Liver Disease and Hepatitis C Infection:

Is it the Virus or is it the Host?

Internal Medicine Grand Rounds

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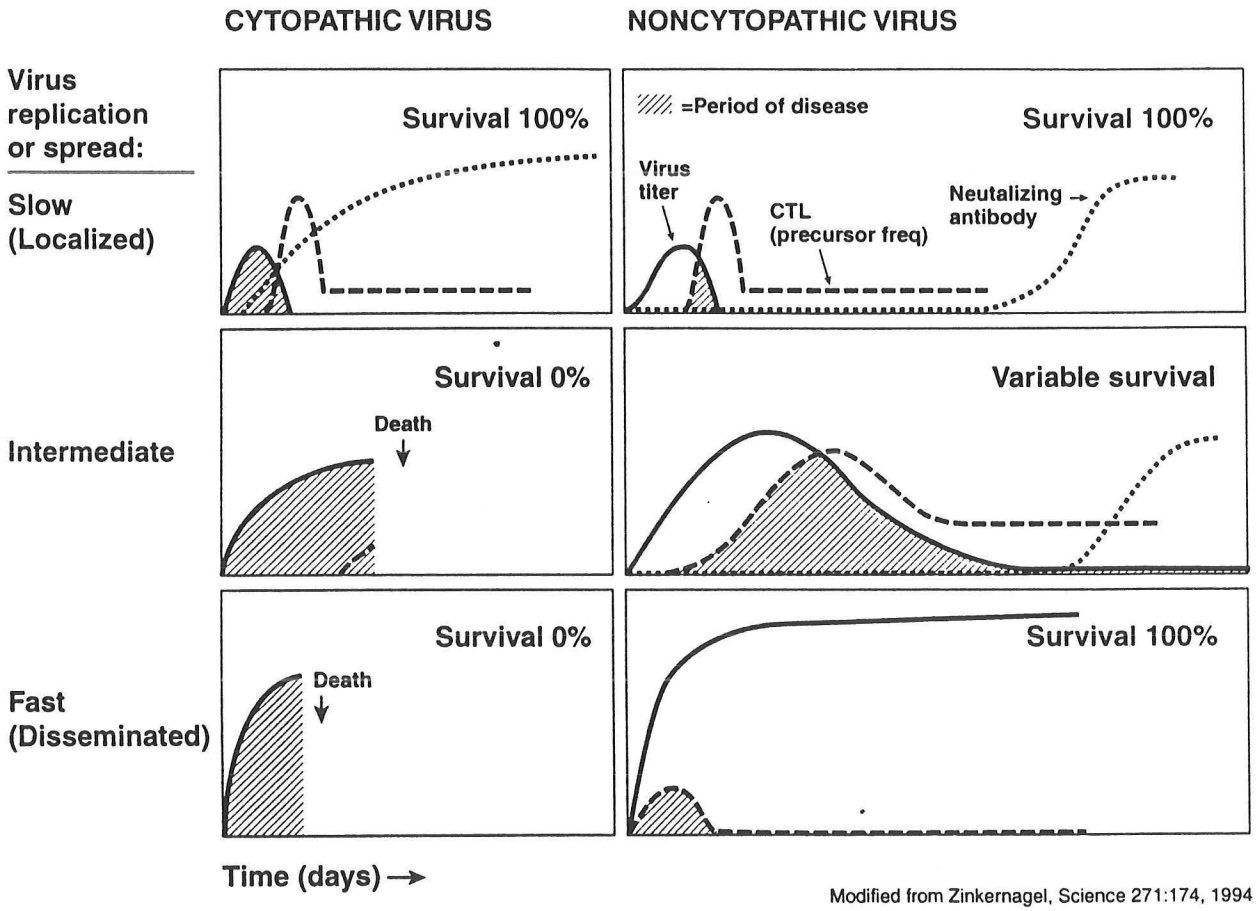
Background

Hepatitis C was first appreciated as a distinct clinical syndrome in the mid-1970's (1). Soon after the existence of a third major hepatitis virus was postulated, it was recognized that hepatitis C / non-A, non-B hepatitis was the most common cause of post-transfusion hepatitis and a frequent cause of hepatitis among injection drug users. A high rate of chronicity was soon appreciated as a hallmark of parenterally transmitted non-A, non-B viral hepatitis. Yet, since most patients with this syndrome seemed to experience at most mild, transient symptoms and were frequently found to have relatively mild histological abnormalities when biopsied 6-18 months after initial infection, physicians initially anticipated that the majority of patients with non-A, non-B hepatitis would experience little in the way of liver disease morbidity (2). By the time of the announcement in 1989 of the cloning and sequencing of hepatitis C virus (HCV) 1a (3), the first isolate from the *hepacivirus* genus of the *Flaviviridae* family, clinicians were well aware of cases of chronic non-A, non-B hepatitis that had evolved into histologically and clinically advanced liver disease.

With the increasing availability of diagnostic and research reagents over the past 9 years, the spectrum of disease associated with hepatitis C virus infection has become increasingly apparent. Current estimates suggest that close to 4 million US residents are infected with this virus which is now listed as the most common cause for liver transplantation (4). In addition to leading to >1000 liver transplants per year in the US, HCV infection is thought to cause 8,000-10,000 deaths per year from liver failure or hepatocellular carcinoma with this annual mortality rate predicted to triple within the next 10-20 years unless more effective therapies are developed (4). Despite these sobering statistics, very disparate pictures have been presented regarding the prognosis of patients infected with HCV. Recently published histologic characterization of a cohort of Irish women infected with HCV genotype 1b during administration of contaminated anti-D immunoglobulin in 1977 revealed that 17-18 years after onset of infection only 10% had developed significant septal or bridging fibrosis and only 2% had incomplete/possible cirrhosis (5). In contrast, histologic studies of patients with post-transfusion non-A, non-B hepatitis / hepatitis C have noted a 20-45% incidence of "early" or definite cirrhosis after < 5 years mean follow-up (6,7). Yet, the largest U.S. study of long-term mortality in patients with transfusion associated non-A, non-B hepatitis (8) has noted no increase in overall mortality during a mean of 18 years of follow-up. During this interval of follow-up, Seeff, et al detected only a small but statistically significant increase in liver disease related mortality from 1.5% in matched controls to 3.3% in subjects with non-A, non-B hepatitis. In contrast, physicians specializing in the care of patients with chronic liver disease have seen a very different picture of HCV associated liver disease. For instance, in a report by Tong, et al (9) symptoms were present in 2/3 of patients initially referred to a tertiary care referral center for evaluation of chronic hepatitis C, with 15% of patients dying of liver disease related causes during relatively short intervals of follow-up (mean 3.9 years). Despite these at times conflicting descriptions of the natural history of chronic HCV infection, better understanding of the host immune response to viral diseases in general and HCV infection specifically along with a growing epidemiologic and clinical experience have begun to provide a clearer picture of the pathogenesis and course of HCV associated liver disease.

Current concepts of virus / host interactions in disease pathogenesis

During the same era that the syndrome of non-A, non-B hepatitis was being characterized, Zinkernagel and Doherty were reporting their seminal observations regarding the nature of specific immune responses to viruses (15a) for which they subsequently received the Nobel prize for physiology or medicine in 1996. In addition to being the first investigators to elucidate the role of Class I MHC antigens in restricting the cellular immune response to intracellular pathogens, these two investigators and their colleagues have conducted extensive studies in animal models of viral infection that have laid a theoretical groundwork for understanding the pathogenesis of human diseases related to viral infections. As detailed in figure 1, there is not always a direct relationship between viral infection and disease. Following infection by viruses that have directly cytopathic effects on cells (examples: smallpox and influenza) there is direct relationship between total viral burden and disease(16). However, many other viruses mediate little in the way of directly cytopathic effects. Following infection by noncytopathic viruses such as the murine lymphocytic choriomeningitis virus (LCMV) or the human hepatitis B virus (HBV), disease appears to be related primarily to destruction of virally infected cells by the host immune system. In cases where host immune responses are deficient, even disseminated infections by noncytopathic viruses are associated with little or no disease.



Modified from Zinkernagel, Science 271:174, 1994

Figure 1

The most important component of the immune response that results in killing of host cells infected by viruses is mediated by cytotoxic T lymphocytes and NK cells. T cell antigen receptors react with short peptide fragments of degraded protein antigens that are bound in the cleft of MHC Class I or MHC Class II molecules on antigen presenting cells (10,11, see figure 2). Antigenic epitopes derived from intracellular pathogens such as viruses are generated by proteolytic cleavage by the proteosome complex that generates 8-9 amino acid long peptides. These peptides are transported into the endoplasmic reticulum where they form a trimolecular complex with MHC Class I molecules and β-2-microglobulin that is then expressed on the surface of the infected host cell. Such antigens usually trigger responses from T cells expressing the CD8 co-receptor that binds to the monomorphic α3 domain of the MHC Class I molecule and facilitates interaction with and signaling through the T cell receptor (TCR) complex. In contrast, extracellular protein antigens or those expressed by micro-organisms that enter into the phagolysosomes of mononuclear phagocytes are degraded within the endosomal compartment of specialized antigen presenting cells that present somewhat longer (typically 13-17 amino acids long) peptides bound to cell surface MHC Class II molecules. Antigenic peptides bound to MHC Class II molecules typically trigger responses from T cells expressing the CD4 co-receptor that binds to the monomorphic β2 domain of MHC Class II molecules and facilitates binding and signaling through the TCR complex. In contrast to T cells, natural killer cells lack clonotypic antigen specific receptors but are equipped with adhesion molecules as well as NK triggering and NK inhibitory receptors that permit these cells to identify and kill targets that both express appropriate ligands for NK adhesion molecules and NK triggering receptors and also lack "self" MHC Class I molecules that engage the NK inhibitory receptors (12). Thus, in contrast to T cells that respond to "non-self" antigens, NK cells respond to "absence of self" in that these cells are especially effective in killing cells that lack the self MHC molecules reactive with NK inhibitory receptors.

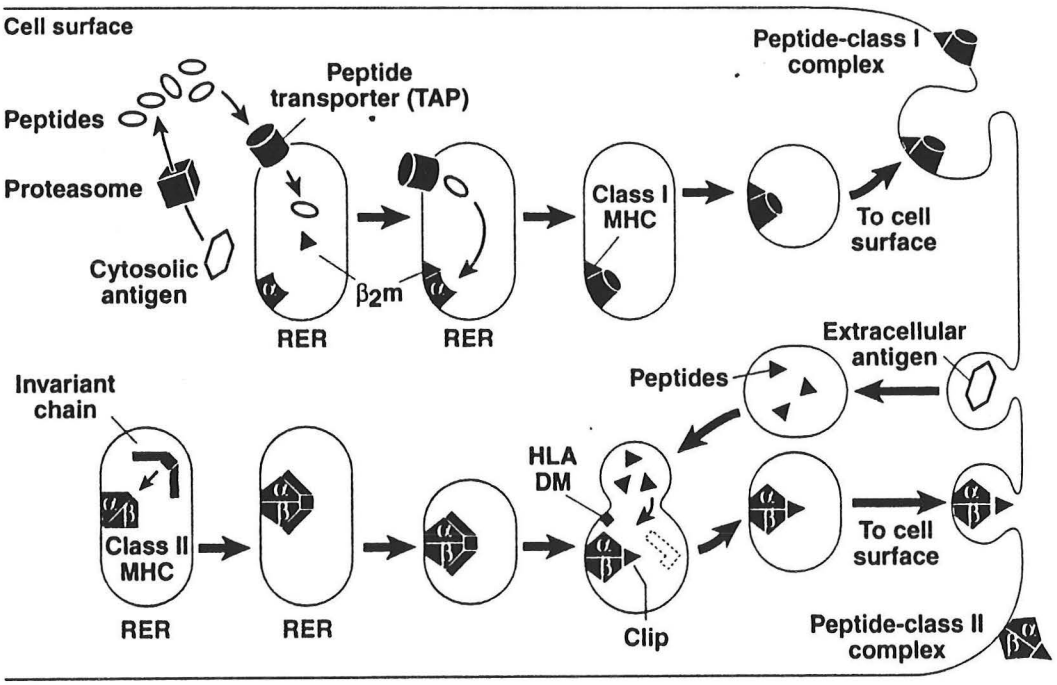


Figure 2

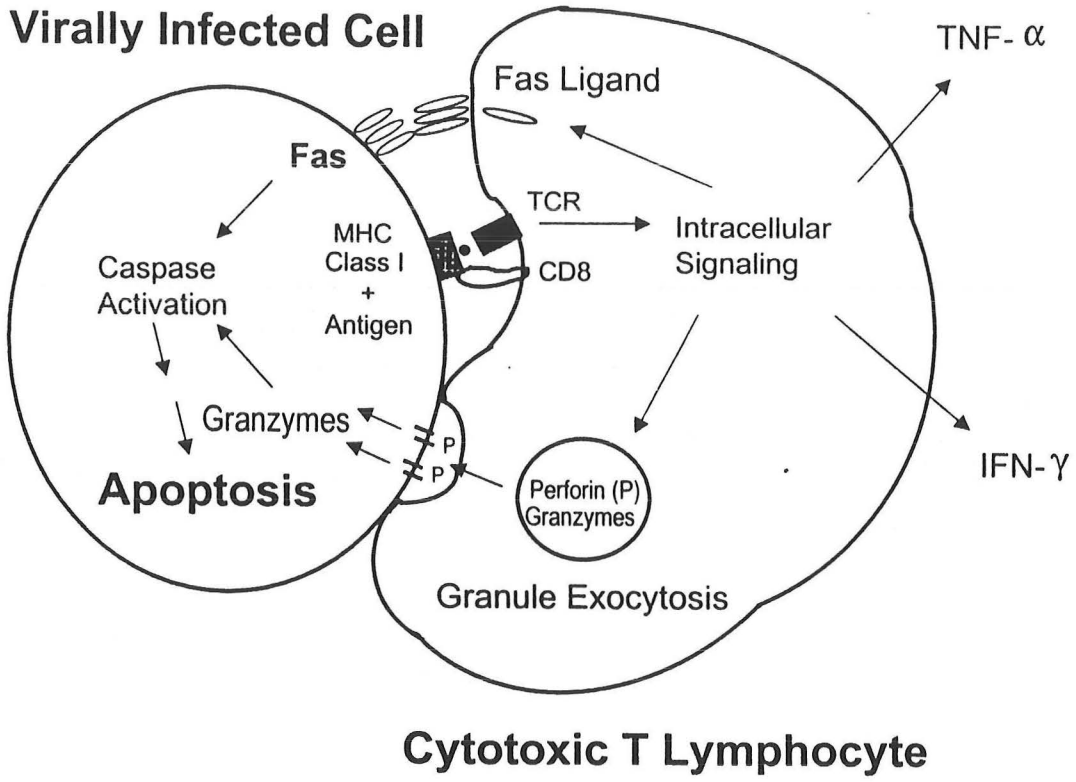


Figure 3

Although the specific target recognition mechanisms utilized are different, both T and NK cells employ similar cytotoxic effector mechanisms. As detailed in figure 3, after antigen specific triggering of a CTL, a variety of responses are elicited that leads to death of the antigen bearing target within a matter of minutes or hours (13). The bulk of rapid target cell lysis is mediated by one of two complementary mechanisms that are highly dependent upon cell-cell contact between the CTL and its antigenic target. One killing pathway is mediated by directional movement of specialized cytolytic effector granules to the region of the CTL membrane that is closely associated with the target cell. Cytolytic granules fuse with the CTL membrane and discharge their contents into the extracellular cleft between target and effector cell. Another distinct pathway is present that permits induction of apoptosis in target cells that express the Fas receptor. Although only Fas expressing cells are susceptible to this second pathway of rapid cell mediated cytotoxicity, Fas expression is induced in many cell types by inflammatory cytokines. Of special note, hepatocytes constitutively express Fas and Fas expression is further upregulated by inflammatory stimuli (15). Fas induced apoptosis can also be triggered under experimental conditions by antibodies to Fas or by trimerized soluble forms of the Fas ligand extracellular domain (14,15). When such Fas triggering molecules are infused in vivo into mice, severe hepatocellular necrosis is observed. Such findings suggest that hepatocytes may be uniquely susceptible to the Fas/Fas ligand pathway of cell mediated cytotoxicity.

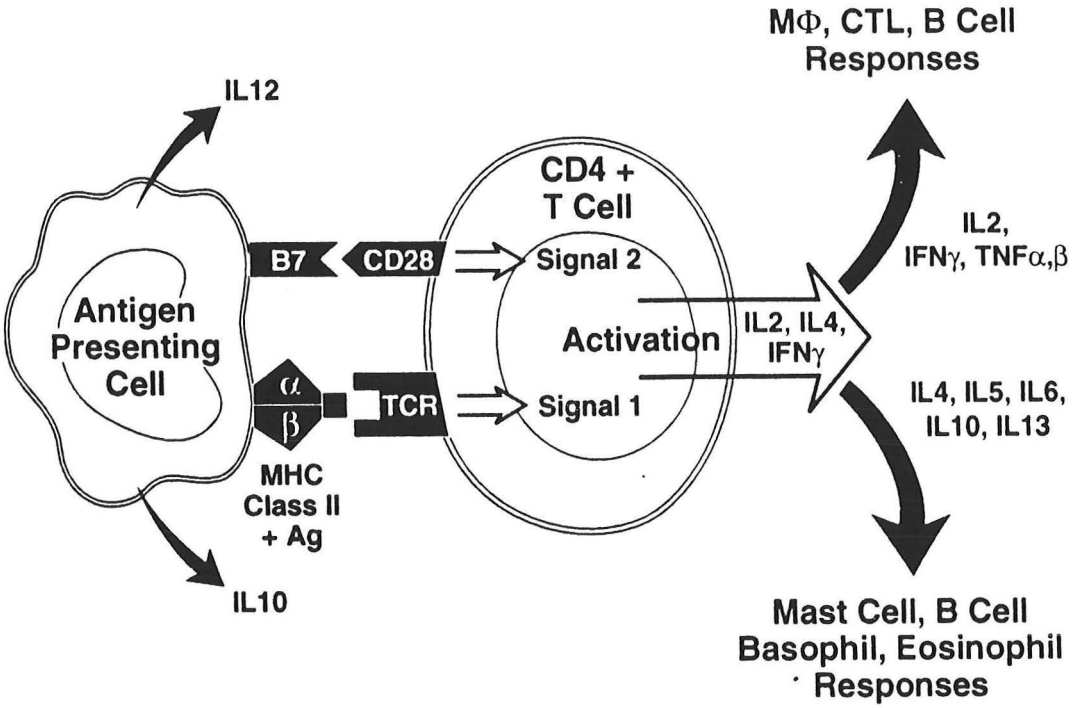


Figure 4

In addition to cell mediated cytotoxicity, both CD4+ T cell responses to extracellular viral antigens (see figure 4) and CD8+ T cell responses to virally infected cells (figure 3) elicit cytokine responses that directly (through production of Type II or γ interferon) or indirectly (via elicitation of Type I or α/β interferon) impair multiple steps involved in viral replication. T cell cytokines and interaction with T cell ligands are also of critical importance in development of highly specific immunoglobulin responses. Unlike T cell receptors, immunoglobulin molecules react with soluble antigens and often recognize complex tertiary structures of foreign glycoproteins. The major impact of antibody responses during viral infections appears to be the role that "neutralizing" antibodies directed against envelope proteins play in blocking binding of viruses to host cell receptors thereby preventing infection of additional cells and facilitating degradation of circulating viral particles.

In clinical situations, it has always been much easier to monitor virus-specific antibody responses than to measure virus-specific T cytokine or CTL responses. In addition, antibody based serologic assays continue to be the most economical although not always the most insightful diagnostic tool in identifying subjects with viral infections. However, it has become clear from more than 2 decades of investigative work performed in animal models that all 3 major components of the immune response to viruses play important roles in host resistance to viral infections. As summarized in table 1, different components of the immune response have been found to play critical or "limiting" roles in different forms of viral infection. Neutralizing antibodies are especially important in controlling disseminated cytopathic viral infections while CTL playing a more prominent role in controlling noncytopathic viral infections (16). Thus, perforin deficient mice that lack one major pathway of cell mediated cytotoxicity (see figure 3) are unable to control infections

Table 1

Critical Immune Effector Mechanisms Responsible for Resolving Viral Infections

Type of Infection	Recovery from 1st Infection	Resistance against Reinfection
Cytopathic Virus, Blood & Lymphoid Organs	T cell cytokines, Neutralizing Antibodies	Neutralizing Antibodies
Cytopathic Virus, Peripheral Organs	T cell cytokines, Neutralizing Antibodies	T cell cytokines
Noncytopathic Virus, Blood & Lymphoid Organs	Cytotoxic T Lymphocytes	Neutralizing Antibodies Cytotoxic T Lymphocytes
Noncytopathic Virus, Peripheral Organs	Cytotoxic T Lymphocytes	Cytotoxic T Lymphocytes

Adapted from Zinkernagel RM, reference 16.

by the noncytopathic LCMV virus but clear infections by cytopathic viruses such as influenza and vaccinia as efficiently as control animals (17). Although of apparently critical importance in clearing noncytopathic viral infections, cell mediated immune responses clearly represent a double-edged sword for the virally infected host since CTL activity is also responsible for host cell injury and much of the morbidity and mortality associated with infection by this class of virus. Thus as detailed schematically in figure 1, after exposure to noncytopathic viral inocula (such as LCMV or HBV) that are modest in titer or pathogenicity, development of an efficient CTL response (and concomitant cytokine and antibody responses) is typically associated with rapid clearance of the foreign pathogen after only a brief interval of "disease" associated with death of virally infected cells. However, if a different balance between viral dose and/or pathogenicity is achieved, a prolonged period of CTL attack on virally infected host cells results in longer duration and greater severity of disease. Paradoxically, in situations under which T cell responses are absent or overwhelmed by large viral loads and the host is immunologically tolerant of viral antigens, the murine host of LCMV (16,17) or the human host of HBV (18) experiences no "disease".

Although B cell or CD4+T cell responses do not appear to be necessary for controlling and eliminating infections by low doses of the murine LCMV virus (16,17,19,20), the absence of antibody responses against LCMV in B cell deficient mice (19,20) or of both T cell dependent antibody and cytokine responses against LCMV or gammaherpesvirus in CD4+ T deficient mice (21,22) renders host animals vulnerable to prolonged viremia following inoculation with intermediate viral doses that are efficiently cleared by control animals. Absence of Type I (α/β) or Type II (γ) interferon receptors also is associated with failure to clear LCMV after intermediate dose challenges (22). In both CD4+ T cell and B cell deficient mice, anti-virus specific CD8+ CTL responses develop but are progressively lost over time as viral replication persists. Similar findings

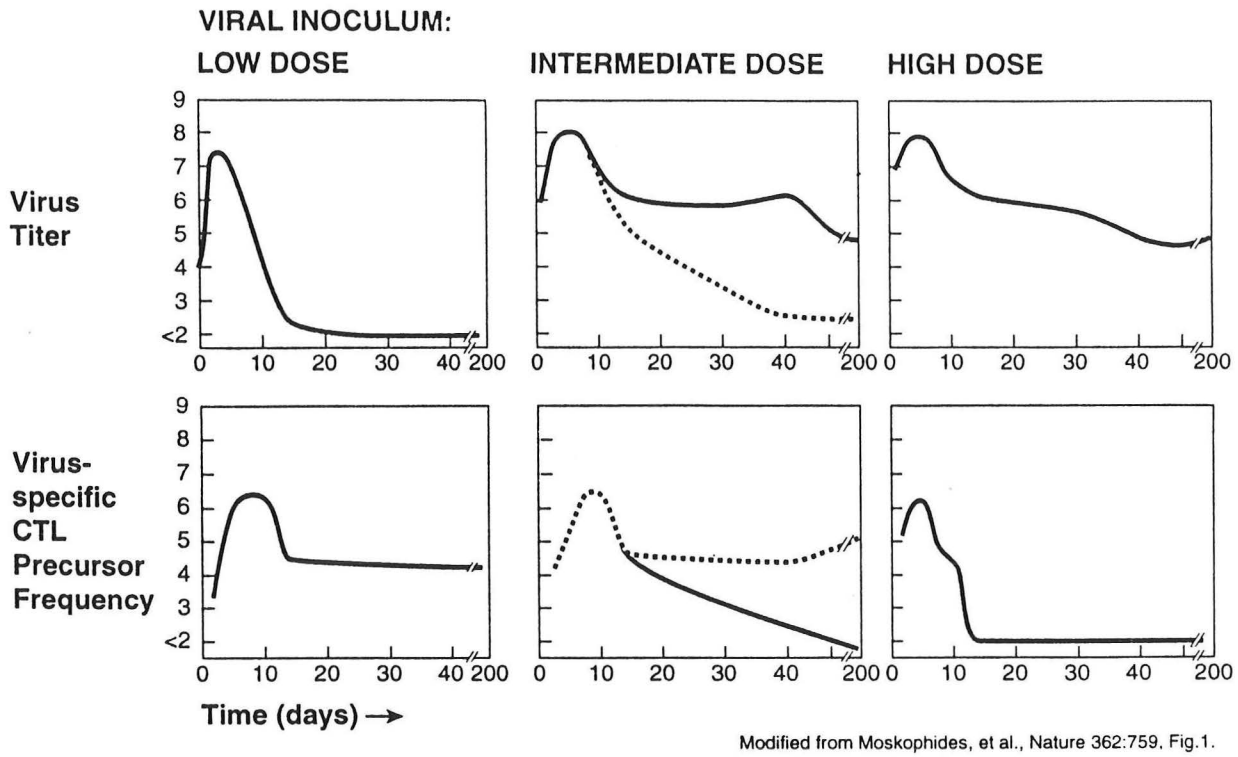


Figure 5

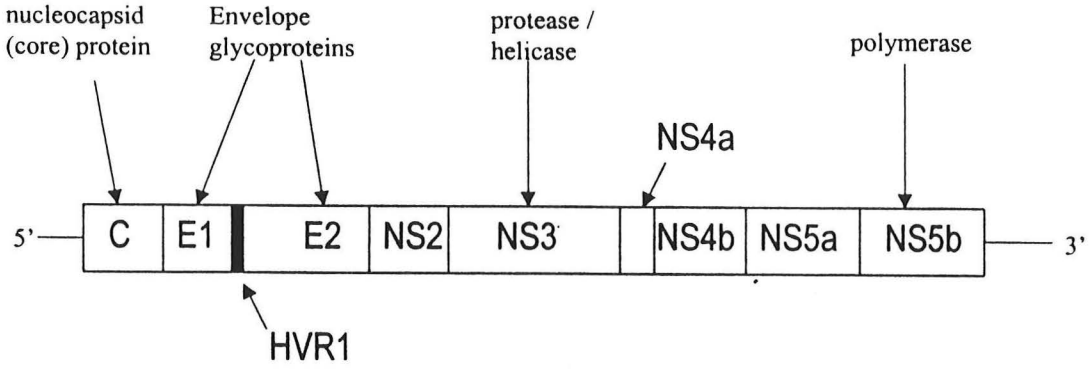
have been observed following inoculation of immunocompetent mice with varying doses of LCMV (23). Thus, in contrast to murine hosts that rapidly clear low dose inocula of LCMV and develop a long lived memory CTL response to LCMV (left panels, figure 5), mice challenged with high dose LCMV inocula exhibit a phenomenon referred to as "CTL exhaustion". In these mice (see right panels, figure 5) after a brisk initial CTL response, all virus-specific CTL appear to disappear while viral proliferation persists. In the case of animals infected with intermediate doses of the same LCMV strain (middle panels, figure 5), variable courses are observed with some animals maintaining persistent CTL responses over long time intervals with gradual control of viral replication while other animals exhibit delayed "exhaustion" of the virus specific immune response associated with persistent viremia. Similar "exhaustion" of some virus-specific CTL clones has also been observed in humans infected with HIV (24). The rapid disappearance of virus specific T cell clones early in the course of overwhelming viral infections has been attributed to high dose tolerance related to a state of maximal stimulation of virus specific T cells that are induced to undergo apoptosis rather than proceeding through additional rounds of the cell cycle and establishing a pool of memory T cells (23,24). Alternatively, it has been postulated that all viral antigen responsive T cells have undergone multiple rounds of replication with secondary telomere shortening and reached a state of replicative senescence that leads to apparent CTL exhaustion (25). It would appear that older individuals would be especially vulnerable to CTL exhaustion mediated by this second mechanism as the bulk of the T cell repertoire develops in the thymus early in life. Thymic export of T cells declines by 90% during the first quarter of the life-span with the human thymus reported to be almost entirely replaced by fat by age 60 (26,27).

Other factors also contribute to age and/or gender related differences in immune responses to viruses. In contrast to numerous observations regarding decline in T cell immune responses with age, B cell numbers and serum immunoglobulin levels remain relatively stable during aging. However, capacity for generation of highly specific antibody responses is impaired apparently because of diminished capacity for CD4+ T cell dependent isotype switching and affinity maturation (28). Although there is little data regarding significant gender differences in cell mediated immune responses, there is abundant observational evidence for enhanced B cell responses to both autoantigens and non-self antigens in females and most reports suggest enhanced anti-viral immunity in females (29). For instance, following parenteral exposure, human adult females are much less likely than adult males to develop chronic HBV (30).

During the course of host immune responses viral pathogens are not static, passive participants. Rather, viruses appear to have evolved multiple strategies to evade the host immune response. RNA viruses, and to a much lesser extent, DNA viruses exist as a population of organisms with similar but not identical genomic sequences. The quasispecies nature of RNA viruses (31) allows for immune selection / viral escape whereby the viral species that are least immunogenic are allowed to preferentially expand in the host. Alternatively viruses have also acquired genetic material encoding proteins that interfere with host immune responses. These include virally encoded proteins that impair MHC Class I expression and presentation of viral antigens to CTL, viral proteins that mimic the MHC molecules that inhibit NK cell killing and viral proteins that block FADD binding to the Fas death domain and thereby block this pathway of CTL induced killing of virally infected cells (32,33).

The Hepatitis C Virus(es)

The viral agents causing hepatitis C infection in humans have recently been classified within a third genus of the *Flaviviridae* family designated *hepacivirus* (34). The six major and multiple minor genotypes of the genus *hepacivirus* currently termed HCV genotypes 1a, 1b, 2a, 2b, 3, 4, 5 and 6 are estimated to have diverged approximately 300 years ago and now exhibit sufficient sequence diversity to merit, in the opinion of some virologists, classification as separate species. However, as will be discussed later in this protocol, there appears to be only modest differences in the clinical manifestations of infection by different HCV genotypes and for most purposes they can be regarded as a single virus. The genome of HCV is a single-stranded linear RNA of positive sense that is unsegmented, approximately 9.5 kb in total size and encodes a single open reading frame of approximately 9000 nucleotides. The approximately 3000 amino acid polyprotein precursor produced from this open reading frame encodes structural (core and envelope) and non-structural proteins (NS2-5b) as detailed in figure 6. As in the case of other RNA viruses, HCV exists in the host as genetically diverse quasispecies (34,35). Among these quasispecies the most highly conserved region of the open reading frame is the nucleocapsid or core gene with the greatest diversity occurring within the approximately 90 nucleotide (30 amino acid) sequence within the 5' end of the second envelope gene termed the hypervariable region 1 (HVR1). Genotype 1b also contains a second, shorter (7 amino acid) hypervariable region termed HVR2 which lies just 3' of HVR1.



Organization of the hepatitis C virus (HCV) genome

Figure 6

Differences in patterns of early biochemical responses to interferon-α therapy of chronic hepatitis B and chronic hepatitis C led many observers to speculate that, unlike HBV, HCV may function as a directly cytopathic virus. However, following development of techniques for quantifying HCV RNA levels, it became apparent that in other clinical situations levels of viremia did not routinely correlate with biochemical or histologic changes (36) and that infection of immunologically immature neonates was not associated with clinically significant disease (37). Thus, HCV infection does not typically produce a clinical course characteristic of a directly cytopathic viruses. One major exception to this generalization is the observation that a small subset (~2%) of HCV infected liver transplant recipients develop rapid cholestatic liver disease and subacute liver failure within weeks to months after transplantation (38). The livers of such patients have been found to have very high levels of HCV RNA yet exhibit a paucity of the lymphocytic infiltrates and other histologic features typical of viral hepatitis (39). It has been postulated that this group of patients represent cases of directly cytopathic injury induced by very high levels of HCV replication in immunocompromised hosts. Yet HCV infected liver transplants as a whole have an overall 5 year survival rate indistinguishable from that of other liver transplant recipients (38) and despite the almost uniform infection of new liver grafts in previously infected hosts, severe acute hepatitis is extremely rare. This clinical picture again suggests that in the majority of human hosts, HCV exists as a relatively noncytopathic virus.

The Human Immune Response to HCV Infection

Following development of serologic and virologic tests for HCV infection, it was soon appreciated that the previously estimated 40-50% chronicity rate after non-A, non-B hepatitis infections was a serious underestimate of the propensity of this virus to establish chronic infection. When sensitive and specific assays are used to identify cases of non-A, non-B hepatitis that truly represent HCV infections, 60-70% of acutely infected individuals are found to have both chronic

viremia and ALT abnormalities that persist for > 12 months after presentation (40, 41). In addition, half or more of subjects with self-limited biochemical features of hepatitis have persistent viremia despite normalization of ALT values (40,41). Thus, at present it is estimated that only about 15% of humans infected with HCV resolve this infection spontaneously (4,41). Even these patients appear to not develop protective immunity (42,43) as examples have been found of second HCV infections by the same or different genotypes of HCV in individuals that cleared HCV RNA after their first infection. Moreover, in research studies performed in chimpanzees, re-challenge of chimpanzees with the same or different HCV strain consistently produced new infections in both chronic carriers or in subjects that had cleared an initial HCV infection (43).

Initial attempts to understand human immune responses to HCV focused on use of assays for antibody formation. The often long delay in development of antibodies to the C-100 epitope used in the first generation anti-HCV assay led many observers to conclude that HCV simply did not elicit the same level of antibody responses observed in the course of infections by other hepatotropic viruses. However, with the development of a broader spectrum of recombinant antigens including recombinant envelope glycoproteins produced in mammalian cells, it has become apparent that nearly all humans develop antibodies to multiple HCV antigens very early in their clinical course (44-46). Moreover, in contrast to serologic patterns noted in patients acutely infected with other hepatitis (18) or flaviviruses (47-49), development of anti-envelope antibodies in patients with HCV infection is not usually associated with clearance of viremia (46). Indeed, with use of appropriately glycosylated antigen reagents derived from both the E1 and E2 region of the HCV genome, nearly 100% of humans chronically infected are found to have putative envelope reactive antibodies (46). With development of assays assessing blocking of HCV virion binding to human tissue culture cells, estimates of neutralizing antibody titers have been made (50,51). Such studies suggest that true neutralizing anti-envelope antibodies are generally low in titer (50) and antibodies capable of neutralizing the original HCV inoculum are more likely to be found early in the course of infection (51,52). To some extent, neutralizing antibodies appear to be directed at epitopes shared by multiple HCV genotypes (50). However, a major portion of neutralizing antibody reactivity appears to be directed against epitopes within the HVR1 of E2 (50,51). In immunocompetent patients followed longitudinally, alteration in HVR1 sequence is observed as are changes in the specificity of HVR1 reactive antibodies (35,53). The titers of anti-HVR1 antibodies usually reach maximal levels several months after initial isolation of an HCV clone having the specific HVR1 sequence (53). The absence of significant genetic drift in HVR1 region sequences following HCV infection in agammaglobulinemic hosts (54) suggests that accumulation of mutations in this region is a result of genetic pressure exerted by neutralizing antibodies that develop in immunocompetent hosts (34,35). Thus, current understanding of the human antibody response to HCV infection suggests that while most immunocompetent hosts develop neutralizing antibody responses, rapid genetic drift in the HVR1 sequences of the HCV envelope protein permits continued replication of immune escape variants and evolution of chronic viremia in the vast majority of humans.

Despite the failure to develop clinically effective neutralizing antibody responses in most humans exposed to HCV, recent analysis of point source outbreaks of HCV infection in young women following post-partum administration of HCV contaminated lots of anti-D immunoglobulin have revealed a statistically significant correlation between development of early anti-HVR1

antibodies and clearance of the virus (55). Partial protection from symptomatic non-A, non-B hepatitis has been observed if normal immune globulin is administered prior to blood transfusion (56-58). Finally, when second generation anti-HCV screening was first employed to screen anti-HCV positive units of plasma from pools of plasma used to prepare lots of intravenous immune globulin, the resultant lots were associated with a high incidence of HCV infection (34, 59,60). Thus, there is at least some hope for use of neutralizing antibodies or vaccines designed to elicit neutralizing antibodies as immunoprophylaxis against HCV infection. However, in vaccination trials completed thus far, HCV E1-E2 based vaccines have provided protection against only the homologous virus from which the antigenic sequence was obtained with subsequent failure to prevent infection by other HCV isolates of the same genotype (34,61).

The development of IgG responses to multiple HCV antigens indicates that the CD4+ T cell response necessary to produce IgG responses specific for peptide antigens must be elicited in al or nearly all humans. However, *in vitro* assays designed to detect and quantify these response have usually only detected responses directed against individual HCV antigens in a minority of acutely or chronically infected individuals (61a). Nevertheless, results of such studies have detected more dramatic correlations between clinical outcomes and amplitude of CD4+ T cell responses than has been noted with use of antibody assays (36). In a study evaluating patients during the first 9 months of HCV infection (62), significantly higher and more frequently detectable T proliferative responses to synthetic HCV core, E1, E2, NS3, NS4 and NS5 antigens were apparent in 12 patients who normalized ALT levels (and cleared HCV RNA in 10/12) after acute HCV infection when compared to T cell responses in subjects who developed chronic hepatitis C (i.e. persistent ALT elevations). In another cross-sectional analysis of T proliferative responses in anti-HCV positive patients with either persistently normal ALT values or with biochemical evidence of chronic hepatitis, responses to HCV core antigen was found to correlate with a benign course (i.e. nl ALT) of chronic HCV infection (63). Since both studies used soluble recombinant protein antigens, the measured responses were thought to detect CD4+ T cell responses to antigenic peptides presented by Class II MHC antigens. It is unclear however, whether the observed clinical correlations relate to the role of CD4+ T cells in facilitating B cell of CD8+ T cell responses or in production of interferons or other cytokines that directly control HCV proliferation.

Because CD8+ T cells respond to Class I MHC presented antigenic peptides derived from endogenously synthesized proteins, strategies for assessing CD8+ T cell responses are much more complicated in outbred subject populations since an appropriate MHC compatible cell infected or transfected with HCV genes must be generated for each subject. Nevertheless, a number of laboratories have developed techniques that have identified HCV-specific CTL in the livers of some but not all individuals with chronic HCV infection (64-66). As in the case of CD4+ T cell responses, CD8+T cell responses appear to be directed at multiple HCV encoded antigens and immunodominant epitopes are not apparent (65). With identification of conserved regions of the HCV genome that elicit CD8+ T cell responses in multiple HCV infected individuals, longitudinal and cross-sectional assessments of CD8+ T cell responses have been conducted and are beginning to provide insights into immune mechanisms responsible for different clinical outcomes. In patients followed longitudinally, appearance in the peripheral blood of an increased precursor frequency of HCV-specific CD8+ T cells has been noted to precede or coincide with rises in serum ALT levels

(66). Another study assessing intrahepatic HCV-specific CTL in patients with chronic HCV infection evaluated prior to interferon-α therapy noted that patients with detectable intrahepatic HCV-specific CTL activity had significantly higher ALT levels, lower HCV RNA levels and were more likely to have a complete initial response (normal ALT, undetectable HCV RNA) to interferon therapy (67,68). Some degree of inverse correlation between serum HCV RNA levels and HCV-specific CTL responses have been noted in studies from two other labs as well (69,70). Thus, while CD8+ T cell responses appear to play a role in diminishing HCV viral load, in most patients the host immune response is unable yo achieve complete viral clearance and persistence of infection is associated with a CD8+ T cell response that causes ongoing hepatocellular injury. Indeed, a striking feature about the CTL response to HCV is that it appears intermediate between that observed in asymptomatic HBV carriers in whom HBV specific CTL responses are usually undetectable (18,69) and patients with resolved viral infections in whom CTL precursor frequencies are usually much higher (18,66,69).

Cytotoxic T lymphocyte escape variants have been detected in subjects with persistent HCV infection (70,71). However, such CTL driven selection of HCV sequence variants appears to proceed at a relatively slow rate relative to the apparent B cell driven selection of HVR1 variants (70). In addition, as both CD4+ and CD8+ T cells appear to respond to multiple epitopes encoded within relatively conserved regions of the HCV genome, persistence of HCV infection cannot be attributed solely to the quasispecies nature of this RNA virus. Rather, other as yet undetermined factors appear to limit but not prevent T cell responses to HCV.

Although studies thus far of the human immune response to HCV have been relatively limited, a number of features of host virus interactions that contribute to the unique spectrum of disease associated with HCV infection have been identified. The lack of a protective antibody response to HCV appears largely related to unique characteristics of the virus and more specifically the tremendous sequence variability within the HVR1 of the E2 protein. The inability of T cell responses to clear HCV infection is likely related in part to the absence of an adequate neutralizing antibody response which usually plays an ancillary role in this process (18-20) but may also relate to other characteristics of the virus that limit T cell responses. The presence in most HCV infected humans of persistent ALT abnormalities over long time intervals appears to relate to the unfortunate co-existence of persistent viremia and persistent host CTL responses.

Factors Influencing the Clinical Course of HCV Infection

Many studies have attempted to identify characteristics of HCV genotype and quasispecies variants that influence the clinical outcome in HCV infected individuals. Early assessments of correlations between genotype and disease outcome found that patients infected with genotype 1b were more likely to have advanced disease (73). However, in Japan and Europe where genotype 1b is especially prevalent, shifts in genotype prevalence appeared to have occurred over time with older patients with longer duration of infection more commonly infected with genotype 1b and younger patients with shorter duration of disease more commonly infected with non-Type 1 genotypes. Recent large European studies that have considered both genotype as well as likely duration of infection and other patient characteristics have found no significant difference among HCV

genotypes with respect to propensity to develop cirrhosis (74,75). However, recent reports have suggested that extrahepatic manifestations of chronic HCV such as cryoglobulinemia (76), non-Hodgkin lymphoma (77) and monoclonal gammopathies (78) occur much more frequently among patients infected with HCV genotype 2 .In addition, patients infected with either genotype 1a or 1b have been found to be less likely than patients infected with genotypes 2 or 3 to achieve a sustained response (nl ALT, absent HCV RNA > 6 months after therapy) to various interferon-α containing regimens (79,80). Other putative virologic factors that have been reported to be predictive of therapeutic response to interferon therapy include quasispecies diversity in the HVR1 (79-83) of the envelope region and serum level of HCV RNA. However, as detailed earlier in this protocol quasispecies diversity in the HVR1 is likely to be as much related to duration of infection and host B cell responses as to any unique features of the original HCV inoculum. The fact that increased HVR1 diversity inversely correlates with response to interferon therapy is thus entirely consistent with reports that long standing infection / advanced histologic disease (cirrhosis) is associated with significantly decreased sustained responses to interferon therapy (79,80). In addition, the inverse correlation between vigor of host responses and levels of viremia that has been noted in HCV and other viral infections suggests that serum HCV RNA levels are likely to be a surrogate marker for overall level of host immune control of viral replication rather than simply an intrinsic feature of individual viral species.

Multiple host factors have been identified that appear to influence the clinical course of HCV infection. While immunologically immature neonates and organ transplant recipients as a whole do not appear to have unusually rapid progression of liver disease following HCV infection (37,38), a number of reports suggest that other forms of immunodeficiency may be associated with unusually rapid clinical progression of liver disease following HCV infection. Initial analysis of life expectancy in patients co-infected with HIV and HCV noted no adverse effect of HCV co-infection on overall mortality rates in HIV infected populations comprised largely of gay males (84). In this study all deaths appeared related to AIDS associated illnesses. However, there have been reports of clusters of HIV infected individuals who progressed to symptomatic cirrhosis within unusually short time intervals after acute non-A, non-B hepatitis infection (85). In addition, recent analysis of large cohorts of multitransfused hemophiliacs has indicated that subjects with HIV/HCV co-infection are much more likely to progress to liver failure than were subjects with HCV infection alone (86). However, in these studies, all complications of liver disease developed more than 20 years after receipt of first blood product infusion. Other cross-sectional studies appear to confirm that HIV/HCV co-infected individuals progress to cirrhosis at a more rapid rate than those subjects infected with HCV alone (87). Yet, the majority of HIV/HCV subjects in all large studies have not developed significant liver disease. Hypogammaglobulinemic patients who have acquired HCV infection from contaminated lots of intravenous gammaglobulin have also been reported to progress to cirrhosis and complications of chronic liver disease at unusually rapid rates (88-90). The absence of severe acute liver disease in either group argues against a directly cytopathic mechanism for HCV induced liver disease such as that proposed for organ transplant recipients with severe cholestatic liver disease (38,39). Of note, both immunodeficiency syndromes associated with accelerated progression of HCV associated liver disease have deficiencies primarily or exclusively in cytokine and antibody mediated anti-viral immune responses with relative preservation of CD8+ T cell responses. It therefore seems plausible that, as has been observed in animal models of noncytopathic

virus infection, highly levels of persistent viremia in the face of ongoing CD8+ T cell responses may be responsible for the increased severity of chronic liver disease in these patients.

In human subjects free of co-existent immunodeficiency, rate of liver disease progression has been found to correlate with three major host factors - gender, age at time of initial infection, and ethanol use (75, 91-93). In the largest study examining such host factors, Poynard and colleagues analyzed liver biopsies from more than 2200 patients enrolled in various French HCV studies. An extensive epidemiologic data base available from these studies allowed the investigators to not only determine risk factors by multivariate analysis but also provide estimates of rates of progression in various host populations. These studies utilized modern liver biopsy staging criteria to grade independently stage of fibrosis (0 = no fibrosis, 1 = portal fibrosis without septa, 2 = few septa, 3 = multiple septa without definite cirrhosis and 4 = cirrhosis) and histologic activity (A0 = no histologic activity, A1 = mild hepatitis, A2 = moderate hepatitis and A3 = severe hepatitis). The numerical values assigned to each stage of fibrosis were used to calculate rates of disease progression that were expressed in terms of fibrosis units per year. As illustrated in figure 7, the men examined in this study appeared to progress to cirrhosis at a median rate that is about 40% faster than for women and results in development of cirrhosis after a median of 26 years in men versus 36 years

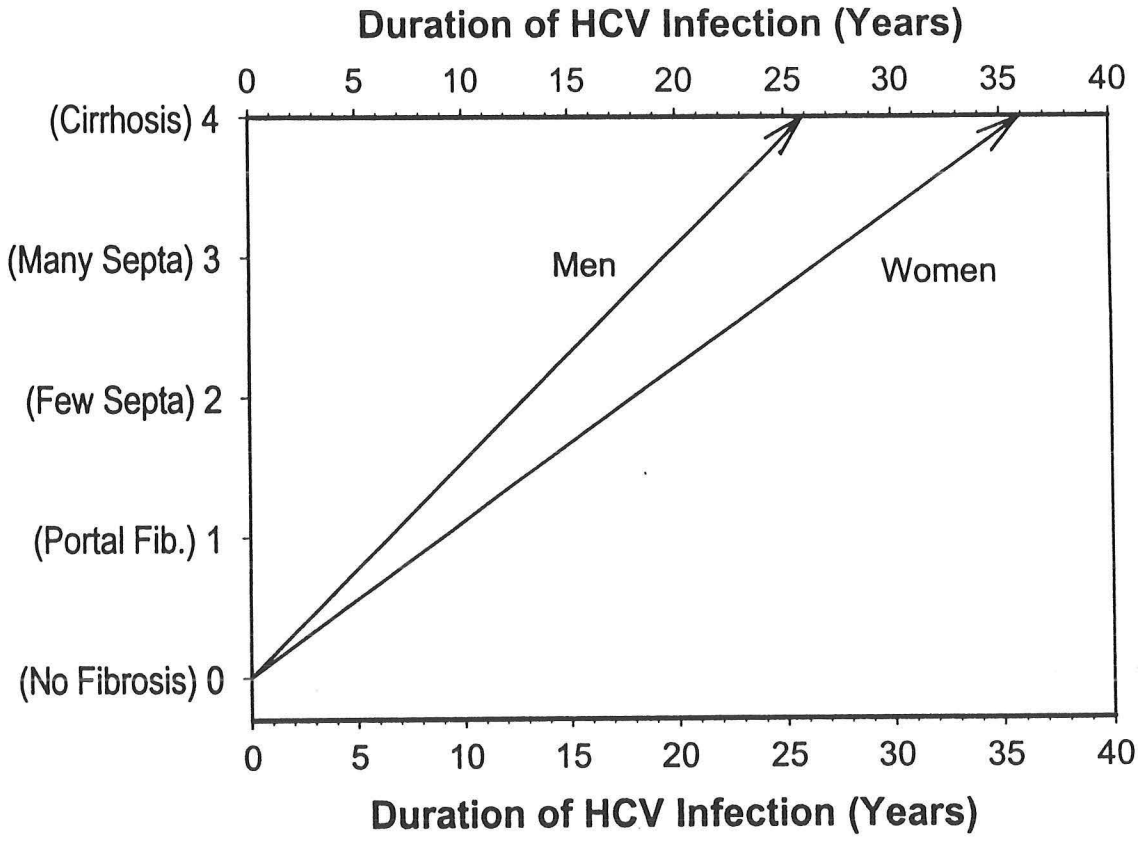


Figure 7. Gender Differences in Median Rate of Progression to Cirrhosis. Median rates of progression to cirrhosis for all women (0.111 fibrosis units/yr) and for all men (0.154 fibrosis units per year) are derived from Poynard, et al Lancet 349:829, 1997.

in women (75). As gender differences were found to be highly significant after multivariate analysis these differences appear unrelated to other life-style factors leaving gender related differences in immune responses to HCV and/or gender differences in hepatic regenerative capacity as the most likely explanation for this observation. As women are actually more susceptible than men to development of alcoholic liver disease (94) and are not known to differ from men in severity of disease induced by other toxic agents such as copper overload resulting from Wilson's disease (94a), it seems most plausible that gender differences in immune responses account for the more benign course of both HCV and HBV infections in women (30,75).

For more than a decade it has been noted that individuals infected with HCV after age 30 or 40 are more likely to develop cirrhosis during short periods of follow-up than those originally infected at younger age (91-93). However, because younger adults more commonly have contracted HCV from injection drug use and older adults more frequently develop post-transfusion HCV (91) it remained unclear whether route of infection contributed to this difference. However, in the large analysis by Poynard, et al (75) route of transmission was not found to be a significant factor in disease course. Yet, age at onset of infection was highly correlated with rates of progression of fibrosis (75). However, as illustrated in figure 8, if median rates of progression defined in this study are used to plot histologic progression for individuals initially infected at different ages, the age at which cirrhosis eventually develops is surprisingly similar. The extent to which age associated

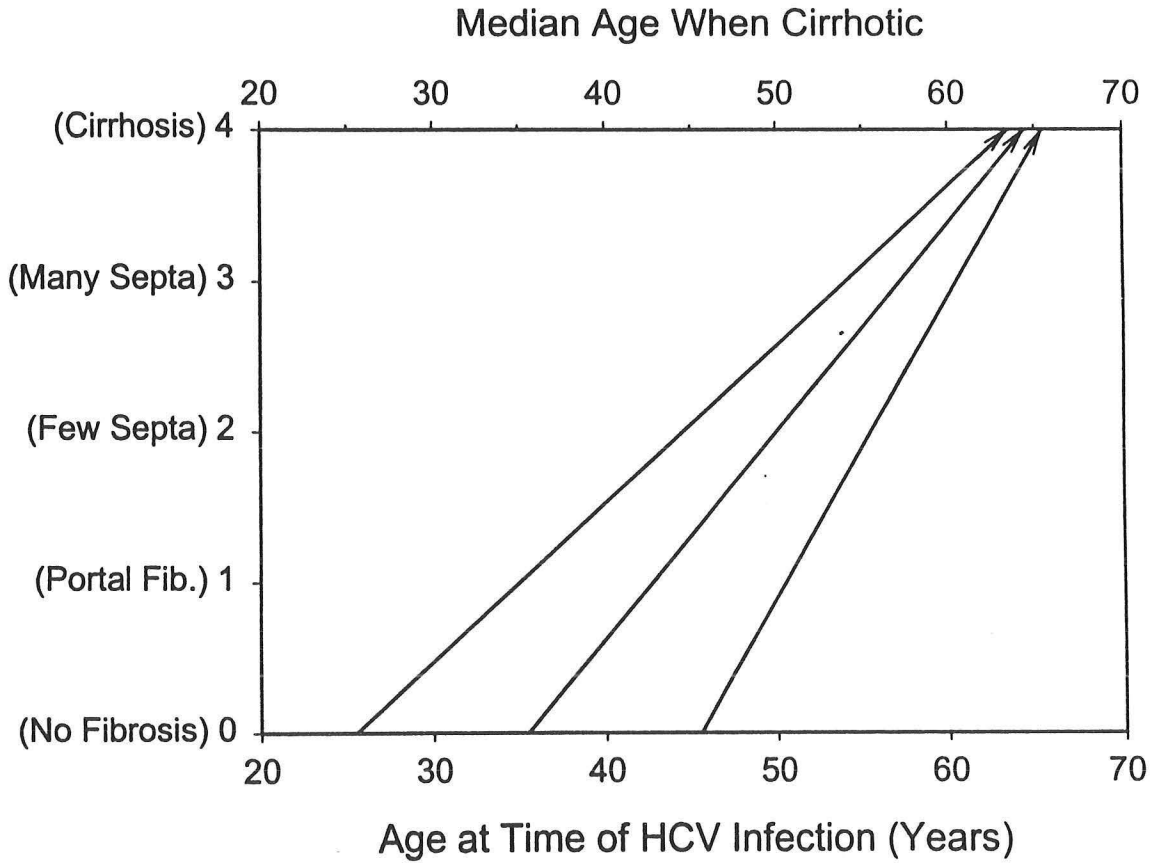


Figure 8. Effect of age at onset on rate of progression to cirrhosis and age at which cirrhosis develops. Median rates of progression to cirrhosis for all subjects with age at onset of 21-30 yrs (0.105 fibrosis units/yr), 31-40 yrs (0.138 fibrosis units/yr) and 41-50 yrs (0.200 fibrosis units/yr) are derived from Poynard, et al, Lancet 349:829, 1997.

decline in T cell dependent immune responses accounts for the effects of age on disease progression is difficult to directly determine but such a mechanism is plausible in light of previous observations regarding outcome of other infectious diseases in older adults (26,27).

The final and most significant co-factor in rate of progression of chronic hepatitis C is level of alcohol consumption (75, 93). Since alcoholism alone presents a significant risk for development of cirrhosis, it is hardly surprising that patients consuming large amounts of alcohol and infected with HCV have significantly higher rates of cirrhosis than those who do not consume alcohol. However, as illustrated in figure 9, a dose dependent effect of alcohol consumption was observed which suggested that accelerated rates of progression to cirrhosis are seen even at more moderate levels of alcohol consumption that are well below the threshold commonly associated with increased risk for alcoholic liver disease (94). Other studies have also suggested that even moderate levels of alcohol consumption are associated with accelerated rates of progression to cirrhosis in patients infected with HCV (93,95). In addition, the largest prospective study of liver disease progression in patients with post-transfusion non-A, non-B hepatitis noted that increased rates of HCV associated liver disease mortality during the first 18 years of follow-up occurred almost exclusively

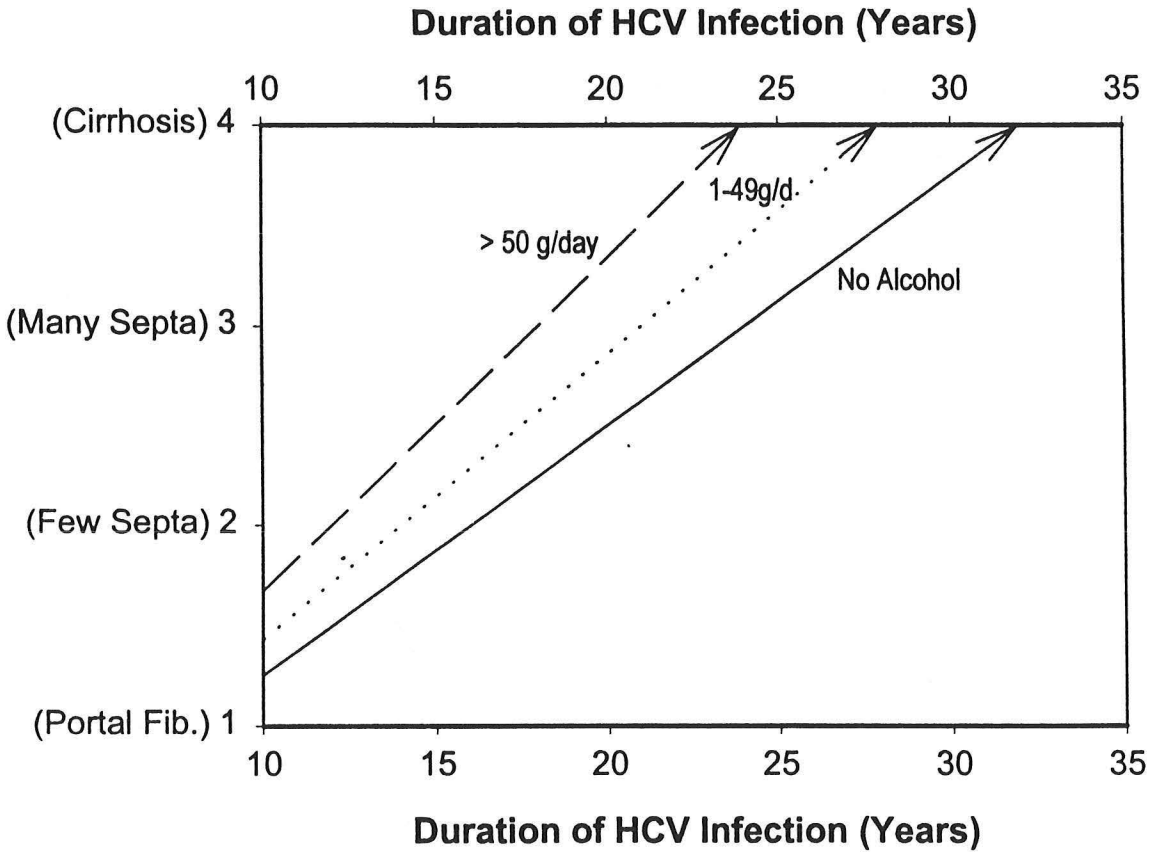


Figure 9. Effect of Alcohol on Median Rate of Progression to Cirrhosis. Median rates of progression for all subjects with no daily alcohol consumption (0.125 fibrosis units/yr), with 1-49 g/d (0.143 fibrosis units/yr) or with > 50g/d consumption (0.167 fibrosis units/yr) are derived from Poynard, et al, Lancet 349:829, 1997.

among patients who were also labeled as alcoholics (8). Finally, two recent reports have identified alcohol as an important risk factor for disease progression among patients with initial biochemical and/or histologic features predictive of benign disease (96,97). In a study assessing 20 Swedish patients with chronic hepatitis C for evidence of histologic disease progression 9-16 years after initial liver biopsies revealing only minimal or mild chronic hepatitis, overall histologic activity indexes (HAI) increased in 14 and improved in 6 (96). Occasional heavy drinking was identified as a risk factor for progression and when patients without significant drinking history were analyzed separately, no statistically significant changes in overall HAI were appreciated. In a larger study assessing histologic progression among 102 anti-HCV (+) patients with persistently normal ALT levels (97), 80% of patients had at least some histologic abnormality although only 6% had fibrosis scores >2 (i.e. either bridging fibrosis or cirrhosis). In patients with known age at onset of infection, rates of fibrosis progression were calculated and were found to be relatively low (0.05 fibrosis units per year) compared to results in patients with persistently abnormal ALT values (0.13). In addition, among patients with normal ALT levels rate of progression to cirrhosis was found to correlate with alcohol consumption and all cirrhotic patients with normal ALT levels were noted to be heavy drinkers (97). In this study, median time interval from HCV infection to cirrhosis for patients with normal ALT levels was calculated to be 80 years for the group as a whole and significantly >80 years for non-drinkers.

The mechanisms underlying the synergism between alcohol consumption and HCV infection in causing hepatic fibrosis remain unclear with multiple plausible explanations. However, results of studies examining the effect of alcohol consumption on levels of HCV viremia and response to interferon therapy suggest that alcohol does modulate the course of HCV infection at least in part by altering the balance between host immune responses and viral replication. In both the large study of Poynard and colleagues (75) and earlier studies from Japan (98) and Australia (99), a dose dependent effect of alcohol consumption on serum HCV RNA levels has been noted with ongoing or recent alcohol consumption significantly associated with higher HCV RNA levels. Of additional interest, cessation of alcohol consumption has been found to be associated with a fall in serum HCV level (99). Patients with heavy alcohol consumption immediately prior to interferon therapy have been noted to have diminished rates of sustained response to interferon therapy (98,100,101) whereas abstinence from alcohol for several years prior to initiation of therapy was associated with an improved response (100). The apparently reversible effect of alcohol consumption on levels of viremia and rate of viral clearance during interferon therapy suggest that alcohol consumption may impair host anti-viral responses. Indeed, an effect of alcoholism on host immunity has been previously suggested by multiple clinical observations indicating increased rates of active tuberculosis and other infections (102) in alcoholics and investigative reports suggesting that alcohol consumption impairs both innate and specific immunity (102,103).

Implications for Clinical Management

The heterogeneity of clinical outcomes appreciated in patients with chronic HCV infection has proven frustrating for both physicians and patients attempting to make decisions regarding clinical management. The results of the large epidemiologic studies completed in France by Poynard, et al have served to re-enforce the concept that outcomes in untreated patients are very

heterogeneous. Thus, it has been estimated that among HCV infected patients with persistently abnormal ALT values, 33% will progress to cirrhosis within 20 years of infection, 36% will progress to fibrosis over 20-50 year intervals and an additional 31% will either never develop cirrhosis or will do so after a >50 year latency interval (75). As the median time interval between initial diagnosis of cirrhosis and development of complications of chronic liver disease in HCV infected patients is > 10 years (104), it seems likely that few if any of the subset of patients with slow progression rates and only a subset of those with intermediate progression rates will ever experience life threatening complications of chronic HCV. While assignment of individual patients to each of these clinical risk groups remains an inexact science, improved understanding of disease pathogenesis and the role of the host in determining disease outcome allows for a set of specific recommendations that remain quite similar to those established as part of an NIH consensus conference held in the spring of 1997 (4).

Among patients with initial HCV infection only about 15% will spontaneously clear the virus. Despite increasing evidence that such individuals have enhanced immune responses to HCV, they remain vulnerable to re-infection. Patients who clear HCV infection following anti-viral therapy also remain vulnerable to re-infection while chronically infected subjects may experience second infections with new HCV strains. Therefore, identification and future avoidance of risk factors must be emphasized in all patients. In addition, among patients with chronic HCV infection, it seems futile to invest in prolonged and expensive therapy in patients who persist with high risk behavior such as injection drug or nasal cocaine use as those fortunate enough to achieve a response may simply be re-infected.

Among patients with chronic HCV infection, approximately 1/3 (105) will have persistently normal ALT levels. An increasing body of literature indicates that while most (~80%) will have at least some histologic abnormality on liver biopsy, progression to cirrhosis occurs at an exceedingly slow rate with histologically advanced lesions observed largely in those who consume excess amounts of alcohol. In addition, while such subjects have been largely excluded from large prospective therapeutic trials, in small studies attempts to eradicate HCV viremia in these patients have had at best mixed results (106). For these reasons, management of such patients should focus on life-style modifications that include abstinence from alcohol, avoidance of parenteral risk factors and periodic monitoring of liver function tests.

The majority of humans initially infected with HCV develop chronic biochemical and histologic hepatitis. However, even without intervention, the estimates provided by Poynard, et al suggest that approximately half of these individuals will complete their natural life expectancy prior to developing life threatening complications and thus will not benefit greatly from therapy. In light of the disappointing response rates and high cost and morbidity of current therapeutic regimens (4), it would be desirable to identify these individuals so that therapy can be directed at those most likely to benefit. In trying to ascertain need for therapy, multiple risk factors including age at time of initial infection, gender, alcohol use and presence of immunodeficiency or immunosuppressive therapy can all be considered. While some of these factors are only of predictive value, others such as alcohol use clearly can and should be altered prior to launching into therapy. Thus all patients within this category should be advised to abstain from alcohol and any attempts at therapy should be delayed

until after a prolonged interval of abstinence. If a patient likely to face future immunosuppression is to ever undergo successful therapy, it is more likely to be accomplished if therapy is initiated prior to undergoing organ transplantation (107)or prior to decline in CD4 count in HIV infected individuals (108).

Once issues such as alcohol use, ongoing injection drug use or concomitant immunosuppression have been addressed, the most import aspect of "staging" a patient with chronic HCV, is to determine whether a patient is a "slow progressor" who is unlikely to develop complications of liver disease during a normal life expectancy or if disease is progressing at a moderate to rapid rate suggesting that therapy will be of greater clinical benefit. The stage of fibrosis and histologic activity on a liver biopsy coupled with an estimate of duration of infection may permit the clinician to make reasonable predictions in at least some patients. For instance, a 45 year old female patient with a history of injection drug use between ages 20 and 25 with only portal fibrosis (stage 1) and mild hepatitis on a current biopsy is demonstrating a pattern of slow progression that is likely though not guaranteed to never result in clinically significant liver disease. It is not unreasonable to defer therapy in such an individual until such time as there is evidence of disease progression or more effective therapies with fewer side effects become available (4,109). In contrast, an alcohol abstinent HCV infected individual with septal fibrosis and active hepatitis on liver biopsy and a reasonably long life expectancy should be evaluated as a potential candidate for therapy (4). Clearly, assessing prognosis in individual patients with chronic HCV infection remains an inexact science. However, until such time as highly effective therapies with minimal side effects become available, it will continue to be prudent to carefully consider each patient's place in the spectrum of HCV associated liver disease before making therapeutic decisions.

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