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Review

Immune responses and immunity in hepatitis C virus infection

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Introduction

Hepatitis C virus (HCV) is a parenterally transmitted RNA virus that causes chronic hepatitis and liver disease. In contrast to infections with other hepatitis viruses, the onset of HCV infection is clinically inapparent in the majority of cases, and the diagnosis of hepatitis C is therefore typically made only after persistent infection is established. HCV persistence occurs in 70% of patients and fewer than half of those are likely to respond to the current antiviral therapy of interferon and ribavirin. Because there are so many HCV-infected patients, it is estimated that the incidence of complications from chronic hepatitis, namely, liver cirrhosis and hepatocellular carcinoma, will increase in the next few decades.[1] These prospects necessitate the search for vaccines to prevent new infections and improved immunomodulatory therapies to cure persistent infections.

Many studies published to date have demonstrated that HCV-specific humoral and cellular immune responses are readily detectable in most, if not all, infected patients. It is, however, still unknown if natural sterilizing and protective immunity against HCV exists and if it could be therapeutically induced. This review provides a detailed discussion of the individual components of the HCV-specific immune response and addresses this question in view of the recent literature.

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HCV and HCV infection

The hepatitis C virus is an enveloped, positive-strand RNA virus, approximately 50 nm in diameter[2,3] and similar in genomic structure to Flavi- and Pestiviridae. Its genome consists of approximately 9600 nucleotides and contains a single open reading frame encoding a polyprotein of approximately 3000 amino acids. Both host and viral proteases cleave this polyprotein into three structural proteins—core, E1, and E2—and six nonstructural proteins—NS2, NS3, NS4A, NS4B, NS5A, and NS5B. The function of p7, located between structural and nonstructural proteins is currently not known (for a detailed review of the HCV genome structure, replication and proteins functions, see references 4 and 5).

HCV enters a susceptible host either directly, through transfusion of contaminated blood products or injection with contaminated needles, or, less efficiently, by crossing over an epithelial barrier, as exemplified by perinatal or sexual transmission. The virus reaches the liver via the hepatic artery or the portal vein and enters hepatocytes, its preferred site of replication. Sharp serum alanine aminotransferase (ALT) elevations may occur between 10 and 16 weeks after infection, but acute hepatitis is rarely diagnosed, because the majority of patients are not icteric and do not develop any symptoms other than fatigue and mild pain in the right upper abdomen. Circulating HCV-specific T cells have been demonstrated as early as 3–4 weeks after infection, whereas HCV-specific antibody responses occur much later, between 7 and 31 weeks after infection.[6]

Innate immune response

The first line of defense against any viral agent is antigen nonspecific, and the earliest response is probably elicited by virus-infected cells. One of the earliest and

common viral products in infected cells is double-stranded RNA, and most cells respond with synthesis of type I interferons, i.e., IFNα and β,[7] which are cytokines characteristic of the innate immune response. Type I IFN can inhibit the replication of many viruses, including the hepatitis B virus, as demonstrated in transgenic mice and in infected chimpanzees,[8] and the hepatitis C virus, as recently demonstrated in an in-vitro replicon system.[9,10] Intracellularly, IFN activates the Mx protein, the 2′5′ oligoadenylate synthetase-induced RNAse L that degrades viral RNAs, and the double-stranded RNA-dependent protein kinase (PKR) that inhibits the synthesis of cellular and viral proteins. Finally, type I IFN stimulates the effector functions of natural killer (NK) cells and CD8+ T cells and facilitates virus-specific immune response by the upregulation of MHC expression on target cells of the adaptive immune response.

Immune cells that provide innate responses in infected tissues are granulocytes, NK cells, macrophages, and dendritic cells. NK cells might be of particular interest for the defense against hepatitis viruses, because the human liver contains significantly higher numbers of NK cells than the peripheral blood or any other organ.[11] NK cells display stimulatory as well as inhibitory receptors and may distinguish infected from uninfected cells by recognizing alterations in MHC class I expression or changes in cell-surface glycoproteins induced by viral infection. They are also activated by type I IFN (IFNα/β), produced in the very early phase of viral infections as described above, and by interleukin (IL)-12, produced by macrophages and dendritic cells. NK cells exert various effector functions within the first several hours to days after infection. First, they release granule contents, inducing apoptosis. Second, they produce cytokines, such as IFN-γ and tumor necrosis factor (TNF-α), and a variety of other chemokines, such as macrophage inflammatory protein-1α (MIP-1α), MIP-1β, and IFN-inducible protein-10 (IP-10).[12] MIP-1α, MIP-1β, and IP-10 promote the accumulation of even more NK cells within the liver because they are potent inducers of NK cell chemotaxis. IP-10 also attracts intrahepatic lymphocytes. In addition to NK cells, natural killer T (NKT) cells constitute a large cell population in the liver. These cells express both NK cell markers such as CD56 and T cell markers such as CD3 and are also called natural T (NT) cells. Although the natural ligand for the NKT cell receptor has not been identified yet, it is known that NKT cells exert cytotoxic functions and produce IFNγ as well as IL-4.

Macrophages and dendritic cell precursors (pre-DC2s) are also capable of producing type 1 IFN in response to viruses,[13,14] in addition to nitric oxide and other pro-inflammatory cytokines that support the initiation of adaptive host-responses.[15–17] The induction of these responses may depend on the route of viral infection. Specifically, large numbers of macrophages are found at potential pathogen entry sites, such as the connective tissues associated with the gastrointestinal tract, the lung, the spleen, and along certain blood vessels in the liver, where they are known as Kupffer cells. In the case of epidermal exposure via injured skin, specialized dendritic cells of the epidermis take up antigen locally, undergo changes of activation status, surface marker, and chemokine receptor expression[18] and enter regional lymph nodes through the high endothelial venules. There, they induce specific cellular immune responses and activate naive T cells.

Evidence for a role of the innate immune response for clearance of HCV has recently been gained by analysis of gene expression in liver biopsies from the early phase of HCV infection.[19] As soon as a few days after HCV inoculation of chimpanzees, and several weeks prior to the peak in serum ALT activity, changes in the expression level of a large number of cellular genes were observed.[20] While these changes affected metabolic genes, cell-cycle regulation genes, apoptotic markers, and immune response genes, the earliest changes were demonstrated for IFN-response genes. These included proteins with antiviral effects, such as OAS, PKR, and Mx proteins, as well as STAT1, a transcription factor necessary for IFN responses. Other IFN response genes with changes in expression level encoded cytokines that are chemotactic for neutrophils, and that enhance the proliferation and cytolytic activity of NK cells. In accordance with the clinical observation that acute HCV infection is mostly clinically inapparent and not associated with significant disease in infected patients, these changes did not coincide with maximum serum ALT levels and were not associated with evidence of a significant lymphocytic infiltrate. Indeed, the gene expression of T lymphocyte surface markers, T cell receptors, MHC II, or proteasome components increased at much later time points, i.e., week 14 after infection, even after the peak of serum ALT activity.[19]

Priming of HCV-specific immune responses

Simultaneously, or soon after the innate immune responses, virus-specific adaptive immune responses are induced. Because HCV-infected hepatocytes cannot prime T cells, as a consequence of the low expression of adhesion and costimulatory molecules and the absence of class II proteins, it is assumed that the virus-specific immune response is primed in the regional lymph nodes. Viral antigen is most likely delivered to the lymph nodes by dendritic cells. These cells present phagocytosed and processed antigen on MHC II proteins to CD4+ T cells and, via cross-priming on MHC

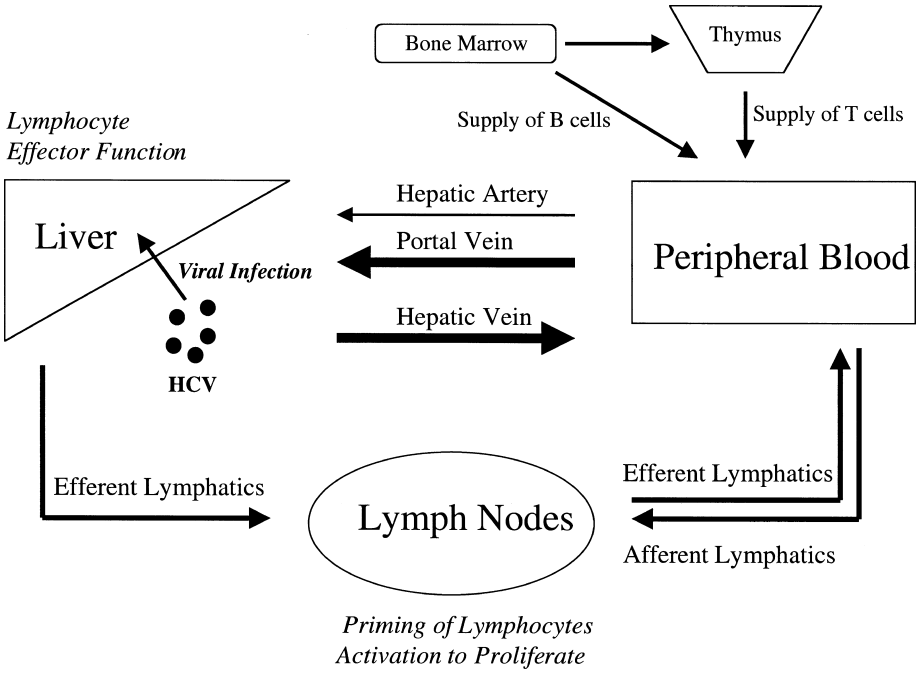

Fig. 1. Homing of effector lymphocytes to the liver during hepatitis C virus (*HCV*) infection

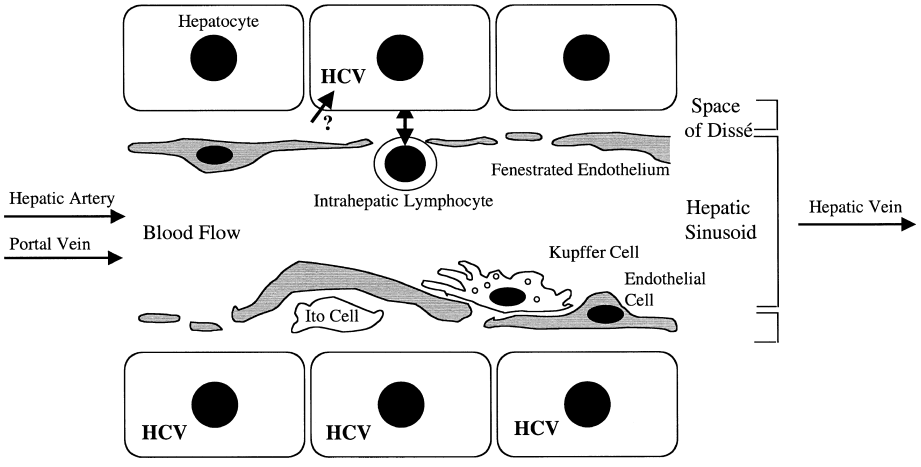

Fig. 2. Infiltration of effector lymphocytes into the liver parenchyma

class I, to CD8+ T cells.[21] To date, there is no direct evidence that HCV may replicate in antigen-presenting cells, but it has been reported that the allostimulatory function of antigen-presenting cells from HCV-infected patients differs from that of healthy controls.[22]

Activated HCV-specific T cells leave lymph nodes as effector cells through efferent lymphatic vessels, circulate in the blood, and enter the liver via the large portal vein and the small hepatic artery (Fig. 1). Notably, even the healthy, uninfected liver contains an estimated number of 2–8 $\times$ 10^9 lymphocytes,[23] equivalent to approximately 1%–4% of the total lymphocyte pool of adults. Therefore, lymphocyte entry into the liver appears to be, to some extent, a random event that might not be strictly antigen-dependent, but facilitated by the slow blood flow in the liver sinusoids and the fenestrated endothelial membrane (Fig. 2). Under these conditions, contact of lymphocytes with endothelial cells can readily be established via adhesion molecules such as very late activation antigen-4 vascular cell adhesion molecule-1 (VLA-4/VCAM-1) or lymphocyte function-associated antigen-1/intercellular adhesion molecule-1 (LFA-1/ICAM-1). In fact, it has recently been demonstrated, in a transgenic mouse model, that the selective retention of circulating T cells that recognize antigens expressed in the liver occurs within minutes.[24] In addition, inflammation of the portal and periportal areas is a common feature in chronic hepatitis C, and gradients of chemokines such as MIP-1α,1β, factor regulated on activation, normal T cell expressed and secreted (RANTES), and IP-10 may facilitate the attraction of liver-infiltrating lymphocytes via endothelial cell junc-

tions into the space of Dissé and the parenchymal tissue (Fig. 2). This hypothesis is supported by a recent observation that hepatic chemokine levels correlated with ALT levels in chronic HCV infection.[25]

Adaptive immune responses (CD4, CD8, B cells)

During the acute phase of HCV infection, adaptive immune responses are mediated by HCV-specific antibodies, CD4+ T helper cells (Th cells), and CD8+ cytotoxic T cells (CTLs). Virus-specific antibodies constitute a major arm of the adaptive immune response to many infectious agents. They have been reported to remove virions from the circulation, to opsonize them for uptake by macrophages and presentation to T cells, to block viral entry into host cells, to label virus-infected cells for complement-mediated lysis, and even to prevent the release of new virions.[26] With respect to HCV infection, several studies suggest that an early antibody response to the amino-terminus of the hypervariable region 1 (HVR-1) of the HCV envelope 2 glycoprotein is associated with a self-limited course of infection,[27–29] and that more complex initial quasispecies distribution is associated with HCV persistence.[30,31] The role of antibodies in protective or sterilizing immunity, however, has been difficult to evaluate because of the lack of an in-vitro system supporting HCV replication and the lack of in-vitro neutralization assays. In the absence of such systems, a neutralization of binding (NOB) assay has been developed to detect antibodies that inhibit the binding of the HCV E2 ectodomain to the candidate HCV receptor CD81 on Molt-4 cells.[32,33] Interestingly, high titers of these antibodies were present in sera of patients who resolved chronic hepatitis C,[34] and in chimpanzees that were protected from low-dose homologous HCV challenge after vaccination with recombinant envelope proteins expressed in mammalian cells.[35]

Direct evidence for a protective role of HCV-specific antibodies derives from limited in-vivo studies in which chimpanzees were protected against HCV infection after in-vitro neutralization of the HCV inoculum.[36,37] Thus far, the hypervariable region 1 (HVR-1) and other regions of the HCV envelope glycoproteins have been proposed as targets for neutralizing antibodies,[35,37,38] and the high degree of the HVR-1 variability has been attributed to immune selection pressure. HVR-1, however, is not randomly variable. It contains both invariant and variable positions and is highly conserved with regards to its chemicophysical properties and conformation.[39] Furthermore, two independent studies have demonstrated that HVR-1 sequence changes are not required to establish HCV persistence in infected chimpanzees.[40,41]

Several studies have demonstrated a significant association between a strong and maintained HCV-specific cellular immune response and viral clearance in acute hepatitis C.[42–44] CD4+ T helper cells recognize short antigenic peptides that are endogenously processed from phagocytosed proteins and displayed in the context of MHC class II molecules on the cell surface. T helper cells fall into two functional classes, Th1 and Th2, according to the cytokine profiles they secrete to activate other cells and to modulate the immune response. Specifically, Th1 cells produce IL-2, IFN-γ, and TNF-α, and stimulate a number of effector cell types.[45,46] Th2 cells produce IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13, and activate B cells to produce antibodies.[47] In addition, in other viral infections, CD4+ T cells have also been shown to exert direct effector functions, such as Fas-dependent cytotoxicity.[48]

Cytokine analysis in the acute phase of HCV infection demonstrated a Th1-dominated T helper cell response.[44] Tsai et al.[49] reported that peripheral blood mononuclear cells of patients with self-limited acute hepatitis C displayed a Th1 cytokine profile upon stimulation with HCV antigens, while a Th2 cytokine profile was observed in patients who developed chronic hepatitis. These findings suggest that a Th1 rather than a Th2 response may be associated with an effective HCV-specific immune response in the early phase of infection.

Although several mechanisms have been proposed and discussed, the precise factors that determine whether Th1- or Th2-mediated immune responses prevail in the early phase of infection are still unknown. Both Th1 and Th2 cells develop from naive CD4+ T cell populations. In the mouse model, this differentiation process is influenced by several factors, including the type of cytokines produced in the very early phase of infection, the HLA haplotype, the quantity and quality of antigen, and so on. For example, peptide/MHC class II complexes that strongly interact with the T cell receptor (TCR; strength being defined as TCR affinity, length of TCR occupancy, and presence of costimulatory signals) induce Th1 cells, whereas weak binders induce Th2 cells.[50] More recently, a role of NKT cells has also been described as a result of their ability to suppress Th1-associated cell-mediated immunity through the production of IL-4, IL-10, and/or transforming growth factor (TGF)-β.[51–54] Vα14 TCR transgenic mice with a tenfold increase of NKT cell numbers demonstrate elevated levels of the Th2 cytokine IL-4 in the blood, and in-vivo NKT cell activation drove Th2-directed immune responses.[55–57] Although the role of NKT cells in HCV infection has not been analyzed yet, their ability to rapidly produce both IL-4 and IFN-γ could play a role in Th1/Th2 differentiation and, potentially, in the outcome of HCV infection.

Strong and vigorous CD8+ T cell responses have also been associated with viral clearance in acute hepatitis C.

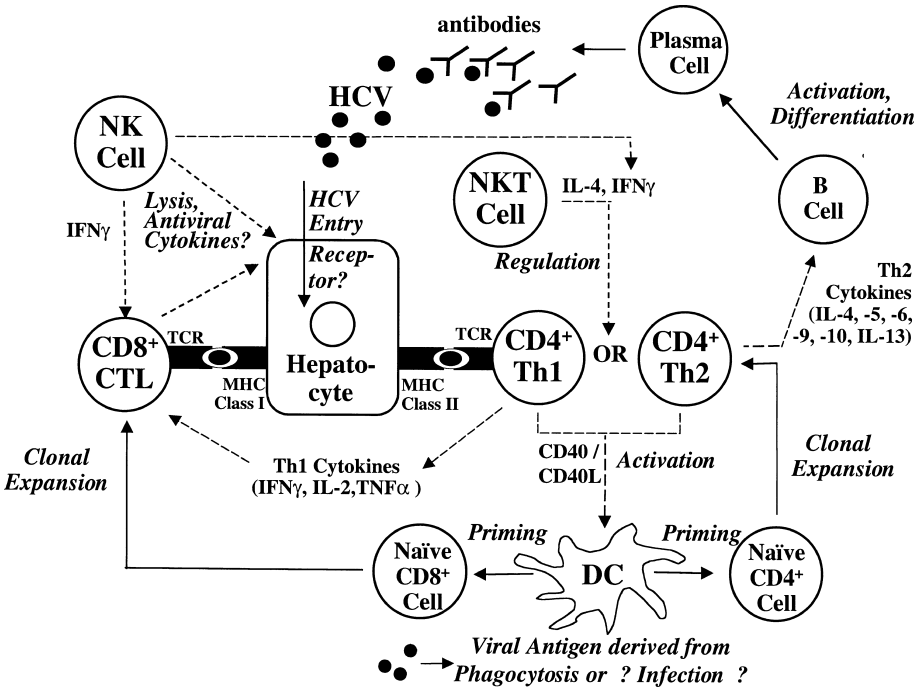

Fig. 3. Components of the HCV-specific immune response. *Th*, T helper cell; *CTL*, cytotoxic T cell; *TCR*, T cell receptor; *MHC*, major histocompatibility complex; *NK*, natural killer cell; *NKT*, natural killer T cell; *IL*, interleukin; *DC*, dendritic cell; *HCV*, hepatitits C virus; *IFN*, interferon; *TNF*; tumor necrosis factor; *dashed lines*, regulatory effect; *solid lines*, differentiation

HCV-specific, cytotoxic T cells are thought to play a particularly important effector role in the host defense against intracellular noncytopathic viruses such as HCV because of their ability to scan the cell surface for viral peptides and to lyse these cells prior to the release of new virions. Accordingly, HCV-specific CTLs have been expanded from the liver of HCV-infected patients[58–60] and chimpanzees[61] and viral clearance correlated with the presence of a strong and multispecific CTL response against multiple HCV proteins.[61] In addition, it was shown that this CTL response appeared more rapidly in chimpanzees that resolved the infection than in those that did not.[61] Similar results have also been reported from human studies; those patients that mounted a strong and persistent CTL response in the blood resolved the infection, while those with a weaker CTL response were unable to clear HCV.[62,63] A summary of all known components of the HCV-specific immune response is shown in Fig. 3.

Viral factors contributing to HCV persistence

The natural history of hepatitis C has been a controversial issue, because, for many patients, the time point of infection is unknown and the evaluation of the long-term outcome of infection is therefore difficult. Approximately 70% of patients are estimated to develop persistent infection, whereas only 30% may clear spontaneously. Because most infected patients are immunocompetent adults, who are able to clear other infectious pathogens, including other hepatotropic agents such as the hepatitis A virus or the hepatitis B virus, it has been argued that HCV must have developed a means either to not induce or to actively antagonize immune responses.

One of the generally discussed hypotheses is that HCV avoids the induction of a strong innate or adaptive immune response during the early phase of infection. The intensity of the immune response is influenced by the dose, form, and route of infection, as well as by the cytopathic effects of the virus. HCV is thought to be a noncytopathic virus that is able to establish persistent infection without eliciting any symptoms of acute hepatitis. The frequency of CD8+ T cells specific for a given HCV epitope has been shown to be generally low, in the range of 0.01%–1.2% of all peripheral blood CD8+ T cells in chronically infected patients,[64] and 2%–8% in acute phase in acutely infected patients.[62] By comparison, 7%–44% of peripheral blood CD8+ T cells have been demonstrated to be Epstein-Barr virus (EBV)-specific in acute EBV infection, and 50%–70% of activated CD8+ T cells were lymphocytic choriomeningitis virus (LCMV)-specific during the acute phase of LCMV infection. However, to obtain a complete picture of the total number of all HCV-specific T cells, all potential epitopes, in the context of all MHC molecules of a given patient, need to be analyzed; as well, their functional properties and specific distribution in the HCV-infected liver need to be evaluated.

Another characteristic feature of HCV that may also affect the host immune response is its high degree of

sequence variation and quasispecies nature. It has been reported that the outcome of acute hepatitis C is predicted by the evolution of the viral quasispecies.[31] Mutations in the HCV genome are estimated to occur at a rate of 0.4 to 1.2 $\times 10^{-3}$ base substitutions per site per year.[65–67] as a consequence of the high error rate of the viral RNA-dependent RNA polymerase. This continuous generation of mutations may have an impact on recognition by the humoral and/or cellular immune system.

With respect to cellular immune responses, mutations could alter the binding of viral epitopes to the MHC class I molecules or to the T cell receptor, or the recognition by specific T cells. Importantly, partial antagonism can downregulate the CTL response against the wild-type epitope. Specifically, it has been proposed, in other viral systems, that small changes in the sequence of immunogenic peptides (agonists) can result in the loss of activation signals and in the inhibition of T cell response.[68–70] Even small quantities of antagonist peptides are sufficient to inhibit the wild-type specific response when simultaneously presented with the wild-type peptide, because such presentation of both antagonist and agonist prevents the multiple TCR engagements necessary to reach the threshold to activate T cells,[71] and thus inhibits appropriate T cell stimulation. Such natural antagonist variants have been reported in infections with other viruses, such as HBV[72] or HIV.[73] Notably, it has recently been reported that mutations in HIV CTL epitopes are maintained upon the infection of new hosts, and thus may accumulate during viral evolution.[74]

In HCV infection, the existence of antagonist variants for both CTLs[75–77] and Th cells[78] has been observed. Indeed, the inability to maintain an appropriate HCV-specific CD4+ T cell response has been associated with recrudescence of the virus.[44] In particular, CD4+ T cells are thought to be at the center of the adaptive immune response, because CD4+ T cell help is required for the maturation of B cells and production of neutralizing antibodies, the induction and maintenance of effective CTL responses, and the stimulation and activation of dendritic cells via the CD40 ligand (Fig. 3). Thus, CD4+ T cells interact with all components of the adaptive immune response, and viral escape in CD4+ T cell determinants would affect several arms of the adaptive immune response. In addition, many CD4+ T cell epitopes bind promiscuously to many MHC proteins,[43] and may therefore exert more immune selection pressure. The presence of viral variants that are not recognized by, or that antagonize T cell responses has been demonstrated in HCV infection,[75–78] and future studies will need to evaluate the significance of these variations and mutations for the outcome of infection and disease pathogenesis.

Similar to findings in other viruses that have adapted ways to circumvent or inhibit innate immune responses, it has been discovered that two proteins of the hepatitis C virus interfere with the function of PKR. PKR is activated by double-stranded RNA and phosphorylates the α-subunit of the eukaryotic translation initiation factor 2 (eIF2α), thereby inhibiting protein synthesis. Interestingly, one of the two HCV envelope proteins, E2, exhibits homology with the phosphorylation sites of the IFN-inducible protein kinase PKR and with eIF2α.[79] Another HCV protein, the nonstructural protein NS5A, blocks PKR dimerization. Furthermore, the expression of all HCV proteins in a continuous human cell line interfered with type I IFN-induced signal transduction through the Jak-STAT pathway.[80]

Another mechanism to suppress host immune responses might be employed at the T cell level by the HCV core protein. Specifically, it has been shown that HCV core protein inhibits T cell proliferation in vivo,[81] and that it inhibits proliferation and IL-2 and IFN-γ production in vitro[82] through the binding to amino acids 188 to 259 of the complement gC1q receptor expressed on the cell surface of T lymphocytes.[82] Remarkably, nanomolar concentrations of HCV core protein are sufficient to inhibit in-vitro responses.[82] Hence, multiple mechanisms may contribute to HCV's ability to survive in otherwise immunocompetent hosts and to establish persistent infection and chronic hepatitis.

Immune status after recovery from hepatitis C virus infection

Even two decades after a patient's recovery, HCV-specific CD4+ and CD8+ T cells may still be detectable in the peripheral blood and provide evidence of prior HCV exposure and infection. These cells are detectable in the blood of recovered patients with direct ex-vivo techniques,[63,83–86] and have also been expanded from liver biopsies of chimpanzees that recovered from hepatitis C.[61] In particular, the persistence of strong, virus-specific responses by CD4+ T helper cells appears to be critical, because the loss of this immune response has been linked to the recrudescence of viremia.[44]

How is this HCV-specific T cell response maintained? First, memory T cells can be more readily activated than naive or effector T cells, and it has been suggested that they may be maintained by cytokines even in the absence of specific antigen.[87] Second, a small amount of antigen is probably retained for long periods, in the form of immune complexes on follicular dendritic cells in lymphoid follicles. Third, even intact virions might persist at this site, or in liver in the case of hepatotropic viruses. For example, studies with acutely HCV-infected chimpanzees have demonstrated that clearance

of viremia from the blood may occur 6 to 8 weeks earlier than clearance of HCV from the liver.[19] Furthermore, in a patient, recrudescence of HCV in the blood was observed several months after HCV was initially cleared from the circulation. In this patient, the reappearance of HCV was associated with the loss of an HCV-specific CD4+ T cell response, and the patient proceeded to develop chronic hepatitis C. Thus, it is possible that the HCV-specific cellular immune response and, potentially, other host factors, control HCV load and limit spreading, even without being able to completely eradicate it.

In the case of HBV infection, the transplantation of liver grafts from donors who have recovered from HBV infection transmits hepatitis to the immunosuppressed recipient. In patients who have recovered from HBV infection, a long-lasting T cell response, predominantly mediated by CD4+ and CD8+ T cells with the phenotype of recently activated cells,[83,88] correlated with the presence of minute amounts of persisting virus and, together with HBs-specific antibodies, might control viral spreading. In contrast to HBV, no transmission of HCV has been reported by livers from donors who were anti-HCV positive but HCV-RNA negative.[89] Consistent with this, the majority of HCV-specific T cells in the blood of recovered patients did not display an activated phenotype, indicating that they were not activated by replicating virus.[62,90] These data suggest that, at least in some patients, HCV might be completely eradicated after recovery. This is in accordance with the observation that HCV-specific antibodies may decrease or even completely disappear after resolution of the disease.[83] Because the half-life of serum immunoglobulin and immunoglobulin-producing plasma cells is very short,[91–93] the continuous differentiation of long-lived memory B cells[94] into plasma cells is required to maintain antibody titers. This process is generally dependent on the repetitive stimulation of those cells by either trace amounts of persisting virus or by antigen-antibody complexes, which may persist on follicular dendritic cells of germinal centers.[2,13–15] The absence of circulating HCV-specific antibodies therefore suggests that persisting HCV antigen is also absent.

Would an immune response that clears HCV infection protect against reinfection upon reexposure to the virus? This question obviously can only be answered using an animal model of infection. Two recent studies demonstrated that accelerated viral clearance following rechallenge with HCV was observed in chimpanzees that had not been exposed to HCV for over 16 years, suggesting that protective immunity was not only possible but also long-lasting. While sterilizing immunity was not observed, the course of transient reinfection was clinically milder and shorter, and resulted in clearance of the challenge virus in all animals. Clearance of the challenge virus was associated with an early and strong recall CD4+ T cell response to HCV nonstructural proteins,[95,96] transiently increased levels of IFNα in the blood,[96] and an HCV-specific cytotoxic T cell response in the liver.[96] At the same time, the antibody response to HCV proteins increased by over 1000 fold. Clearance of the challenge virus occurred much more rapidly than in the primary infection, and was not associated with extensive hepatocellular damage, because the chimpanzees maintained either normal or only slightly elevated serum ALT activities.[95] Furthermore, protective immunity also extended to different genotypes, because an animal that was previously infected with genotype 1a also cleared the challenge virus of genotype 1b,[95] and an animal that had recovered from HCV genotype 1b infection rapidly cleared the HCV 1a challenge virus.[96] Thus, despite the absence of sterilizing immunity, reinfection was only transient and associated with significantly reduced viremia, minor ALT elevations, and recovery within a relatively short period of time. Because it might be possible to induce or to therapeutically enhance the immune responses that mediate these events, these results have promising implications for the development of HCV vaccines. Furthermore, even though sterilizing immunity might not exist, these data support the interesting hypothesis that HCV-specific immune responses can be protective and can prevent the development of chronic persistent infection.

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