To C or Not To C: These Are the Questions

By Harvey J. Alter

Cloning of the Hepatitis C Virus (HCV) in the absence of an observed particle, a tissue-culture system, an established genomic sequence, or a serologic assay was a remarkable accomplishment that has illuminated the perplexing entity long known as non-A, non-B (NANB) hepatitis.1-2 However, despite this illumination, the discovery of HCV has raised innumerable questions, only some of which were contemplated in the dark ages of NANB hepatitis research. This review will focus on the questions and the biologic and clinical issues they engender.

What Are the Structural and Functional Components of HCV?

Within 1 year of the detection of the first HCV-specific clone (5-1-1), the entire viral genome was sequenced and many of its structural and functional relationships were defined.3 HCV is a single-stranded, positive-sense RNA virus of ~9,500 bases coding for ~3,000 amino acids. It has no DNA intermediate and, hence, cannot integrate into the host genome, but does use a negative-strand RNA in its replicative cycle within the liver. HCV has similarities to the family of Flaviviridae, sharing ~40% sequence homology in the conserved 5' region and having similar hydrophobicity patterns. The virus is now considered to be a new member of the Flaviviridae having the closest relationship to the pestiviruses that include hog cholera virus and bovine diarrheal virus.

The similarity of HCV to the Flaviviridae and the established sequence have allowed the deduction of a crude structural model as shown in Fig 1. The 5' untranslated region (UTR) extends from the N-terminus. The strong sequence conservation of this region suggests that it has a major regulatory role in viral transcription or translation, but this role has not been defined. Downstream from the 5' UTR are the structural sequences of the genome, coding for the core (nucleocapsid) and then for sequences designated E1 and E2/NS1 that code for glycosylated proteins presumed to represent the viral envelope. It is unclear whether the NS-1 region is part of the envelope or the first nonstructural (NS) protein. It is important to note that the nucleocapsid is relatively well conserved from strain to strain whereas the envelope shows great variation among strains, between individuals and even within the same individual over time. Downstream from the structural coding regions are a series of NS genes designated NS-2 to NS-5 that code for proteins with enzymatic functions including a polymerase, replicase, and helicase as indicated in Fig 1. Overall, HCV has one open reading frame that encodes a single polyprotein precursor that is subsequently cleaved into functional proteins by the combined action of host-encoded and virus-encoded proteases.

What Are the Mechanisms of Viral Persistence?

The most striking feature of HCV is its ability to persist in the host. Previous estimates of persistence based on chronic alanine aminotransferase (ALT) elevations considerably underestimated the extent of viral persistence. Classically, chronic non-A, non-B/HCV infection has been thought to occur in 50% of infected individuals. More recent estimates, based on long-term follow-up in the National Institutes of Health (NIH) cohort of patients with transfusion-associated hepatitis, indicate that chronic hepatitis occurs in ~70% of infected individuals. Measurement of HCV RNA in serum and liver suggest that the extent of persistent infection is higher than the estimate of chronic hepatitis and may be in the range of 70% to 80% (H.J. Alter, in preparation). Given this high frequency of measurable persistence, there is some question whether any patients completely clear the virus and fully recover from HCV infection.

What then are the mechanisms of such pervasive persistence? What adaptations has the virus made to so effectively evade the host immune response? Clearly, it is not related to viral integration into the host genome as there are no DNA intermediates in the viral life cycle. Rather, the mechanism of persistence appears to reside in the virus' ability to mutate rapidly under immune pressure and to exist simultaneously as a series of related, but immunologically distinct variants, any one of which can become the predominant strain when a coexistent strain comes under immune press-
Mutations may also result in the formation of defective interfering particles that can absorb potentially neutralizing antibodies and prevent such antibodies from reaching the replicative particles. In addition, it appears that HCV can downregulate its replication to escape immune clearance, while persisting in the liver in a quiescent state. These mechanisms, and perhaps others, have allowed HCV to become an efficient machine for survival. In this survival scheme, a master strain is accompanied by a series of subservient strains that under immune pressure can themselves become the master; defective particles are formed that can protect replicative particles, and a low level of replication can allow the virus to both hide from the host and protect its environment by inducing such indolent disease that the cells which nurture the virus are well maintained or only incrementally destroyed. HCV is a survival machine rather than a killing machine and perhaps there is none more efficient in the viral kingdom. It is thus that this agent chronically infects ~1% of the world’s population.

**ARE THERE PROTECTIVE IMMUNE RESPONSES IN HCV INFECTION AND DO THESE PORTEND VACCINE DEVELOPMENT?**

The fact that 80% or more of HCV-infected individuals have persistent infection despite the presence of multiple HCV-directed antibodies suggests that such antibodies have a minimal role in viral clearance. Only recently has it been possible to measure neutralizing antibodies. In a chimpanzee study wherein convalescent sera were mixed with acute phase infectious serum, Farci et al have shown that a serum obtained 11 years after infection had no neutralizing effect, whereas a serum obtained 2 years after infection was able to neutralize the presumed infecting strain. Although these sera differed markedly in their neutralizing response, they both contained antibodies against nonstructural and structural HCV proteins including the envelope antigen. Analysis of sequential viral isolates from this patient showed significant genetic divergence beginning early in the course and persisting throughout. Further, different strains were recovered from different chimpanzees given the same acute inoculum, consistent with the early presence of quasi-species as outlined above. Overall, the study suggested that neutralizing responses are highly strain specific and confirmed the quasi-species nature of the virus that allows other strains to emerge and perpetuate the infection in the face of measurable neutralization.

Shimizu et al have examined neutralization using an in vitro model that employs the HPB-Ma cell line obtained by infecting HPB-ALL cells with murine leukemia virus. A clone from this line has been shown to be sensitive to HCV infection and sufficiently supports HCV attachment and growth to allow for neutralization studies that measure inhibition of both adsorption and intracellular viral proliferation. These studies showed that the infectivity titer, as measured by the detection of HCV RNA within cells, was comparable with the infectivity titers established in the chimpanzee model. Using inhibition of in vitro HCV replication as the endpoint, these studies showed that serum from a chronically infected patient was able to neutralize the original infecting
inoculum for a period of 5 years, but not thereafter. Virus isolated from this patient later in his course was not neutralized by serum obtained early in his course, but was neutralized by serum obtained 1 year after the later isolation. Thus, these in vitro neutralization studies directly confirm the in vivo neutralization studies of Farci et al10 and lead to the same conclusions, namely that neutralizing antibodies do develop, that they are strain specific, that they are ineffective against emerging strains and that persistent infection can be established despite the presence of neutralizing antibody. In sum, host immune responses are generally unable to contain the emergence of neutralization-resistant variants. In line with the above, it has been shown by the cloning and sequencing of virus recovered in chimpanzee cross-challenge studies that an animal can be infected with one strain of HCV and then reinfection with another strain.9 Of greater surprise was the fact that a chimpanzee, after presumably recovering from one episode of hepatitis, was reinfected with the same strain of HCV when rechallenged. Hence, in chimpanzees, there appeared to be no protective immune response against either heterologous or homologous challenge. Similar data has now accrued from multiply transfused patients with thalassemia who, by genetic sequencing, have been shown to sustain two separate episodes of HCV infection.10 Each of the above studies have been directed toward humoral responses to HCV infection. Little is known about cytotoxic T-cell responses to this agent. However, Koziel et al11 has performed elegant studies in which liver infiltrating lymphocytes from patients with chronic hepatitis C have been tested for HCV-specific cytolytic activity using autologous target cells infected with vaccinia virus expressing recombinant HCV core and envelope antigens. HCV-specific, HLA class I–restricted cytotoxic lymphocytes were identified. These studies have great relevance both for the pathogenesis of HCV-induced liver cell injury and for the potential to induce T-cell responses that would clear HCV from its replicative sites.

Overall, given the transient efficacy of neutralizing antibodies to HCV, the quasi-species nature of the virus, the high frequency of mutation in critical regions of the envelope protein, the potential for escape mutation, the extraordinarily high rate of persistent infection and chimpanzee and human studies showing reinfection with both heterologous and homologous strains, the prospects for vaccine development seem tenuous at best. Nonetheless, major efforts are underway to develop a vaccine that would bypass these obstacles and provide protective immunity. In preliminary studies,12 Chiron Corp has produced a vaccine that uses glycosylated E1 and E2 complexes as the immunogen. Seven chimpanzees were vaccinated with this preparation and five appeared to be protected from an HCV challenge that infected four of five controls. The caveats to this encouraging experiment are that the challenging dose (10 CID50) was small, that a previous challenge with 100 CID50 had failed, and that the challenge was administered exactly at the time of peak antibody response whereas antibody levels diminished rapidly thereafter. It is probable that the challenge dose would not have been neutralized if given 1 to 2 weeks later. More definitive studies with this vaccine are in progress.

An exciting new approach to vaccination is that of gene transfer by the injection of plasmids containing key segments of the viral genome resulting in the in vivo production of key epitopes and the induction of cytotoxic T-cell and neutralizing antibody responses. Such an approach has been used successfully for heterologous protection against influenza13 and is being strongly considered as an avenue to HCV immunization.

HOW DO CURRENT SEROLOGIC AND MOLECULAR BIOLOGIC ASSAYS REFLECT THE NATURAL HISTORY OF HCV INFECTION AND HOST IMMUNE RESPONSES?

Humoral responses to HCV are vigorous and are directed to a multiplicity of antigenic sites. As shown in Fig 1, the original clone produced a protein designated 5-1-1, which was then expanded and fused to form the c100-3 antigen that served as the basis of first generation anti-HCV assays. This early assay was highly effective in identifying HCV carrier blood donors. Retrospective analyses of stored samples from donors whose blood was transfused to prospectively followed patients who developed hepatitis C showed that the first generation assays had the potential to prevent ~80% of such cases. Indeed, this 80% reduction was subsequently proved in a prospective study conducted in Barcelona.14 The problem with the anti-c100-3 assay was that antibody often did not appear for a protracted period after exposure, creating a window of infectivity that ranged from 12 to over 26 weeks. Indeed, some HCV-infected patients never developed anti-HCV antibody. The second generation assays added two critical epitopes to both the screening enzyme immunoassay (ELA) and the confirmatory recombinant immunoblot assay (RIBA). These were a core protein designated c22-3 and an NS3 protein designated c33c. Antibodies to these new epitopes generally appear much earlier than anti-c100-3 and the window period in which a donor might be seronegative has considerably narrowed. In the NIH prospective study, it was shown that 41% of HCV-infected individuals had specific antibody detected by second generation assays within 10 weeks of exposure, 80% had antibody within 15 weeks, and all patients had antibody within 6 months. Second-generation assays were licensed in March 1992. It was predicted from retrospective analyses of prior prospective studies that second generation assays would prevent ~90% of HCV transmissions. This prediction appears to be valid based on an ongoing prospective study at NIH (see below) and a recent study from Japan.15 Third-generation ELA tests, which incorporate an antigen in the NS-5 region and delete the c100 antigen, have been introduced in Europe and are pending licensure in the United States. These assays appear to provide a slight increase in sensitivity,16 but will provide only incremental benefits in disease prevention because existing assays are already highly sensitive. Unfortunately, the third-generation ELA does not provide increased specificity. Nonspecificity has been a significant problem with both first- and second-generation assays, primarily because of nonspecific reactivity to the 5-1-1 and c100-3 antigens. Nonspecificity has been associated with aged sera, hyperglobulinemia, rheumatoid factor–positive sera, and se-
rum from persons recently vaccinated for influenza, but generally is unexplained.

Studies are in progress to evaluate the use of the envelope proteins E1 and E2. These proteins are produced in mammalian cells rather than yeast so as to allow for glycosylation and complex formation. The E1/E2 proteins thus produced appear to be the most antigenic of the HCV recombinant proteins reacting with the sera of ~90% of HCV-infected individuals. The order of reactivity in tested populations appears to be E1/E2 > c22-3 > c33c > c100-3 > 5-1-1 > NS5, with reaction rates ranging from 90% down to about 70%. In combination, these antigens react with near 100% of HCV-infected individuals, once beyond the early window period. Antibodies, particularly to 5-1-1, c100-3, and E1, may disappear spontaneously, during immunosuppression, or after successful antiviral therapy. Antibodies to c22-3 and c33c rarely disappear in those chronically infected and only infrequently disappear even in those with apparent recovery. In most individuals, anti-HCV antibodies persist for very long periods, perhaps for a lifetime. The prevalence of anti-HCV antibody using second-generation EIA, confirmed by RIBA, in US blood donors ranges from 0.2% to 0.4%. Similar rates have been observed in Europe and higher rates in Japan and other eastern countries. These rates in highly selected blood donors are underestimates of the prevalence in the general population.

Because of the problem of nonspecificity, it is important to confirm EIA reactivity with a supplemental assay. The only licensed supplemental test is an RIBA that displays the key epitopes in a linear form on a nitrocellulose strip. These strips are overlaid with patient serum and, after incubation, are treated with anti-gamma globulin and substrate that results in visible bands when specific antibody is present. A positive result requires reactivity in at least two band positions. Using second-generation assays, ~40% of EIA reactive samples are confirmed by RIBA in a low-risk blood donor setting. Among high-risk populations or EIA reactive donors who have elevated ALT, the confirmation rate is much higher (60% to 80%). In the licensed supplemental assay, ~30% of EIA positive samples are classified as indeterminate because only one band appears on the strip. A third-generation RIBA assay now under review for licensure replaces the recombinant proteins c22-3 and c100-3 with synthetic peptides representing only a portion of the respective coding regions and replaces 5-1-1 antigen with recombinant NS5. It is anticipated that this new format will resolve many RIBA-2 indeterminate patterns. There is very good, but not absolute correlation between a positive RIBA result and polymerase chain reaction (PCR) documentation of HCV RNA.

The most sensitive, but least practical way to detect the presence of HCV is the measurement of HCV RNA by PCR. PCR has shown that HCV RNA is almost universally detected in the early phase of HCV infection. Animal studies with very frequent sampling have shown the appearance of serum HCV RNA within days of HCV exposure and almost always within 2 weeks of exposure.17 Most humans also have HCV RNA in the serum within 1 to 2 weeks of exposure. Thus, HCV RNA detection precedes the increase in serum ALT, sometimes by as much as 10 to 12 weeks. In addition, it has been shown by quantitative PCR or by the branched DNA assay that the highest levels of HCV RNA are early in the course of infection, generally preceding or coinciding with the first significant ALT elevation.18 In persons who appear to recover from HCV infection, HCV RNA generally declines or disappears just before or at the peak of ALT elevation. However, in the vast majority (at least 80%) of individuals, HCV RNA persists, generally with associated fluctuating ALT elevations, but sometimes even in the absence of biochemical abnormalities. The implications of these temporal relationships are that persons may be most infectious before they have signs or symptoms of acute hepatitis, that chronic HCV infection is more frequent than indicated by ALT elevations alone, and that there may be a small segment of infected individuals that are true asymptomatic carriers with normal serum ALT and minimal histologic abnormalities in the liver.

Typically, in the chronic phase of infection, HCV RNA levels are lower than in the acute or preacute stage.18 However, RNA levels may fluctuate dramatically and sometimes show a temporal relation to fluctuations in serum ALT. In these cases, a rise in HCV RNA immediately precedes the rise in ALT and a reduction in HCV RNA precedes the reduction in ALT.18 This suggests that bursts of viral replication may be directly responsible for liver damage, either by direct cytotoxicity, by immune complex formation, or by stimulating a rapid cytotoxic T-cell response. It is difficult to sort out these possibilities because the temporal relation between RNA level and ALT level is often absent, because immune complexes exist at a high level even in the absence of significant hepatocellular inflammation, and because cytotoxic T-cell responses have been difficult to measure.

The above considerations allow for a schematic depicting the typical course of acute resolving and chronic hepatitis C (Fig 2). The essential features of viral replication, humoral immune responses, hepatocellular inflammation, and clinical outcome are described above and in the figure legend. It must be stressed that these are representative diagrams of classic cases and that there is considerable variability in these patterns from case to case. The most consistent feature is that viral replication can be detected very soon after exposure; that peak viremia occurs in the acute or preacute phase, both in patients who recover and those who progress to chronic hepatitis; that progression to chronic hepatitis cannot be predicted by the time of onset or peak level of viremia; and that antibodies to the envelope, core, and NS3 regions tend to appear earlier than those to NS4 and NS5 and tend to persist in all those with chronic hepatitis and, generally, even in those with apparent recovery.

**HOW SERIOUS ARE THE ACUTE AND CHRONIC CONSEQUENCES OF HCV INFECTION?**

Acute hepatitis C is generally a benign event. In the transfusion setting, where the acute onset is best documented, 70% to 80% of cases are anicteric and asymptomatic. In the NIH series of 86 consecutive posttransfusion hepatitis cases, only 30% had a bilirubin greater than 2.5 mg/dL and the mean peak ALT was 708 U/L. The vast majority of patients...
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Fig 2. The biochemical, serologic, and molecular biologic profile of acute and chronic TAH C virus infection is shown. Acute, resolving hepatitis C is shown in A and chronic hepatitis C in B. Resolving disease cannot be distinguished from progressive disease, based on the time of onset of detectable HCV RNA by PCR, the magnitude of HCV RNA elevation as measured by branched DNA assay, the interval to the first ALT elevation, the magnitude of ALT elevation in the acute phase, or the interval between exposure and the first appearance of antibody. Hence, progression to chronic disease cannot be predicted in the acute phase and the only distinguishing features in these patterns are the persistence of ALT elevation and the persistence of HCV RNA in those who develop chronic hepatitis C. The acute, resolving pattern (A) may be seen in 10% to 15% of patients with TAH C and the chronic pattern (B) in 85% to 90%. Other points of note are as follows: (1) HCV RNA is detectable very early after exposure. In the cases shown, PCR was positive in the 2-week postexposure sample, but it may become positive even sooner. (2) Detectable HCV RNA by the branched DNA assay may appear coincident with PCR reactivity, but may be delayed as in these cases. (3) The major peak of viral replication, as assessed by HCV RNA level, occurs before the first rise in ALT and hence, before any clinical or biochemical evidence of hepatitis. It is presumed that persons might be most infectious in this preacute-phase interval. (4) In acute resolving infection, HCV RNA levels decrease rapidly and the decrease precedes the decline in serum ALT. (5) In chronic infection, the level of HCV RNA diminishes and may either persist at low level, fluctuate, or become undetectable. As shown in B, sometimes HCV RNA levels show a periodicity that parallels the fluctuations in ALT. In this case, the increase in HCV RNA shortly precedes the increase in ALT and the decrease in HCV RNA precedes the decrease in ALT. (6) Second-generation anti-HCV assays considerably diminish the seronegative window in HCV infection as compared with first-generation assays. Nonetheless, anti-HCV was not detectable for 12 to 15 weeks after exposure and for 6 to 7 weeks after the first significant increase in ALT. (7) Antibody to HCV, as detected by second-generation assays, almost always persists in chronic cases and, generally, even in acute resolving cases; antibodies detected in the first-generation assay (anti-c100, anti-5-1-1) generally disappear in resolving cases.

had a very mild illness and none had a protracted, severe acute illness. In the setting of community-acquired acute HCV infection, where cases are identified and defined by their presentation with overt clinical illness, 70% were icteric; 4% had an ALT level between 2.5 and 5 times the upper limit of normal (ULN); 22% had an ALT level 6 to 15 times the ULN; and 74% had ALTs that exceeded 15 times the ULN. Because these community-acquired cases had to be sufficiently ill from their hepatitis to seek medical care, and since the total hepatitis C–infected population from which such cases were derived is unknown, they cannot be used to define the clinical spectrum of acute HCV infection. However, these cases do confirm that HCV infection can present as an acute, overt hepatitis that cannot be clinically distinguished from acute cases of hepatitis A or B. By extrapolation from transfusion studies, it can be estimated that such clinically apparent acute illness occurs in no more than 25% of HCV infections.

Documented fulminant hepatitis C is extremely rare. Among transfusion recipients, the occurrence is so rare as to justify publication of individual case reports. Farci et al have extensively studied three such cases and using PCR have unequivocally established the acute appearance of HCV RNA coincident with the onset of fulminant disease. Quantitation of HCV RNA using the branched DNA assay showed high levels of viremia in each case (10^7 HCV RNA equivalents/mL). The HCV genotype and sequence was not unique in these cases, suggesting that the fulminant nature of the disease related either to viral burden or to a unique host response. In hepatitis B, it has been postulated that fulminant hepatitis relates to a massive humoral and cell-mediated host response resulting in the rapid destruction of virtually all HBV-infected hepatocytes. In HCV infection, the mechanism of liver cell destruction in either acute or chronic infection is largely unknown and it is still unclear if the virus is directly cytopathic, in which case the viral burden would seem to be a critical determinant of fulminant disease, or whether there is an important immune-mediated component via either cytotoxic T cells or immune complex formation. It has been shown that acute HCV infection of liver grafts in immunocompromised transplant recipients can result in subfulminant hepatitis and also that hepatitis is more severe in the immunocompromised chimpanzee. These observations favor the hypothesis that HCV is directly cytopathic rather than that the liver injury results from the immune assault on the virus. However, these hypotheses are not mutually exclusive and both may be playing an important role in HCV-related liver injury.

Given the rarity of fulminant disease and the general benignity of the acute illness, the significance of HCV infection resides in its penchant to become persistent and induce chronic liver disease. However, even here the significance of chronic infection has been a controversial issue. There was initial skepticism as to whether NANB hepatitis represented anything more than a nonspecific, transaminitis, but evidence continued to accrue that NANB hepatitis evolved into chronic liver disease in ~50% of infected individuals and that among those biopsied, the vast majority had chronic active hepatitis (CAH) or cirrhosis. Later, an association with hepatocellular carcinoma (HCC) became evident and the potential severity of NANB hepatitis was increasingly accepted. However, the controversy has now reemerged because a carefully controlled study by Seeff et al has shown that after a mean of 18 years of follow-up, patients with transfusion-related NANB/C had no increase in mortality.
compared with control patients who were similarly transfused, but did not develop hepatitis. The study enrolled and then followed transfusion-associated hepatitis cases and controls from the five major prospective studies that defined posttransfusion NANB hepatitis in the 1970s. Although it is clear that some patients with NANB/C hepatitis have a severe outcome, this very well-designed, large-scale study provided little evidence that this clinical entity often resulted in severe, life-threatening liver disease. This conclusion is tempered somewhat by the fact that, although there was no difference in overall mortality, there was a small but statistically significant excess of liver-related mortality in patients with CAH, whereas clinical cirrhosis and HCC, Kiyosawa et al has shown in Japan that patients with chronic persistent hepatitis (CPH) tend to remain in that histologic category. In contrast, of 22 patients with CAH, 5 (23%) developed cirrhosis and 11 (50%) progressed through cirrhosis to HCC. Evolution to HCC seems more common in Japan than in Western nations that are currently unclear.

In the NIH transfusion study, 92 consecutive cases of hepatitis C have been enrolled since 1970. Six of these cases of apparent transfusion-associated hepatitis (TAH) were subsequently shown to have preexisting HCV infection; it is not certain at this time whether the hepatitis observed in these cases was an exacerbation of prior disease or a second HCV infection. Of these 92 cases, 33 (36%) have had biopsy specimens procured on one or more occasions. The majority had mild to moderate CAH and 8 of 33 (24%) had cirrhosis on the initial or subsequent biopsy. During long-term follow-up, 3 of 8 with cirrhosis died of end-stage liver disease and three had very severe liver disease when they died of other causes; 2 of these 3 had unsuccessfully sought liver transplantation. The seventh patient with cirrhosis died early in follow-up from unrelated causes and the eighth patient is alive and well 20 years after histologically documented cirrhosis. The overall liver-related mortality in the 23-year NIH series is 3% (3 of 92) and could have been as high as 6% if three patients with near-end stage liver disease had not died of a coexistent disease process.

In the Centers for Disease Control (CDC) sentinel counties study of community-acquired hepatitis, chronic hepatitis developed in 60 of 97 (62%) HCV-infected patients followed for 9 to 48 months. Of note is the fact that although biochemical evidence of hepatitis was observed in only 62%, HCV RNA was detected in 15 of 15 patients that had seemingly resolved their hepatitis. Hence, in community-acquired (sporadic) hepatitis just as in TAH, the frequency of chronic hepatitis C is more severe than that acquired by other routes. As noted above, Sanchez-Tapias et al found no difference in severity related to the route of transmission. In the CDC study, 5 of 5 transfusion recipients who underwent liver biopsy had CAH whereas only 3 of 16 (19%) with no parenteral risk factor had lesions of this severity ($P < .01$).

Another means to assess severity of HCV infection is to determine the proportion of cases that evolve into end-stage liver disease requiring liver transplantation. It is difficult to derive these numbers in a prospective fashion, but it is clear that chronic HCV infection is now the primary indication for liver transplantation. Although there are many variables that favor the selection of these cases for transplantation and, thus, inflate the proportion of patients so classified, the transplant statistics nonetheless attest to the fact that many HCV-infected patients develop end-stage liver disease and survive only by virtue of transplantation.
Chronic hepatitis C is now also the leading indication for treatment with interferon α (IFNα). Again, this represents a selection bias because IFN is only used for viral-induced liver disease and because patients with hepatitis C respond better and with lower dosages than do patients with hepatitis B. Despite this bias, the large number of patients being treated with IFN attests to the large number of HCV-infected persons showing serious or potentially serious liver lesions. In the US multicenter trial of IFN,36 45% of enrollees had CAH and 55% had active cirrhosis.

Another way to assess the severity of HCV infection is to evaluate asymptomatic persons in whom HCV infection was first detected as an incidental finding at the time of blood donation or during population surveys. In a remarkable study, designated the Dionysos study,31 the entire population of two towns in Northern Italy were asked to enroll in a study to determine the prevalence of chronic liver disease; 6,917 of 10,150 (69%) citizens enrolled in the study. Analysis showed that 17.5% of the population had persistent evidence of chronic liver disease including 1.1% with cirrhosis and 0.07% with HCC. The prevalence of anti-HCV in the entire population was 3.2%, three-fold higher than the prevalence of HBsAg. HCV infection was the second leading cause of chronic liver disease in this population, accounting for 16% of cases. Although alcohol was the leading cause of chronic liver disease, the combination of HCV and alcohol resulted in a ten-fold increase in cirrhosis and a six-fold increase in HCC compared with alcoholics with no evidence of viral hepatitis. Among the 78 patients that had cirrhosis, 28% were related to HCV; 26% to alcohol and 8% to alcohol in combination with HCV.

Two studies have performed liver biopsies in asymptomatic individuals found HCV positive at the time of blood donation. In a study of HCV-positive donors in Barcelona,32 all donors whose anti-HCV status was confirmed by RIBA had abnormal histologic findings; 8% had minimal changes, 23% CPH, 60% CAH, and 9% active cirrhosis. There was a significant correlation between ALT level and histologic activity and of the 54 patients with CAH or cirrhosis, 89% had elevated ALT levels. In a similar NIH study,33 31 asymptomatic, anti-HCV-positive donors had biopsy specimens taken. All were RIBA positive and 97% were HCV RNA positive. Biopsy specimens were abnormal in all subjects and showed more severe lesions with increasing levels of serum ALT. Among 12 persons who had ALT levels greater than two times the upper limit of normal, 91% had CAH and 9% cirrhosis compared with 43% CAH and 0% cirrhosis in those who had persistently normal ALT. The degree of liver injury did not seem related to the level of viremia as measured in the bDNA assay, but may have been influenced by concomitant alcohol consumption, especially in those with moderate ALT elevations.

Lastly, as alluded to above, there is an apparent strong association between HCV and HCC. Even before the discovery of HCV, several case reports34 and a chimpanzee study documented that NANB hepatitis could evolve into liver cell cancer. The advent of HCV serology showed a strong correlation between HCC and the presence of anti-HCV35; in Japan, 70% of HBsAg-negative HCC cases are anti-HCV positive, and HCV may have causally contributed to other cases that were both HBsAg and anti-HCV positive.36 Lower rates of anti-HCV (25%-50%) have been observed in HBsAg-negative HCC in the United States and Africa. Lending credence to these serologic findings has been the detection of HCV RNA in both cancerous and noncancerous tissues of such patients.37 Because of its global presence and persistence, HCV is thought to be a major agent in the etiology of HCC throughout the world and in many areas HCV poses a greater liver cancer risk than HBV. Almost all HCV-related HCC occurs in the setting of cirrhosis, and it is currently unclear whether the virus is directly oncogenic or mediates its effect by inducing cirrhosis and the mitotic events that accompany attempts at rapid regeneration in the chronically damaged cirrhotic liver.

The existing question in chronic HCV infection is why some patients, even those with cirrhosis, do very well over long intervals whereas others progress to a fatal outcome in less than 10 years and sometimes less than five years. Do the more severe cases harbor a more virulent strain of HCV? Do they have higher levels of virus in the liver? Do they have different immune responses or genetic susceptibilities or do they have cofactors that enhance the severity of HCV infection? The answers to these questions are not known. One speculation is that alcohol is an important cofactor and that patients with current and even those with remote alcoholic liver injury may be more prone to develop the more serious manifestations of HCV infection. There is already evidence that HCV infection and alcoholism may result in liver injury that is more than additive.38 There is need to be firm in providing advice that patients with chronic hepatitis C should not consume alcohol or do so only on very rare occasions. The distinction between viral and host factors in the evolution and severity of HCV infection remains the most fundamental issue in understanding the pathobiology of this ubiquitous and confounding disease.

**HOW SIGNIFICANT ARE HCV GENOTYPES IN DETERMINING DISEASE PROGRESSION AND RESPONSE TO THERAPY?**

Nucleotide sequences from specific regions of the HCV genome have been compared in a large number of HCV isolates from around the world. Phylogenetic analysis showed clustering of sequences into six major groups.39 Sequence similarities between members of the different groups ranged from 55% to 72% in the NS-5 region. More closely related variants within these groups, designated subgroups, have sequence similarities of 75% to 86% (mean 80%). This sequence analysis has allowed for a classification HCV variants into six major types, each with one to three subtypes. In a recently proposed nomenclature, the types are designated by an Arabic numeral and the subtypes by a lower case letter. These have been related to the former nomenclature40 such that type 1 is now 1a, II is 1b, III is 2a, IV is 2b and V is 3a. Having established the phylogenetic basis for this typing, genotypes can now be more easily determined by performing reverse transcriptase PCR (RT-PCR) with type-specific primers or by performing RT-PCR followed by type-specific restriction endonuclease digestion.
The full significance of these HCV genotypes is not known, but there are clear differences in genotype prevalence in various geographic regions. For instance, in the United States, type 1a (I) predominates, whereas in Japan, type 1b (II) and 2a (III) predominate. The prime question is whether these genotypic differences might explain differences in clinical severity of cases in Japan versus those in the United States, chronic disease appearing to be more severe in Japan. In Japan, HCV-infected patients of genotype 1b (II) have been shown in several studies to be significantly more likely to have elevated ALT and biopsy evidence of chronic liver disease than patients of genotype 2a (III).

Despite these initial reports of an interplay between genotype and disease severity, the relationship is not firmly established, and patients with the same genotype can have very different clinical outcomes. Hence, if strain differences as reflected in genotypes are important to disease outcome, they represent only one piece of a complex interaction between host and virus. In addition, the effect of genotypes may be indirect in that they may reflect differences in viral burden rather than differences in strain virulence. There is more convincing evidence that genotypes influence response to interferon therapy and this relationship will be discussed below.

HOW EFFECTIVE ARE CURRENT TREATMENTS FOR ACUTE AND CHRONIC HEPATITIS C?

The end of the past decade brought not only the cloning of HCV and the development of specific assays, but also the first substantive treatment for NANB/C hepatitis, IFN. After initial encouraging results in an open trial at NIH, IFN α 2b was used in controlled trials both in the US and Europe. Response to treatment was initially defined by normalization or near normalization of serum ALT. Subsequently, other parameters were assessed including improvement in liver histology and the loss of HCV RNA. After a 6-month course of 3 million units (MU) of IFN given thrice weekly, from 43% to 71% of patients with chronic hepatitis C were considered complete responders in these three clinical trials. The response rate in the US multicenter trial of 55 patients was 53% as compared with 9% in the placebo group (P < .001). These results were extremely encouraging, but early enthusiasm was tempered somewhat by follow-up studies that indicated that 50% of initial responders had a biochemical relapse after cessation of IFN therapy. Thus, the sustained response rate appeared to be in the range of 25%. Many additional clinical studies have confirmed this general finding and with accumulating data, it is now felt that the sustained biochemical remission rate is closer to 20%. Although this relatively low rate of biochemical remission is discouraging, it still far exceeds the spontaneous remission rate and is often accompanied by the loss of HCV RNA and by improved liver histology. Many alternate IFN regimens have been used in an attempt to improve treatment outcome including doses up to 10 MU, extension of therapy to 48 or 60 weeks, and the use of alternate forms of IFN, particularly IFNβ. Indeed, the permutations in treatment schedules are so numerous as to preclude a complete review. As a generalization, increasing dose has not improved outcome, and side effects increase markedly above doses of 5 MU; initial dosing of 3 MU thrice weekly is still the standard dose and is generally well tolerated. There are indications that more prolonged administration may be beneficial, and it is clear that treatment must be sustained for at least 6 months. There is increasing tendency to treat for 1 year and studies are in progress to determine if chronic low-dose (1 MU) maintenance therapy will improve the sustained remission rate. There are advocates of each IFNα and IFNβ with α 2b being most commonly used in the United States.

When HCV genotyping methods were developed, many studies were performed, particularly in Japan, to relate genotype to treatment response. Indeed it was shown that patients with genotypes II (1b) and III (2a) responded better than those with the common US genotype, I (1a). This suggested that viral strains differed in their susceptibility to the antiviral effects of IFN. However, more recent data suggests that response is primarily a function of viral burden and that the effect of genotype is only indirect to the extent that it reflects the level of HCV RNA; responsive genotypes tend to be associated with lower levels of HCV RNA in the serum, presumably reflecting lower rates of viral replication. The predominant US genotype, 1a (I), tends to be associated with higher levels of HCV RNA and poorer responses to IFN. Using branched DNA technology for quantitation of HCV RNA, it was shown that nonresponders had a mean HCV RNA level more than doubled that of those who had a sustained response. Hence, the pretreatment HCV RNA level is perhaps the best prognosticator of treatment response. Posttreatment determination of HCV RNA is also useful in predicting relapse; treated patients who normalize ALT, but do not completely clear HCV RNA are likely to relapse. However, there is great variation in these parameters and some patients will have sustained ALT normalization despite persistence of low-level HCV RNA and others will clear HCV RNA only to have detectable levels return at a later time. Despite these variations, patients who have a sustained loss of HCV RNA after therapy are generally in true remission and perhaps cured, and patients who significantly diminish their level of HCV RNA, even if still detectable by PCR, will generally show lessened degrees of hepatocellular inflammation. Conversely, patients who do not lose or sustain low levels of HCV RNA after therapy will almost invariably relapse and have no lasting benefit from their treatment.

It is now apparent that the best candidate for IFN therapy is the patient with low titers of HCV RNA, low-level ALT elevations, and generally less severe histologic lesions. Conversely, patients with higher levels of virus and more severe disease are less likely to respond. This raises an interesting paradox. Is this treatment most beneficial to those who do not need to be treated at all? It is apparent from the earlier discussion that most patients with chronic hepatitis C will do very well for a prolonged time and indeed that most will probably succumb to another illness or to natural causes before they develop clinically severe hepatitis C. Do such patients need to be treated? It is only a subgroup of patients with chronic hepatitis C that have more severe disease and progress to cirrhosis and hepatic decompensation. It is this latter group that needs treatment the most and responds the
least. Thus, who should be treated? Should we treat patients who respond well, but may not require treatment, or those who desperately need treatment, but respond poorly, or should we treat both? This is a controversial issue. I believe there is unanimity of opinion that persons with compensated liver disease who have sustained biochemical evidence of chronic hepatitis and have chronic active hepatitis or active cirrhosis on liver biopsy should be treated even though the anticipated response rate is only 20%. Most also agree that patients with inactive cirrhosis or decompensated liver disease should not be treated. Controversy resides in treating the milder cases. Some feel that any HCV carrier should be treated irrespective of the ALT level or extent of liver damage in an attempt to clear the virus and prevent potential late sequelae. Others feel that patients with mild disease should be observed over time and that treatment should be withheld until there is a therapeutic intervention with better efficacy and less toxicity or until there is biochemical, clinical, or histologic evidence of worsening liver disease. I concur with the latter view and believe this is the predominant view among hepatologists, most of whom only treat HCV carriers when there is liver biopsy evidence of CAH and/or significant hepatitis-related symptomatology.

At present, IFN is licensed in the United States only for the treatment of chronic hepatitis B and C. However, there is rationale for using it in acute infection as well and studies to test the efficacy of IFN in acute hepatitis C have been conducted and additional studies are in progress. Two early studies46,47 showed only marginal benefit of early treatment in preventing the progression to chronic hepatitis C. In both studies, less patients in the treated group had elevated ALT after 12 to 18 months of follow-up, but the differences were not significant in these relatively small trials. Encouraging in the trial performed in Italy47 was the fact that at the end of 18 months, HCV RNA was absent and the ALT normal in seven treated patients (39%), but in none of the controls. More encouraging results were obtained in a prospective, controlled study in Japan.48 Eleven patients were treated with IFN/ for an average of 30 days during the acute phase of hepatitis C. At the end of 3 years, 10 of 11 had normal ALT and absent HCV RNA compared with 3 of 14 controls that normalized ALT and only one of 12 controls that lost HCV RNA. These differences were significant and suggest the need for large-scale clinical trials to assess the efficacy of acute-phase therapy. Given the extraordinarily high rate of persistent HCV infection and associated progression to chronic hepatitis and given the overall poor response to treatment in the chronic phase, early intervention has inherent logic and appeal and should be actively pursued with appropriate randomized, controlled studies.

**IS HCV SPREAD BY SEXUAL, PERINATAL, OR OTHER NONPARENTERAL ROUTES?**

As the genomic and immunologic characteristics of HCV unfolded, it seemed that the epidemiologic patterns of viral transmission would also become readily apparent. Instead, the epidemiology of HCV has become increasingly labyrinthine, as conflicting data have amassed regarding the existence and magnitude of nonparenteral transmission modes.

Although it is evident in a variety of clinical settings and in chimpanzee transmission studies that HCV is readily spread by percutaneous inoculation, studies of community acquired hepatitis have also shown that many cases have no demonstrable parenteral exposure.49 In the CDC sentinel counties studies50 wherein clinical cases of hepatitis are intensively questioned regarding sources of exposure, it has been shown in recent years that blood transfusion accounts for only about 5% of such cases, that the prime identifiable source is intravenous drug abuse (IVDA) accounting for 40% of cases and that an equally large share of cases (40%) have no identified exposure source.

The unexpectedly large number of cases with no identified parenteral exposure has given rise to the strong suspicion that NANB/C hepatitis is sexually transmitted. However, evidence to support sexual transmission has been scant and difficult to compile. The primary data favoring sexual transmission came from a case control study conducted by M. Alter et al51 at CDC. In this study, 52 patients with NANB hepatitis were matched to 104 uninfected controls and extensively questioned regarding potential sources of HCV exposure. Only two responses achieved statistical significance in the multivariate analysis; these were “did you have two or more sexual partners in the preceding six months?” and “was there a history of hepatitis in a household or sexual contact?”; 12% of patients with NANB hepatitis answered affirmatively to both questions compared with 1% of controls (P < .05 and .06, respectively). To date, this single statistical association remains the strongest evidence favoring sexual contact as an important vehicle for NANB/C transmission. In contrast, there are considerable data to suggest either that sexual transmission does not occur or occurs with a very low level of efficiency. In multiple studies of male homosexuals,51,52 anti-HCV is found in only 4% to 8% after correction for coexistent IVDA. Although this prevalence is above the background prevalence established in blood donors (0.3% to 1%), it is far below the 60% to 80% prevalence of antibodies to other viruses, such as hepatitis B virus and human immunodeficiency virus (HIV), known to be sexually transmitted in this population. Further, anti-HCV prevalence in homosexuals generally does not correlate with sexual practices that foster viral transmission.53 In addition, when the specific sexual partners of HCV-infected homosexuals are tested, they tend to be anti-HCV negative unless they have their own parenteral risk factor.54 Virtually, the same findings have been observed in studies of female prostitutes and their male clients54 where anti-HCV prevalences again range from 4% to 8%. Finally, it has been shown in the United States that the true population prevalence is approximately fivefold higher than the highly selected blood-donor population and, hence, not significantly different from that reported in non-drug-using homosexuals and prostitutes.

In summary, there are three factors that mitigate against sexual transmission of HCV in promiscuous populations: the relatively low frequency of antibody when corrected for IVDA and when compared with established sexually transmitted diseases; the absence of a correlation between anti-HCV and sexual practices that enhance viral transmission; and the absence of infection in sexual partners of infected index cases if the partner has no independent risk factor.
The most direct way to assess sexual transmission is to test specific heterosexual partners of those known to be HCV infected. There have been many such studies and almost all conclude that there is minimal evidence for sexual transmission. In five European studies, less than 4% of exposed heterosexual partners were anti-HCV positive. In an ongoing NIH study of HCV positive donors, 6 of 73 partners (8%) were anti-HCV positive. However, 5 of the 6 had independent parental risk factors and one was an unreliable historian. Hence, the possible risk of sexual transmission in this population is 1.4% (1/73) and the probable risk is zero.

An interesting observation in hemophiliacs and their partners sheds light on the sexual transmission of HCV. Eyster et al. studied 235 hemophiliacs and their sexual partners. Among the 170 female partners of HIV+/HCV+ hemophiliacs, 20 (12%) were infected with HIV and 7 (4%) were infected with HCV. In contrast, of the 30 female partners exposed to HIV+/HCV+ hemophiliacs, none were HCV infected. The data suggest that HIV is more readily transmitted sexually than HCV and that HCV is poorly transmitted except in the presence of coexistent HIV infection. It is tempting to postulate that the immunodeficiency that accompanies HCV infection facilitates the replication of HCV to levels that can be transmitted by sexual and perhaps other nonparenteral routes. This hypothesis is now supported by quantitative measurements of HCV RNA in patients coinfected with HIV and HCV and by animal studies showing an increase in HCV titer following experimentally induced immunosuppression.

These findings suggest the following conclusions regarding sexual transmission of HCV: (1) HCV is neither spread by sexual routes or is spread with very low efficiency as compared with other established sexually transmitted agents; and (2) transmission may be dependent upon viral burden in the index case and this burden may be higher in the presence of coexistent HIV infection or other immunodeficiency state. It is probable that HCV can be spread sexually under conditions of high-level viremia or particular sexual conditions that foster the entry of virus through mucosal barriers. The critical question is whether even a very low level of sexual transmission can account for a large number of cases of HCV infection, given the number of persons who engage in sex and the frequency with which it is engaged. Because of the uncertainties and complexities of this issue, there are currently no specific guidelines from CDC regarding changes in sexual practice relating to HCV infection. Persons with multiple sexual partners should follow existing guidelines for safe sexual practice including the use of barriers to prevent contact with body fluids. Persons in monogamous relationships should have the untested partner tested. If found negative, the partners should be advised of the uncertain risks of sexual transmission of HCV and then allowed to make their own decision regarding the use of condoms or other protective devices.

Most of the confounding variables and data sets that complicate interpretation of the role that sexual activity plays in HCV transmission, also confound our interpretation of vertical transmission. Indeed, the data are even more conflicting in this setting. The vast majority of infants born to anti-HCV positive mothers are themselves anti-HCV positive when first sampled. Serial samples almost invariably show a loss of antibody over time indicating that this represents passive transfer of maternal antibody rather than active infection. However, there are some examples where antibody persists in the infant for more than 1 year or where antibody first appears 3 to 12 months after delivery. The determination of neonatal HCV infection is now primarily based on the finding of HCV RNA by PCR. Even in studies using stringent criteria for PCR interpretation, there is major discordance in the results and their interpretation. Most studies show minimal evidence for mother to infant transmission with infection rates ranging from zero to 5%. In contrast, Giovanni and others showed anti-HCV seroconversion or persistent anti-HCV in HIV+/HCV+ infants born to anti-HCV positive mothers; each of the 11 were coinfected with HIV suggesting, as in sexual transmission, that HIV infection may facilitate the nonparenteral transmission of HCV. In a composite of published studies through 1993, 10 studies showed zero to <5% of maternally exposed infants to be HCV infected, two showed infection rates of 10% to 15%, three studies showed rates between 40% and 50% and two studies had HCV neonatal infection rates near 90%. Although there was some correlation with HIV status, this alone did not account for the divergent findings. Differences may reflect differences in the maternal populations, differences in viral strains or genetic susceptibility, or differences in PCR methodology resulting in either false-positive or false-negative results. At present, these discrepancies in reported data on vertical transmission cannot be resolved. It is probable that, as with sexual transmission, there is a generally inefficient spread of this virus that is enhanced when maternal viral burden is high as might occur in HIV-infected individuals. Indeed, studies that use quantitative assessments of HCV RNA have now confirmed that transmission is more likely when maternal viral burden is high. There are currently no specific CDC or World Health Organization recommendations that address vertical transmission and no established preventive measures to protect the neonate. Similarly, there are no recommendations advising against pregnancy based on anti-HCV status.

Lastly, in considering nonparenteral modes of HCV transmission, one needs to consider more obscure modes by which blood may be transported from individual to individual; such covert spread may be an important determinant of HCV transmission and may be compounded by the failure of individuals to be truthful about prior IVDA. In a study performed in an area of Japan where HCV infection is hyperendemic (anti-HCV prevalence, 20%), it has been shown that the primary cause of the high rate of HCV infection is neither sexual or perinatal spread, but rather the use of unsterilized needles in the practice of folk medicine. Indeed, both folk medicine (including acupuncture and related techniques) and traditional medicine, in which economic conditions dictate the reuse of needles, may account for a large segment of HCV-infected individuals throughout the world. Furthermore, there is increasing evidence that IVDA is vastly underreported. In an ongoing study at NIH in which HCV-positive donors are recalled for an extensive
medical history and laboratory evaluation, 44% admit to intravenous drug use with shared needles despite having denied such use at the time of donation. These individuals were not current drug addicts, but had experimented with drugs at some point in their life, usually from age 18 to 30 and usually for only a brief duration. Nonetheless, this observation points to the frequency with which such practices exist and the need for very careful, in depth questioning before concluding that no parenteral exposure exists. In the NIH study, 80% of HCV carriers had apparent or covert parenteral exposures and this still may be an underestimate.

Thus, the epidemiology of HCV infection remains perplexing and will take additional large-scale studies to sort out. The difficulty of unequivocally establishing the existence of sexual or perinatal transmission and the proven occurrence of covert parenteral spread invokes a level of caution in the final interpretation of transmission patterns. Overall, it is probable that parenteral transmission accounts for the majority of HCV infections, but that nonparenteral transmission occurs in certain settings. Although nonparenteral transmission may be very inefficient, it might cumulatively account for a large number of infected persons because of the frequency of potential exposures.

WHAT IS THE RISK OF TAH IN THE AFTERMATH OF ANTI-HCV DONOR SCREENING?

The impact of HCV testing on TAH incidence has been remarkable. As soon as first generation assays became available, they were applied to stored sera from previously conducted prospective studies of TAH. In an analysis of donor-recipient relationships in the multicenter transfusion-transmitted virus study, it was shown that an anti-HCV-positive donor was associated with 81% of hepatitis C cases and, hence, that this proportion of cases might have been prevented had these assays been in place in the 1970s when these studies were originally conducted. The later retrospective application of second generation EIA to this same cohort and a correction for background transaminis, showed that second-generation assays would have prevented 93% of TAH. Similar findings came from the NIH prospective series wherein it was found that first-generation assays would potentially have prevented 80% of type C TAH and second-generation tests, 88% of such cases. That these retrospective projections were accurate was shown in a prospective study conducted in Barcelona. Immediately prior to the introduction of anti-HCV screening, the incidence of TAH in Barcelona was 9.6%. One year after the exclusion of anti-HCV-positive donors, the incidence had fallen to 1.9%, an 80% reduction attributed solely to anti-HCV testing. In a large study of anti-HCV seroconversion among transfusion recipients, Donahue et al showed a per unit risk reduction of 80% to a residual risk of 0.07% per unit transfused.

It can be calculated from the prevalence of HCV carriers (0.3%), the number of blood products transfused (15,000,000 annually), and the infectivity of anti-HCV-positive, RIBA-positive blood (90%) that the introduction of first-generation assays initially prevented 111 transfusion-associated HCV infections per day in the United States alone or ~40,000 infections per year. A subsequent analysis showed that second-generation assays would detect one additional true-positive donor in every 1,000 tested. This could have prevented an additional 10,000 to 13,000 HCV transmissions per year.

The efficacy of HCV screening has now been further shown in the ongoing NIH prospective posttransfusion study. In the ~300 recipients that have been prospectively followed since second-generation testing was introduced in March 1992, there has not been a single case of hepatitis. Although the study is not large enough or bold enough to project that the blood supply has achieved zero risk, the upper bound of the confidence interval indicates that the risk of hepatitis per transfusion episode does not exceed 1.2% and the probability is that the current risk is much closer to zero than to the upper bound. This is a remarkable achievement given that hepatitis rates at NIH were 33% in the 1960s and still 10% to 12% in the 1980s. Although anti-HCV screening has been a very critical determinant in this risk reduction, it has been only one of many interventions since 1985 that have increased blood safety. Other measures include the more judicious use of blood products by physicians and a concerned recipient population, the increasing use of autologous blood by predeposit or intraoperative salvage, intensive donor questioning and interdiction based on high-risk behavior, the shift to single-donor platelets, the introduction of surrogate assays, HIV and human T-cell lymphotropic virus testing and viral inactivation of clotting-factor concentrates. Pending procedures are the inactivation of frozen plasma and plasma products by solvent-detergent and the potential to virally inactivate platelets by photochemical decontamination.

Blood transfusion is now extraordinarily, though not absolutely, safe and has the realistic potential to be hepatitis free in the foreseeable future.

ARE THERE HEMATOLOGIC OR OTHER NONHEPATIC SYNDROMES ASSOCIATED WITH HCV INFECTION?

Hepatitis-associated aplastic anemia. The association of aplastic anemia and viral hepatitis has been repeatedly recognized and well documented. Typically, the aplasia occurs weeks to months after the episode of hepatitis and tends to be severe and irreversible. In contrast, the hepatitis tends to be mild and often clinically inapparent. Almost uniformly, the cases have been found to be unrelated to hepatitis A or B viruses and, thus, have generally been classified as NANB hepatitis. When the primary agent of NANB hepatitis was shown to be HCV and this agent was found to be related to flaviviruses, agents known to infect hematopoietic stem cells, it seemed logical to assume that HCV would prove to be the cause of hepatitis-associated aplastic anemia. However, this assumption was not confirmed and the bulk of evidence now suggests that HCV is not the cause of this hematologic syndrome. Although the prevalence of anti-HCV in such cases is much higher than the general population, the prevalence does not differ in patients with aplastic anemia who do or not have a history or biochemical evidence of hepatitis. Furthermore the frequency of antibody has been shown to be proportional to the extent of prior transfusion. Pol et al has shown that when patients were matched for transfusion volume, there was no difference in the prevalence of anti-
HCV or HCV RNA among patients with hepatitis-associated aplastic anemia and those with aplasia of other etiologies. In a study by Hibbs et al., 58% of 12 patients with hepatitis-associated aplastic anemia who had received 21 or more units of blood were PCR positive for HCV RNA compared with 19% of 16 patients that had received lesser amounts of blood (P < .05). Further, HCV was not found in the bone marrow or peripheral blood of three patients tested before transfusion therapy or in the transplanted livers of three patients who developed aplastic anemia after liver transplantation for NANB hepatitis. We have inoculated chimpanzees with both serum and liver homogenates from the above NIH patients and did not induce hepatitis C. These negative HCV studies have raised the speculation that there is an additional human hepatitis agent, tentatively designated non-A, non-B, non-C. There is currently only inferential evidence that such an agent exists, but extensive studies are in progress to define non-A, non-B, non-C through molecular approaches similar to those that were so successful in the elucidation of HCV. The aforementioned chimpanzee study, using pedigreed inocula from hepatitis-associated aplasia cases, did not transmit any form of viral hepatitis and, thus, did not substantiate the presence of a transmissible agent separate from HCV. Nonetheless, there is early evidence for a non-A, non-B, non-C agent that can be transmitted to tamarins. If confirmed, sera from hepatitis-associated aplastic anemia cases should be inoculated into this animal model.

**Essential mixed cryoglobulinemia.** The availability of specific HCV assays has established a probable causative association between HCV and type II essential mixed cryoglobulinemia, a vasculitis characterized by cryoglobulins consisting of complexes of polyclonal IgG and monoclonal IgM rheumatoid factors. Although the association of HCV with this entity now seems unequivocal, it is a surprising finding because clinical cryoglobulinemia or vasculitis is not usually associated with established HCV infection. Nonetheless, Agnello et al. found anti-HCV antibody in 42% of 19 patients with type II cryoglobulinemia and HCV RNA in 84%. Controls with type I cryoglobulinemia were negative for these markers. Further, HCV antibody and HCV RNA were concentrated 10- and 1,000-fold, respectively, in the isolated cryoprecipitate. Marcellin et al. also reported four cases of cryoglobulinemia associated with vasculitis in patients with chronic hepatitis C. HCV RNA was found in the cryoglobulin of all four patients. In one patient who normalized ALT on IFN therapy, the cryoglobulin disappeared, whereas cryoglobulinemia persisted in another patient who did not respond to interferon. In a larger series, Ferri et al. found RIBA confirmed anti-HCV in 54% of 52 unselected patients with mixed cryoglobulinaemia, only 1.2% of healthy controls, and none of 64 patients with rheumatoid arthritis, systemic lupus, or systemic sclerosis.

Although the association of HCV with essential mixed cryoglobulinemia seems unequivocal and although it appears that immune complexes involving HCV are intimately involved in cryoprecipitate formation, the pathogenetic pathways of this interaction are yet to be elucidated.

**Porphyria cutanea tarda (PCT).** As in mixed cryoglobulinemia, there is now a strong association between HCV infection and the development of PCT. This disease, believed to be caused by reduced hepatic uroporphyrinogen decarboxylase activity, appears to require extrinsic factors such as drugs and alcohol to manifest clinical illness. It now appears that HCV may serve as one of these extrinsic factors perhaps accounting for the long-established association of PCT with chronic liver disease. Fargion et al. studied 74 patients with PCT in Italy and found anti-HCV in 82% and HCV RNA in 66%. Liver biopsy performed in 42 patients showed CPH in 17%, CAH in 52%, fibrosis in 7%, and cirrhosis in 24%, a distribution typical of chronic hepatitis C. All of those with CAH were HCV infected. Similarly, Decastro et al. found anti-HCV in 62% of patients with PCT compared with 0.8% in blood donors, 17% in patients with alcoholic liver disease and 6% in hospitalized patients without liver disease. Of 15 anti-HCV-positive biopsy samples, 11 had chronic hepatitis or cirrhosis. Whether or not HCV infection is integral to the skin lesions characteristic of PCT is problematic, but it does seem apparent that HCV infection is the primary cause of the liver disease that commonly accompanies PCT.

**CONCLUSION: THE OTHER QUESTIONS**

In this review, I have selected questions that I considered to have the most clinical relevance. There is a definite selection bias and many important issues have not been addressed or have been addressed in insufficient detail, particularly in the arena of molecular biology. Virologic and immunologic questions that require continued and more intense scrutiny include the following: What is the mechanism of HCV-induced liver injury? What role does cell-mediated immunity play in pathogenesis and recovery? Is HCV directly oncogenic or associated with liver cancer only through the intermediary of cirrhosis and random malignant degeneration? Are there mechanisms other than immune pressure that induce the quasi-species nature of HCV replication? Do some persons truly recover from HCV infection and, if so, do such individuals show less strain variation in circulating virions?

In the area of blood transfusion therapy, one could ask: should surrogate tests be retained in the wake of sensitive specific assays? Can residual TAH be eradicated by viral inactivation of cellular as well as plasma products? Given the current low risk of transfusion transmitted hepatitis and AIDS will such measures be cost effective? Can we develop practical assays for the detection of viral nucleic acids that could be used in donor screening instead of or in addition to antibody testing? Is there an additional blood transmissible, human hepatitis agent (non-A, B, C)?

In the realm of treatment and prevention: How important are nonparenteral transmission routes in the global spread of HCV infection? Are there viable options to IFN therapy? Given the side effects and relatively low cure rate of IFN, should HCV carriers with normal or only mildly elevated ALT levels be treated? What are the critical epitopes for virus neutralization and can these be parlayed into an effective vaccine? Is a DNA vaccine a viable approach?

It is to be emphasized that the pivotal discovery underlying this review was not the cloning of HCV, but the molecular-biologic approach to the unearthing of infectious agents that have not been visualized, grown in culture, sequenced.
TO C OR NOT TO C: THE QUESTIONS

or immunologically defined. It is this generic approach to the recognition of infectious agents, potentially responsible for a wide array of diseases whose etiology is currently obscure, that will be fundamental to the Nobel prize almost certain to be awarded to the discoverers of HCV. No question!

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To C or not to C: these are the questions

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