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Immunopathogenesis of hepatitis C virus in the immunosuppressed host

Key words:
hepatitis C virus; immunosuppression; immunopathogenesis; organ transplantation

Abbreviations:
HCV, hepatitis C virus; HCC, hepatocellular carcinoma; HIV, human immunodeficiency virus; OLT, orthotopic liver transplantation; BM, bone marrow; RNA, ribonucleic acid; NS, non-structural; PBMC, peripheral blood mononuclear cells; ALT, alanine aminotransferase; CTL, cytotoxic T lymphocyte; HBV, hepatitis B virus; IL-2, interleukin 2; IFN-γ, interferon gamma; TNF-α, tumor necrosis factor alpha; NKT, natural killer T; NK, natural killer; HLA, human leukocyte antigen.

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Abstract: The prevalence of chronic hepatitis C virus (HCV) infection among various groups of immunosuppressed patients is high. These groups include patients co-infected with human immunodeficiency virus (HIV), recipients of organ transplants, and those with hypogammaglobulinemia. The liver disease in the immunosuppressed host is typically severe with an unusually rapid progression to cirrhosis. This is somewhat paradoxical, as the classical model for HCV-induced liver disease assumes that cell-mediated immune responses induce liver injury. It is likely that a combination of viral-related factors and host-related factors plays a role in this accelerated natural history of HCV. Data are accumulating in immunocompromised hosts that address the immunopathogenesis of liver injury, although there are still fundamental gaps in our understanding of this process. In this review, we will focus on our current understanding of the mechanisms of liver injury and how it relates to the accelerated liver disease progression in immunocompromised hosts.

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In the United States, it is estimated that the hepatitis C virus (HCV) infects approximately 1.8% of the population. Following infection 85% of healthy adults will develop persistent infection. Acute HCV infection is typically mild and often subclinical, yet there is a high rate of chronicity after HCV infection, with at least 50–70% of those infected developing chronic elevations in transaminases (1). Some individuals with persistent viremia have normal transaminases, which might suggest complete recovery; however, more than half of these individuals will have abnormal liver biopsies, including the presence of cirrhosis (2). In immunocompetent individuals, chronic active hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) develop approximately 10, 21, and 29 years after infection, respectively. It is estimated that the number of individuals who have been infected for more than 20 years, thus facing the highest risk of liver cancer, will triple by the year 2010 (3). In fact, a recent study documented a doubling of the rate of HCC between the period of 1991–1995 compared to 1976–1980, with a 41% increase in the mortality rate (4). The prevalence of chronic HCV infection among different groups

Prevalence of hepatitis C virus (HCV) infection and time to development of cirrhosis in representative cohorts of immunocompetent and different groups of immunosuppressed HCV patients

	Prevalence of HCV infection (%)	Time to cirrhosis[1] - median/range (years)	References
Immunocompetent	1.8	30/13–42	(5, 6)
Hypogammaglobulinemia	21.5	8.8[2]/4.5–15	(7)
HIV-HCV co-infection	72.7	10[3]/6–30	(8, 9)
BM transplantation	45	10.1/1.2–24.9	(10)
Kidney transplantation	29	Not available	(10)
Liver transplantation	> 95	2/1–4	(11, 12)

[1]Defined as time from exposure to contaminated immunoglobulin or from transplantation to clinical or histological diagnosis of cirrhosis in patients with hypogammaglobulinemia or transplant recipients, respectively, and as time from diagnosis of HCV to diagnosis of cirrhosis in immunocompetent or co-infected patients.
[2]Represents mean value.
[3]Dependent upon CD4 count; CD4 < 200 associated with more rapid disease progression.

Table 1

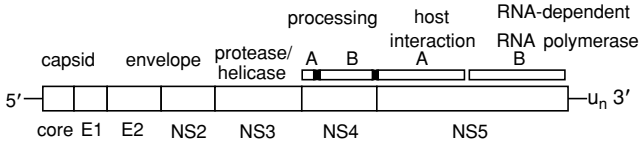

Fig. 1. **HCV genome**. HCV is a positive single stranded RNA virus that consists of approximately 9400 nucleotides, encoding a polyprotein of 3011 amino acids. The structural proteins include a core protein and two envelope proteins. The non-structural (NS) 3 region encodes a protease, which cleaves the polyprotein into individual proteins. The NS5 region is probably an RNA-dependent RNA polymerase.

of immunosuppressed patients is high (Table 1). The disease in immunosuppressed hosts is typically severe with an unusually rapid progression to cirrhosis (Table 1) (5–12).

HCV is a positive strand ribonucleic acid (RNA) virus that consists of a single open reading frame of approximately 9400 nucleotides, encoding a polyprotein of 3011 amino acids (Fig. 1) (13). The structural proteins include a core protein, as well as two envelope proteins, which likely form a heterodimer *in vivo*. The non-structural (NS) 3 region of the virus is important for post-translational processing of the polyprotein into individual proteins, and the NS5 region is probably an RNA-dependent RNA polymerase (13). Six major genotypes have been identified on the basis of nucleic acid sequencing. Furthermore, different quasispecies evolve in a given host, due to the high error rate of the viral polymerase during replication (13). The primary site of HCV replication is the hepatocyte, although cells of the macrophage/monocyte lineage in bone marrow and peripheral blood mononuclear cells (PBMC) also appear to support viral replication *in vivo* (14).

The available knowledge of the mechanisms of liver damage and virus clearance in the immunocompetent host is restricted due to a number of technical limitations. These are particularly important in trying to interpret the available literature in immunocompromised hosts. First, HCV infects only humans and chimpanzees, and thus, hypothesis-driven studies of immune responses are difficult to perform. Second, even in the immunocompetent host, the natural history is highly variable, which makes it difficult to associate any single host or viral factor with outcome, in the absence of very large samples sizes and long periods of follow up. Third, due to the lack of a robust tissue culture system, it is also difficult to evaluate virus-host interactions. Fourth, in clinical studies, the available literature is often limited by the lack of histologic information. Unfortunately, there are no accepted surrogate markers of disease progression or liver fibrosis for HCV, analogous to the use of CD4+ T cell counts or HIV-1 viral loads in HIV disease progression. HCV viral load and alanine aminotransferase (ALT) levels have been used by many investigators as markers for disease severity, but are inadequate for this purpose (1). Liver biopsy with histopathology remains the most important and accurate tool in assessing prognosis, but has increased morbidity in many immunosuppressed hosts. Finally, in the immunocompromised host it is difficult to quantify immunosuppression, making comparison studies in patient cohorts very difficult. Most of the studies in the available literature on immune responses in immunocompromised hosts have been performed using samples from liver transplant recipients. It remains uncertain how accurately these studies reflect the pathogenesis in other groups of immunocompromised hosts.

Viral factors in liver injury

Two models that are mutually non-exclusive explain the liver injury related to HCV. The first suggests that a direct viral cytopathic effect is

the mechanism for liver injury, while the second suggests that the presence of high levels of expression of HCV is injurious to the cells. HCV viremia is higher in liver allograft recipients (15) and in patients co-infected with HIV (16, 17) than in immunocompetent patients. HCV viral load, however, is not directly correlated with liver injury in immunocompetent patients (18); its correlation with liver injury in the setting of immunosuppression is questionable. Some studies in liver and kidney allograft recipients suggest that a high serum level of HCV-RNA is associated and coincides with the development of acute lobular hepatitis (19) and fibrosing hepatitis with or without cholestatic features (20–22). Moreover, high HCV-RNA titers before orthotopic liver transplantation (OLT) are strongly correlated with poor patient survival (23). In contrast, other studies have demonstrated no correlation between viremia and histologic liver injury either post OLT (24–26) or in co-infection with HIV (27). Genotype does not seem to play a significant role in the pathogenesis of HCV, as most studies have shown that differing genotypes do not account for a different degree of liver injury in either immunocompetent (28) or suppressed hosts (29).

Supportive evidence for a direct viral role in HCV-induced liver injury comes from the use of osteosarcoma cell lines, in which expression of HCV proteins causes severely reduced proliferation (30). Furthermore, expression of HCV proteins may promote programmed cell death (apoptosis) in cell lines, although such effects have been highly variable depending on the cell line and the stimulus to apoptosis (31). Expression of HCV proteins, especially the core protein, or full-length HCV in either cell lines or mice has resulted in a variety of other manifestations of cellular injury, particularly the accumulation of fat (steatosis) (32, 33). This intracellular steatosis is reminiscent of that seen in liver biopsies from patients with hepatitis C infection. The speculation that somehow the high-level expression of HCV directly injures liver cells is attractive, but is difficult to test directly in the absence of a robust tissue culture system. Furthermore, it does not exclude other mechanisms responsible for liver injury, as high viral load could reflect increased release from destroyed hepatocytes in the severely injured liver.

There is contradictory evidence regarding the diversity of quasispecies in the setting of immunosuppression. Most studies have demonstrated lower quasispecies diversity in immunosuppressed HCV patients when compared with immunocompetent HCV patients or the pre-transplant state. This lower diversity has been shown in patients with hypogammaglobulinemia (34), bone marrow (BM) (35), allograft recipients, liver transplant recipients (36), and HIV-HCV co-infected individuals with low CD4 counts (37). Several studies have shown that development of severe histological recurrent hepatitis and liver fibrosis progression post-transplant is associated with decreased quasispecies diversity (20, 38, 39). There are several potential explanations for these observations, depending on whether the virus or the host immune system is viewed as inducing the liver injury. The viral variants selected in these rapid progressors might be more aggressive or "fit" in terms of induction of liver disease. Decreased quasispecies diversity might also reflect the decreased ability of the host immune system to control the virus. In contrast, one could argue that higher complexity indicates generation of more viral variants that could escape from recognition by antibody or CTLs. This fundamental conundrum awaits further prospective study of the complex relationship between the virus, host immune response, and liver injury.

Host-related factors in liver injury

The second model suggests that liver injury is due to HCV-specific T-cell destruction of hepatocytes. Antibody, CD4 + helper T-cell, and CD8 + cytotoxic T-cell responses constitute the acquired immune responses against HCV (Fig. 2). As the classic understanding of HCV-mediated liver injury focuses on cellular immune responses, this review will concentrate on these aspects of the immune response.

CD4 + T cells

In acute HCV, a vigorous and multispecific PBMC CD4 + T-cell response is correlated with viral clearance and self-limited disease (40). The role of these responses in chronic infection, however, is less clear; classically these cells are thought to be protective against progression of disease by limiting viral replication. A weaker CD4 + response during the initial phase of infection is associated with more rapid histological progression in the setting of HCV and schistosomiasis co-infection, but these data need to be confirmed in other groups of hosts (41). Certainly, the proliferative response of CD4 + T cells is decreased in immunosuppressed HCV patients. Multispecific CD4 + T-cell responses to at least one HCV antigen are found in the PBMC of 40% of liver transplant patients with HCV infection with minimal to no histological abnormalities. However, no proliferative response occurs in patients with severe HCV recurrence, despite similar responses to control antigens in both patient populations (25).

Similarly, the proliferative response of PBMC-derived CD4 + T cells in response to HCV proteins, but not to other recall antigens, is lower in HIV-HCV co-infected patients than in HCV mono-infected individuals (17). It remains unclear, however, whether this lower response is correlated with liver injury in the co-infected group. One significant limitation of these studies is the fact they were derived from PBMC rather than intrahepatic T cells. In one study in liver transplant recipients, HCV-specific CD4 + T-cell responses were more vigorous in the liver compared to PBMC, but did not appear to be associated with disease severity (42).

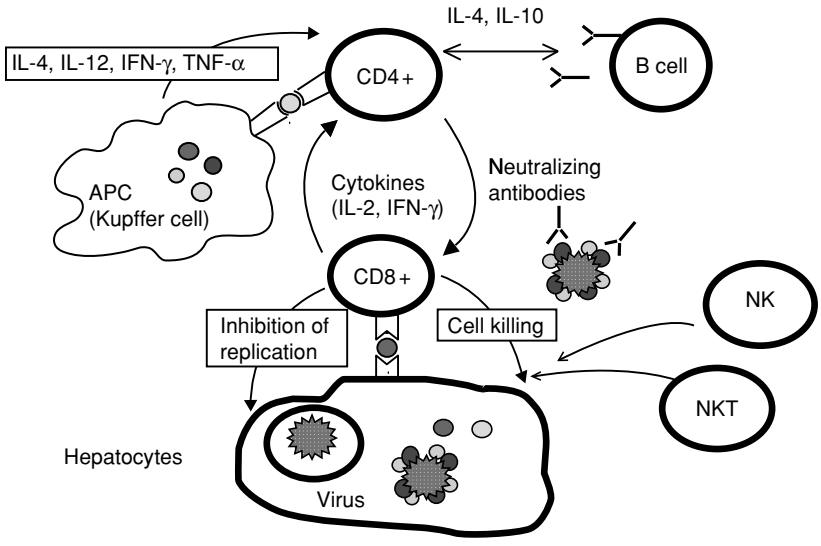

Fig. 2. **Immune responses in HCV**. Antigen-specific cellular acquired immune responses are the predominant immune mechanism against HCV. CD8 + cytotoxic T cells inhibit viral replication and at the same time remove infected cells, causing liver damage. CD4 + T cells are thought to be protective against progression of disease by limiting viral replication. Type 1 cytokines, such as IL-2 and IFN-γ, produced by CD4 + and CD8 + T cells prime and maintain these cellular immune responses, whereas, type 2 cytokines, such as IL-4 and -10, produced by CD4 + T cells, activate B cells and stimulate production of neutralizing antibodies. Innate immune mechanisms, such as NKT-cells and NK-cells responses, are likely involved in controlling viral replication and liver damage as well.

CD8 cytotoxic T cells

CD8 + cytotoxic T lymphocyte (CTL) cells have dual roles, which are to control viral replication, often through the production of cytokines, and to remove infected cells. In both acutely infected animals and humans, a polyclonal HCV-specific CTL response is protective against persistent infection (43, 44). The role of these cells in chronic infection is less clear. Our understanding of the role of these responses in chronic infection is further limited by the fact that there appears to be compartmentalization of immune responses, such that responses measured in PBMC may not accurately reflect ongoing events in the liver (45). The relationship between the presence or absence of CTL responses in the chronic phase of HCV and liver injury is controversial, with some studies suggesting a protective role and others failing to demonstrate such a function (46, 47).

The classic understanding of the pathogenesis of liver injury in viral hepatitis is that it is mediated by virus-specific CTL, which recognize infected cells and lyse them using a variety of effector molecules. This role of CTL is supported by studies in murine models of viral hepatitis (48). For example, adoptive transfer of hepatitis B virus (HBV)-specific CTL into mice transgenic for HBV proteins leads to liver injury (48). There have not been comparable studies in HCV. Nevertheless, there is some indirect evidence for the role of viral-specific and non-specific cell-mediated immune responses in liver injury in HCV. First, transgenic mice expressing HCV proteins do not develop inflammatory liver injury characteristic of chronic HCV infection (49). Second, CD8 + T cells isolated from liver tissue are capable of lysing autologous hepatocytes (50). Third, treatment of an HCV-infected patient with anti-CD8 monoclonal antibodies resulted in improvement of serum transaminases, suggesting that the CD8 + T cells were the cause of liver damage (51). Therefore, the finding that immunosuppressed hosts, many of whom are presumed to have global defects in T-cell number and function, have a more rapid disease progression is somewhat paradoxical.

Little literature exists on CD8 + T-cell function in the setting of immunosuppression despite their putative role in liver injury. CD8 + T cells are the major cells in the inflammatory hepatic infiltrate in hepatitis post OLT as well as in immunocompetent hosts (26). Furthermore, the immunohistochemical expression of CD69 (a marker of lymphocyte activation), β2-microglobulin, and intercellular adhesion molecules is equally increased in severely inflamed livers from patients with post-OLT hepatitis and immunocompetent hosts, when compared with mildly inflamed specimens (26). It is not known, however, what proportion of these cells are truly HCV-specific versus non-specifically activated cells. Thus, clearly more work needs to be performed in this area in order to understand the role of CD8 + T cells in hepatic injury during HCV infection.

Cytokines

Activated CD4 + and CD8 + cells display a number of effector functions, including cytokine production. Type 1 cytokine responses are characterized by production of interleukin 2 (IL-2) and interferon gamma (IFN-γ), which prime and maintain antigen-specific cellular immunity. As a result, they are important in defense against intracellular pathogens such as viruses (52). Type 2 responses are typically characterized by production of IL-4, -10, and -13. In viral infections, a type 1 response is often viewed as a correlate of strong cellular immunity, and thus as "protective." In chronic hepatitis C, however, the level of expression of type 1 cytokines within the liver is correlated with the extent of liver injury (53). This finding is in line with a growing body of evidence that suggests that cytokines mediate liver damage in viral hepatitis. Liver-infiltrating CTLs from HCV-infected individuals with chronic hepatitis produce cytokines, such as tumor necrosis factor alpha (TNF-α) (54), which initiate a cascade of events resulting in hepatic fibrosis. The mechanisms of cytokine-induced liver damage include recruitment of antigen non-specific inflammatory cells (55), induction of apoptosis in hepatocytes (56, 57), and up-regulation of extracellular matrix protein production leading to fibrosis (58). TNF-α production may be one of the means by which virus-specific CTLs amplify the damage to nearby non-infected cells (59). Data are limited, but a single published study suggests that type 1 responses are present in both PBMC and liver of patients post OLT (60).

Innate immune mechanisms

Only limited information is available about the role of innate immune mechanisms in HCV-induced liver injury even in immunocompetent hosts, although these cell types represent a majority of the intrahepatic lymphoid compartment. There is some evidence to suggest that natural killer T (NKT; CD3 + CD16/56 + CD161 ±) cells and natural killer (NK; CD3 − CD16/56 +) cells are involved in liver damage. It has been recently shown that liver lymphocyte lines from patients with chronic HCV have high levels of CD1d-reactive NKT cells, when compared to PBMC (61). These cells exhibit proliferative and type 1 cytokine responses, and thus are potentially pro-inflammatory and capable of damaging hepatocytes (61). Renal allograft recipients with HCV have a higher proportion and number of NKT cells in their PBMC than immunocompetent HCV patients. Liver injury is associated with higher numbers of NKT cells in the immunosuppressed group (62). These data need to be interpreted cautiously though, as NKT cells were identified by phenotypic and not by functional markers.

The data concerning NK cells are even more limited. NK cells sense recognition molecules expressed on the surface of most nucleated cells, and respond with cytotoxic effector functions and cytokine production. No study has looked into the correlation of the function of intrahepatic NK-cells with HCV-induced liver injury in either immunocompetent or immunosuppressed hosts; *in vitro* HCV appears to impair NK function (63). A lower NK-cell number in PBMC is correlated with worse liver disease in immunocompetent HCV patients (62). However, in renal transplant HCV patients, a lower number of PBMC NK cells is paradoxically associated with absence of liver disease by histology (62). Whether NK cells indeed contribute to the liver injury in the immunosuppressed group is yet to be determined. It also remains to be found whether differences truly exist in the pathogenic mechanisms responsible for liver injury between this group and the immunocompetent one.

Other factors

Immunosuppression

The effect of different immunosuppressive regimens on HCV-induced liver injury after organ transplantation is not fully understood. Development of cirrhosis is significantly associated with the load of initial immunosuppressive therapy after OLT, as it is more frequent in patients treated with triple or double agents than in those treated with a single agent regimen (64). Several studies have demonstrated no differences in viremia and histological HCV recurrence rates between tacrolimus (FK506) and cyclosporine-based regimens as primary immunosuppression after OLT (22, 65). In contrast, azathioprine-containing regimens significantly reduce histological recurrence and progression of HCV post OLT (65). Intravenous courses of methyprednisolone or use of OKT3 as an anti-rejection treatment are associated with worse liver histology in liver and kidney graft recipients (66, 67). The duration of the immunosuppressive therapy also seems to play a role, as patients treated with steroids for longer duration have higher HCV-RNA levels a year after OLT (22). The viremia in these patients is strongly correlated with the extent of liver fibrosis (22). The exact mechanisms by which differences in type of immunosuppression relate to the clinical course, however, are not well defined.

Low CD4 count is one of the quantitative immune alterations found in patients infected with HIV or those receiving immunosuppressive therapy (68). Interestingly, CD4-cell count of less than 200 cells/mm^3 is associated with liver fibrosis (27) and is an independent predictor of progression to cirrhosis in HIV-HCV co-infected patients (8). Immunosuppressive therapy and co-infection with HIV also induce qualitative immune impairments, such as impaired cellular responses by down-regulation of major histocompatibility complex (MHC) class I and II molecules (30, 69). Thus, accelerated liver injury might not be a result of loss of HCV-specific responses, but rather qualitative differences in immune function leading to pro-inflammatory immune responses and/or to an increase in viral load.

Conclusion

Innate and acquired immune responses, in particular T-cell responses, account for HCV-induced liver injury in immunosuppressed hosts. However, these may not be the sole mechanism, as viral variables seem to play an important role in liver injury. Fundamental gaps still exist in our understanding of the pathogenic mechanisms leading to the accelerated disease progression in the immunosuppressed host. We hope that further characterization of these mechanisms will lead to novel therapeutic strategies, improved organ allocation, and improved patient survival.

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