



Review

Evasion of Host Immune Surveillance by Hepatitis C Virus: Potential Roles in Viral Persistence

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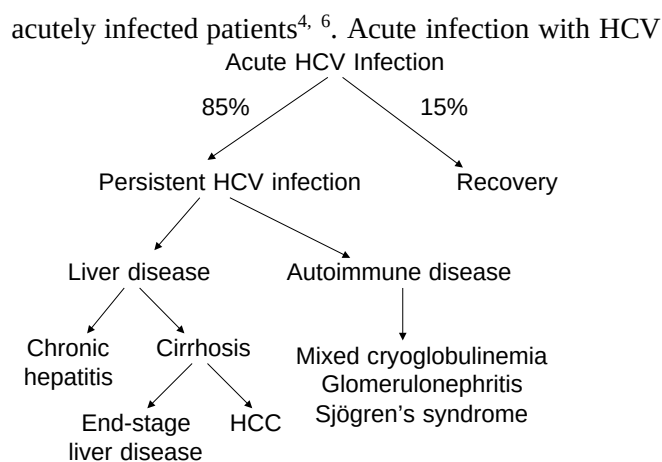
Abstract. Hepatitis C virus (HCV) is a major human pathogen that causes mild to severe liver disease worldwide. This positive strand RNA virus is remarkably efficient at establishing chronic infections. In order for a noncytopathic virus such as HCV to persist, the virus must escape immune recognition or evade host immune surveillance. Immune escape via the hypervariable region of the E2 envelope protein has been postulated as one mechanism for HCV persistent infection. Such hypervariability within the E2 protein may be under selective pressure from protective B cell or T cell responses and be able to escape immune recognition by rapid mutation of antigenic site. In addition to antigenic variation, HCV may also suppress immune response, leading to dampening of cellular immunity. This is supported by recent studies in our laboratory demonstrating that the HCV core protein can suppress host immune responses to vaccinia virus by downregulating viral specific cytotoxic T lymphocyte (CTL) responses and cytokine production. An understanding of the mechanisms behind HCV persistence will provide a basis for the rational design of vaccines and novel therapeutic agents targeting human HCV infection.

Key words: hepatitis C virus; persistence; viral quasispecies; immune evasion.

Introduction

Hepatitis C virus (HCV) infection occurs in 1% of the world's population and is a major cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma^{2,3}. As shown in Fig. 1, the most remarkable feature of HCV infection is its ability to evade host immune responses, resulting in persistent HCV infection in over 85% of

Fig. 1. A diagrammatic representation of the clinical spectrum and potential outcomes of HCV infection. HCV infection leads to over 85% of persistent infection. Persistent HCV infection is associated with the development of hepatocellular carcinoma and autoimmune diseases. HCC – hepatocellular carcinoma



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is subclinical in the majority of acutely infected patients⁴¹. Although the serum alanine aminotransferase level is minimally elevated in acutely infected patients, this measurement is neither predictive of viremia and hepatitis nor of the eventual course of the disease^{21, 37}. Chronic HCV infection is similarly asymptomatic until the development of liver cirrhosis, hepatocellular carcinoma, or autoimmune diseases such as mixed cryoglobulinemia and glomerulonephritis⁵. This persistent infection leads to the development of cirrhosis in up to 35% of patients and, as such, end-stage liver disease from HCV infection is the chief indication for orthotopic liver transplantation worldwide¹².

HCV is a single-stranded RNA virus belonging to the *Flavivirus* family^{9, 10}. The genome consists of ~9500 nucleotides encoding a single large polyprotein precursor that is cleaved to form both the structural and non-structural proteins necessary for viral propagation. The structural proteins, core, E1 and E2 are located in the N-terminal quarter, whereas the non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) are translated from the remaining portion of the polyprotein. At least 6 genotypes and 30 subtypes have been identified differing primarily in the region of core, E1 and NS5^{39, 43}. Despite the lack of an efficient tissue culture system to allow the analysis of viral replication and gene function, a great deal of data have been accumulated through the use of several established expression systems, leading to the identification of important interactions between viral and host proteins.

Unfortunately, no vaccine or effective treatment for HCV infection is currently available beyond interferon alpha (IFN- α) therapy, with the vast majority of patients relapsing upon cessation of treatment^{22, 34, 35}. More recent studies combining IFN- α treatment with ribavirin, an antiviral agent, suggest that this regimen leads to sustained responses in approximately 50% of patients^{1, 11, 31, 36}. Long-term response to therapy has been seen more often in patients with short duration of disease, low serum HCV RNA, and minimal liver pathology²¹. In addition, viral genotype appears to be important for response to IFN- α treatment, as indicated by infections with HCV genotype 1b being resistant to IFN- α treatment^{22, 29}. Resistance to IFN therapy has been associated with a specific region in the NS5a protein¹³.

An understanding of the mechanism(s) involved in the establishment of persistent HCV infection is crucial in the design of effective therapeutic agents against HCV infection. From a fundamental standpoint, the establishment of a persistent HCV infection may be due to either host factors, such as the inability of the im-

mune system to induce protective responses, or to viral factors, such as the ability to escape from host immune responses. Multiple potential mechanisms have been postulated for persistent HCV infection and are summarized in Table 1. It is clear that viral integration of HCV into host DNA (i.e. latency) does not occur and, thus, other viral properties interfering with the host immune responses must be considered. Studies with other

Table 1. Potential mechanisms involved in the establishment of HCV persistence

1. Viral load and immune tolerance
rapid viral replication ³²
possible immune tolerance
2. Variability of viral genome
presence of viral quasispecies circulating in blood of HCV-infected patients ²⁸
identification of hypervariable region within the E2 glycoprotein ¹⁹
variation in NS5 protein relating to the resistant phenotype of IFN- α treatment ¹³
3. Evasion of host immune surveillance
interaction between core protein and the tumor necrosis receptor family members ^{8, 30, 45}
suppression of host immune response by the core protein ²⁷
interference of the antigen presentation pathway by HCV gene product(s)

persistent viruses have shown immune escape by infection of immune-privileged sites, interference with antigen processing and presentation, alteration of cytokine expression and survival pathways, and decreased immune recognition by viral gene mutation. In this review, we will focus on addressing the potential mechanisms that HCV may facilitate a persistent infection from an immunological point of view and review recent data obtained from our laboratory suggesting a role of HCV core protein in modulation of the host immune responses.

Viral Overload and Immune Tolerance

One possibility for the ability of HCV to persist involves the induction of immune tolerance due to an overwhelmed immune system in the face of massive viral replication. This is supported by recent studies on the viral dynamics of HCV infection in chronically infected patients³². The studies on determination of viral kinetics indicate that viral production was on a scale of up to 10¹² virions/day, larger than estimates for HIV-infected patients. If the kinetics of virus production

exceed the generation of sufficient CD8⁺ cytotoxic T lymphocyte (CTL) effectors to destroy virus-infected target cells, viral replication will be sustained and viral persistence will ensue. Alternatively, the waning of the CTL response to infection in chronically infected patients suggests a role for immune tolerance of virus specific T cells in these individuals, leading to T cell deletion, although no definitive studies have addressed this issue.

Immune Escape Mutation

Like many RNA viruses, HCV demonstrates considerable genetic heterogeneity due to a low-fidelity RNA polymerase lacking the proof-reading capability of a 3'-5' exonuclease activity^{15, 20}. The rate of nucleotide misincorporation in the HCV genome has been estimated at up to 10⁴ base substitutions per genome during one year of infection³³. This antigenic variation leads to the presence of multiple and closely related viral variants, designated as quasispecies, within a given patient²⁸. The most variable genes appear to be the envelope glycoproteins, E1 and E2^{18, 25}, with the greatest degree of diversity in the hypervariable region 1 (HVR 1) located at the N-terminal region of the E2 protein¹⁹. The degree of viral quasispecies in HCV-infected patients is well correlated with the severity of HCV-induced liver disease²⁴. Recent studies on HCV viral dynamics³² suggest that the rate of production of HCV virions is quite high, perhaps explaining how quasispecies might emerge so rapidly. Interestingly, patients with long-term infections tend to have higher numbers of quasispecies within their genotype isolate^{15, 20}, and the presence of increasing levels of these quasispecies has been associated with clinical treatment failures²⁴.

In addition, HCV elicits neutralizing antibodies to at least some of the strains present in a quasispecies swarm^{14, 38}. The HVR 1 in particular appears to be a major target for such neutralizing antibodies, but, as noted above, it represents significant degrees of sequence diversity^{16, 18, 40}. This suggests that this diversity may be in part due to selective pressure against the host immune response. The emergence of mutants within the HVR1 will allow escape from immune recognition. This is supported by chimpanzee studies demonstrating that anti-serum to the HVR1 was able to prevent HCV infection with a virus of this specificity, but was unable to prevent infection with pre-existing quasispecies variants^{14, 16}. Furthermore, the activity of neutralizing antibodies is weak and short-lived, thus providing no protection against re-infection of the same strain of HCV²³.

Given the quasispecies nature of HCV infection, viral mutants may emerge following clearance of a wild-type virus and contain changes within the immunodominant site recognized by CTL. CTL play a crucial role in viral pathogenesis by recognizing viral antigens as peptides bound to class MHC I molecules on the surfaces of antigen-presenting cells. Virus-infected cells are destroyed by CTL that recognize the MHC/peptide complexes. It has been reported that the magnitude of virus-specific CTL response in chronic HCV patients correlates with disease status and viral load⁷. The mutational loss of this immunodominant epitope would render viral variants invisible to CTL recognition, and indeed such a CTL escape mutation has been described in a chimpanzee with chronic HCV infection⁴². Several lines of evidence, however, argue against this being a true immune escape mechanism. The initial CTL response to HCV infection is polyclonal and multispecific⁷, such that it is unlikely that the loss of a single epitope would allow escape mutants to survive. In addition, a given CTL clone can often recognize other residues within the same epitope and should be able to target such a virally infected cell.

Immune Modulation of Host Immune Responses

In addition to the emergence of viral quasispecies during HCV infection as a predominant mechanism of immune escape, HCV gene product(s) may evade the host immune surveillance. While it is likely that CTL can adequately clear virally infected cells, several lines of evidence suggest that CTL might suppress viral replication non-cytolytically by expression of antiviral cytokines. Such a suppression of viral replication has been shown to be crucial in HBV transgenic mice following injection of HBV-specific CTL, perhaps being particularly important in the local, *in vivo* environment¹⁷. CTL clones derived from the liver have been shown to secrete IFN- γ and TNF- α ²⁶, and it is possible that resistance to these facilitates HCV persistence. This is supported by the lack of a durable response of the majority of patients to IFN- α therapy²².

Given the importance of an adequate cytokine response, we and other investigators have focused on the possibility of selective immunosuppression during acute HCV infection. The HCV core protein appears to be a multifunctional protein that can interact with several members of the tumor necrosis factor receptor (TNFR) family that modulate immune responses. HCV core interactions with the lymphotoxin-beta receptor (LT β R) modulates cytotoxicity mediated by its physio-

logical ligand^{8, 30}. The HCV core also appears to interact with TNFR1⁴⁵ and may dysregulate NF κ B signaling, perhaps facilitating survival of an infected cell⁴⁴. We have also found that the HCV core is physically associated with the cytoplasmic domain of Fas molecules and this interaction increases the susceptibility of the T lymphocyte cell line, Jurkat, to Fas-mediated apoptosis (manuscript submitted for publication). In addition, overexpression of HCV core in Jurkat cells dramatically induced apoptosis that was dependent on the activation of both caspase 8 and 3, suggesting modulation of TNFR family member signaling pathways by this protein (MOORMAN, manuscript in preparation). Strikingly, the increased apoptosis of core-expressing cells was independent of the expression of Fas ligand. Currently, we are further characterizing the molecular mechanism of increased Fas-mediated apoptosis induced by the HCV core.

In addition, we have developed a murine model to examine the role of HCV gene product(s) in the regulation of host immune responses²⁷. Using vaccinia virus recombinants expressing various HCV viral gene product(s), we infected BALB/c mice and examined whether HCV gene product(s) may modulate the host immune responses against vaccinia virus. Interestingly, the expression of core protein led to prolonged viremia of vaccinia virus and increased mortality of mice by suppressing the host immune responses. These mice downregulated the production of both IL-2 and IFN- γ , key cytokines during the early host immune response against viral infection. Furthermore, core expression led to a > 5 fold reduction in the *in vivo* primary CTL response and a decreased frequency of CTL precursors in the experimental murine model. These data support a key role for the core protein of HCV in suppressing an initial immune response to viral infection²⁷.

Summary and Conclusions

A great deal of progress has been made in understanding HCV immune modulation and pathogenesis since its discovery in 1989. The development of tests to detect anti-HCV antibodies in blood has significantly reduced the incidence of post-transfusion HCV infection. The main challenges of this field involve the development of a vaccine and new drugs to combat viral infection. These goals will be largely achieved by an understanding of the potential mechanisms involved in the establishment of persistent HCV infection.

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