasispecies (123). Similarly, in other experiments, convalescent animals could be reinfected by either heterologous or autologous challenge (124). Therefore, it appears that although neutralizing antibodies are formed, evolution of the virus may allow escape from this response. This is not unexpected because the envelope proteins are not highly conserved. E2 contains two HVRs in which nucleotide substitutions are largely unconstrained and wide genetic differences evolve. It has been suggested that antibodies to these regions (especially HVR1) neutralize existing strains of the virus and drive genetic drift, but this is controversial and most recent evidence weighs against such an effect (125–128).

TABLE 30.2. SUMMARY OF COMPONENTS OF THE HOST IMMUNE RESPONSE TO THE HEPATITIS C VIRUS

HUMORAL
- Activated B cells in lymphoid aggregates
- Peripheral expansion of $CD5^+$ B-cell population
- Antibodies to structural and nonstructural peptides
- No evidence for antibody-dependent cellular cytotoxicity

CELLULAR
- Innate
  - Inflammatory cytokine and IFN release
  - Natural killer cell activation
  - Natural T-cell activation
- CD4 lymphocytes
  - Early response
    - Augments antibody production by B cells
    - Primarily act peripherally
    - Possible role to control infection and protect liver
    - Stimulates CD8 cells
  - Later response
    - Compartmentalization to liver
    - May target different HCV peptides from peripheral CD4
- CD8 lymphocytes
  - Compartmentalization in liver
  - Predominant infiltrating lymphocyte
  - Probable role in controlling replication and causing liver injury
  - Activity possibly results from
    - upregulation of adhesion molecules to recruit CD8 cell
    - upregulation of Fas proteins may facilitate CD8 target killing
    - induction of local cytokine release (IFN-γ, TNF-α)
- Cytokine response
  - T_H1 response early in infection and persists locally in liver
  - T_H2 response may be a peripheral autoregulatory response to T_H1 activation

HCV, hepatitis C virus; IFN, interferon; TNF, tumor necrosis factor.

Antibodies may also direct the destruction of their bound target through activation of other mechanisms, specifically the complement-mediated antibody-dependent cell-mediated cytotoxicity (ADCC). However, for these antibodies to contribute to cell injury, they must recognize HCV antigens on the hepatocyte cell membrane. Although HCV antigens (core, E1, E2, NS3, and NS4) have been detected in the cytoplasm of infected hepatocytes, membranous antigens have not been observed (129). Even when intracellular expression of HCV viral proteins is driven to very high levels by a recombinant vaccinia system, neither HCV antigens nor immunoglobulins could be detected on the cell membrane (130). These data suggest that ADCC is unlikely to play an important role in mediating hepatocellular damage.

Although evidence suggesting a role for the humoral response in HCV liver disease is still lacking, the antibody response may be associated with other manifestations of infection (See "Extrahepatic Manifestations"). It has been suggested that binding of HCV to the CD81 receptor on B cells could activate these cells, thereby facilitating the production of antibodies (131,132). Indeed, patients with chronic HCV infection commonly develop autoantibodies (133). One of these, the antibody to the human-derived epitope GOR, appears to result from molecular mimicry between the HCV core sequence and GOR (134). This is supported by the rarity of anti-GOR in patients infected with HCV genotype 3, which has amino acid differences within the proposed molecular mimicry site. However, there is no evidence to suggest that autoantibodies have clinical significance or a role in disease pathogenesis (135).

Humoral activation during HCV infection is not limited to antibody production. More than half the patients with chronic HCV infection show expansion of $CD5^+$ B lymphocytes in peripheral blood (136). Again, this might be related to binding of HCV to B-cell CD81 receptors (131,132). Activation of CD5 B cells has previously been associated with autoimmune diseases such as rheumatoid arthritis (137), and it is possible that a similar mechanism plays a role in the development of B-cell lymphomas in patients with HCV infection (138). HCV is also associated with the development of mixed essential cryoglobulinemia, in which deposition of immune complexes composed of inmmunoglobulin G (IgG) and rheumatoid factor precipitates in small blood vessels (139). This appears to be due to antigen-driven benign proliferation of B cells. The mechanism may be through the CD81 mechanism discussed earlier or due to impaired ability of hepatocytes to endocytose HCV–very low density lipoprotein (VLDL) complexes containing apo E2 through the LDL receptor. Retention of the complexes in the circulation may then stimulate rheumatoid factor production (139).

Cellular immune response

The cellular immune response to viral infection involves innate nonspecific mechanisms, such as those described in the preceding text and NK cell activity, and adaptive antigen-specific mechanisms, including cytotoxic T lymphocytes with accompanying inflammatory cytokine release (Table 30.2). Although nonspecific cellular responses may act early to limit some infections, eradication of infection appears to also require specific and classical $CD4^+$ and $CD8^+$ cytotoxic T-lymphocyte (CTL) responses. However, as previously described, activation of endogenous antiviral mechanisms and adaptive cellular responses may be linked.

In contrast to the humoral response triggered by the binding of unprocessed extracellular antigens to B-cell immunoglobulin receptors, the cellular (T-cell) immune response is triggered by peptides that have been processed within the cell cytoplasm and expressed on cell membranes in conjunction with a major histocompatibility complex (human leukocyte antigen

[HLA]) molecule. Processed peptides are generally presented to $CD8^+$ T cells by HLA class I molecules, which are expressed on virtually all cells, or to $CD4^+$ T cells by the HLA class II molecules, which are found on specialized antigen-presenting cells. Although the subsequent events that lead to the death of the infected presenting cells are not entirely clear, both direct cytolysis and secreted antiviral factors (tumor necrosis factor-α [TNF-α] and IFN-γ) can be implicated.

Innate cellular response

The liver has a dense population of lymphoid cells representing approximately 10% of all lymphocytes and 35% of T lymphocytes in the host (140). These cells comprise a highly regulated immune environment within the liver for monitoring and implementing responses to foreign stimuli. The innate response is the earliest phase of immune defense to viral infection and helps regulate the subsequent adaptive responses of the host. Over a third of hepatic lymphoid cells are NK cells that can be nonspecifically activated by a variety of signals and are able to kill targets in the absence of antibody or antigenic stimulation. Histocompatibility antigen expression appears to inhibit NK activity. In the case of viral infection, downregulation of endogenous class I histocompatibility antigens occurs, possibly to assist the virus in evading CTLs, and this may result in early NK cell activation (141). NK cells appear to exert their activity against HCV by releasing IFN-γ (142), but it appears that they are only able to control acute infection at low levels of replication (143). Natural T (NT) cells (also called innate T cells) are $CD3^+$, common in the normal liver, and prevalent in HCV-infected liver (144). These cells produce IL-4 and probably play a role in modulating both early and subsequent immune responses. Finally, memory HCV–specific T cells are sometimes present before HCV exposure because of cross-reacting antigens. For example, immunodominant HCV-specific CTL response to the NS3 epitope can be induced by influenza A infection because of sequence homology between NS3 and the neuraminidase protein (145). Although these latter two cell types are active in HCV infection, their role in the initial control of infection is not known.

$CD4^+$ T-lymphocyte response

The $CD4^+$ T-cell response to viral proteins is critical for host protection. It occurs early, augments antibody production by B cells, and is a prerequisite for subsequent $CD8^+$ T-cell responses, including those that are specific for virus-infected cells (146,147). Therefore, its role has typically been viewed as a protective one. $CD4^+$ T-cell responses to viral infection have traditionally been determined by measuring the ability of these peripheral lymphocytes to proliferate or produce IFN-γ when exposed to viral proteins. No one viral antigen is responsible for this $CD4^+$ response, although peptides derived from core and NS4 result in the strongest proliferative responses (148). Interestingly, proliferative $CD4^+$ responses are most robust in infected individuals in whom acute infection resolves (149), who have persistent infection without histologic evidence of liver damage (150), or who have chronic hepatitis that responds to IFN (151). These observations suggest that a vigorous $CD4^+$ response to HCV infection provides early control of infection and protects against subsequent hepatocellular damage.

The studies of peripheral blood proliferative responses must be considered with some degree of skepticism because these circulating $CD4^+$ cells may not accurately reflect the immunologic climate at the site of infection. Indeed, it appears that HCV-specific proliferative responses tend to home in on the liver once chronic infection is established (152). Although studies of intrahepatic $CD4^+$ responses have been limited, proliferative responses to core, E1, and NS4 have been reported (152,153). Several striking differences from peripheral $CD4^+$ responses have been noted. First, the reactive $CD4^+$ clones do not always react to the same HCV peptides that are recognized peripherally (152), and second, proliferative response appears to correlate with more active liver disease (153). Finally, intrahepatic $CD4^+$ T cells differentiate into both T-helper cell 1 (T_H1) (IFN-γ) and T_H2 (IL-4) populations, although the former predominate (154).

In summary, $CD4^+$ cells play a role early in infection, perhaps by local cytokine production, providing help for B cells and protection of the hepatocyte from injury. $CD4^+$ cells seem to compartmentalize to the liver after the resolution of the acute phase, produce predominantly T_H1 cytokines, and may even generate or directly develop HCV-specific CTL activity (146). However, $CD4^+$ activity is typically weak in patients with chronic infection, and this may impair the development of an effective $CD8^+$ response. It has been proposed that HCV core binds to the complement receptors of $CD4^+$ T cells and dendritic cells, thereby impairing the production of IL-2 and IFN-γ that are required to generate T-cell effector functions (155).

$CD8^+$ T-lymphocyte response

The $CD8^+$ arm of the cellular immune system has been shown to be important in the control of viral infections and pathogenesis of cell injury in vivo. Several lines of evidence suggest that these cells also play an important role in HCV infection. First, immunophenotyping studies have demonstrated that a significant proportion of the activated cells in the livers of patients with chronic hepatitis C are $CD8^+$ T lymphocytes (156,157). Second, expression of adhesion molecules,

one pathway for recruitment and priming of T cells, is upregulated in the inflamed hepatic portal tracts (158). Third and most important, HCV-specific cytotoxic $CD8^+$ T lymphocytes have been isolated from both liver and peripheral blood in a significant proportion of patients with chronic HCV infection (159–161). These cells are restricted by HLA class I molecules, suggesting that HLA subtypes might present different processed viral peptides to lymphocytes and affect the severity of cell injury. Indeed, target epitopes from within both structural and nonstructural regions have been identified (162–164). The immunodominant CTL epitopes are most commonly located within the HCV structural antigens (core/E1/E2); CTL responses to nonstructural regions occur in a smaller subset of patients (161). However, multiple epitopes may be targeted by the same patient, and the magnitude of the CTL is variable both within the same patient and between different patients (165). Interestingly, HCV-specific CTL cannot usually be detected in peripheral blood without HCV-specific stimulation (161). The estimated frequency of HCV-specific CTL in the peripheral blood mononuclear cells of patients with chronic hepatitis C is only 1 per 30,000 to 1 per 300,000 (162). This tissue-dependent prevalence suggests compartmentalization of HCV-specific CTL activity, similar to what was described earlier for $CD4^+$ cells (152). Of course, it is not surprising that the liver, the primary and perhaps only site of HCV replication, is the recruiter of HCV-specific CTL. However, although HCV-specific $CD8^+$ cells may be more frequent in the liver, they appear to be dysfunctional, with impaired ability to secrete IFN-γ compared to peripheral cells (166). The mechanism for this is not clear but could be related to the viral inhibition of IFN production (107).

Nonetheless, HCV-specific CTL appears to have an important role in the control of viral replication and promotion of hepatocellular damage in chronic HCV infection. Using an in vitro protocol to expand liver-derived $CD8^+$ cells without HCV-specific stimulation, Nelson et al. (161) have measured HCV-specific CTL activity in bulk-expanded $CD8^+$ T cells isolated from liver. Patients with measurable HCV-specific CTL activity had higher serum alanine aminotransferase (ALT) levels and more histologic activity in the liver biopsy. Furthermore, these patients also had significantly lower HCV RNA levels, suggesting that HCV-specific CTL activity may be important in regulating HCV replication. A similar role for virus-specific CTL has also been reported for lymphocytic choriomeningitis virus (LCV) infection (165). In fact, in LCV infection, $CD8^+$ CTL may lead to viral clearance through direct lysis of infected cells or cytokine-mediated viral inhibition (167). Current evidence suggests that HCV-specific T-cell responses do not lead to viral escape mutations (168).

The means by which the HCV-specific CTL mediates these effects remains largely speculative. CTL may reduce viral replication by the local production of lymphokines such as IFN-γ and TNF-α (135–137), although some studies have shown that this response is impaired compared to the response to other viruses such as influenza (166). Apoptosis may be the most important mechanism of hepatocyte death by CTL (169). This is likely to occur by a variety of apoptotic pathways. CTLs can be directly cytopathic to their targets by inducing the formation of pores in the target cell membrane. This occurs through a sequence of events resulting in the secretion of the pore-forming protein perforin, a series of granule serine proteinases (granzymes), and other molecules (170). CTL can also induce apoptosis by facilitating the interaction of the Fas/Apo 1 antigen with its receptor ligand on the surface of the activated $CD8^+$ cells (171). In fact, hepatic expression of c-Fas is increased in patients with chronic HCV infection, and Fas-bearing cells are more sensitive to CTL killing (172,173). HCV core expression may be important in making cells susceptible to Fas-mediated apoptosis (174).

Cytokine response

The high replication rate of HCV and the large number of infected hepatocytes present a formidable challenge to the cellular immune system. The fact that this infectious burden exceeds the capacity of the CTL response is apparent from the persistent nature of the infection. However, other mechanisms may assist in controlling the infection. Cytokines are regulatory molecules that play an important role in orchestrating several physiologic and pathologic processes. Cytokine responses are referred to as T_H1, T_H2, or the inactive T_H0, after the original description of the cytokine profiles produced by subsets of the $CD4^+$ T_H cells (175). T_H1-like responses include IL-2, TNF-α, and IFN-γ secretion and are required for CTL generation and NK cell activation during the host's antiviral immune response. T_H2-like responses produce IL-4 and IL-10, which help augment antibody production and inhibit the development of the T_H1 response (176).

Patients with chronic HCV infection have an activated T-cell response pattern and have been reported to have elevated serum levels of both T_H1 and T_H2 cytokines (177,178). Peptide stimulation of either peripheral blood- or liver-derived HCV-specific T-cell clones results in a predominantly T_H1 cytokine response with release of IFN-γ and TNF-α (179). Furthermore, IFN-γ and IL-2 messenger RNA (mRNA) levels are increased in the livers of patients with chronic hepatitis C, suggesting that these cytokines are produced locally by resident $CD4^+$ cells (180). The levels of IFN-γ and IL-2 mRNA correlate with fibrosis

and portal inflammation, suggesting that T_H1 cytokines play a role in mediating hepatocellular damage. To further support this hypothesis, elevated plasma levels of TNF-α appear to be associated with more severe hepatocellular damage (181). However, others have reported a predominantly T_H2 profile with elevated serum IL-4 and IL-10 levels and some T_H2 cell markers in hepatic inflammatory infiltrates (116,177). IFN treatment appears to reduce this T_H2 cytokine response in parallel to the reduction in viral levels (182). Given the ability of IL-4 and IL-10 to inhibit immune cell function, T_H2 cells may provide an autoregulatory mechanism through which the host is able to partially offset the potentially detrimental effects of the T_H1 response.

From the existing data, it appears that the T_H1 response is activated in the liver in response to HCV infection. This may be an attempt to control viral replication, and its persistence results in hepatocyte injury. The T_H2 cytokine response probably represents an autoregulatory response that originates outside the liver and attempts to confine the T_H1 response to the site of infection (the liver) to prevent systemic effects.

Direct viral cytopathicity

Some viruses can kill cells directly without invoking immune-mediated pathways. This may occur through the ability of some viruses or viral gene products to increase lysosomal permeability, alter cellular membranes, or interfere with normal cellular functions such as the synthesis of cellular macromolecules. Characteristic morphologic alterations of cellular architecture such as cell rounding and shrinkage and nuclear pyknosis suggest a cytopathic effect.

It has been difficult to determine whether HCV is directly cytopathic because cell culture systems that allow replication of unmodified HCV have not yet been perfected. However, several lines of evidence support a cytopathic role for HCV. First, other members of the Flaviviridae family, such as the yellow fever virus, cause direct cytopathic injury to infected cells (183). Second, histologic examination of HCV-infected livers occasionally reveals dying hepatocytes without adjacent inflammation (184,185). Third, serum aminotransferase levels and hepatic inflammation decline relatively parallel to viral levels during treatment with antiviral agents such as IFN (186). And finally, some studies have found a correlation between serum HCV RNA levels and the degree of hepatocellular damage (187,188). In fact, high-level cellular expression of HCV has been seen in some patients with severe hepatic injury. This was first reported in an immunosuppressed heart transplant recipient who acquired acute HCV infection from the donor organ (189), but a similar picture has since been reported in other immunosuppressed patients particularly after liver transplantation (190,191). In many of these cases an unusually high proportion of the liver cells contain HCV, and liver biopsies reveal an atypical histologic picture of pericellular fibrosis, marked intracellular cholestasis, and only mild inflammation. This pathology is similar to the fibrosing cholestatic hepatitis sometimes seen in highly viremic immunosuppressed patients with chronic hepatitis B (192). If HCV is indeed cytopathic at high levels, one would anticipate that high-level expression of HCV proteins in vitro might alter hepatocellular structure or function. Indeed, evidence supporting this hypothesis was recently provided in cell lines expressing high levels of HCV structural proteins. These cells showed mitochondrial and endoplasmic reticulum proliferation, distension of the endoplasmic reticulum, and hepatocellular ballooning similar to those seen in the infected transplant recipients described in the preceding text (193).

On the other hand, there is also evidence to suggest that HCV is not directly cytopathic. In the overwhelming majority of patients, particularly immunocompetent patients, biochemical or histologic markers of disease activity do not correlate with serum viral levels or the amount of HCV RNA or antigen in the liver (194,195). In fact, many patients with HCV infection have persistently normal serum ALT levels and minimal liver injury despite the presence of detectable HCV RNA in serum (196). Furthermore, a transgenic mouse model with high-level expression of HCV structural proteins does not demonstrate cytopathic changes in the liver, and this has called into question the cell culture findings discussed in the preceding text (197). Taken together, these observations suggest that direct cytopathic injury due to expression of HCV structural proteins is not typical but might be possible when the unusually high levels of the virus exceed a certain threshold. However, such injury is not predictable and therefore is probably dependent on factors other than simple expression of virus or protein.

Diagnostic Tests

SCREENING AND SUPPLEMENTAL ANTIBODY TESTS

The screening diagnostic test for HCV infection is a sensitive and unique enzyme immunoassay (EIA) in which antibodies to several different viral antigens (anti-HCV) are simultaneously detected. This commercially available assay is simple to perform, reproducible, and relatively inexpensive. A supplemental recombinant antigen immunoblot assay (RIBA) is available to resolve false-positive results in the screening tests, although this assay is rarely necessary today. Three versions of the anti-HCV EIA test have been developed over the last

TABLE 30.3. HEPATITIS C VIRUS PEPTIDES IN IMMUNODIAGNOSTIC ASSAYS

Peptides	EIA-1	RIBA-1	EIA-2	RIBA-2	EIA-3	RIBA-3
5-1-1 (NS4)		X		X		
C100-3 (NS3-4)	X	X		X		X
C33c (NS3)				X		X
C200 (fusion C100/C33)			X		X	
C22-3 (core)			X	X	X	X
NS5					X	X

EIA, enzyme immunoassay; RIBA, recombinant antigen immunoblot assay; NS, nonstructural.

several years. The antigens included in the tests and the sensitivity of the different versions are important to recognize for historical purposes (Tables 30.3 and 30.4).

The first-generation test (EIA-1), introduced immediately after the discovery of HCV (21), was an important first step in diagnosis, particularly for the screening of blood donors to reduce post-transfusion hepatitis (22,198). However, because the assay used only a single target antigen, it lacked sensitivity. In fact, only 80% of infected patients were antibody positive by this test (199). However, false-positive results were also common. Therefore, the supplemental RIBA test that used the same and sometimes additional HCV peptide antigens affixed to a solid phase was often required to resolve the specificity of the EIA result (Table 30.3) (200). This assay allowed visualization of the peptide target of the reactive serum of the patient, confirming or refuting whether the serum reacted specifically against HCV antigens. The importance of the supplemental RIBA test was particularly evident in a low HCV prevalence setting, such as with screening healthy blood donors. In this situation, 50% to 70% of the positive EIA-1 results were subsequently shown to be false-positives when tested by the supplemental assay (199). It is important to recognize that the RIBA assay is not as sensitive as the EIA test and, therefore, should not be used for screening purposes. It is most appropriately used to confirm positive EIA results in low-prevalence settings such as with healthy blood donors and anti-HCV–positive patients with normal ALT levels. Because most infected patients are EIA positive, confirmation by RIBA is generally not necessary when the serum ALT level is elevated or risk factors for infection are present.

The first-generation anti-HCV test was replaced in 1992 by a multiantigen test, EIA-2 (201), which had HCV antigens from the core and third and fourth nonstructural regions (NS3 and NS4) (Table 30.3). This provided greater sensitivity and specificity. EIA-2 detects anti-HCV in at least 95% of infected patients, which is a great advantage over EIA-1 (199). Because the test is more sensitive, it is capable of identifying new infections at an earlier time (mean, 10 weeks postinfection vs. 16 weeks with EIA-1) (201). Finally, the more sensitive EIA-2 improved the identification of potentially infectious blood donors and resulted in a further reduction in the incidence of post-transfusion hepatitis C (202,203). The use of multiple target peptides also appeared to improve the specificity and reduce the false-positive rates in low-prevalence groups to 40% to 50% (204,205).

EIA-3 was approved for screening blood products in the United States in 1997. Although the EIA-3 test contains reconfigured core and NS3 antigens, as well as an NS5 peptide not included in earlier versions of the assay (Table 30.3), these changes result in only a small increase in sensitivity (206). The major advantages of EIA-3 are earlier identification of acute infection (7 to 8 weeks in up to a third of patients) and fewer false-positive tests in low-prevalence populations (204,206).

A third-generation supplemental test (RIBA-3) consists of the antigens added to EIA-3 and the peptides already present in RIBA-2 (Table 30.3). RIBA-3 is more specific than RIBA-2 (correlates better with HCV PCR) and has fewer indeterminate results (207,208).

TABLE 30.4. SENSITIVITY AND PREDICTIVE VALUE OF SEROLOGIC TESTS FOR HEPATITIS C VIRUS INFECTION

Assay	Sensitivity (%)	Positive predictive value		Time to positive (wk)[a]
		Low prevalence (%)	High prevalence (%)	
EIA-1	70–80	30–50	70–85	16
EIA-2	92–95	50–60	88–95	10
EIA-3	97	25	98	7–8

[a]Time after infection.
EIA, enzyme immunoassay.
Adapted from Carithers RL, Marquardt A, Gretch DR. Diagnostic testing for hepatitis C. *Sem Liver Dis* 2000;20:159–172.

MEASUREMENT OF VIRAL RIBONUCLEIC ACID

Detection of HCV RNA in blood by a highly sensitive assay is important for confirming the diagnosis of hepatitis C and for assessing the antiviral response to IFN therapy. Qualitative HCV RNA tests such as the reverse transcription PCR (RT-PCR) or transcription-mediated amplification (TMA) are particularly useful as a diagnostic tool in confirming the presence of infection in special situations such as in immunocompromised individuals in whom antibody is less likely to be present or in seropositive patients with a normal serum ALT level (209). In the latter the HCV infections may either have resolved (i.e., HCV RNA negative) or be viremic (HCV RNA positive) despite normal liver enzyme levels. Although the natural history and management of such patients remains controversial, testing of HCV RNA seems medically prudent so that patients with viremia may be counseled about the potential risks of virus transmission, disease progression, and the possibility of antiviral treatment. Qualitative testing is also important to confirm the clearance of HCV after antiviral therapy. Quantitative HCV RNA tests, by either PCR or signal amplification, are used to guide antiviral therapy. In this role, HCV RNA testing can provide a reference point before initiation of therapy. Monitoring HCV RNA during therapy allows early prediction of response (See "Prevention and Treatment").

Qualitative hepatitis C virus ribonucleic acid tests

Qualitative assays for HCV RNA simply determine whether the virus is present. These tests have the potential to be extremely sensitive (Table 30.5). There are two methods that are widely available in commercial laboratories for testing clinical samples—RT-PCR and TMA. Both methods require extraction of nucleic acid from the specimen. RT-PCR then involves reverse transcription of the sample RNA to its cDNA using specific primers based on highly conserved sequences of the virus genome. The DNA product is then repeatedly amplified with a bacterial DNA polymerase until the amount of product reaches a level that can be detected by autoradiography, ethidium bromide staining, or colorimetric testing. Most of the early RT-PCR assays for HCV RNA were designed by individual laboratories (so-called home-brew tests), had limited sensitivity (1,000 to 2,000 copies per milliliter), and were subject to error related to lack of standardization and sample contamination (205). The development of RT-PCR tests by commercial diagnostic laboratories has employed standardized methodology, easier assay formats, and routine assay controls. These measures have eliminated many of the problems experienced with the early assays. The widespread availability of commercial PCR tests for HCV RNA such as the Amplicor assay (Roche Diagnostics, Nutley, NJ) has made diagnosis and clinical management of hepatitis C much easier.

TMA is an extremely sensitive method for detecting HCV RNA. The test involves a three-step process (target capture, amplification, and detection) and has the advantage of being performed in a single tube, thereby eliminating the concern about sample contamination (210). Nucleic acid is released from the sample by a lysis agent and isolated by hybridization to capture oligonucleotides that bind to magnetic microparticles. Amplification of the viral RNA is performed by autocatalytic, isothermal production of RNA transcripts using two enzymes (reverse transcriptase and T7 RNA polymerase) and two primers, one of which contains a T7 promotor. The promotor-containing primer hybridizes to viral RNA, and cDNA is synthesized by reverse transcriptase. The RNA of this complex is degraded by the RNase H activity of the reverse transcriptase,

TABLE 30.5. CHARACTERISTICS OF COMMERCIALLY AVAILABLE HEPATITIS C VIRUS RIBONUCLEIC ACID ASSAYS

Test	Manufacturer	Method	Application	Dynamic range (IU/mL)[a]		Conversion factor (copies/IU)
				Lower limit	Upper limit	
Amplicor	Roche	PCR	Qualitative	42	N/A	N/A
UltraQuant	NGI-LabCorp	PCR	Qualitative	30	N/A	N/A
Versant-TMA	Bayer	TMA	Qualitative	5	N/A	N/A
Amplicor Monitor 2.0	Roche	PCR	Quantitative	600	500,000	2.4
SuperQuant	NGI	PCR	Quantitative	60	1,500,000	3.4
QuantaSure[b]	NGI	PCR	Quantitative	2	2,000,000	2.5
Versant Quant 3.0	Bayer	bDNA	Quantitative	520	8,300,000	4.8
LCx	Abbott	kLCR	Quantitative	23	2,300,000	4.3
Heptamax	Quest	kPCR	Quantitative	50	50,000,000	2.7
TaqMan ASR	Roche	kPCR	Quantitative	25	5,000,000	0.6

[a]Limits of detection are approximated on the basis of published data.
[b]This test increases the lower limit of detection by centrifugation of a larger sample.
PCR , polymerase chain reaction; N/A, not applicable; NGI, National Genetics Institute; TMA, transcription-mediated amplification; bDNA, branched deoxyribonucleic acid; LCx, ligase chain reaction; kLCR, kinetic ligase chain reaction; kPCR, kinetic PCR also known as *real time PCR*.

and a second primer then binds to the cDNA already containing the promotor sequence. Therefore, new DNA can be synthesized by reverse transcriptase. The RNA polymerase recognizes the T7 promotor sequence in the double-stranded DNA molecule and synthesizes numerous RNA transcripts. Each of the newly synthesized RNAs reenters the TMA process and serves as a template for a new round of replication, resulting in exponential amplification of target RNA. The RNA amplicons are detected by a hybridization protection assay with amplicon-specific chemiluminescent probes and compared to an internal control standard. The sensitivity of TMA is in the range of 25 to 50 copies per milliliter. According to the World Health Organization HCV RNA standard, the sensitivity is 96% (5 IU/mL) and 100% (10 IU/mL) (211).

Quantitative tests for hepatitis C virus ribonucleic acid

Two different technologies have evolved to quantitate HCV RNA levels. These include target amplification methods that use PCR-based technology and signal amplification technologies such as branched DNA (bDNA) assay (212–214).

Target amplification methods typically spike the initial reaction mixture with a known amount of a tag that is amplified along with the sample. The ratio of the initial tag to the amount in the final reaction mixture can then be used to estimate the original amount of sample RNA. The major limitations of early PCR tests were sensitivity and the potential for contamination (false-positives). Standardization of commercial tests reduced these problems. Recently, real- time PCR techniques such as TaqMan have been developed. These assays are based on the cleavage of fluorescent dye-labeled probes by the 5′ → 3′ exonuclease activity of the DNA polymerase during PCR. Measurement of fluorescence intensity by a sequence-detection system provides a measure of RNA over time (cycles) and optimizes detection across a wider dynamic range of virus levels. These assays are rapid, are sensitive, have broad dynamic ranges, provide precise quantitation of viral load, and are done in a closed-tube system that prevents crossover contamination by PCR products (215).

Signal amplification is a novel methodology that involves capturing sample nucleic acid in a microtiter well by hybridization with a number of primers targeted to different conserved regions of the genome. An amplification multimer (the branched label probe or bDNA) then hybridizes to the captured RNA complexes, and its signal can then be further amplified and detected by a chemiluminescent reaction. The test is currently marketed by Bayer Diagnostics as the Versant version 3.0 (Table 30.5). The test is highly standardized and reproducible and is accurate over a wide range of viral levels (187,216).

Limitations of hepatitis C virus ribonucleic acid measurement

A major problem and limitation of quantitative HCV RNA testing has always been standardization. This was especially problematic with early tests. A blinded survey of 31 laboratories in Europe demonstrated that only 16% correctly identified all samples in the coded test panel (217). A similar series of laboratory surveys in the United States found accurate identification ranging from 12% to 95% (218). Several factors may have explained these inaccuracies. Delayed serum separation, inadequate storage conditions, and specimen contamination can reduce the amount of nucleic acid in the specimen (219). The sensitivity of the actual assay is limited by the design of amplification primers, length of the amplicon, efficiency of reverse transcription, utilization of reaction substrates and other inefficiencies during amplification, dilution steps, and efficiency of postamplification detection systems (220,221). Even small inefficiencies are exponentially amplified during PCR, and therefore significant errors can be seen. A 12% reduction in efficiency early in the process (e.g., during primer hybridization) results in a 10-fold reduction of the product, and a 24% reduction in efficiency results in a 100-fold decrease (43). Primer hybridization efficiency may also be affected by viral heterogeneity, even within the well-conserved 5′ UTR region used for PCR amplification (87,95), and the use of synthetic RNA transcripts can result in different amplification efficiency for the various HCV genotypes (43). Although this problem potentially affects all PCR-based assay and bDNA systems, appropriate modifications of the reaction mixtures and primers have since corrected the differences in genotype sensitivity in the commercial tests (43,213). Despite the early problems with both qualitative and quantitative HCV RNA assays, testing has been made easier and more reliable by the availability of commercial assays that use familiar test formats that are easily adaptable to hospital laboratories.

Differences in methods lead to variability in quantitation of both the target RNA and standard. Therefore, results with different assays are generally not comparable (222–224). Incorporation of a World Health Organization standard into all assays and use of international units were implemented in the hope of resolving these differences, but have not. Therefore, it is important that clinicians understand the technology and limitations of the assays they or their laboratory choose to use. Because levels of HCV RNA measured by different assays are not necessarily the same, care should be taken to ensure that the same assay is used when

is important to document changes in virus levels (e.g., during treatment).

HEPATITIS C VIRUS GENOTYPING

HCV is a remarkably heterogeneous family of viruses, with at least six distinct genotypes and numerous subtypes of HCV identified throughout the world (86,87). The methods used to determine genotypes have been described in the preceding text (See "Genotyping"). Currently, the reverse hybridization line probe assay (LiPA, Bayer Diagnostics, Emeryville, CA) (97) is the most commonly used genotyping method in practice. HCV genotype is highly associated with response to antiviral therapy and is essential in determining the optimal treatment duration (See "Prevention and Treatment").

HISTOLOGY

The morphologic features of chronic viral hepatitis are similar regardless of the etiology. These features are described in detail in Chapter 26 of this book. In general, the degree of inflammation in chronic hepatitis C is mild to moderate. Severe bridging necrosis and confluent necrosis are unusual in chronic hepatitis C. A few histologic features are highly suggestive of chronic hepatitis C, although they are not entirely pathognomonic. These include epithelial damage of small bile ducts, lymphoid aggregates and sometimes lymphoid follicles in portal tracts, and microvesicular or macrovesicular steatosis (225,226). These histologic features are rarely seen in chronic viral hepatitis B or autoimmune hepatitis and therefore strongly suggest the diagnosis of chronic viral hepatitis C. The pathogenesis of these characteristic changes has not been determined, but the presence of lymphoid aggregates in portal tracts and lymphocyte infiltration of lobules and bile ducts suggest that immune mechanisms play a role in the mediation of cell injury.

Coexistence of chronic viral hepatitis C with other causes of liver diseases is not uncommon and this may modify the morphologic appearance of the liver biopsy. Alcoholic liver disease and chronic viral hepatitis C frequently coexist, and alcoholic liver damage may play an important role in the progression to cirrhosis and hepatocellular carcinoma (HCC) in these patients (227–229). Iron overload is more common in patients with chronic viral hepatitis C than in the normal population but does not seem to influence the severity of necroinflammatory activity or fibrosis of the disease (230). Other conditions include coinfection with other viruses, particularly hepatitis B (231,232), and nonalcoholic steatohepatitis. Occasionally, epithelioid granulomas of unknown etiology have been observed in the nodular parenchyma of patients with HCV-related cirrhosis (233).

Characterization of the degree of histologic injury is usually performed using one of several scoring systems that utilize descriptive terminology for inflammatory activity and fibrosis in an attempt to group similar degrees of histologic severity. The inflammatory (grade) portions of these different systems typically utilize combinations of numerical assessment for portal, periportal, lobular, and focal inflammation and therefore ignore the specific implications of inflammation in different portions of the lobule. However, inflammation is typically mild to moderate in patients with chronic hepatitis C and is therefore less likely to be important in determining change over time or in making clinical decisions. On the other hand, fibrosis is important in assessing prognosis and has become a critical piece of information in making treatment decisions. Therefore, a quantitative classification of fibrosis (stage) is important. The different staging systems are summarized in Table 30.6. The Knodell system was the first staging system but is noncontiguous and no longer used (234). The Ishak system uses four of its total of seven stages to subdivide the extent of fibrosis between periportal septae and marked bridging (235). This may be helpful in clinical studies when fine gradations of fibrosis pattern are required to demonstrate change, but this system is not commonly used in practice. The Scheuer and Metavir systems are essentially the same and each has five stages (236,237). Metavir is the most commonly used system in practice today. The accuracy of grading and staging is dependent on the adequacy of

TABLE 30.6. STAGING SYSTEMS FOR HEPATIC FIBROSIS PATTERN IN PATIENTS WITH CHRONIC HEPATITIS C

Score	Knodell	Ishak	Scheuer	Metavir
0	None	None	None	None
1	Portal	Portal	Portal	Portal
2		Periportal	Periportal	Septae
3	Bridging fibrosis	Focal bridging	Architectural distortion without cirrhosis	Bridging fibrosis
4	Cirrhosis	Diffuse bridging	Cirrhosis	Cirrhosis
5		Extensive bridging		
6		Cirrhosis		

the specimen and the experience of the pathologist, but considerable sampling error can exist as well (238,239).

Epidemiology

ACUTE INCIDENCE AND POPULATION PREVALENCE

Estimates of the incidence of acute hepatitis C infection in the United States have been extrapolated from age-specific prevalence data and indicate that the new infection rate was low (18/100,000) before 1965, increased rapidly for the next 15 years, and remained high (130/100,000) throughout the 1980s (Fig. 30.6) (240). Prospective surveillance studies conducted by the CDC in four sentinel counties in the United States have indicated a dramatic decline by more than 80% over the last decade (24,241). These rates correspond to 240,000 annual cases in the 1980s and approximately 28,000 to 35,000 cases per year since then (242,243).

The incidence of new infections is most common in young people (aged 20 to 39), especially Hispanics, with a slight predominance of men. The most common risk factor for new infection, accounting for 60% of cases, is intravenous drug use (Fig. 30.7) (243). Identifiable risk factors are present in most acute cases of hepatitis C. Between 10% and 40% of patients do not have a recent or readily identifiable risk factor for infection, although a history of remote high-risk behavior is common in them because such patients tend to underreport such behavior (244,245). Although the incidence of transfusion-associated hepatitis declined dramatically after 1984 because of the testing of potential donors with surrogate markers such as ALT and anti-HBc, and later anti-HCV, this had little impact on the more recent incidence of infection (241). In fact, the recent slowing of new cases is due to a precipitous fall in the occurrence of infections in intravenous drug users (246). This is surprising in the absence of specific public health changes, preventative therapy, or widespread testing for infection.

The prevalence of HCV infection throughout the world is low, averaging 2% to 3% or 170 million persons (21,247–251). These estimates are often based on volunteer blood donor prevalence rates and may therefore underestimate the true population prevalence (242). Nonetheless, these estimates provide some idea of the worldwide pattern of infection. Overall and age-specific prevalence varies considerably from country to country. Wasley and Alter suggests that this is due to the predominant risk factors (242). For example, in the United States and Europe, the prevalence is low and concentrated in young men who predominantly acquire infection in early adulthood from intravenous drug use (see subsequent text). By contrast, infection is most common in older persons in Japan and Italy, indicating a risk in the distant past. Furthermore, the high prevalence across all adult age-groups in Egypt indicates a common risk factor, namely medical injections (242,252,253). The antibody prevalence is low (0.01% to 0.1%) in the United Kingdom and Scandinavia; slightly higher (0.2% to 1%) in the United States, western Europe, Australia, and parts of South America and Africa; and intermediate (1% to 5%) in eastern Europe, the Mediterranean, Middle East, Indian subcontinent, Brazil, and parts of Africa and Asia (242). The highest prevalence is in northern Africa (6% in Zaire, 7% in Libya, and 17% to 26% in Egypt) (242,252,253). The

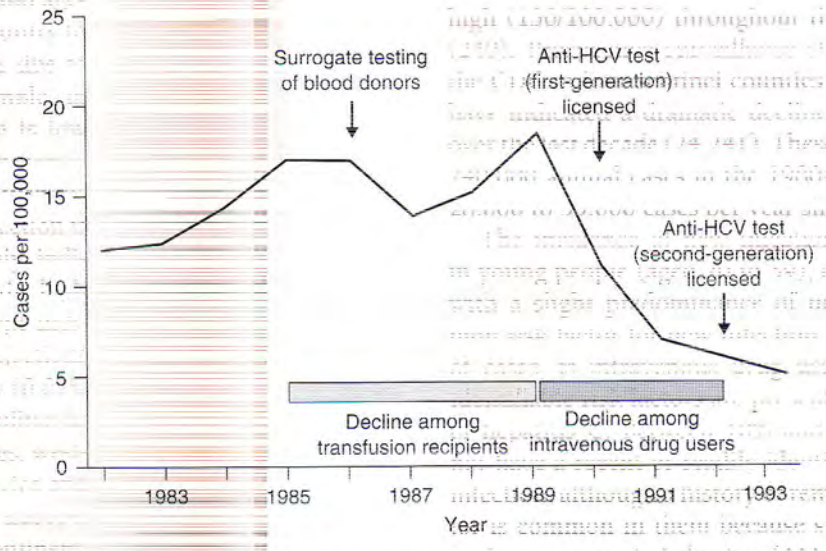
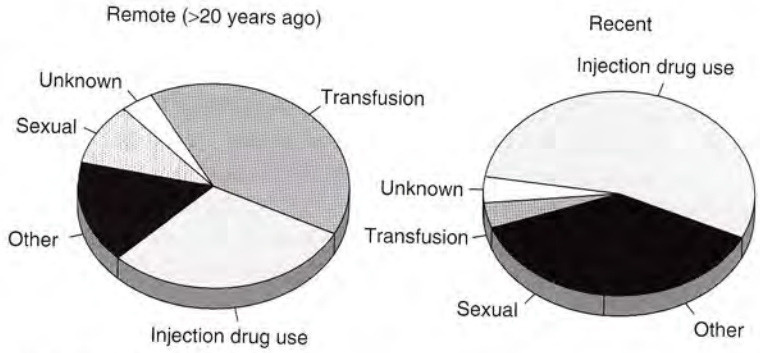


Figure 30.6 The incidence data in this chart indicate reported cases in the Centers for Disease Control and Prevention Sentinal County study of Acute Viral Hepatitis and are not indicative of the national incidence. (See reference 240 for further explanations of incidence calculations.)



■ **Figure 30.7** Sources of acute hepatitis C virus infection over time. (Modified after data from the Centers for Disease Control and Alter MJ. The epidemiology of acute and chronic hepatitis C. *Clin Liver Dis* 1997;1:559–568, with permission.)

prevalence is also high in some areas of Saudi Arabia (254) and isolated communities in Japan (255).

Although the prevalence of antibodies to HCV is approximately 0.6% in blood donors, the National Health and Nutrition Examination Survey, conducted during 1988 to 1994, found the prevalence of anti-HCV to be 1.8% in the general population of the United States (251,256). Given that approximately 80% of these are probably infected, this accounts for more than 4 million infected persons in the United States. However, some high-risk and high-prevalence populations have not been included in many prevalence surveys. There are currently more than 2 million incarcerated persons in the United States (257), and the prevalence of infection in this group is nearly one in four (258). Therefore, these prevalence estimates may underestimate the true rate. Prevalence begins to rise over the age of 20 and is highest among men aged between 30 and 49 years. African Americans and Hispanics have a higher prevalence of HCV infection than do whites, but the prevalence of HCV infection varies in the population on the basis of risk factors for infection (246). In contrast to what is currently observed in patients presenting with acute hepatitis, transfusion was a more important risk factor in the past (Fig. 30.7). Therefore, transfusion is a common identifiable risk factor only in patients older than 50 years (259).

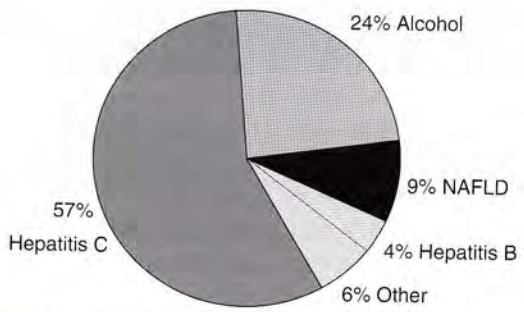
Chronic hepatitis C is the most common cause of chronic liver disease in the United States, accounting for 40% to 60% of cases (Fig. 30.8) (243,260). This results from the high incidence of acute hepatitis before 1990 and the propensity of acute infection to persist. Because progression to cirrhosis mainly depends on the duration of infection (see subsequent text), each year an estimated 8,000 to 10,000 deaths result from HCV-associated chronic liver disease (243). Most patients who develop cirrhosis have had infection for more than 20 years (261). In the year 2000, approximately 30% of patients with chronic hepatitis C had a history of infection for at least this long (262). This proportion will increase in the future with the cohort of chronically infected patients' age. It is estimated that by the year 2010, more than 60% of patients will have had infection for more than 2 decades (262,263). This has obvious and significant implications for the prevalence of cirrhosis in the infected population. Mathematic models estimate that the proportion of HCV-infected patients with cirrhosis will increase by more than 50% (from 22% of infected cases to 35%) and that complications of cirrhosis such as liver failure and HCC will nearly double (263).

ROUTES OF TRANSMISSION

HCV can be transmitted through a variety of routes. Infection is most efficiently transmitted by large or repeated percutaneous exposures such as through transfusions, transplantation from an infected donor, or sharing illicit drug paraphernalia. Transmission of HCV may also occur from exposures to infected contacts through sexual activity, household contacts, perinatal exposure, and parenteral exposures in the health care setting (264).

Intravenous drug use

Intravenous drug use has been the most common risk factor for acquiring HCV infection for more than



■ **Figure 30.8** Etiology of newly diagnosed cases of chronic liver disease. NAFLD, nonalcoholic fatty liver disease. (See reference 260.)

25 years and currently accounts for 60% to more than 90% of new infections (Fig. 30.7) (265,266). Sharing of needles and other paraphernalia during parenteral drug use is an extremely efficient means of transmitting infection (243,267). Although both the incidence and prevalence of HCV infection remain high in this group, the incidence of acute hepatitis C among intravenous drug users has declined dramatically since about 1989 (Fig. 30.6). HCV is rapidly acquired after initiation of drug injection behavior; 50% to 80% of users became anti-HCV positive within 12 months of initiation of drug injection behaviour (268), and nearly all seroconvert by 8 years (269). The risk factors for acquisition of HCV include frequent use, shared paraphernalia, injecting cocaine, sharing with an older user, and long duration of use (270,271). However, it has also been suggested that intranasal cocaine use may be a risk factor (272). Although this route of infection might be possible (e.g., sharing of blood-contaminated straws), it is likely a surrogate for injecting behavior. In addition, two other factors appear to be important in facilitating exposure in this group. Although about a half of infected drug users are either aware of or willing to admit seropositivity, and many continue high-risk behavior despite knowing that they have infection and are at a risk of spreading it to others (244,273).

Health care workers; other percutaneous routes

Health care workers have increased exposure to patients infected with HCV. A serologic survey of emergency department patients found that 18% were infected with HCV (274). The proportion with HCV infection was even higher in patients with a history of intravenous drug use (83%), past blood transfusion (21%), or a male homosexual lifestyle (21%) (274). Although all potential routes of transmission of HCV infection to hospital workers are not obvious, needle-stick injuries probably account for a large proportion of cases. Cases of needle-stick transmission of HCV have been clearly documented (275). Skin exposure to blood is not thought to be a risk factor (276). A recent review of follow-up studies of health care workers who sustained percutaneous exposure to blood from anti-HCV–positive patients found that anti-HCV seroconversion after accidental needle-stick/sharp exposures averaged only 1.8% (range, 0% to 6.6%) (Table 30.7) (243,277,278). The risk is greatest with the hollow needles used to draw blood, as compared to hollow infusion needles (278). In another study, an incidence of 10% was found on the basis of detection of HCV RNA by PCR (279). In these prospective studies, none of the infections was associated with mucous membrane or exposure to nonintact skin, although there have been case reports of the transmission of HCV from a blood splash to the conjunctiva (280,281). Several points deserve comments with regard to the risk of transmission by isolated percutaneous exposure. First, the reported risk of transmission by needle stick is greater for HCV than for human immunodeficiency virus (HIV) or HBV (assuming that hepatitis B immunoglobulin is given) (282,283). Second, even a low risk of infectivity has grave implications, given the high risk of progressing to chronic infection.

Although health care workers have increased risk of being exposed to HCV infection, it is debatable whether this occupational exposure results in more than an occasional infection. There were several early reports with seroprevalence rates in health care workers ranging from 0.6% to 4.5%; in all surveys these exceeded the rates in blood donors from the same institution by as much as 4.5-fold (284–286). For example, dentists and oral surgeons had seroprevalence rates of 1.75% and 9.3%, respectively, compared with 0.14% in their patients (287). However, other surveys have not found an increased prevalence among health care workers, even in those with regular exposure to blood, including surgeons, dentists, and early responders (276,282–288).

The risk of nosocomial transmission of blood-borne infections has been dramatically reduced by the use of screening of blood and organ donors, effective

TABLE 30.7. RISK OF HEPATITIS C VIRUS TRANSMISSION AFTER EXPOSURES TO ANTI–HEPATITIS C VIRUS–POSITIVE BLOOD

Exposure	Number tested	Number seroconverted (%; range)
Needle sticks/sharps	911	16 (1.8; 0–6.6)
Hollowbore	331	4 (1.2)
Other	105	0
HCV RNA–positive	68	7 (10.3)[a]
Mucous membrane	114	0
Nonintact skin	165	0
Total	1,302	16 (1.2; 0–6.6)

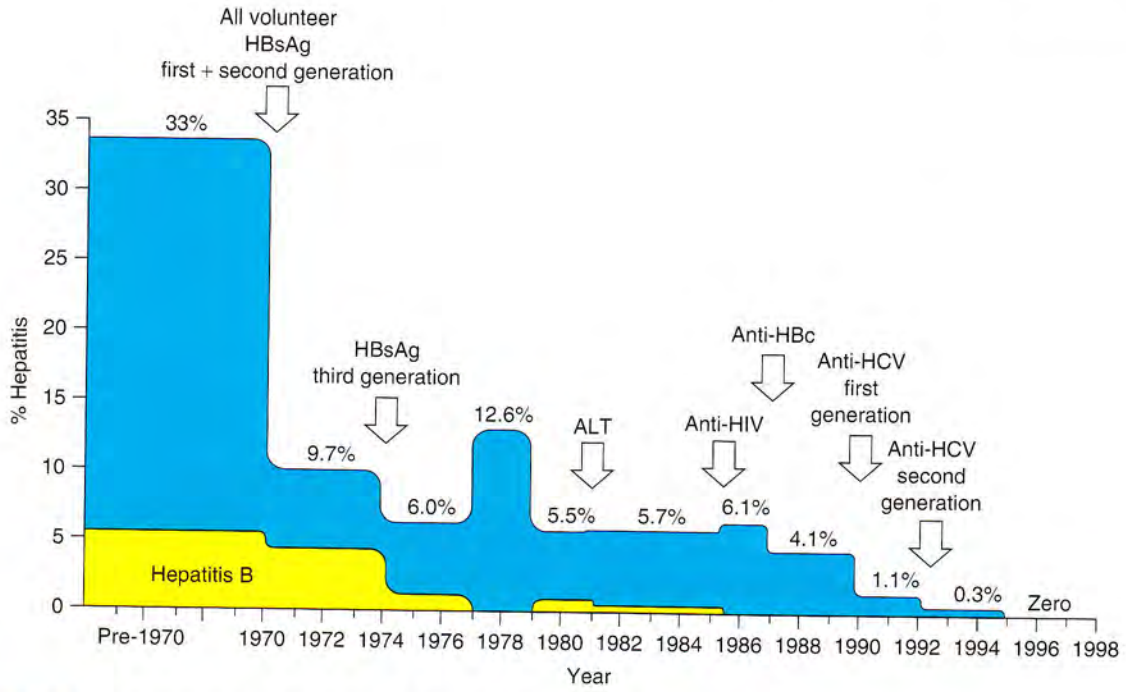
[a]HCV RNA–positive.
HCV, hepatitis C virus; RNA, ribonucleic acid.
See Alter MJ. The epidemiology of acute and chronic hepatitis C. *Clin Liver Dis* 1997;1:559–568.

disinfection protocols, disposable equipment, and universal precautions. Transmission of HCV from medical procedures or personnel is exceedingly rare when these precautions are followed. Nonetheless, some cases of nosocomial HCV infection have been reported, although they have usually been associated with breaks in usual technique, such as reusing equipment or multidose drug vials in different patients (See "Hemodialysis") (289). The risk of transmission from an infected health care worker is very low and there does not appear to be sufficient evidence to restrict the practice of these workers. Modeling projections estimate that the risk of transmission from an infected surgeon to a patient is 0.014% or approximately 1:10,000 (290). Nonetheless, there have been two reports of transmission from infected cardiothoracic surgeons. The first, from Spain, involved transmission to five patients over a 6-year period, but the factors responsible for transmission were not identified (291). The other report, from the United Kingdom, found transmission to 1 of 277 at-risk patients during a 1-year period (292).

In countries other than North America and western Europe, unsafe injections, especially involving reusable needles during routine or mass inoculations, have been an important route of nosocomial transmission of HCV. Such practices probably explain the high seroprevalence in Egypt, although these practices appear to have improved (252,253). In many areas, unsafe injection practices, such as reuse of syringes and needles or unnecessary injections, continue (293). Although unsafe injection practices may still be typical in some areas, the lack of available medical supplies is also a factor. Other causes of percutaneous transmission of HCV have included contaminated instruments, equipment, and supplies that were used during the performance of procedures involved in traditional medicine, folk medicine, tattooing, body piercing, and commercial barbering (294–299). These routes of transmission have not been documented in the United States.

Transfusion associated

HCV is easily transmitted by blood and blood products (22,300,301). In the past, HCV was the major cause of post-transfusion hepatitis, accounting for at least 85% of cases (22,302). As a result, transfusion has often been thought to be the predominant route of transmission of the infection. Early epidemiologic studies supported this because 37% to 58% of patients with chronic hepatitis C, particularly those older than 50 years, gave a history of blood transfusion before 1990 (259). However, the risk of acquiring post-transfusion hepatitis has declined dramatically in recent years (Fig. 30.9) (243,267,303–305). The largest drop in post-transfusion hepatitis incidence resulted from adoption of a volunteer donor system and, to a lesser degree, mandatory testing of donor units for hepatitis B surface antigen (HBsAg). Introduction of testing for surrogate



■ **Figure 30.9** Risk of post-transfusion hepatitis due to non-A, non-B/hepatitis C in transfusion recipients over the last 3 decades. HbsAg, hepatitis B surface antigen; ALT, alanine aminotransferase; HIV, human immunodeficiency virus; HBc, hepatitis B core; HCV, hepatitis C virus. (See reference Alter HJ, Houghton M. Clinical Medical Research Award. Hepatitis C virus and eliminating post-transfusion hepatitis. *Nat Med* 2000;6:1082–1086, with permission.)

markers of non-A, non-B hepatitis (serum ALT and antibodies to the hepatitis B core [anti-HBc] antigen) reduced the risk of transfusion-associated hepatitis by nearly two thirds (305). Further reduction in the risk of post-transfusion hepatitis was observed after institution of testing for antibodies to HIV and screening of donors for a history of risk factors. Finally, introduction of donor testing for antibodies to HCV was initiated on May 2, 1990, shortly after the discovery of the virus. Retrospective testing of sera from donors and patients who participated in the multicenter Transfusion-Transmitted Viruses Study between 1974 and 1979 predicted that screening of donors for anti-HCV alone would reduce the risk of hepatitis to a level comparable with that of the nontransfused control population (300). Indeed, the incidence of transfusion-associated HCV infection is currently estimated at 0.01% to 0.001% per unit transfused sera (306).

In the past, multiply transfused recipients of blood and blood products had an extremely high risk of acquiring HCV infection. The likelihood of multiply transfused patients developing HCV infection before institution of anti-HCV donor screening was 8.3% in a trauma intensive care unit setting (307) and 18% in a burn unit (308). Patients with transfusion-dependent hemolytic disorders such as thalassemia (309) or hemophilia were at particularly high risk (310–312). The risk in patients with hemophilia stemmed from the requirement that factor concentrates be prepared from plasma pooled from hundreds of individuals, who, in many cases, were commercially paid donors (311,312). Before initiation of donor screening, the seroprevalence of anti-HCV ranged from 10% to 16% in paid donors as compared to less than 0.5% in volunteer donors (22,313). Before 1990, between 60% and 90% of factor-dependent patients with hemophilia had serologic evidence of HCV infection (314). HCV infection was more prevalent in those who received greater volumes of concentrate, especially unpasteurized products, and was virtually nonexistent in patients who either had not required factor transfusion or had received exclusively vapor-treated factor concentrates (311). The recent move of vapor-heat sterilization of pooled plasma concentrates and recombinant clotting factors has nearly eliminated the risk of acquiring hepatitis C from replacement therapy (315,316). However, an outbreak of hepatitis C 10 years ago was reportedly associated with contaminated intravenous immunoglobulin (317). This outbreak involved recipients of a single product produced from the plasma that had been screened by the second-generation anti-HCV assay but on retrospective testing remained positive for HCV RNA (318). Intramuscular immunoglobulin has never been associated with the transmission of any infectious disease in the United States. Currently, all immunoglobulin products (intravenous and intramuscular) commercially available in the United States must undergo an inactivation procedure and be HCV RNA–negative before release.

Hemodialysis

HCV infection is common in patients on dialysis and, currently, is present in approximately 8% of patients in the United States (22,248,319–323). This may, however, be an underestimate because approximately 4% to 15% of infected patients have falsely negative antibodies to HCV (209,321,322). Most dialysis patients are already infected when they present with end-stage renal disease, but acute infection is common in dialysis centers (320,323–325). The annual risk of acute HCV infection is currently estimated by the CDC to be 0.15% in hemodialysis patients and 0.03% in continuous ambulatory peritoneal dialysis patients (relative risk, 5.7). Others have estimated the annual incidence to be as high as 0.44% to 1.7% (320). Chronic HCV infection develops in most of those who are acutely infected (70% to 90%) (319). About a third of acute HCV infections are acquired outside the dialysis unit (319,325). However, nosocomial outbreaks have been reported in hemodialysis centers and have been confirmed by molecular sequencing and mapping (326,327). Such infections are usually related to breaks in infection control procedures. Dedicated dialysis machines might reduce much of this risk (328), but this is not common practice in the United States. Other factors such as reuse of multiuse vials and care by specific health care workers have all been implicated (327,329). The cause in many cases remains elusive (329). The improved safety of the blood supply, the availability of recombinant erythropoietin, and the phasing out of pretransplant "immune conditioning" transfusion protocols have significantly reduced the risk of hepatitis C in these patients.

Transplantation

The magnitude of risk assumed by receiving an organ from a donor with HCV infection is subject to some debate. However, HCV infection is not uncommon in cadaveric donors (330,331). In a large study of cadaveric donors evaluated by eight procurement centers in the United States, 4.2% were anti-HCV positive and 2.4% of these were HCV RNA positive by PCR (332). There is no doubt that recipients of organs from these HCV RNA–positive donors are likely to develop infection and liver disease. Some studies have shown nearly universal transmission from anti-HCV–positive donors to recipients (333), whereas others have been unable

to demonstrate such a strong association (331,334). Pereira et al. reported that 75% of the 29 recipients of organs (19 kidneys, 6 hearts, and 4 livers) from 13 anti-HCV–positive donors became anti-HCV or HCV RNA positive (333). By contrast, Roth et al. found post-transplantation liver disease in only 13 of 46 (29%) recipients of kidneys from RIBA-positive donors. HCV RNA levels were not checked (331). Despite the latter study, concern over the risk of transmission is great. Most procurement organizations and transplantation centers have now developed policies for selective utilization of organs from anti-HCV–positive donors (335,336). Modeling strategies have demonstrated that patients without HCV infection who receive an organ from an infected donor incur high cost and have a poor outcome (336). Therefore, donor screening is a necessity. Exclusion of all anti-HCV–positive or HCV RNA–positive donors would incur high costs through the loss of up to 4% of donors and extended waiting times (336). The most cost-effective strategy appears to be transplantation of HCV-positive organs into patients already infected with the virus (336). Most studies have shown that HCV-positive patients who are recipients of an HCV-positive graft have the same graft and recipient survival as those who receive an HCV-negative organ (335,337,338). Furthermore, the waiting time is usually shorter for those who elect to receive an HCV-positive organ (339). After transplantation, either the recipient or the donor strain may become predominant after a few weeks, and this strain then generally persists indefinitely (340). Although the limited data in humans to date does not suggest a genotype advantage in such cases, genotype 1b appears to overtake other genotypes in chimpanzees (341). Despite these many reports to the contrary, however, one recent study has questioned the advisability of using HCV-positive donor organs for kidney transplantation in patients with chronic HCV infection (342).

Sexual transmission

Although sexual transmission is frequently listed in the epidemiology literature as a common risk factor for acquisition of hepatitis C, the data supporting this is poor. Although cases of probable sexual transmission have indeed been reported (343), the extent to which sexual transmission of HCV occurs is not known and it is likely to be exceedingly uncommon during normal sexual activity (264,344–351). The risk of transmission in prospective studies of monogamous heterosexual couples is estimated to be near zero (347–350). Although these prospective studies have documented a few cases of HCV infection in spouses, these were either phylogenetically unrelated or the spouse had ongoing risk factors in addition to sexual exposure.

The likely explanation for most cases attributed to sexual or so-called sporadic transmission is underreporting of risk factors. One and often many risk factors are present in at least 80% of carefully questioned patients with chronic hepatitis C, but sexual behavior has not been implicated with any certainty as a sole risk factor (352). The risk of overestimating the importance of sexual transmission by not carefully assessing other risk factors has been emphasized (353). Indeed, although the prevalence of infection is usually increased in groups with high-risk sexual practices, it is difficult to exclude all other risk factors for infection. Furthermore, among sexually active individuals without other apparent risk factors attending sexually transmitted disease clinics, the risk does not appear to be increased (344,345).

This being said, there does appear to be circumstantial evidence for sexual transmission among those who practice high-risk sexual behavior. There is a higher seroprevalence of antibodies to HCV, ranging from 0.8% to 22%, in men who have sex with men (MSM) (248,259). In population surveys, antibody prevalence is sixfold higher in MSM than in heterosexuals (259). The risk for HCV appears to be related to the number of sexual contacts, acquisition of other sexually transmitted diseases, use of noninjection drugs during sexual activity, and traumatic sexual practices, particularly anal intercourse (354,355). Heterosexual intercourse, particularly with multiple exposures, is also likely to play a role, although probably a small one, in the transmission of HCV infection. Antibodies to HCV is common in sexual partners of intravenous drug abusers (6%), prostitutes and their clients (3.5% to 9% and 16%, respectively), and heterosexuals with multiple sexual partners (4% to 6%) (343,354). Nonetheless, direct documentation of HCV transmission by high-risk sexual practices has been difficult to obtain. Furthermore, the transmissibility of HCV by this route appears to be extremely low by comparison with HBV, HIV, and other sexually transmitted diseases (354). In one study of sexual partners of HIV–HCV coinfected individuals, 29 pregnancies, one HIV seroconversion, and no cases of HCV infection occurred during an estimated 5,800 unprotected vaginal or anal contacts (351). Importantly and not to be forgotten, barrier contraception with condoms has been shown to reduce the risk of transmission of sexually transmitted infections.

Perinatal transmission

Perinatal transmission is known to occur, but the predominant route (i.e., intrauterine, intrapartum, or perinatal) is not known (356). Antibodies to HCV usually passively transferred from the infected mother to the infant and may remain detectable in the baby for

up to a year (357,358). The risk of transmission of HCV from viremic (HCV RNA–positive) mothers to their infants is 3.2% (356,359). The risk of transmission in viremic women coinfected with HCV and HIV (HCV RNA positive) is 7.9% (359). This higher rate of transmission may, in part, relate to higher levels of HCV RNA in coinfected women; however, some studies have not shown a relationship between risk and viral levels (356,358,360,361). The rate of mother-to-infant transmission is similar for vaginal and caesarean delivery (356). However, prolonged duration of membrane rupture appeared to increase the risk of infection in the infant in one study (362). HCV is not transmitted by breast-feeding (363). Finally, despite development of infection (HCV RNA positive) in the infant, occurrence of abnormal serum ALT levels or liver disease is unusual, with a weighted rate of 1.7% (356–359).

Sporadic hepatitis

Large public health surveys have suggested that up to 40% of patients with acute hepatitis C have no identifiable risk factor (24,241,267,284). These cases have been called *sporadic* or *community-acquired hepatitis*. The implication is that such cases result from previously unidentified modes of transmission. However, the fact that nearly all anti-HCV–positive patients with chronic liver disease have identifiable risk factors suggests the possibility that the number of sporadic cases is not as great as that reported, and the lack of identifiable risk factors may be more dependent on the patients' recognition of or willingness to reveal their high-risk behaviors (353,364).

The prevalence of HCV infection in the population and the difficulties in clarifying its epidemiology make it obvious that a search for other routes of transmission is important. Although some studies have failed to identify nonparenteral routes of transmission (346), familial and community clustering of hepatitis C cases with seroprevalence of antibodies to HCV ranging from 0% to 34% have been reported (365,366). Although sexual partners appear to be at increased risk in such surveys (366,367), a similar antibody prevalence has been noted in nonsexually exposed family members including children, parents, and siblings, particularly in the older individuals exposed for a longer period (367,368). Sharing of hygiene items such as combs, razors, toothbrushes, and nail scissors has been proposed as a possible means of transmission in such cases (369). Saliva has been implicated as a vector of transmission (370), and indeed, infection through saliva has been documented in chimpanzees (371) and by a human bite (372). However, the existence of infectious virus in saliva is controversial (370,373,374). Occasional casual contact is not likely to transmit infection, and only 3% of patients with acute HCV infection give such a history (284).

Natural History

ACUTE HEPATITIS C VIRUS INFECTION

Clinical presentation

Acute hepatitis C is typically asymptomatic and unrecognized. When identified prospectively, cases present with an elevation of the levels of serum aminotransferases anywhere from 2 to 26 weeks after exposure (375). The mean incubation period is intermediate between that for hepatitis A and hepatitis B, with a peak onset of 7 to 8 weeks after infection. Eighty percent of cases occur between 5 and 12 weeks (376). It is not known whether the route of infection, inoculum size, viral genotype, or other factors influence the variability in the incubation period. However, a shorter incubation period was noted in several early reports of hepatitis after transfusion of pooled blood products (377,378). Serum ALT levels are usually high, with about three fourths of patients having elevations more than 15 times the upper limit of normal (379). HCV RNA is present in the blood within days of exposure and usually remains detectable throughout the infection (380). By contrast, anti-HCV is often not detectable until 5 to 6 weeks after exposure (204,206). The mean times to detection of anti-HCV by the second- and third-generation tests are 10 weeks and 7 to 8 weeks, respectively (204,206). Symptoms occur in less than 30% of patients and are usually so mild that they do not interfere with the daily routine (24). When present, the symptoms of acute hepatitis C are nonspecific and do not differ from other forms of hepatitis. The most common symptoms are flu-like and include anorexia, weight loss, abdominal pain, myalgia, arthralgia, and fatigue. Less common symptoms include fever and rash. Jaundice occurs in less than one third of all patients (24,379) and is most common in symptomatic patients (375). The symptoms associated with acute hepatitis usually resolve within 1 to 3 months.

Fulminant hepatic failure due to HCV is extremely uncommon (381). Although some early studies reported detection of serum HCV RNA in up to 60% (mean approximately 10%) of fulminant hepatitis cases without an obvious etiology (382–386), most clinicians feel that HCV accounts for only rare cases. Large studies in the United States and Japan have failed to identify any case (387,388).

Risk of chronicity

Early reports based on observations in transfusion recipients suggested that about half of the patients with

acute non-A, non-B hepatitis recovered spontaneously; later reports challenged that this event occurred in only 10% to 20% of cases (376,389,390). It is clear that there is considerable variability in the risk of progression to chronic infection, with a range from 50% to 90% (390,391). Resolution of acute infection appears to be more common in young people and women but may also be related to viral genotype (391). Clearance of acute infection is documented by persistent loss of detectable virus because liver test results may normalize or the virus may become transiently undetectable in some patients who go on to develop chronic infection (392). Indeed, chronic viremia persists in at least half of the 30% to 40% of cases in whom the serum ALT level returns to normal (375). Overall, approximately 80% of acutely infected patients develop chronic infection (viremia) and most of these (80% to 90%) have chronic hepatitis with elevated ALT levels (248,393–399). Factors that determine viral clearance or persistence are not clear. Although several retrospective serologic studies have identified anti-HCV–positive patients who are HCV RNA negative, suggesting the possibility of late viral clearance, it is not possible to exclude the possibility that in these cases virus clearance occurred during acute infection (396,400,401). Indeed, most data suggest that spontaneous eradication of chronic infection is extremely unusual (402).

CHRONIC HEPATITIS C VIRUS INFECTION

Clinical presentation

Most patients with chronic hepatitis have asymptomatic elevations of serum aminotransferase levels and do not have physical signs of liver disease (403). Only about 6% have symptomatic liver disease (404). Fatigue is the most common symptom, but its onset is insidious and is usually mild (403). Dull right upper quadrant pain, which is often intermittent and positional, is also common. Less common symptoms include anorexia, nausea, pruritus, arthralgia, and myalgia. Importantly, although symptoms are more common in patients with fibrosis or cirrhosis, the correlation with the histologic severity of disease in individual patients is poor (403). The physical examination may be more helpful once cirrhosis has developed. In these patients, a palpable firm liver is present in 79%, splenomegaly in 34%, and stigmata of chronic liver disease in 31% (405).

Serum ALT levels are usually only mildly elevated. Up to one third of patients have normal serum ALT levels, whereas only about 25% have a level more than twice normal (261,405,406). However, there is a wide variability in enzyme level elevation when patients are followed up over time. In those with ALT level elevations, the levels are persistently elevated in only 26% of cases and elevated in most determinations in 22%. ALT levels fluctuate down to the normal range in 17%. Finally, 30% to 40% of cases have only occasional ALT level elevations over a 12- to 18-month period of testing (375,376). Furthermore, although in group analyses higher ALT levels suggest more hepatic inflammation, the variability is marked and ALT has little predictive value in individual patients (261,403,407,408). Only a 10-fold or greater elevation of serum ALT level is predictably associated with significant piecemeal necrosis (408). Therefore, ALT alone should not be used to estimate disease severity, prognosis, or necessity of treatment.

Histologic and clinical progression

Although chronic hepatitis C develops in approximately 80% of acutely infected individuals, the disease progresses slowly, if at all, in most patients. However, the true rate of histologic progression has been the subject of debate and uncertainty. Some authorities have suggested that progression to severe end-stage liver disease is inevitable provided the infected person does not succumb first to another lethal illness, whereas others have concluded that disease progression is extremely unusual and restricted to a limited few. These opposing views can be accounted for by the slow pace of progression, the usual lack of symptoms in chronically infected patients, and the limitations of available natural history data (390). For example, studies based on exposed or acutely infected patients such as the post-transfusion hepatitis studies of the 1970s report few significant sequelae of infection (see subsequent text), whereas short-term prospective and retrospective studies of patients who already have established liver disease clearly show a significant risk of progressing to cirrhosis, liver failure, and HCC (409–411). The limitations of each of these types of studies have been discussed (411). Interestingly, mathematic models of the natural history of chronic hepatitis C appears to resolve this debate (412). These clearly demonstrate that both perceptions of the natural history are accurate, but progression is more easily observed in studies focused on the later stages of the disease.

Several long-term follow-up studies of recipients of contaminated blood products show that few patients with acute hepatitis C progress to liver failure and liver-related death. The classic study by Seeff reported the outcome in patients who had participated in five transfusion surveillance studies from 1968 to 1980 and had been prospectively followed up after the development of acute transfusion-associated non-A, non-B hepatitis (mostly hepatitis C) (404,413–417). Approximately half of these individuals were known to have developed chronic hepatitis with elevated aminotransferase levels, and, of these, slightly more than 30% had developed cirrhosis (411). Although more than 40% of patients with cirrhosis had some

evidence of hepatic decompensation, all-cause mortality was not significantly different from that of transfusion recipients who had not developed hepatitis (404). Most deaths were due to cardiac disease, which was not unexpected because most subjects received their transfusions during cardiac surgery. Nevertheless, liver-related mortality after 18 years was higher among patients with hepatitis (3.2%) than in controls (1.5%). A follow-up report 7 years later found a liver-related mortality of 4.1% versus 1.3% in controls (418). In both groups, liver-related mortality was strongly correlated with alcohol consumption. A similar study among HIV-negative, transfusion-dependent patients with hereditary bleeding disorders found liver-related mortality of just 3.4% after 25 years (396). A major limitation of transfusion studies is the high (50% to 67%) all-cause mortality related to the comorbid conditions that necessitated transfusion in the first place (404,418,419). However, long-term retrospective data are available in healthy young women who received HCV-contaminated lots of immunoglobulin products more than 20 years ago (420–422). More than 400 of the 53,178 (0.8%) recipients of eight HCV-contaminated lots of anti-D immunoglobulin in Ireland in 1977 were found to be anti-HCV positive on subsequent testing 17 years later (420). Liver biopsies revealed mild or moderate hepatitis in 93%. Although 15% already had bridging fibrosis, only 2% had cirrhosis. In a similar study from Germany, 160 of 2,533 (6.3%) women who received anti-D immunoglobulin between 1978 and 1979 became anti-HCV positive (421). Slightly more than half developed chronic hepatitis, but a large proportion appeared to recover completely. The low transmission and high recovery rates in these studies are quite atypical and may relate to the size of the inoculum, age of recipients, or both. Nonetheless, taken together, the studies in transfusion and immunoglobulin recipients confirm that most patients with acute hepatitis infection do well and are not at high risk of developing cirrhosis or liver failure, even after 20 to 25 years.

In contrast to these studies, some prospective studies of patients with acute post-transfusion hepatitis C have reported cirrhosis in 16% to 24% after follow-up of just 8 to 14 years (262,409,423–425). The prevalence of cirrhosis may increase to more than 40% after 40 years (426). Furthermore, the subset of patients who present with established chronic hepatitis C appears to have an even higher rate of progression to cirrhosis, liver failure, and liver-related death, although a self-selection bias is probably responsible for this observation (261,427–429). Evidence to support this derives from the estimated duration of infection in patients presenting with complications of liver disease; it is similar to the period of observation in the transfusion studies described earlier. Retrospective studies of patients with cirrhosis have suggested that the mean duration of infection before the development of cirrhosis is about 21 years, although this interval may occasionally exceed 50 years (261,430).

The rate of progression to cirrhosis has been estimated in several studies but is probably not linear in most individuals and depends upon several host and environmental factors (see subsequent text). The annual rate of fibrosis progression is estimated by dividing the fibrosis score (Metavir stage) by the estimated number of years after infection (431,432). Using this method, the average rate of fibrosis accumulation is 0.133 units per year (95% CI, 0.125 to 0.143) in patients with elevated serum ALT levels (432,433). Therefore, if progression is linear, it will take an average of 30 years to develop cirrhosis. However, many factors may influence the rate of progression in individuals, and therefore, it has been proposed that most patients fall into one of three groups with fibrosis rates that are rapid (less than 20 years to cirrhosis), intermediate (late cirrhosis), or slow (no cirrhosis or after 50 years) (433). Patients with persistently normal liver test results fall into the latter group because their mean fibrosis rate is only 0.05 units per year, which extrapolates to a mean time to cirrhosis of 80 years (434). Hepatic inflammation also influences the rate of progression (428,433). The 10-year risk of cirrhosis is less than 10% to 13% among patients with minimal or mild chronic hepatitis and 44% to 100% in those with moderate hepatitis (Table 30.8) (428,433).

There are several environmental, host, and viral cofactors that accelerate disease progression. The most important of these cofactors is alcohol intake (227,435). The association between HCV infection and alcoholic liver disease was first noted in early epidemiologic surveys of anti-HCV prevalence (435–437). It is now apparent that regular alcohol intake, particularly heavy intake, may accelerate liver injury in persons with chronic HCV infection (435–441). The risk of progressing to cirrhosis appears to be 1.5 to 3 times higher in those who consume alcohol (435,438,439,442–445). Furthermore, the effect appears to be dose–dependent, with a 15-fold higher

TABLE 30.8. LIKELIHOOD OF HISTOLOGIC PROGRESSION TO CIRRHOSIS

Initial histology	Risk of cirrhosis (%)		
	5 y	10 y	20 y
Minimal-to-mild hepatitis	7	7	30
Moderate hepatitis	25	44	85
Severe hepatitis	68	100	100
Bridging fibrosis	58	100	100

Adapted from Yano M, Kumada H, Kage M, et al. The long-term pathological evolution of chronic hepatitis C. *Hepatology* 1996;23:1334–1340.

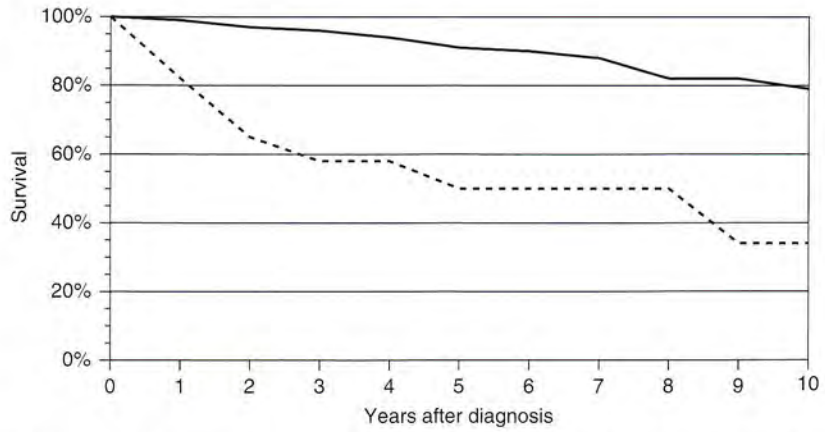
risk in the heaviest consumer compared to teetotalers (446). Alcohol presents greater risk for progression than duration of infection, age, gender, or coinfection with either HBV or HIV (444). Finally, patients who die from liver disease are more likely than others to be alcohol users (447). It is not yet clear whether the mechanism for this outcome is increased HCV replication or an additive injury from both the virus and the alcohol (228,435,448). Heavy alcohol use has been shown to inhibit hepatic expression of Bcl-2, an inhibitor of apoptosis, and to increase oxidant exposure in patients with chronic hepatitis C (449,450). Regardless of the mechanism, the outcome of alcohol use is clear and its use in persons with chronic HCV infection is to be strongly condemned. Evidence supporting a role for other environmental factors, such as toxins, in the progression of HCV infection is limited.

Other host-related factors are also important. The rate of disease progression seems to increase if HCV infection is acquired after the age of 50 to 55 years (261,428,432). Age may also play a role in those already infected chronically and might explain the more rapid progression that sometimes occurs as the disease advances beyond the second decade (428,432). The low rate of both acute and chronic infection in the contaminated anti-D immunoglobulin studies suggests the possibility that female gender, possibly in combination with young age, might reduce the risk of infection (420,421). Other host-related factors, particularly ethnic background, deserve further study because there is some evidence that disease progression and the development of HCC is more common in Japan and Italy (451). African Americans appear to have histologically somewhat less severe liver disease than do Hispanics or whites (452). Finally, host immune status may influence progression. Some studies suggest that HLA DRB1 might be important in the susceptibility to infection (453), and other phenotypes might be important in defining the host's ability to regulate viral replication (454). Furthermore, immunosuppression from HIV infection or immunosuppressive drugs after organ transplantation may accelerate disease. HIV-infected patients have higher HCV levels (455) and a higher rate of fibrosis progression, although there may be other confounding factors involved (400,456). Progression to cirrhosis is threefold higher in HIV-infected persons and is more common in patients with low CD4 counts (457). Liver transplantation and the necessity for exogenous immunosuppression results in accelerated liver injury. HCV infection persists in almost all patients who undergo transplantation for chronic hepatitis C and results in severe and progressive chronic hepatitis in many (458,459). Virus levels increase 10- to 15-fold after transplantation (459). The actuarial risk of developing cirrhosis is 3.7%, 8.5%, and 28% after just 1, 2, and 5 years of transplantation, respectively (460). More than 40% of these patients with cirrhosis will decompensate within 1 year, and survival is only 41% within a year of the decompensation event (461). Surprisingly, however, rapid progression and decreased early survival is not present in recipients of other solid organs or bone marrow (462–464).

There is currently insufficient data to suggest that viral factors influence the progression of HCV infection. Early studies suggested that genotype 1 caused more severe disease than genotype 2 (465) and that 1b was more harmful than 1a (466,467). However, the relationship of genotype 1 to more severe disease may be related to a longer duration of infection, and more recent studies suggest that genotype plays no role in influencing disease outcome (468,469). However, viral genotype significant influences treatment response and may therefore have considerable impact on the long-term disease outcome (see subsequent text).

Liver-related morbidity and mortality among HCV-infected persons is directly related to complications of cirrhosis including hepatic failure and HCC (405,428,429). Liver-related mortality in those with compensated cirrhosis is low before the first episode of decompensation supervenes or the onset of synthetic dysfunction (Fig. 30.10) (405,470). Among patients with compensated cirrhosis, the annual risk of decompensation is 3.9% (Fig. 30.11). Decompensation is most commonly manifested by the development of ascites (48%) or variceal hemorrhage (22%), although multiple complications may occur simultaneously (17%) (405,470). At least one third of deaths in patients with cirrhosis occur as a consequence of these two complications. When ascites first presents, it can usually be managed quite easily with diuretics and salt restriction. Varices can often be managed effectively with endoscopic therapy. However, one should not be lulled into complacency with patients who can be managed medically because the excess mortality is still 10% to 33% after 3 years (471). Therefore, such patients should be considered for transplantation at the time when ascites or bleeding varices first appear. Survival in patients with decompensated cirrhosis is poor, with only approximately 50% surviving for more than 5 years (Fig. 30.10) (405). Hepatic synthetic dysfunction alone without other complications of cirrhosis has less impact on survival. Elevated bilirubin level, decreased albumin level, or thrombocytopenia results in a 16% to 19% decrement in 10-year survival compared with those with normal synthetic function (405). However, an elevated prothrombin time is a poor prognostic sign associated with a 39% reduction in 10-year survival compared with patients with cirrhosis who have normal synthetic function (405).

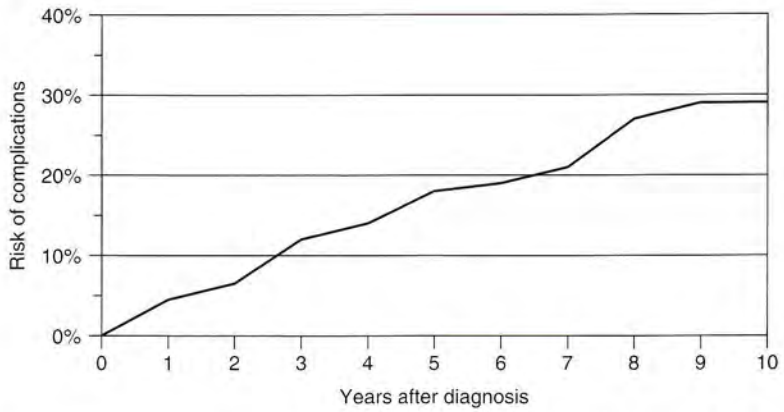
HCC is the fifth most common cancer worldwide. HCC is a significant complication of HCV infection and HCV is the most common etiologic factor in the United



■ **Figure 30.10** Survival in patients with cirrhosis due to chronic hepatitis C. Effect of complications and decompensation (*top line*, compensated cirrhosis; *bottom line*, decompensated cirrhosis). (See Fattovich G, Giustina G, Degos F, et al. Morbidity and mortality in compensated cirrhosis type C: a retrospective follow-up study of 384 patients. *Gastroenterology* 1997;112:463–472, with permission.)

States (472). Its incidence is also increasing in Europe (473). HCV-related HCC rarely occurs in the absence of cirrhosis (474,475). The annual risk of HCC is 1.4% to 3.3% in patients with cirrhosis in the United States, most of Europe, and Australia (261,405,476–478). It is estimated that the risk of HCC will double over the next 10 years (263) and may approach the higher risk rates (2.6% to 6.9%) observed in Japan and Italy (439). The mechanism for development of HCC in HCV disease is not known. Associations with small cell dysplasia and mutations in the protein kinase receptor binding domain, serine protease region, or CD81 genes have been reported (479–481). There is no association with mutations of tumor suppressor genes, virus levels, or viral genotype (482,483). The mean duration of HCV infection in patients with HCC is 28 to 29 years (261,405). HCC is more common in the presence of the following factors: Longer disease duration, hepatic synthetic dysfunction, cytopenia, male gender, and alcohol use (438,476–485). HCC appears to occur earlier in HIV-coinfected patients (486). Diagnosis of HCC is by imaging examinations and α-fetoprotein (AFP). AFP level is elevated in a variable proportion of patients with HCC and the risk of HCC is increased 30-fold when the AFP level is elevated (485,487). However, AFP is not specific and its level may be elevated in 30% to 45% of patients with chronic hepatitis C without HCC, although it is usually less than 100 ng/mL (405,487–490). Magnetic resonance is the most sensitive screening test, followed by computerized tomography and ultrasonography (490). However, the more sensitive imaging modalities are quite expensive. Imaging is most sensitive when performed every 6 months (490). Treatment of HCC is discussed in Chapter 44 of this book.

Currently, hepatic decompensation and HCC related to chronic hepatitis C are common indications for liver transplantation. HCV is the leading indication for liver



■ **Figure 30.11** Risk of developing decompensated liver disease among patients with stable cirrhosis due to chronic hepatitis C. (See Fattovich G, Giustina G, Degos F, et al. Morbidity and mortality in compensated cirrhosis type C: a retrospective follow-up study of 384 patients. *Gastroenterology* 1997;112:463–472, with permission.)

transplantation in the United States, accounting for approximately half of the nearly 6,000 liver transplantations performed each year. Liver transplantation for hepatitis C is described in detail in Chapter 53 of this book.

Prevention and Treatment

PUBLIC HEALTH, PASSIVE PROTECTION, AND VACCINES

Public health

Health care professionals must be informed about appropriate medical management of HCV-infected patients, known and potential risks for infection, the need to identify risk factors in their patients, appropriate evaluation of high-risk patients, and recommendations for prevention. All anti-HCV–positive patients should be considered infectious and informed of the possibility of transmission to others, although no tests are available to reliably determine infectivity. Counseling recommendations to prevent transmission of HCV were published by the U.S. Public Health Service in 1998 and were incorporated, in part, into the 2002 National Institutes of Health (NIH) Consensus Development Conference report on the management of hepatitis C (491,492). These recommendations are summarized in Table 30.9.

Passive protection

Neutralizing antibodies directed against immunodominant epitopes of HCV should protect the susceptible hepatocytes and other target cells from HCV infection. Experimental studies in chimpanzees have shown that plasma from a patient with chronic hepatitis C was capable of neutralizing isolate-specific HCV in vitro, thereby preventing infection (123). Neutralizing antibodies may be acquired passively by the administration of immunoglobulin preparations containing these antibodies. Before identification of HCV and elimination of anti-HCV–positive patients from the donor pool, it would be reasonable to assume that antibodies directed against HCV envelope proteins were present in the immunoglobulin prepared from pooled donors. On the basis of this assumption, passive immunization with conventionally prepared immunoglobulin was attempted to reduce the risk of transfusion-associated non-A, non-B hepatitis. Four prospective, randomized (three of which were placebo-controlled) clinical trials of immunoglobulin for the prevention of transfusion-associated non-A, non-B hepatitis provided conflicting results (415,417,493,494). Piazza et al. found that only 1 of 450 heterosexual partners of patients with HCV antibodies who received immunoglobulin enriched with anti-HCV became infected compared to 6 of 449 partners who received placebo (495). Feray et al. demonstrated indirect evidence that passive immunization may prevent HCV infection in liver transplant recipients. Among 218 HBsAg-positive patients who had HCV coinfection before transplantation, the prevalence of HCV viremia after transplantation was lower in those receiving hepatitis B immunoglobulin prepared before 1990 when screening of donors for anti-HCV began (25 of 46 [56%] patients) compared to others who received immunoglobulin free of HCV antibody (162 of 172 [94%] patients) (496). The 3-year actuarial rate of recurrent hepatitis C was also lower in those who underwent transplantation before 1990 (10%) than in those who underwent transplantation after 1990 (61%). Among patients who did not receive immunoglobulin therapy, the proportion who developed hepatitis C after transplantation was similar to that in patients who underwent transplantation before (63%) or after 1990 (71%) (496).

These studies suggested the possibility that pretransfusion administration of immunoglobulin containing antibodies to HCV would reduce the risk of non-A, non-B hepatitis, but the results are far from conclusive. Furthermore, these studies have much less relevance today. First, plasma collected for fractionation is screened for anti-HCV, and it is therefore likely that HCV-neutralizing antibodies are either absent or present in exceedingly low titers in currently manufactured conventional immunoglobulin. Second, the risk of post-transfusion hepatitis has nearly been eliminated by donor screening (306). In summary, postexposure prophylaxis with standard immunoglobulin is not effective in preventing HCV infection and is not recommended by the Advisory Committee on Immunization Practices (277).

It might be possible to produce immunoglobulin containing high titers of HCV-neutralizing antibodies. In fact, hyperimmune serum prepared against a synthetic peptide derived from the HVR of the HCV envelope protein is capable of neutralizing HCV and preventing infection when mixed with HCV in vitro before inoculation into chimpanzees (497). Postexposure prevention of HCV infection has also been studied by using a human HCV hyperimmunoglobulin prepared from anti-HCV–positive plasma that tested negative for HCV RNA (498). Intravenous infusion of this polyclonal product into a chimpanzee within 1 hour of inoculation with an infectious dose of HCV did not prevent infection but did appear to delay the onset of liver injury. Similar preparations have now been used in humans (499,500). Both studies infused antibody at the time of and after liver transplantation in patients with chronic hepatitis C. Neither study demonstrated any effect on HCV RNA levels or recurrence in the graft.

TABLE 30.9. GUIDELINES FOR HIGH-RISK AND HEPATITIS C VIRUS–INFECTED INDIVIDUALS FROM THE 2002 NATIONAL INSTITUTES OF HEALTH CONSENSUS DEVELOPMENT CONFERENCE ON MANAGEMENT OF HEPATITIS C (491,492)

HIGH-RISK BEHAVIOR

- Get vaccinated against hepatitis A and B
- Persons who continue to use or inject illegal drugs should
  Never reuse or share syringes, needles, water, or drug
  Use only new and sterile syringes and water obtained from a reliable source
  Use new or disinfected containers and filters to prepare drugs
  Clean the injection site with alcohol
  Dispose of syringes after one use
- Persons at risk for sexually transmitted diseases should
  Have sex only with a single uninfected partner or abstain, which is the best way to avoid infection
  If having high-risk sexual activity, use latex condoms correctly and every time

HEMODIALYSIS PATIENTS

- Use dedicated dialysis stations, chairs, and beds; clean after each use
- Avoid sharing ancillary nondisposable supplies; clean after each use
- Medication and supplies should not be shared and medication carts should not be used
- A central medication and supply prep area should be used
- Separate clean and contaminated areas

HCV-INFECTED PERSONS

- Consider all anti-HCV or HCV RNA–positive persons to be potentially infectious
- Do not donate blood, organs, tissues, or semen
- Avoid sharing of household items such as toothbrushes and razors
- Changes in sexual practices are not required within a monogamous relationship (however, a low potential for sexual HCV transmission exists; anti-HCV–positive persons and their partners should be informed of the potential risk of sexual transmission and an informed decision regarding the need for precautions should be made)
- Vaccinate against hepatitis A and B
- Avoid exposure to risk factors for transmission to others
- Avoid alcohol consumption

PERSONS REQUIRING ROUTINE TESTING FOR HCV INFECTION

- Persons who have at any time injected illegal drugs regardless of number of events
- Persons who received clotting factor concentrates before 1987
- Persons who were at any time on long-term hemodialysis
- Persons with persistently abnormal alanine aminotransferase levels
- Persons who received a transfusion or organ transplant July 1992
- Persons with needlestick, sharp, or splash exposure to HCV-positive blood
- Children born to an HCV-positive mother
- Routine testing is *not* recommended for:
  Health care, emergency medical or public safety workers
  Pregnant women
  Household contacts
  General population

HCV, hepatitis C virus; RNA, ribonucleic acid.

Because of the lack of an effective immunoglobulin, persons with percutaneous exposure to HCV should be closely monitored for infection. At a minimum, this requires serial testing of serum ALT and anti-HCV levels at baseline and 4 to 6 months postexposure (491). However, others have suggested serial HCV RNA determinations by PCR to identify early acute infection that might prove more amenable to IFN treatment (see subsequent text) (501).

Vaccination

Development of an effective vaccine against HCV has faced several obstacles. First, it appears that neutralizing antibodies against the hypervariable envelope proteins, although potentially protective, are largely isolate specific and therefore likely to provide only temporary protection against a heterogeneous virus such as HCV (126). Second, little is yet known about the ability of HCV-specific cellular immune responses to induce protection. Finally, it will be difficult to test the efficacy of a potentially protective vaccine when the risk of acute infection is so low. Nonetheless, some progress has been made in developing hepatitis C vaccines. A recombinant E1E2 heterodimer vaccine administered four times over a year to healthy volunteers induced antibodies that bound to E1E2 and

CD81, the putative HCV receptor (502). Furthermore, the antibodies neutralized E1E2-encoated vesicular stomatitis virus pseudovirions and induced lymphocyte proliferation. In another study, a recombinant E1 vaccine was given to 24 HCV-infected patients in repeated courses. All but three patients developed a significant de novo E1-specific T-cell response. Anti-E1 antibodies increased and higher levels correlated with the decrease in total Ishak score in 9 of the 24 subjects (503).

ANTIVIRAL THERAPY

The major goal of treatment of HCV infection is to prevent the development of decompensated liver disease and death. This can be accomplished by preventing new infections, reducing the chance of acute infection progressing to chronic hepatitis, or effectively treating chronic infection. The goals in treating chronic hepatitis should include eradication or prolonged suppression of viral replication, reduction of hepatic inflammation, and ultimately, slowing the rate of progressive liver injury. Not all these goals may be achievable in every patient. However, eradication of chronic HCV infection is now possible in half or more of treated patients (504).

Evolution of treatment for chronic hepatitis C

Increasingly more effective treatment regimens for chronic hepatitis C have evolved rapidly over the last 2 decades (Fig. 30.12). In the mid-1980s, IFNs became the first agent studied for the treatment of what was then called *chronic non-A, non-B hepatitis*. IFNs are glycoproteins produced in vivo by leukocytes in response to viral infection. IFNs can be commercially manufactured by cell culture or recombinant technology and have been commercially available for the treatment of chronic hepatitis for more than a decade. IFNs inhibit the replication of many viruses, including hepatitis viruses, through a variety of mechanisms including direct antiviral mechanisms (inhibition of virus attachment and uncoating, and induction of intracellular proteins and ribonucleases) and amplification of specific (CTL) and nonspecific (NK cell) immune responses (505). The specific mechanism(s) of action for IFN in chronic hepatitis C infection remain(s) poorly understood. However, viral kinetic modeling in IFN-treated subjects shows a biphasic decline in HCV RNA level after IFN treatment (506). The first 24- to 48-hour phase is characterized by a rapid decline in virus level, which is thought to represent degradation of free virus, while replication of new virions and infection of naïve cells is inhibited (506). The second phase has a slow exponential decline in viral levels and is thought to represent loss of residual infected hepatocytes (506).

Recombinant IFN was first approved by the U.S. Food and Drug Administration (FDA) for the treatment of non-A, non-B hepatitis in 1991. Initially, the recommended treatment course was 6 months, but it was subsequently shown that prolonging treatment to 12 months doubled the sustained response rate, and this longer regimen was subsequently approved as the standard of care (507–509). Sustained biochemical (normalization of the serum ALT level) and virologic (loss of detectable serum HCV RNA) responses were relatively uncommon with IFN monotherapy, ranging from 6% to 15% after a 6-month course of IFN to 13% to 25% after 12 months of therapy (509). More recent studies that have carefully assessed virologic endpoints of treatment with different regimens have clearly shown that sustained responses to IFN monotherapy are at the lower end of these previously described ranges (510,511).

Ribavirin was added to the treatment regimen almost 10 years later. Ribavirin (1-β-D-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide) is a synthetic nucleoside analog that structurally resembles guanosine (512). Ribavirin has in vitro activity against several DNA and RNA viruses, including Flaviviridae (513). The mechanism of action in HCV infection is not clear, but the

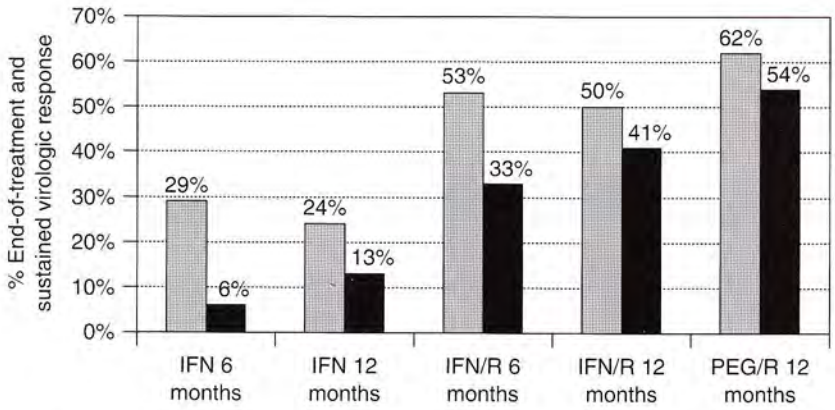


Figure 30.12 Changes in the end-of-treatment and sustained response rates with different interferon (IFN) treatment regimens (*shaded bar*, end-of-treatment response; *solid bar*, sustained response). IFN/R, interferon and ribavirin; PEG/R, polyethylene glycol and ribavirin.

predominant current opinion is that ribavirin induces lethal mutations in viral genome, a mechanism known as *viral error catastrophe* (512–514). Early studies of ribavirin alone found that serum ALT levels fell to within the normal range in 40% of treated patients, but virus levels did not significantly change (515–517). However, when combined with IFN, ribavirin reduces relapse after response during treatment, and this results in a dramatic improvement in the sustained virologic response (SVR) rate (510,511,518,519). The results of two similarly designed large randomized controlled trials comparing combination therapy to IFN monotherapy have been combined and reported (520). These studies compared 6- and 12-month courses of combination therapy (3 million units of IFN-α-2b thrice weekly plus 1,000 to 1,200 mg oral ribavirin daily) to similar duration courses of IFN alone (510,511). The combined results showed sustained viral-negative responses in 41% and 33% of subjects treated with 12 and 6 months of combination therapy, respectively, compared to 16% and 6% in those treated with IFN alone for 12 and 6 months, respectively (520).

Long-acting pegylated IFNs increase host exposure to IFN and double the response seen with standard IFN preparations (521–523). These formulations replaced standard IFNs after their approval by FDA in 2001. Pegylation involves the attachment of a large inactive molecule (polyethylene glycol [PEG]) to a protein to reduce clearance. This process results in some variable loss of activity of the native protein that is dependent on the size and site of attachment of the PEG molecule (524). In the case of IFNs, pegylation results in a 10-fold increase in drug half-life and a corresponding decrease in clearance (525,526). It is this longer half-life that allows large doses of the drug to be administered less frequently (once weekly instead of three times per week). The PEG molecule is cleaved after binding of the complex to the IFN receptor and cleared. Short PEG molecules, such as the 12-kDa tail attached to the IFN α-2b protein, are renally cleared while longer molecules, such as 40-kDa tail on the α-2a drug, are hepatically metabolized (527). At the present time, there is no evidence that either PEG molecule has any deleterious effects.

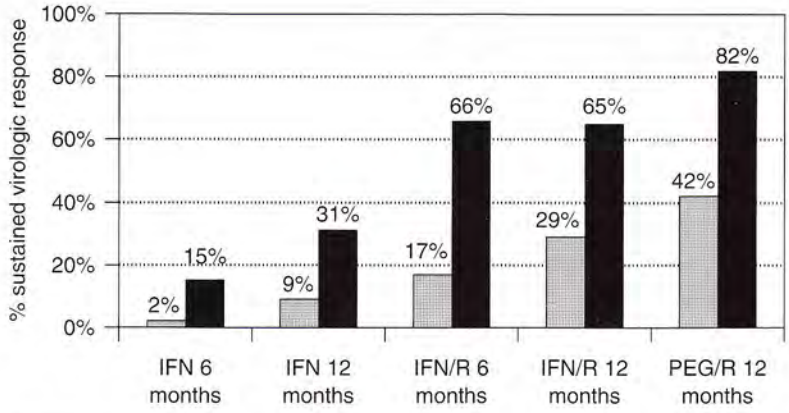
Pegylated IFNs are more effective than standard IFN, and there is a clear dose response with increasing doses of the PEG-IFNs (521–528). Studies of monotherapy with pegylated IFN α-2a at a fixed dose of 180 μg weekly for 48 weeks resulted in an SVR rate twice that observed with the standard IFN α-2a thrice-weekly dosing control group (39% vs. 19%) (522). Similarly, studies of pegylated IFN α-2b using weight-based dosing of 1.0 or 1.5 μg/kg weekly for 48 weeks demonstrated an SVR rate twice that observed with the standard IFN α-2b control group (25% and 23%, respectively, vs. 12%) (523). It is important to point out that the study groups were not comparable and the sustained response rates cannot be directly compared; it is likely that any differences in efficacy between the two drugs, if they exist at all, are minimal.

Neither pegylated IFN preparation alone has clinical efficacy approaching the combination of standard IFN and ribavirin. Therefore, the clear role of pegylated IFNs is in combination with ribavirin, and this combination is currently the standard of care for chronic hepatitis C. Large international randomized controlled studies confirmed the advantage of the pegylated combinations over standard IFN combinations (528,529). PEG-IFN α-2b 1.5 μg/kg once weekly plus 800 mg daily ribavirin led to an SVR rate of 54%, although the dose of ribavirin in this study was suboptimal (528). Sustained response was 42% in patients infected with genotype 1 and 82% in those with genotype 2 or 3 (528). In another trial, PEG-IFN α-2a 180 μg once weekly plus 1,000 to 1,200 mg of ribavirin per day resulted in an SVR rate that was 56% (529). Sustained response was 46% in patients infected with genotype 1 and 76% in those with genotype 2 or 3 (529). In both studies, tolerance including cytopenia and discontinuation of drug was similar to that of standard IFN and ribavirin, although fever, nausea, and injection site erythema were seen more commonly.

Optimal treatment regimens

The sensitivity of different HCV genotypes to IFN-based therapies varies considerably (Fig. 30.13). Therefore, determination of the virus genotype before treatment remains a critical step, and the optimal dosing regimens for the predominant genotypes have been more clearly defined (Table 30.10). For patients with genotype 1, the optimal regimen includes 1 year of PEG-IFN plus ribavirin. Although the approved dose of ribavirin is 1,000 to 1,200 mg/day for those with weight less than or greater than 75 kg, respectively (530), an extended weight-based dose ranging from 800 to 1,400 mg/day is commonly used (Table 30.11). It is not known whether such dosing improves response or is appropriate for other genotypes. Recently, some investigators have suggested that "rapid viral response" (RVR) (undetectable HCV RNA after 4 weeks of treatment) in patients with genotype 1 identifies a small subgroup (approximately 20% of treated patients) who may be treated for only 24 weeks and still achieve an SVR rate of 73% to 91% (531). Others have found a lower SVR rate with 24 weeks of treatment, so this needs to be confirmed before this regimen is made standard practice (532).

Patients with genotype 2 or 3 respond as well with doses of 800 mg/day and just 6 months of treatment as they did with higher doses and a longer duration (530). Recently, studies have suggested that some patients with genotype 2 or 3 infection who respond rapidly to



■ **Figure 30.13** Influence of viral genotype on sustained virologic response to interferon (IFN)-based treatment (*shaded bar,* genotype 1; *solid bar,* genotype 2 or 3). IFN/R, interferon and ribavirin; PEG/R, polyethylene glycol and ribavirin.

treatment can be treated for as little as 14 to 16 weeks with excellent outcomes (537).

Patients who are infected with genotype 4 have viral response rates similar to or perhaps slightly better than those infected with genotype 1 and, like genotype 1, some achieve good responses with only 24 weeks of therapy (533). Genotypes 5 and 6 have SVR rates approaching those achieved with genotypes 2 and 3, although they require a full year of therapy (534,535).

Patient selection, drug administration, and monitoring therapy

Rational treatment management decisions are based on a clear understanding of the epidemiology and natural history of chronic hepatitis C, as well as the factors that influence the response to treatment. These decisions require that both the patient and the physician be fully informed about the disease. In the individual patient, general health factors such as age, comorbid disease, psychosocial circumstances, compliance, desire to be treated, contraindications to treatment, and financial issues must be weighed in arriving at an initial decision about whether treatment is appropriate. Counseling about transmission, natural history, treatment options, and possible treatment risks and outcomes is indicated regardless of what intervention, if any, is ultimately decided on. All should be counseled about the significant risks of alcohol use with this disease (538). Patients should also clearly understand the risks of complications should they or their sexual partners become pregnant during or shortly after the treatment course. All patients, particularly those with a history of depression, should be forewarned of the risk of depression and the need to treat it early. If treatment is not considered at this time, the importance of long-term follow-up must be emphasized. If the physician and patient agree to proceed with the treatment, assessment of the status of the disease (as determined by liver biopsy) and infection (as determined by viral genotype and HCV RNA level) should be made. This allows a more accurate estimate of the prognosis and chance of response to available treatment. It also allows the physician to use these patient characteristics that may independently influence treatment response to personalize the treatment strategy to achieve the optimal response. Although most hepatologists use only viral genotype and histology to choose the best treatment duration (539), others have recommended a more complex "a la carte" method that also incorporates gender, age, and viral level into the equation (540). Retrospective analysis suggests that such algorithms might improve

TABLE 30.10. INTERFERON AND RIBAVIRIN DOSING ACCORDING TO VIRAL GENOTYPE (530,533–536)

Genotype	Interferon dose (/wk)	Ribavirin dose (mg/d)	Duration (wk)	SVR (%)
1	180 μg PEG α-2a *or* 1.5 μg/kg PEG α-2b	800–1,400 weight based	48	41–42
2	180 μg PEG α-2a *or* 1.5 μg/kg PEG α-2b	800	24	60–84
3	180 μg PEG α-2a *or* 1.5 μg/kg PEG α-2b	800	24	60–84
4	180 μg PEG α-2a *or* 1.5 μg/kg PEG α-2b	1,000–1,200	48	55
5	180 μg PEG α-2a *or* 1.5 μ/kg PEG α-2b	1,000–1,200	48	64
6	180 μg PEG α-2a *or* 1.5 μg/kg PEG α-2b	1,000–1,200	48	63

SVR, sustained virologic response; PEG, polyethylene glycol.

TABLE 30.11. DOSING GUIDELINES FOR COMBINATION THERAPY WITH PEGYLATED INTERFERON AND RIBAVIRIN (536)

Body weight in kilograms (pounds)	Peg-IFN α-2a dose (μg)	Peg-IFN α-2b dose (μg)	Ribavirin dose (≥13 mg/kg)
<40 (<88)	180	50	800
40–50 (88–100)	180	64	800
50–65 (112–141)	180	80	800
65–75 (143–165)	180	112	1,000
76–85 (167–187)	180	120	1,000
86–104 (189–229)	180	150	1,200
>105 (>231)	180	150	1,400

- U.S. Food and Drug Administration–approved dosing for pegylated IFN α-2b and ribavirin is 1.5 μg/kg once per week and 800 mg/d in divided dose, respectively (454,460).
- Traditional doses of ribavirin based on licensed recommendations with nonpegylated regimens are 1,000 mg/day if body weight is less than 75 kg or 1,200 mg if weight is more than 75 mg.

SVR rates to 50% to 83%. However, the wisdom of complicated algorithms that would, in effect, treat a higher proportion of infected cases with a longer and more costly regimen is controversial and has not been confirmed prospectively.

Baseline assessment of liver tests, complete blood counts, and HCV RNA level are important to later determine treatment response and drug-related toxicity. The patient should be instructed in injection techniques and forewarned about what drug-related symptoms are expected. Treatment tolerance is improved if the patients are educated about the potential side effects of therapy and what they might expect. Some easy measures such as evening dosing, exercise, adequate hydration, and use of acetaminophen at the time of each IFN dose will reduce anxiety, side effects, and noncompliance. Reinstitution of antidepressants should be considered in patients with a significant past history of depression. Physician extenders such as nurses, pharmacists, and commercial treatment support services are extremely helpful in this respect.

Standard treatment of chronic hepatitis C should consist of the combination of pegylated IFN α- 2b (PEG-Intron™, Schering Plough, Kenilworth, NJ, at a dose of 1.5 μg/kg body weight) or pegylated IFN α-2a (Pegasys, Roche, Nutley, NJ, at a fixed dose of 180 μg) administered subcutaneously once per week and 13 to 15 mg/kg (minimum dose, 800 mg) of oral ribavirin per day in divided doses (Table 30.11). Pegylated IFN α-2b is dosed on the basis of body weight because it has a larger volume of distribution than pegylated IFN α-2a (20 L vs. 8 L), which has a volume of distribution approximately equivalent to plasma volume and can therefore be administered as a fixed dose (529). The listed doses of ribavirin are based on retrospective analyses of ribavirin dose response in clinical trials (528–530,536,540). Although this extended weight-based dosing method has become common, the advantage of extended dosing method appears to be marginal in comparison to the previous limited weight-based dose (1,000 or 1,200 mg) (536). Nonetheless, a recent study demonstrating that even higher doses of ribavirin may improve response rates justify the use of this dosing method (541,542). Viral genotype determines the duration of therapy (Table 30.10).

Monitoring response and potential drug toxicity is essential. Symptoms related to treatment rarely necessitate dose adjustments. However, hematologic alterations, particularly anemia, can be significant and clinically important during the first few weeks. Therefore, blood counts including hemoglobin, white count, differential, and platelet count should be repeated 2 and 4 weeks after starting therapy. Transient IFN dose reduction is indicated only for a white count less than 1,500/mL, a neutrophil count less than 750/mL, or platelets less than 50,000/mL. Ribavirin should be reduced if the hemoglobin level falls to less than 10 g/dL. The amount of dose reduction required to reverse cytopenia has not been established. Although the labeling of the drugs calls for reducing doses by half, this is usually not necessary. Temporary dose modifications are common in patients treated with combination therapy. In fact, in large controlled trials dose modifications were required at least transiently in 34% to 42% of patients, respectively (510,511,528,529). Between 8% and 13% of patients require reduction of the ribavirin dose for anemia, usually during the first 4 weeks of treatment (543). Ribavirin-induced anemia is dose dependent, and therefore, anemia usually stabilizes or improves with dose reduction. Occasionally transfusion or support with erythropoietin is necessary, although it generally takes 4 to 8 weeks before the hemoglobin level increases after erythropoietin is started (544). The hemolytic anemia is accompanied by a vigorous reticulocytosis that usually serves to maintain stable levels of hemoglobin after the first few weeks of treatment and result in its return to baseline within 4 weeks of stopping treatment. Approximately 15% to

20% of patients treated with pegylated IFN require dose reductions for neutropenia (528). Growth factor support is rarely required. Significant thrombocytopenia necessitating dose reduction is uncommon because the anemia caused by ribavirin induces a reactive thrombocytosis. Therefore, platelet counts tend to remain relatively stable throughout combination therapy, even when the pretreatment count is low. IFN should be permanently stopped only if symptoms are incapacitating, the absolute neutrophil count is less than 500/mL, or the platelet count is less than 25,000/mL. Discontinuation of therapy for cytopenia is uncommon if patients have been monitored and dose adjusted appropriately. It is extremely important that treatment not be stopped prematurely or for decreases in blood counts that do not meet the criteria stated earlier. Inappropriate dose reduction and discontinuation significantly reduces the likelihood of a treatment response. Early discontinuation of treatment can reduce the likelihood of a sustained treatment response by 80% (545). Management of other side effects is discussed later.

Side effects of treatment

The safety and tolerability of combination therapy have been reviewed in detail elsewhere and will only be highlighted here (543,546). Overall, IFN-based therapies are reasonably well tolerated. Most patients experience flu-like side effects including fatigue, fever, headache, myalgia, and arthralgia (528,543). These are most severe when treatment is initiated and often abate to a large degree as treatment is continued. Gastrointestinal symptoms including nausea, vomiting, or diarrhea occur in about a third of patients but are rarely severe. Psychiatric symptoms such as depression, impaired concentration, irritability, and insomnia occur in about a third of cases but are also common in untreated patients with chronic hepatitis C (510,528,547). Dermatologic signs and symptoms occur in about a quarter of patients, but injection site erythema is seen in 30% to 60% of cases and is more common and pronounced with pegylated IFNs (528).

As described in the preceding text, ribavirin causes a predictable dose-related hemolysis. Therefore, the drug should be used with great caution or avoided completely if there is preexisting anemia, a hemolytic disorder, coronary artery disease, or hypoxia. Because ribavirin is renally excreted, it can cause profound hemolysis in patients with renal failure and should generally be avoided. Careful consideration should be given to the potential effects of an acute anemia in each patient in whom combination treatment is considered. The mean fall in hemoglobin is level 2 to 3 g/dL (510,511,517). The decline occurs gradually during the first 4 weeks of treatment and the hemoglobin level usually remains relatively stable thereafter.

Severe adverse events, including severe psychiatric symptoms, suicide attempts, and profound cytopenia, are extremely uncommon, being reported in fewer than 1 in 1,000 treated cases (546). Development of immune-mediated disorders such as thyroid disease, diabetes, dermatologic conditions, neuropathy, and other autoimmune-like signs was seen in about 1% in a large retrospective series (546). Development of autoantibodies is not necessarily associated with autoimmune disease. Autoantibodies are common in patients with HCV infection and may be more common during IFN treatment (133,548). Finally, ribavirin has embryotoxic and teratogenic effects in animals and should be avoided in patients with childbearing potential unless adequate contraception is assured. Trial and postmarketing surveillance data suggests that most patients or spouses of patients who become pregnant during or within 6 months of treatment will spontaneously abort if the pregnancy is not otherwise terminated (GL Davis, unpublished data, 2002).

Assessing treatment response

Treatment responses are defined by changes in the HCV RNA level during and after treatment; these are listed and described in Table 30.12. Early studies also utilized serum ALT level to assess the response to therapy, but virologic endpoints are now most appropriate. Serum ALT level does not always reflect on treatment response because ribavirin may normalize serum ALT level in the absence of a virologic response and ALT level may occasionally be elevated despite a virologic response, especially in those receiving pegylated products (517,528,529).

The implications of RVR are described in the preceding text, but this is a new concept and requires confirmation before influencing treatment duration in most patients. Early virologic response (EVR) is used to assess nonresponsiveness to treatment during the first weeks of therapy. EVR is based on the concept that the slope of the second-phase decline in HCV RNA levels during treatment correlates with the likelihood of eventual virus clearance (82,549). Obviously, it requires a quantitative HCV RNA assay with a wide dynamic range to assess this decline (See "Diagnostic Tests"). Genotype 1–infected patients who do not achieve at least a 2-log reduction (99%) in HCV RNA level after 12 weeks of treatment have less than 1% chance of reaching an SVR with continued therapy (Fig. 30.14) (550). This has served to justify discontinuation of therapy after 12 weeks in the 20% or so of patients without EVR. Patients with genotype 2 or 3 almost always reach an EVR, so it is usually not helpful to assess HCV RNA levels in them during treatment (550).

Measurement of HCV RNA at the end of therapy is helpful in identifying those in whom the virus has

TABLE 30.12. DEFINITIONS OF TREATMENT RESPONSES

Response	Time to assess	Definition	Implication
RVR	4 wk	HCV RNA undetectable by PCR or TMA	Higher chance of SVR; may respond as well with only 24 wk of treatment
EVR	12 wk	HCV RNA decreased by ≥two logs from baseline or HCV RNA undetectable	Failure to achieve EVR associated with almost no chance of SVR and treatment can usually be stopped
ETR	End of treatment	HCV RNA undetectable by PCR or TMA	On treatment response; observe for SVR
SVR	24 wk after treatment	HCV RNA undetectable by PCR or TMA	Eradication of virus

RVR, rapid viral response; HCV, hepatitis C virus, RNA, ribonucleic acid; PCR, polymerase chain reaction; TMA, transcription-mediated amplification; SVR, sustained virologic response; EVR, early virologic response; ETR, end-of-treatment response.

cleared (end-of-treatment response) and who require subsequent screening to confirm the durability of the response. Approximately 15% of patients with end-of-treatment response will relapse during the first few months after treatment is stopped (528,529). SVR is confirmed by the absence of detectable HCV RNA by a sensitive molecular test 6 months after completing therapy. SVR is the major goal of treatment and is durable.

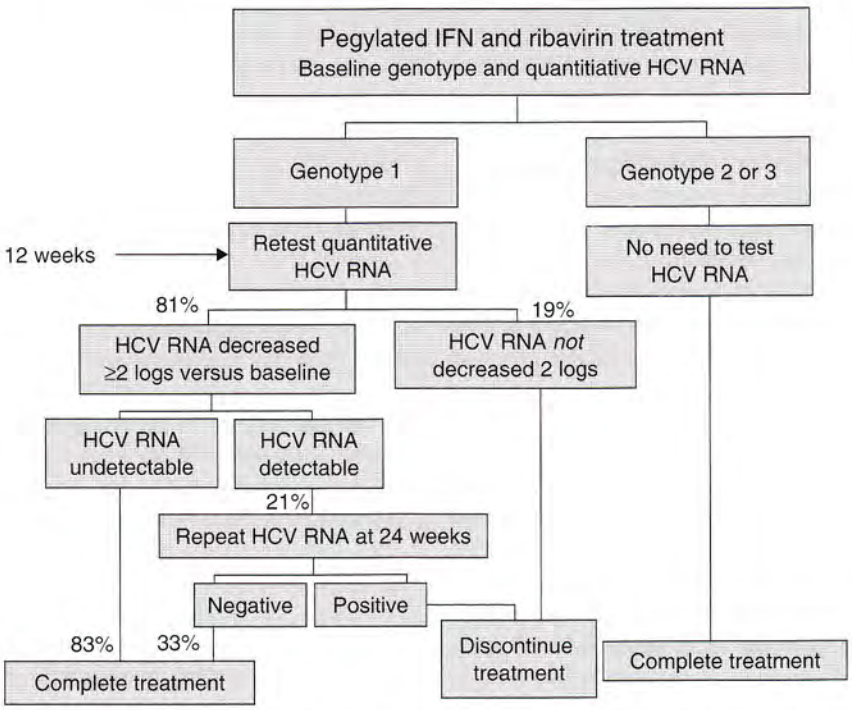
Clinical implications of sustained virologic response

SVR is the primary goal of treatment. It is durable and connotes eradication of infection (551). Furthermore, SVR results in long-term histologic improvement with fibrosis regression at a rate of 0.28 Metavir units per year (552–555). By contrast, nonresponders to IFN-based therapy continue to have progression of fibrosis at a rate of 0.10 Metavir units per year (555). It is reasonable to assume that these benefits would translate into a reduction in disease-related morbidity and mortality. Indeed, patients with fibrosis or cirrhosis who achieve SVR have gradual improvement in quantitative hepatic function, reduction in portosystemic shunting, significant reduction in the risk of developing HCC, and improved survival (556,557).

Treatment in special patient groups

Fibrosis or cirrhosis

The presence of bridging fibrosis or cirrhosis negatively impacts treatment response to IFN-based regimens. SVR among patients with stage 3 or 4 fibrosis is 41% to 44% compared to 54% to 55% in those with stage 0 to 2



■ **Figure 30.14** Algorithm for determining early virologic response. IFN, interferon; HCV, hepatitis C virus; RNA, ribonucleic acid.

(528,529). The justification for treating patients with advanced fibrosis is clear because these patients have a greater risk of progressing to develop HCC or complications of cirrhosis. Patients with fibrosis or cirrhosis who achieve SVR have a reduced risk of developing these complications (557).

Higher doses of IFN may overcome some of the lack of response to IFN. In a trial using a dose of 3.0 μg/kg of pegylated IFN α-2b, SVR was the same in patients with and without advanced fibrosis, although this was not the case with the standard IFN dose (558). Finally, it has been suggested that chronic low-dose IFN might also delay progression of fibrosis and reduce complications of liver disease in patients with fibrosis or cirrhosis who have failed to reach an SVR with standard antiviral therapy. This is currently being evaluated in prospective clinical trials (See "Viral Nonresponders").

Treatment of patients with decompensated cirrhosis can be considered in the hope of eradicating infection before liver transplantation. SVR in this group usually prevents recurrence of HCV infection after transplantation (559). However, such treatment is extremely difficult. Most patients have cytopenia that prevents them from receiving the optimum doses of medications, and severe complications usually require dose reductions or discontinuation of therapy (559,560). These problems may be partially avoided by initiating treatment at low doses and escalating as tolerated (559). Despite the problems, SVR is possible in approximately 25% of patients. Treatment in this group of patients is best managed by experienced transplantation hepatologists.

High viral load

High levels of HCV RNA are associated with a decreased chance of clearing virus, at least with genotype 1 infection (528,529). SVR in patients with more than 2 million copies per milliliter achieved SVR in 30% to 39% compared to 56% to 68% in those with lower levels (528,529). Furthermore, patients with low levels of HCV RNA before treatment are also more likely to reach an RVR that may allow them to discontinue treatment early (531). It does not appear that higher doses of IFN are able to overcome the impaired response in patients with high viral loads.

African Americans

SVR rates have consistently been lower in African Americans than in whites in trials of IFN-based therapy. Recent studies with pegylated IFN and ribavirin in previously untreated patients with genotype 1 infection observed SVR rates of 19% to 26% in African Americans versus 39% to 52% in whites (561,562). Several factors including the higher proportion with genotype 1, higher body weight, and a slower decline in HCV RNA in viral kinetic studies might at least partially explain this observation. However, the apparent ability of higher doses of IFN or ribavirin to improve responses suggests that the explanation may lie in the impaired ability of intracellular antiviral mechanisms to be turned on at standard doses of these medications (558,563).

Human immunodeficiency virus–hepatitis C virus coinfection

HCV infection is common in HIV-infected individuals (564). Coinfected patients tend to have high HCV RNA levels, and some studies suggest that they have more severe liver disease, more rapid disease progression, and a higher prevalence of hepatic fibrosis (455,565–568). Liver disease due to hepatitis B, hepatitis C, or alcohol is second only to acquired immunodeficiency virus as a cause of death in patients with HIV (569). Several clinical trials of pegylated IFN and ribavirin have recently been reported (570–572). Treatment responses vary considerably in these studies because of differences in design and compliance. However, SVR in genotype 1 patients was low (14% to 29%) while it was reasonably intact in genotype 2 and 3 subjects (62% to 73%). Overall, SVR rates appeared to be 10% to 15% below what would be expected in an HIV-negative population. Several points are worth making. First, SVR is possible in the HIV–HCV coinfected patient and treatment should be considered. Second, failure to achieve EVR reliably predicts lack of response in this group and this should be discussed before treatment is started because the treatment goals are different from those of HIV. Finally, patients receiving highly active antiretroviral therapy (HAART) or with low CD4 counts appear to be more likely to experience adverse effects from treatment, including drug hepatotoxicity (564,573). Severe hepatotoxicity, especially related to ritonavir, is almost four times higher in patients with HIV who are coinfected with HCV, having a risk of approximately 12% (573). However, 88% do not develop severe hepatotoxicity. Furthermore, ribavirin may increase phosphorylation of zidovudine and dideoxyinosine and potentiate toxicity of these drugs (574,575). However, despite a case report implicating ribavirin in multiorgan failure and lactic acidosis, most HIV physicians have not found the potential drug interaction to be a clinical problem.

Obesity and fatty liver disease

Obesity and hepatic steatosis are both associated with a reduced response of HCV-infected patients to IFN-based treatments (528,529,576). For genotype 1 and 2, hepatic steatosis is associated with a fall in SVR from 57% to 25% and 96% to 86%, respectively (576). Genotype 3 infection induces steatosis in and of itself, and

this is not associated with reduced SVR. The mechanism(s) by which obesity or steatosis reduce(s) SVR is (are) not known, but it has recently been suggested that it may be related to insulin resistance and hyperinsulinemia. Sanyal et al. studied the effect of insulin on the action of IFN in an HCV replicon system composed of a Huh-7 hepatocellular carcinoma cell line stably transfected with full-length HCV RNA (577). IFN predictably inhibited HCV replication in this model. However, addition of insulin to the media resulted in a significant increase in HCV replication and a marked blockade of IFN's ability to decrease HCV RNA. The mechanism of insulin interference appeared to be impairment of activation of the STAT pathway with resulting blockade of the IFN-mediated increase in protein kinase R and IRF-1. Other mechanisms may also be involved.

Acute hepatitis C

Acute hepatitis C has a high propensity for progression to chronicity in the absence of antiviral therapy. Over the last 15 years, there have been numerous studies assessing the efficacy of IFN for the prevention of chronic infection. These studies vary considerably in design, type and dose of IFN, use of ribavirin, duration of therapy, and presence of controls. Nonetheless, it is clear that acute hepatitis C is extremely sensitive to the effects of IFN and most treated patients resolve the infection regardless of the regimen utilized (397–399,509,578–582). More recent studies have utilized either daily doses of a standard IFN or weekly doses of pegylated IFN for 4 to 24 weeks (579–582). SVR rates ranged from 75% to 98%. One study randomized patients to immediate treatment or a delay until 1 year after onset (582). Chronicity was reduced from 60% to 13% by early intervention.

Although these results are quite encouraging, treatment of patients with acute HCV infection must be considered in the proper perspective. The incidence of acute hepatitis C in the United States has fallen dramatically to about 28,000 infections per year (243). Only a small proportion of these cases are recognized by either the patient or physician. Furthermore, prospective surveillance to identify acute infection in those at risk (e.g., intravenous drug users) is impractical. Therefore, most cases of acute hepatitis will never come to medical attention. Nonetheless, treatment of identified cases with 6 months of pegylated IFN should be strongly considered. There is no data suggesting that ribavirin is required.

Extrahepatic manifestations

Mixed cryoglobulinemia is the most common extrahepatic manifestation in patients with chronic hepatitis C. It typically manifests as cutaneous vasculitis, glomerulonephritis (GN), neuropathy, or systemic vasculitis (583,584). IFN alone, or in combination with ribavirin, is able to suppress cryocrits in most patients with mixed essential cryoglobulinemia, and treatment may be indicated solely on the basis of the presence of clinical complications of cryoglobulinemia, regardless of the presence or severity of the liver disease (583–587). The fall in cryocrit observed during treatment usually correlates with a drop in serum HCV RNA levels, normalization of complement levels, and clinical improvement (583,586,587). However, polyneuropathy appears to be relatively resistant to treatment (585,586). Although results in this small series vary considerably, SVR after discontinuation of IFN-based treatment appears to be lower than that in patients without cryoglobulinemia (587).

GN has been associated with chronic HCV infection (588,589). Most of these cases present with proteinuria, which may reach nephrotic syndrome range, and have cryoglobulinemia (588–590). The histologic lesion is usually one of membranoproliferative GN, so-called cryoglobulinemic GN, although other histologic forms such as mesangial proliferative GN (usually IgA nephropathy) and membranous GN may also occur (588–592). In contrast to membranoproliferative GN, the latter two histologic lesions are typically not associated with cryoglobulinemia (588,591). Membranous GN responds poorly, if at all, to IFN (588,592). Furthermore, a recent report cautions that GN not caused by HCV may worsen under IFN treatment (593). Finally, ribavirin is renally excreted and should be used with great caution, if at all, in patients who have developed significant renal insufficiency as a consequence of their GN (578).

Post-transplantation

Complications of chronic hepatitis C including HCC and decompensated cirrhosis are the most common indication for liver transplantation, accounting for more than 40% of transplantations performed in the United States and Europe. If HCV RNA is detectable at the time of transplantation, infection will always recur (458). HCV RNA levels increase rapidly after transplantation as a consequence of immunosuppression (594–597). These patients often have very high HCV RNA levels, which may result in sometimes rapidly progressive chronic hepatitis, or, less commonly, an aggressive cholestatic hepatitis, which leads to liver failure (458,598–601).

Several options for treatment should be considered in the setting of liver transplantation including treatment of listed patients awaiting transplantation, preemptive therapy for patients shortly after transplantation before histologic recurrence is noted

or treatment once recurrent disease is apparent (458,559,560,602,603). Treatment of such patients is predictably difficult regardless of which strategy is chosen because most patients have genotype 1 infection and high viral loads, have previously failed to respond to or tolerate IFN-based therapy, often have preexisting cytopenia and renal insufficiency, and have drug interactions that increase the risk of cytopenia or symptoms. Although antiviral treatment is successful in some patients with recurrent hepatitis C, it is extremely difficult to administer and requires dose reductions in most cases (458,604). Furthermore, acute and chronic rejection may ensue during or after treatment (604). Nonetheless, approximately 30% of treated patients can achieve SVR if both the patient and physician are committed to therapy (458,603).

IFN-based antiviral therapy should generally be avoided in recipients of other solid organ transplants because of the risk of rejection (605). However, treatment appears to be safe in autologous and allogeneic bone marrow transplant recipients (606).

Normal alanine transaminase level

Approximately 15% to 20% of patients with chronic HCV infection have persistently normal serum ALT levels (264). Although most of these patients have some degree of inflammation on liver biopsy, most studies have shown that few have significant fibrosis. In one review of 11 published studies, cirrhosis was found in only 0.3% (607). Others have reported fibrosis in up to 10% (608). However, in the absence of alcohol use, the rate of fibrosis progression is very low in patients with persistently normal serum ALT levels (609,610). Virologic response to IFN-based therapy appears to be the same as that in patients with elevated ALT levels (611). Therefore, treatment can be considered on an individual basis in these patients, balancing the cost and difficulty of therapy with the estimated likelihood of progression. Although it may be hard to justify the cost and side effects of therapy in many patients, it is easy to make an argument for treating patients with hepatic fibrosis or genotypes 2 and 3.

Viral relapsers

Virologic relapse occurs in 45% to 80%, 35% to 40%, and 15% of those who lose detectable HCV RNA during IFN monotherapy, standard combination therapy, or pegylated combination therapy, respectively (186,510,511,528,529). There is little or no benefit in retreating patients with the same treatment regimen (612,613). However, treatment of relapsers to monotherapy with higher doses or a longer duration of IFN, or combination therapy, may achieve a sustained response (614–617). However, patients previously treated with suboptimal regimens should always be given the current standard of care regimen if they are retreated. A recent study found that half of patients who relapsed after standard IFN and ribavirin achieved SVR when retreated with pegylated IFN and ribavirin (618).

Viral nonresponders

Mathematically, one would predict that approximately 10% to 20% of viral nonresponders to IFN monotherapy or standard combination therapy might achieve a sustained response when retreated with pegylated combination therapy. Indeed, prospective trials have confirmed this to be the case (618). However, much of the published literature is quite confusing because of differences in definitions of nonresponse (some include relapsers and partial responders), different retreatment regimens, and inclusion of high proportions of patients with factors that favor response (i.e., patients without cirrhosis, those with genotypes 2 or 3, and those with low HCV RNA levels) (619). Furthermore, the reasons for nonresponse are often not given in such studies. Many patients fail to respond to treatment because of incorrect dosing, early and sometimes inappropriate dose reductions or discontinuations, and poor side effect management. These patients might be expected to respond better to retreatment than those who were given full dosages whose virus was refractory to the drugs. Indeed, patients who have partial HCV RNA response to the initial course of treatment are far more likely to respond to retreatment than those whose virus level did not change (620,621).

Several strategies for improving response to retreatment of nonresponders have been proposed. Several trials with high-dose IFN induction therapy were unsuccessful. A longer duration of treatment might be helpful in patients with slow and partial response. Berg et al. found that although the overall response was similar in patients treated for 48 or 72 weeks, those who had EVR but still had low levels of virus (<50,000 IU/mL) were most likely to benefit from prolonged treatment (622). Another study randomized genotype 1 patients who remained PCR positive at week 4 to 48 or 72 weeks (623). This study found a higher SVR rate among those who received the longer course of treatment, but the study was hard to interpret because it utilized a suboptimal dose of ribavirin and the overall SVR rate was accordingly low. More recently, a few studies have suggested that high doses of antiviral drugs throughout the treatment course might be successful in achieving SVR in patients who were resistant to the effects of standard doses. Leevy et al. initiated 48 weeks of high-dose daily consensus IFN and ribavirin in patients who failed to achieve an early viral response (563). One would expect that almost no patient without EVR would achieve SVR with continued treatment with the initial doses (550). Nonetheless, the SVR rate with

this intensive regimen was similar to that with treatment of naïve subjects (27% in African Americans and 41% in whites). These data are intriguing and suggest that higher drug doses throughout treatment might be effective in some difficult-to-treat subgroups. Indeed, Gross et al. reported the results of the REtreatment of NonrEsponders with Weight-based dosing (RENEW) study in which 704 subjects who had failed to clear HCV RNA during a previous treatment course were treated with either 1.5 μg/kg or 3.0 μg/kg of pegylated IFN α-2b in combination with weight-based ribavirin (800 to 1,400 mg/day) for 48 weeks (558). SVR was significantly higher in the high-dose group (17% vs. 12%). Importantly, while SVR was lower in African Americans and patients with advanced fibrosis who received a standard dose of pegylated IFN, these differences were not seen in the high-dose group. Similarly, Shiffman et al. reported that high-dose ribavirin significantly reduced relapse and as a result almost doubled the SVR rate, particularly in difficult-to-treat patients such as African Americans (624).

Another strategy for retreating nonresponders is long-term IFN maintenance. It is clear that some patients who do not clear HCV RNA during treatment have a reduction in hepatic inflammation and fibrosis (186,426,510,511,528,529). However, it is not known whether this effect persists with maintenance therapy or whether it might result in a lower risk of disease complications such as liver decompensation or HCC. Shiffman et al. found that fibrosis could be reduced with long-term IFN administration in the subset of patients who demonstrated early histologic improvement despite viral persistence (625). Everson reviewed the results of three trials that administered IFN treatment of patients with cirrhosis (626). The overall incidence of HCC was 15% in untreated patients, 4% in IFN nonresponders, and 0% in sustained responders (626–630). Although the accumulated data suggests that IFN might provide a beneficial long-term effect, no prospective studies have confirmed any benefit to date. However, three trials are currently under way to examine the efficacy of long-term pegylated IFN in preventing progression of fibrosis and development of decompensation (the federally sponsored Hepatitis C Long-term Treatment against Cirrhosis (HALT-C) study and the pharmaceutical company–sponsored Colchicine versus PEG-INTRON Long-Term [COPILOT] and Evaluation of PEG-INTRON in Control of Hepatitis C Cirrhosis [EPIC3] studies).

In summary, retreatment should be strongly considered in patients who previously received IFN monotherapy or standard IFN in combination with ribavirin. This is particularly true in patients who already have advanced fibrosis. Retreatment of patients who failed to respond to pegylated IFN and ribavirin is unlikely to be beneficial and cannot be recommended. Several studies with high-dose antiviral therapy need to be confirmed before these aggressive regimens are routinely used. To date, there is no good evidence to justify maintenance IFN therapy outside the clinical trials.

NONPRESCRIPTION AGENTS USED FOR CHRONIC HEPATITIS

The use of alternative medicines is increasingly common in the United States and Europe, with nearly half of surveyed adults using some form of these agents (631). Several agents, including α-tocopherol, bayberry, blessed thistle, milk thistle, blue flag, dandelion root, fringe tree bark, gentian, yellow dock, and various Chinese herbal remedies, have been touted as effective remedies for chronic hepatitis in the lay literature, although there are little data available to support their use. Silymarin, commonly referred to as *milk thistle*, is the most popular of the herbals and is used by approximately 12% of patients with chronic hepatitis C, although this is probably a significant underestimation because nearly 40% of patients fail to report the use of such compounds to their physicians (632). The active ingredients in milk thistle are the flavanoid silymarin and its main structural component silybin. Animal and cell culture studies have demonstrated that these compounds inhibit the lipoxygenase pathway and have antioxidant properties that diminish toxicity induced by a variety of hepatotoxins including *Amanita phalloides*, acetaminophen, and allyl alcohol if the drug is administered before toxin exposure (633,634). However, both silymarin and silybin have been shown to induce cell damage in cell culture systems (635). The popularity of milk thistle among patients with liver disease must be attributed to word-of-mouth, lay literature, and highly effective although misleading Internet marketing ("nature's premier herbal liver tonic with specific protective benefits for liver tissues"; "supports the production of new liver cell to replace the old or damaged ones") because the compound has repeatedly been shown to be ineffective for the treatment of chronic hepatitis C (636–639). Milk thistle does appear to be well tolerated for short periods except for occasional gastrointestinal symptoms and rash (640,641). Nevertheless, all herbal and nonprescription agents should be used with extreme caution, especially in patients with significant liver disease, because the safety profiles of these remedies have not been critically studied.

FUTURE TREATMENT OPTIONS

Future options for combination therapy may include antibodies or envelope inhibitors to prevent binding to receptors and cell entry, oligonucleotides such as interfering RNA or antisense molecules to prevent