Cellular Immune Responses against Hepatitis C Virus

Margaret James Koziel
Infectious Disease Division, Beth Israel Deaconess Medical Center, Harvard Institutes of Medicine, Boston, Massachusetts

Cellular immune responses are typically important in recovery from acute infections, and studies of acute hepatitis C confirm that broadly directed CD4+ and CD8+ T cell responses are associated with spontaneous clearance of infection. However, a major unanswered question is what role the cellular immune response plays in progression of liver disease during chronic infection. Classic models of hepatitis C suggest that cellular immune responses promote liver injury, either by causing direct cytolysis of infected cells or by promoting inflammation. However, clinical evidence suggests that persons with cellular immune dysfunction, such as that due to with human immunodeficiency virus (HIV) infection, have more-rapid disease progression. Recent data suggest that cellular immune responses do serve to limit the progression of liver disease, even if they are ineffective at clearance of virus. There is limited information on the effect of HIV coinfection on the cellular immune response to hepatitis C virus, but further study of this issue might shed light on the pathogenesis of liver disease in both immunocompromised and nonimmunocompromised hosts.

Hepatitis C virus (HCV) infection has been increasingly recognized as a major cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma worldwide. After acute infection, a majority (50%–85%) of healthy adults will develop persistent viremia. This high rate of persistent infection has hindered the ability to determine the correlates of protective immunity, although recent studies have demonstrated the importance of cellular immune responses in spontaneous clearance of HCV infection. In immunocompetent persons, chronic active hepatitis, cirrhosis, and hepatocellular carcinoma typically develop 10, 21, and 29 years, respectively, after infection. However, the likelihood of progression of chronic disease is highly variable, and identification of the host and viral factors that are associated with slow versus rapid progression is an area of intense interest and importance.

MECHANISMS OF CLEARANCE

The role of cellular immunity. Effective clearance of an acute viral infection typically requires the coordinated function of multiple arms of the immune system, including the innate immune system (IFNs and natural killer [NK] and NK T cells), as well as the adaptive or acquired immune response specific to a given pathogen (CD4+ and CD8+ T cells). Although neutralizing antibodies to HCV no doubt exist, there has been no pattern of antibody response that clearly distinguishes between recovery and chronic infection, in contrast to acute hepatitis B, in which the development of antibody to hepatitis B surface antigen heralds the onset of recovery. The exact importance of each arm of the immune response is typically dependent on the pathogen. The liver has a larger proportion of cells that are representative of components of innate immunity (e.g., NK, γδT, and NK T cells) than does the peripheral blood. There is a relative paucity of literature about these cells in acute HCV infection, despite their abundance within the hepatic lymphoid system; therefore, they will not be discussed in detail here.

CD4+ T cells. CD4+ T cell responses are critical to both the generation and maintenance of antiviral immune responses, because they secrete cytokines that augment antibody production by B cells and prime CD8+ cells specific for virus-infected cells. Without CD4+ cells, induction of new immune responses is impaired, and cytotoxic T lymphocyte (CTL) memory cannot be maintained in vivo. In both experimental chimpanzee and natural human infection, clearance of HCV is as-
Figure 1. Role of immune responses in hepatitis C virus (HCV) infection. A, Successful clearance of HCV infection requires the coordinated action of innate immunity and acquired immunity. After infection, there is activation of natural killer (NK) cells, as well as processing of viral antigens by immature dendritic cells (iDCs). After maturation, these mature dendritic cells (mDCs) activate CD4+ and NK T cells. CD4+ cells produce cytokines, such as IFN-γ, that induce cytotoxic T lymphocytes (CTLs). CTLs can control replication by direct lysis of infected cells but also through production of cytokines that can inhibit viral replication. B, Failure to clear HCV infection is due to a failure to initiate immune responses at the appropriate time. Possible mechanisms of chronicity in HCV include failure of NK cells, dendritic cells, and CD4+ cells. This results in inappropriate or ineffective cytokine production that fails to control virus. These cytokines also appear to lead to the accumulation of nonspecific inflammatory cells. Continued viral replication leading to death of infected cells and accumulation of fat within cells, as well as the inflammatory response, are all stimuli to fibrosis.

associated with a strong, polyclonal, and sustained HCV-specific CD4+ T cell response [6–13]. In the chimpanzee model of acute HCV infection, the appearance of these CD4+ T cell responses is temporarily associated with a substantial decrease in viremia, and the accumulation of HCV-specific CD4+ T cells in the liver appears to be essential for clearance of HCV [10, 11, 13]. Persons who have a polyclonal HCV-specific CD4+ cell response are more likely to clear HCV, whereas persons who do not are
As with the CD4+ cell response, polyclonal and multispecific responses have shown that, although CD8+ cell responses are generated were prospective, studied only a few patients. These studies require that large amounts of blood be collected or that so-frequent of such responses in the peripheral blood, which is limited by the subclinical nature of the acute illness and low frequency of infection is associated with relapse of viremia [16], whereas enhancement of CD4+ cell responses is associated with a higher likelihood of clearance of virus after treatment [17]. The kinetics of this response appear to be important: Persons who clear HCV infection have a more rapid and sustained induction of CD4+ cell responses than do persons who develop persistent disease [18].

**CD8**+ T cells. The effector function of CTLs consists of both the cytolyis of infected cells and the production of cytokines that lead to clearance of virus (e.g., TNF-α and IFN-γ) [19, 20]. The importance of these noncytolytic effector mechanisms has been shown for HIV and hepatitis B virus [19, 21, 22]. Recent evidence suggests that IFN-γ but not TNF-α inhibits HCV replication in the replicon model system [23, 24]. As with the CD4+ cell response, polynomial and multispecific CD8+ CTL responses are also associated with spontaneous clearance in both chimpanzee [11, 25–27] and human infection [18, 28, 29]. In the chimpanzee model, resolution of acute hepatitis C was temporally associated with an early polyclonal and vigorous immune response in the liver [25]. Chimpanzees that recovered were found to have an early intrahepatic CTL response directed against multiple protein targets, whereas those that went on to develop chronic infection had a more narrowly focused response during acute infection. HCV-specific IFN-γ–secreting CD8+ T cells are abundant in chimpanzees that recovered, compared with levels in chimpanzees with chronic infection [10, 30]. In chimpanzees that recovered, protection against reinfection was maintained by the presence of memory CD8+ T cells, because depletion of these memory CD8+ cells before rechallenge resulted in reinfection in chimpanzees that had previously recovered [27]. The importance of the CD4+ cell memory response in maintaining an effective CD8+ cell response was demonstrated by Grakoui et al. [5], who found that depletion of CD4+ T cells despite existing memory CD8+ cells was associated with persistence of virus and escape from the immune system.

Prospective studies of the CD8+ cell response in humans are limited by the subclinical nature of the acute illness and low frequency of such responses in the peripheral blood, which requires that large amounts of blood be collected or that sophisticated techniques be applied to freshly collected blood. Therefore, studies to date either were retrospective or, if they were prospective, studied only a few patients. These studies have shown that, although CD8+ cell responses are generated in the majority of acutely infected persons, irrespective of outcome, the distinguishing feature of spontaneous recovery is the ability to maintain such responses over time [9, 29, 31, 32]. Recent studies have suggested that there is functional "stunning" of the immune response in acute HCV infection, with impaired production of IFN-γ by virus-specific CD8+ cells [29, 33], which persists in patients with chronic HCV infection [34]. Taken together, these chimpanzee and human studies suggest that the presence of a broadly directed CTL response is associated with clearance of virus.

**MECHANISMS OF CHRONICITY IN HCV INFECTION**

Failure to eradicate HCV results in the development of chronic HCV infection, with resultant chronic hepatitis, after which a significant proportion of persons will develop cirrhosis and hepatocellular carcinoma. The mechanism by which chronic HCV infection develops and persists in the majority of infected persons remains unclear, but it does so despite the presence of HCV-specific CD4+ and CD8+ T cell responses in the peripheral blood and the liver, which suggests that these responses are, for the most part, ineffective [35–41]. It is likely that HCV has developed a number of means to evade host defenses, although most literature has focused on the role that quasi-species variability plays as a mechanism of escaping immune defenses [42–45]. However, several other potential mechanisms have been reported, which suggests that there are multiple means by which this virus interferes with an effective immune response. These include interference with the endogenous IFN system [46], suppression of host immune responses by HCV proteins through interference with T cell function [47], and possible abnormal dendritic cell function [48], among others.

**THE ROLE OF IMMUNE RESPONSES IN CHRONIC INFECTION**

The mechanisms responsible for tissue injury in acute and chronic infection are not well understood. HCV is not considered to be a cytopathic virus because of the absence of classic cytopathic features in liver biopsy samples, although HCV appears to have important interactions with host cell proteins that might adversely affect hepatocyte survival or regeneration [49]. The classic understanding of the pathogenesis of liver disease is that it is due to the cellular immune response against the virus, specifically that of CD8+ CTLs, which activates hepatic stellate cells and, thus, leads to liver inflammation and fibrosis. However, there are fundamental problems with this simplistic understanding of the pathogenesis of liver injury. Clinical experience with immunocompromised hosts indicates that patients with depressed cellular immunity, such as patients with AIDS or patients who have had orthotopic liver transplantation, are more likely to have progression of liver disease [50]. Studies of the role that immune responses play in the progression of
liver disease are difficult, because liver fibrosis does not develop in the chimpanzee model, at least in the short term. Moreover, the slow and highly variable rate of disease progression in infected persons limits our understanding of the role that immune responses play in chronic infection.

Although it is often stated that immune responses fail to develop in the setting of chronic infection, this is not the case. Instead, responses in chronic infection appear to be preferentially localized to liver tissue. Both CD4+ and CD8+ cell responses are relatively enriched in liver tissue, compared with those observed in peripheral blood [35–38, 41, 51–53]. Indeed, HCV-specific CTLs can be readily expanded from liver tissue of both humans and chimpanzees, without exogenous antigenic stimulation [35, 36, 51, 54], and are detected at higher frequencies in liver than in blood by use of tetramers [37, 41]. Much less is known about the function of these T cells in the liver than about their counterparts in the peripheral blood. Most studies of HCV-specific CD8+ T cells in the liver have relied on T cell lines or clones expanded by in vitro stimulation. Studies of these liver-derived CD8+ T cell clones revealed that their specificity was diverse and comparable to those observed in persons with acute spontaneous recovery, with some specificities found in the liver that were not identified in PBMCs [35, 36, 51, 55]. This may indicate that the frequency of HCV-specific CD8+ T cells is higher in the liver than in the peripheral blood and/or that the specificity and other properties of the CTLs in the liver may differ from those in PBMCs. Recent data suggest that virus-specific regulatory cells that inhibit CD8+ CTL function are specifically increased within the liver in HCV-infected persons [56]. Therefore, to study the HCV-specific immune response in chronic infection, it is necessary to study responses in both peripheral blood and liver.

There is evidence both for and against a role cellular immune responses play in liver disease and fibrosis. The immune response may play a central role in liver injury, if the magnitude, specificity, or effector function is such that viral replication cannot be completely controlled. The role that antigen-specific CTLs play in liver cell damage is well established in murine models of viral hepatitis, including recent studies of mice expressing HCV. Mice that express HCV only under the control of an inducible promoter and in which HCV is expressed after the period of neonatal development develop inflammation of the liver on expression of HCV, whereas mice that are constitutively tolerant to HCV proteins do not develop inflammation [57–59]. It was recently shown that adoptive transfer of HCV-specific CTLs to transgenic mice expressing HCV proteins caused the mice to develop liver injury [60]. In infected persons, the role that cellular immune responses play is more controversial, in part because of the difficulties in measuring low-frequency responses in the blood and correlating these responses with progression of disease over time, which requires large numbers of patients to be followed for years. In a small study that examined only CTL responses against HCV structural proteins, the presence of liver-derived CTL activity was associated with a higher hepatic necroinflammatory score [61]. Reconstitution of the immune system after a period of depressed cellular immune responses, such as that which occurs after successful engraftment of bone marrow after transplantation, can be associated with dramatic increases in inflammatory activity in the liver, presumably on the basis of enhanced HCV-specific immune responses [62]. Additional evidence that CTLs play a role in liver injury comes from a study in which anti-CD8 monoclonal antibodies were administered to a patient, after which improvement in serum transaminase levels was seen [63]. Finally, Nelson et al. [64] have shown that recombinant IL-10 improves hepatic inflammation but also leads to increases in virus load via immune modulation. These ineffective CTLs are thought to exert most of their effect through cytotoxic effects on infected hepatocytes, although indirect effects on nearby, bystander uninfected cells cannot be excluded [65]. Activation of the Fas–Fas ligand system on these activated CTLs may be responsible for a high level of lysis of bystander cells [66]. Production of cytokines by these CTLs may also lead to recruitment of inflammatory cells and activation of stellate cells, although direct evidence for this in hepatitis C is lacking.

Alternatively, the cellular immune response may exert a protective effect against disease progression. As discussed above, patients with depressed cellular immunity have more-rapid disease progression, which suggests that, although some aspects of the cellular immune response are clearly ineffective at clearing virus, they do serve to limit liver damage. Cross-sectional studies of a small number of subjects with HCV infection suggest that a more vigorous CD4+ cell response is associated with less-severe liver histologic abnormalities [67]. Two recent studies by Kamal et al. [68, 69] used the model of Schistosoma mansoni coinfection, in which there is rapid evolution to cirrhosis, compared with patients with HCV infection alone, and a Th2 bias as a result of parasitic infection. The rate of progression of fibrosis was associated with the magnitude of the peripheral and intrahepatic immune response, in that subjects who had a vigorous type 1 response in PBMCs and liver had slower rates of progression of fibrosis [68, 69]. Notably, responses waned in the periphery over time but were maintained in the liver. The role of CD8+ T cells is more controversial, with some studies suggesting that a relationship exists between the presence of HCV-specific CTL responses and a lower virus load [61, 70, 71] and other studies failing to find a relationship [72]. Because virus load is a poor surrogate for liver damage and does not predict progression of disease in hepatitis C, the clinical importance of the former studies is not clear. None of these cited studies demonstrated a relationship between any CD8+ cell responses and liver injury, and the number of subjects...
was relatively small. Therefore, this is another major gap in our understanding of the role of immune responses in the pathogenesis of liver disease.

**THE EFFECT OF HIV ON HCV CELLULAR IMMUNE RESPONSES**

Numerous studies have demonstrated that patients with HIV/AIDS have a higher rate of progression of fibrosis, particularly those with CD4+ T cells counts of <200 cells/mm³ [50, 73, 74]. Before the advent of antiretroviral therapy, persons with both HIV and HCV infections had an ~3.6-fold increase in the risk of developing cirrhosis [74], which is comparable to the risk of liver disease progression among HCV-infected persons who consume large amounts of alcohol. This rapid progression appeared to be most dramatic in persons with CD4+ T cell counts of <200 cells/mm³. Reconstitution of immunity leads to a decrease in the rate of progression of fibrosis and risk of clinical events due to liver disease [75].

The difficulties in understanding the role of cellular immune responses in progression of HCV disease are magnified in the presence of HIV infection. Several studies have shown that both HCV-specific CD4+ and CD8+ cell responses are less frequent than HIV-specific responses in the peripheral blood of persons with both infections [76, 77]. In fact, even by use of sensitive assays, responses are typically detectable in PBMCs only in patients with slow progression of HIV disease, who also have more-vigorous immune responses against HIV [78]. However, when liver tissue is examined, HCV-specific responses in chronic infection are present and are not quantitatively different in coinfected and in persons with HCV infection alone, at least for subjects with only modest immune dysfunction [79]. There appear to be qualitative, rather than quantitative, differences in the types of cytokines produced. Interestingly, these HCV-specific immune responses are negatively correlated with the degree of inflammation and fibrosis, but these relationships may be revealed only if cytokines other than IFN-γ are studied [79, 80]. Given the effect that HCV coinfection may have on morbidity and mortality of HIV-infected persons, better understanding of the pathogenesis of liver disease and the progression of fibrosis is clearly needed.

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**References**


