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Immune responses during acute and chronic infection with hepatitis C virus

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Abstract

Hepatitis C virus (HCV) induces persistent infection and causes chronic liver disease in most infected patients. Vigorous HCV-specific CD4⁺ and CD8⁺ T cell responses against HCV multiple epitopes are necessary for spontaneous viral clearance during the acute phase, but the virus appears to have multiple strategies to evade these defenses. There are relatively few studies on the role of immune responses during the chronic phase of infection. CD4⁺ T cell responses appear to protect against liver injury and may be important to clearance during interferon and ribavirin based therapy. Classic cytotoxic T cells (CTL) may primarily damage the liver in chronic HCV, but there may be subpopulations of T cells that protect against liver inflammation. Resolution of these outstanding questions is important to the development of a prophylactic vaccine as well as improving therapeutic options for those with chronic infection.

Keywords

Hepatitis C virus; CD4 T cells; CD8⁺ T cells; chronic infection; liver injury; persistence; immune escape; immunopathogenesis

Introduction

Hepatitis C virus (HCV) is a leading cause of chronic liver disease worldwide [1], with more than 170 million individuals with chronic infection [2]. Since the advent of highly effective screening of blood and blood products to eliminate HCV, transmission is primarily through intravenous drug use; although cases do continue to occur through iatrogenic, occupational, vertical and sexual routes of exposure. Acute HCV infection is typically subclinical, although if detected it is highly amenable to therapy (approximately 80 % chance of sustained virologic response (SVR), or cure). Spontaneous resolution of HCV infection occurs in approximately 20% of infected individuals, although this rate may vary widely depending on host factors such as age and gender, with young females having a higher

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likelihood of spontaneous resolution than other groups [3]. It is estimated that on average 20 % of those with chronic HCV will develop liver cirrhosis over a 20-year period after infection, although the range is highly variable; and of those who develop cirrhosis between 0 and 3 % will develop hepatocellular carcinoma per year [4]. The rate of disease progression to cirrhosis is highly variable and the host and viral factors that modify the course are poorly understood. Alcohol ingestion is a major cofactor [5], but how other factors, such as male gender and age at acquisition, impact liver injury are at present not understood. Although current therapies can cure HCV infection in approximately one half of infected persons [6], therapy is difficult and not tolerated by many patients.

Characteristics of HCV

HCV belongs to the *hepacivirus* in the *Flaviviridae* family [7]. Other members of this family, such as yellow fever virus, cause acute hepatitis but not chronic infection; since HCV appears to infect only humans and chimpanzees, the lack of a closely related virus that can be studied in a small animal model has hindered studies of pathogenesis. HCV has 6 major genotypes and more than 50 subtypes [8]. HCV is a positive-sense single-stranded RNA virus with a genome of 9600 nucleotides encoding a single open reading frame (ORF) encoding a polyprotein of approximately 3,000 amino acids that is processed during and after translation into at least 10 proteins [9]; three structural proteins and seven non-structural proteins (Figure 1). For full details of the current concepts regarding replication and function of the viral proteins, the reader is referred to several recent reviews [7; 9; 10; 11], although the functions are summarized in Table 1. In brief, the viral genome encodes for structural proteins (core, which forms the viral nucleocapsid, and the envelope glycoproteins E1 and E2, which are glycosylated membrane proteins) as well as the non-structural (NS) proteins, which have essential functions in viral replication. NS3 contains protease, RNA helicase and nucleoside triphosphatase (NTPase) activities, all of which are essential to viral replication. NS5B is an RNA-dependent RNA polymerase (RdRp) of HCV, and a key enzyme for viral transcription and replication [7; 10; 11; 12; 13]. Inhibition of NS3 and NS5b function are key targets for rationale drug design of new antiviral agents. However, all proteins can be targets of the immune response and, as we will discuss, several viral proteins appear to play some role in evading host immune defenses.

The major site of HCV replication appears to be the hepatocyte, although it is not known how many hepatocytes within the infected liver support productive replication. HCV has also been reported to infect numerous other cell types, including B cells, dendritic cells (DC), and other peripheral blood mononuclear cells [14; 15; 16; 17; 18; 19]. However, true replication within these cells, as opposed to passive adsorption of virus, is not universally accepted; and whether or not this affects function of these cells is similarly not clear. HCV may bind to B cells and drive clonal proliferation, which may account for cryoglobulinemia commonly seen in HCV [20; 21; 22], as well as possibly being related to lymphoproliferative disorders [23].

An HCV replication system has only recently been established that can produce virus capable of both *in vitro* and *in vivo* infection [24]. Unfortunately at present this system is limited to a subclone of the hepatocellular carcinoma line Huh-7, and does not produce high

titers of virus. This has limited studies of the interaction between host immune cells and the target of infection. There is no small animal model of HCV infection, although immunodeficient mice manipulated to have destruction of the native liver can be transplanted with human hepatocytes, which can then be infected with HCV [25]. The utility of this model is limited by the high mortality of the mouse strain, lack of an adaptive immune system and, more significantly, the fact that in order to understand interactions of the immune system and HCV these mice would have to be transplanted with immune cells from the same donor as the hepatocytes. Chimpanzees are the only species other than man that can be infected with HCV, and thus have been invaluable in studies of the early events following infection. However, very few naïve animals have been available for study in the past, and due to declining availability of these animals, it is possible that this model may no longer be available for research.

Immune responses in acute HCV infection

Following infection, HCV RNA appears in the peripheral blood within 1–2 weeks after primary infection, and triggers multiple arms of the immune response, including both innate and adaptive immunity [26; 27; 28].

The interferon system

HCV RNA triggers production of type I interferon (IFN) α/β in infected cells (reviewed in [11; 29]). A key role in the IFN α/β system is played by IFN regulatory factor (IRF)-3, which activates the IFN α/β promoter; both IFN α and IFN β are recognized by the IFN receptor, thus providing a positive feedback loop in which there is activation of multiple IFN-responsive genes. Gene expression analysis in acutely infected chimpanzees revealed that HCV triggers expression of type I IFN and IFN-induced genes during the early phase of infection in the liver [30]. Another study in chimpanzees showed upregulation of type I IFN induced double-stranded RNA-dependent protein kinase (PKR), 2'–5' oligoadenylate synthetase (OAS) and Mx genes upon acute HCV infection, which collectively serve to inhibit replication of viruses and induce apoptosis in infected hepatocytes [31]. IFN may also upregulate the expression of HCV antigens on the surface of infected hepatocytes by modulating the generation of immunoproteasomes, which are essential to presentation of antigens to the immune system [32]. HCV appears to use several mechanisms to subvert the endogenous IFN response, as will be discussed later.

Natural killer (NK) and NKT cell

NK cells and NKT cells play an important role in the first lines of defense against viral infection. These cells may comprise up to 20 to 30 % of intrahepatic lymphocytes in the normal liver and can contribute to the control of viral replication through cytolysis of infected cells, production of cytokines that might inhibit viral replication, such as IFN γ , and activation of both DC and T cells [33; 34; 35]. It has been difficult to measure NK function in acutely infected persons given the subclinical nature of most acute HCV infection. Most studies have focused on measurement of NK cell function in chronic HCV infection and compared these to individuals who have previously cleared HCV. Genetic polymorphisms in the activating and inhibitory receptors expressed on these cells have been associated with the

likelihood of spontaneous clearance in some, but not all, cohorts of patients with HCV [36]. There has been no clear consensus about whether the frequency or function of NK cells in chronic HCV infection, in part because of different methodologies used in the published studies. Several studies have reported that the frequency of NK cells in both peripheral blood [37; 38; 39; 40] and liver [41; 42] in chronic HCV compared to controls is decreased. However, Corado *et al.* reported that there were no differences in the frequency of CD3-CD56+ cells [43]. Functional studies of NK cell have also been conflicting. There is *in vitro* data that suggests that binding of the E2 protein to CD81 on NK cells inhibits NK cell function [44; 45]. This is consistent with the observation that patients with chronic HCV infection had lower levels of cytotoxicity of NK cells against sensitive cell lines (e.g. K562) than normal controls [43]. This has been confirmed by some [39; 40], but not all [46] groups. Even if one focuses on the CD56^{dim} population, which comprises the NK subgroup with cytotoxic effector function, it is not clear that there are clear differences in the cytolytic capabilities of these cells when the number of effector cells is taken into account [37; 46]. While NKT cells are abundant in the liver, nothing is known about their functional role in acute HCV infection.

Dendritic cell (DC) function

DCs play an important role as antigen presenting cells (APCs) and link innate and adaptive immune responses. Two major subsets of DC are well known: myeloid DCs (mDCs, DC1), which predominantly secrete IL12 and TNF α following pro-inflammatory stimuli and drive naïve CD4 cells to a Th1 phenotype; and plasmacytoid DCs (pDCs, DC2), which secrete large amount of IFN α in viral infections and drive naïve CD4 T cells toward a Th2 phenotype [47; 48]. *In vitro*, HCV structural and non-structural proteins influence the capacity and differentiation of DC [49]. DCs generated from uninfected individuals and stimulated with HCV core and E1 protein are impaired in their capacity to stimulate allogenic T cell responses [50], and both DC expressing HCV core and NS3 protein have impaired differentiation [51].

Despite these *in vitro* studies, studies on DC function and outcome of HCV infection have produced contradictory results. In general, studies have compared individuals with a remote history of spontaneous resolution of HCV and individuals with chronic HCV infection, and compared these to either each other or to non-infected controls. Some studies have shown that mDCs generated from peripheral blood of chronically infected patients have impaired capacity to stimulate allogenic T cell responses and secrete IL12 [18; 52; 53]. In addition, reduced frequencies and impaired capacity of peripheral blood DCs in both acute and chronic HCV infection have been seen [54; 55]. However, not all studies have seen differences either in frequency [56] or function of pDC [57]. In some studies, pDC and mDC are reduced in number but appear to be functional once differences in cell number are corrected for [58; 59]. Moreover, mDC from chronically infected chimpanzees are phenotypically and functionally normal [60]. These conflicting results may be due to methodologic issues, such as a failure to differentiate mDC and pDC in studies of whole blood, and definitive answers about whether DC dysfunction contributes to persistence in HCV await resolution.

Adaptive immune responses

Adaptive immune responses include humoral immune responses and cellular immune responses. Development of neutralizing antibodies is a hallmark of clearance in many viral infections and induction of these antibodies through immunization with viral subunits or inactivated virus is a classic strategy for the induction of protective immunity. HCV-specific antibodies (Ab) usually become detectable in the serum within several weeks after primary HCV infection, although the range is highly variable. There may be a delayed appearance of antibodies in individuals with persistent infection [61; 62]. Moreover, HCV-specific antibodies appear after cellular immune responses and aminotransferase elevations [63]. The first detectable antibodies against HCV antigens in serum usually target NS3 protein (anti-c33 Ab) and core protein (anti-capsid Ab or anti-22c Ab). Later, the specific antibodies against NS4 protein and envelope glycoproteins (E1 and E2) appear [64]. In particular, the hypervariable region 1 (HVR-1) of HCV E2 glycoprotein is thought to be a major target for neutralizing antibodies. Whether or not the typical antibodies measured in these studies conferred or was a marker of sterilizing immunity is controversial. *In vivo* studies in chimpanzees showed that the HCV-specific antibodies, specific for the HVR and with *in vitro* neutralizing ability, had protective effects against HCV infection [65]. On the other hand, other studies demonstrated that anti-HVR antibodies could not confer protective immunity against reinfection with homologous or heterologous strains [66; 67]. Moreover, spontaneous clearance of HCV also was reported in absence of humoral immune responses in a- or hypo-gammaglobulinemic patients [68; 69].

The recent development of assays based on the use of surrogate HCV particles has permitted re-assessment of humoral immune responses against conformational, as opposed to linear, HCV epitopes. The later studies suggest that antibodies may indeed have some role in spontaneous resolution of HCV and might be useful components of prophylactic vaccines. Two technologies have been used to identify neutralizing antibodies. Virus-like particles (VLPs) are HCV structural proteins (core/E1/E2 proteins) assembled into a structural mimic of the native HCV viral particle [70; 71]. Use of VLPs as immunogens resulted in production of antibodies that prevented HCV entry *in vitro* [72; 73] and *in vivo* [74]. Pseudotyped retroviral particles (pps) are infectious virus, usually members of the lentivirus family, which are decorated with the HCV envelope glycoprotein on the surface. These HCV pps can be used to detect antibodies that inhibit infection and to demonstrate cross-genotype neutralization of HCV by antibodies in patients with chronic HCV infection [75; 76; 77; 78; 79]. The studies with HCV pp in humans and chimpanzees have demonstrated that HCV-specific neutralizing antibodies can be found in the late phase of chronic HCV infection. Moreover, these antibodies had cross-reactive activity, which meant they could response against both homologous and heterologous viral variants or HCV pps [76; 77; 79; 80; 81; 82]. A seminal study using a human liver-chimeric mouse model, in which immunodeficient mice have reconstitution with human hepatocytes, demonstrated the ability of antibodies to provide not only protection from homologous HCV isolated and heterologous HCV strains [83].

T cells in acute HCV infection

CD4⁺ T cells have multiple effector functions in antiviral responses, both via secreting antiviral cytokines and via activating viral specific B cells and CD8⁺ T cells. A strong preponderance of evidence demonstrates that in the acute phase of HCV infection, vigorous, broadly directed and sustained HCV-specific CD4⁺ and CD8⁺ T cell are closely associated with a self-limited course of infection [26; 84; 85; 86; 87; 88]. HCV-specific T cell responses and the induction of IFN γ in peripheral blood and liver coincide with a decrease in HCV RNA titers [26; 30; 89], although there is a notable lag between the onset of viremia and the onset of T cell responses [26; 89; 90]. There is no “immunodominant” response that is the critical determinant of protective immunity, as the immune response is broadly directed within the individual and the population. For example, Day *et al.* demonstrated immune responses against 10 of 37 defined epitopes using a proliferative assay in individuals with resolved HCV infection, whereas a responses against more than one epitope was never detected in those with chronic infection [85]. If the HCV-specific CD4⁺ T cell responses are inefficient (weak, narrow and short-lasting or lost), persistent infection develops [85; 91]. The definitive role of CD4⁺ T cell responses in acute HCV was shown in a recent study in which the generation of HCV-specific CD4⁺ T cell responses was shown to be critical for successful control of HCV; without CD4⁺ T cell responses, HCV-specific CD8⁺ T cell and neutralizing antibodies may develop yet fail to control viremia [92].

Similarly, in both human and chimpanzee studies, HCV-specific CD8⁺ T cell responses in acute spontaneously resolving HCV also vigorous and target multiple epitopes [87; 88], whereas HCV-specific CD8⁺ responses among those individuals with evolution to chronic infection are lower in frequency and target only a few epitopes [93; 94; 95]. A key proof of principle that T cell responses may be critical to vaccine development was a recent chimpanzee study in which elicitation of peripheral and intrahepatic CD8⁺ T cell responses conferred protection against HCV and, of significance, against challenge with a heterologous isolate [96]. Elegant experiments in the chimpanzee model, in which it is possible to deplete specific cell populations, demonstrate the critical role of CD4⁺ and CD8⁺ T cells in the primary protective immunity against HCV [97; 98; 99]. In these studies, CD8⁺ T cells are the primary effector cells in protective immunity. However, without sustained CD4⁺ T cell help, CD8⁺ T cells cannot keep pace with the viral replication and ultimately escape mutations develop [98; 99].

CD8⁺ T cells have both cytolytic and non-cytolytic effector functions, the latter of which is mediated by production of cytokines such as IFN γ and TNF α [33]. Recent evidence in other viral systems implicates non-cytolytic mechanisms as a major effector mechanism of clearance [33], although the mechanisms whereby CD8⁺ T cells might control HCV replication are poorly understood. Drawing upon clear cut data in animal models of HBV infection [33], most investigators have focused on IFN γ as a key cytokine both necessary and sufficient for inhibiting HCV replication. Certainly *in vitro* data suggests that IFN γ , as well as IFN α/β , can inhibit HCV replication [34; 100]. However, IFN γ expression is abundant in within the liver, even in chronic HCV, and there is no evidence that the level of expression is related to HCV viral load; indeed, the level of IFN γ expression correlates positively with the degree of inflammation within liver tissue [101; 102]. A recent study

examined the effect of IFN γ delivery directly into the liver via a gene therapy vector [103] and observed no effect on viral load. Whether other cytokines that might be produced by HCV-specific T cells play a role in controlling HCV replication awaits further study.

Immune escape

HCV has developed sophisticated escape mechanisms to evade host immune systems and it is likely that several different mechanisms are operational. In addition to interference with the endogenous IFN system and the potential for DC dysfunction, there is evidence for viral evasion of the adaptive immune response.

Subversion of innate immunity

Multiple lines of evidence suggest that HCV specifically interferes with the endogenous IFN and Toll-like receptor (TLR) response (summarized in Table 2 and reviewed in [11; 29; 104]). Retinoic-acid-inducible gene I (RIG-I) is an essential molecule for the IFN β promoter [105] and leads to phosphorylation and activation of IRF-3, which promotes type I IFN production in infected cells in turn [11; 29; 104]. HCV NS3/4A serine protease blocks activation of virus induced IRF-3 [106] and inhibits type I IFN induction by disruption of RIG-I signaling [105; 107; 108; 109]. NS3/4A protease also inhibits TLR3 signaling through cleavage of the TRIF protein [110; 111] (Figure 2). Recent *in vivo* studies confirm that IRF-3 activation in the setting of chronic HCV is limited [112], which suggests that the endogenous IFN response is not fully operational.

Newly synthesized IFN β binds to its cognate receptor and activates the expression of numerous IFN-stimulated genes (ISG) via the JAK/STAT pathway. Jak1 and Tyk2 are members of Janus kinase (JAK) family and phosphorylate signal transducer and activator of transcription (STAT) proteins. Phosphorylated STAT-1 and STAT-2 recruit IRF-9 to form a complex known as interferon-stimulated gene factor 3 (ISGF3). The ISGF3 complex locates to the cell nucleus and binds to IFN-stimulated response elements (IRES) in the promoter regions of ISG. This results in the expression of PKR, 2'-5' OAS, ISG-p56, IRF-7 and other genes [11; 29; 104] (Figure 3). HCV core protein inhibits activation or translocation of STAT-1 [113; 114; 115; 116] and increases degradation of STAT-1 [115; 117]. HCV core protein also inhibits the function of ISGF3 by inducing expression of suppressor of cytokine signaling 3 (SOCS-3) [104; 113; 118; 119] and blocks DNA binding by ISGF3 [120]. NS5A represses activation of PKR [121; 122; 123] through binding to PKR and inhibiting formulation of the PKR dimer [122]. NS5A also blocks the PKR-dependent activation of IRF-1 [123]. In addition, NS5A interacts with 2'-5' OAS [124] and induces interleukin (IL) 8 [125], a chemokine that inhibits IFN activity *in vivo* and *in vitro* [126; 127]. The envelope protein E2 also binds PKR and inhibits its kinase activity [128]. Therefore, collectively HCV uses multiple pathways to disrupt a key mechanism of antiviral defenses.

“Exhaustion”/anergy

A key question in HCV immunopathogenesis is why T cells fail. As previously discussed, there is controversial data about whether T cell failure might be due to DC dysfunction, although data from acute infection in the chimpanzee or in humans is limited. Data from

other model viral infections, such as lymphocytic choriomeningitis virus (LCMV), suggests that T cell exhaustion might result from a high rate of viral replication that exceeds the capacity of host immunity [129]. One mechanism of persistence in HCV is failure to maintain CD4⁺ T cells during early infection [91], which may be on the basis of high viral loads. Others have shown that HCV-specific CD8⁺ T cells in chronic infection are functionally impaired with respect to IFN γ production or anergic [93; 130; 131] and, consistent with this loss of function, exhibit phenotypic changes characteristic of early stages of differentiation [132]. In the chimpanzee, HCV-specific CD8⁺ T cells in the acute phase do not produce IFN γ having a so-called “stunned” phenotype. In a later phase of infection, T cells recovered their ability to secrete IFN γ , coinciding with substantial decrease of viral titer and resolution of hepatitis [26]. Whether HCV-specific T cell responses in the liver during chronic infection are functional or not *in vivo* is a matter of active scientific debate. Several studies have shown an impaired capacity of HCV-specific T cells to proliferate and secrete IFN γ against HCV proteins [93; 133]. However, other studies have shown IFN γ production both *in vitro* and *ex vivo* [134; 135; 136] and complete exhaustion with deletion of HCV-specific CD8⁺ T cells does not occur because CD8⁺ T cell responses can be restored with stimulation by IL2 and HCV antigens [137].

Several recent studies suggest that the PD-1/PD-1 ligand (PD-1L) pathway is up-regulated in those with chronic HCV and blockade of PD-1/PD-1L pathway had a beneficial effect on CD8⁺ T cells, restoring their ability leading to viral clearance, such as lymphocyte proliferation, secretion of cytokines and killing of infected cells [138; 139; 140]. However, a recent study demonstrated that expression of PD-1 was independent of whether HCV resolved spontaneously or became chronic [141].

Sequence mutations

Mutational escape from adaptive immune response has been thought to be one of major viral evasion strategies used by HCV. HCV replicates at a high rate, estimated at approximately 10^{10} to 10^{12} viral genome per day, even in the chronic phase of infection [142]. The mutation rate of HCV is estimated to be $1.5\text{--}2.0 \times 10^{-3}$ base substitutions per genome site per year, due to the intrinsic lack of proof reading of the RNA dependent RNA polymerase [143]. These characteristics of HCV contribute to the marked genomic diversity of HCV, with the potential to induce viral variants that can evade immune recognition [143; 144]. Erickson *et al.* first demonstrated immune escape from CD8⁺ T cell in the chimpanzee model [145]. Mutation occurred within multiple MHC class I restricted epitopes but not flanking regions of the HCV genome within the first few months of chronic infection. Mutations within CTL epitopes have also been seen in longitudinal studies of acutely infected patients and in single source outbreaks [146; 147; 148; 149] and by population based approaches, which sequence multiple strains and compare the observed patterns of viral variability with the known epitopes recognized in the context of that population's HLA [147; 148; 150]. HCV mutations also affect virus specific CD8⁺ T cell responses by decreasing binding affinity between epitope and MHC molecule [149], decreasing T cell receptor (TCR) recognition [151] and impairing proteosomal processing of HCV antigens [152]. Mutational escape from CD4⁺ T cell responses has also been demonstrated [149; 153].

Regulatory T cell (Treg)

Recently, several studies have examined the role of regulatory T cells (Treg) in HCV infection and whether alterations in these T cells might influence the likelihood of chronicity. These cells maintain self-tolerance and limit deleterious immune responses [154; 155; 156]. Although there are likely multiple populations of Treg cells [157; 158], the majority of studies in HCV have focused on the CD4+CD25+ population (or “natural” Treg). The frequency of CD4+CD25+ Tregs was found to increase in the peripheral blood of chronically HCV infected individuals in comparison with resolved individuals or uninfected individuals. Depletion of CD4+CD25+ Tregs from peripheral blood led to peptide-specific IFN γ production and proliferation by HCV-specific CD8+ T cells [159; 160; 161; 162]. However, these studies may be limited by the fact that CD25 is expressed on activated T cells as well; definitive identification may require staining of forkhead box protein 3 (*foxp3*), for which reagents have only recently become available, or other markers such as CD127 [157; 158]. Indeed, a recent study in chimpanzees showed no difference in CD4+CD25+*foxp3*+ cells in the periphery or liver irrespective of the outcome of infection [163].

Immune responses in chronic infection: friend or foe?

Many studies confirm that HCV-specific immune responses in the periphery are weak and directed against a limited series of epitopes in chronic infection compared to those responses observed in spontaneous resolution of HCV. However, immune responses appear to compartmentalize very early to the liver tissue, thus complicating studies of pathogenesis of chronic liver injury. Many studies have demonstrated that in the setting of chronic infection both HCV-specific CD4+ and CD8+ T cells are present in the infected liver [133; 134; 135; 136; 164; 165; 166; 167; 168; 169; 170; 171]. The ratio of intrahepatic HCV-specific T cells to peripheral T cells may be 100:1 or more. Little is known about the process whereby HCV-specific T cells home to the liver, although there is likely a series of events triggered by the endogenous IFN response. IFN α/β produced by hepatocytes can induce the production of monocyte chemoattractant protein-1 (MCP-1) by the resident liver macrophages, which leads to the recruitment of other macrophages that produce macrophage inflammatory protein-1 alpha (MIP-1 α). MIP-1 α recruits NK cells, which in turn serve as a source of IFN γ ; this cytokine may be antiviral but also leads to the production of monokine induced by IFN γ (MIG or CXCL9), which recruits T cells to the site of inflammation [172]. IFN-inducible protein-10 (IP-10), which correlates with the degree of inflammation, is also increased in chronic HCV [173]. HCV itself may upregulate critical homing molecules involved in T cell recruitment such as interferon-inducible T cell alpha chemoattractant (I-TAC) [174], the expression of which correlates with the degree of hepatic inflammation [175]. This data is consistent with the observation that HCV transgenic mice, despite constitutive tolerance to HCV proteins, may develop inflammatory liver lesions [176; 177].

Clinical data strongly suggests that patients with depressed cellular immunity, such as those with liver allografts or human immunodeficiency virus, have an accelerated course of HCV, and may develop cirrhosis much more rapidly than non-immunocompromised hosts [178; 179]. Prospective studies done early in the course of HCV infection demonstrate that the

magnitude of HCV-specific CD4⁺ T cell responses clearly determine the rate of progression of chronic liver disease [180]; this was confirmed in later cross sectional analysis of CD4⁺ T cell responses using IFN β linked ELISpot [181]. Similarly, the magnitude of the CD4⁺ T cell response in the liver appears to be inversely proportional to the degree of liver injury [181]. Although most investigators have approached Treg as a potential factor in mediating chronicity, a recent study demonstrated that CD4⁺CD25⁺ Treg may have an inverse relationship to liver inflammation [182]. Therefore, CD4⁺ responses in general appear to be protective in the setting of chronic infection, even if they do not completely prevent disease.

Conflicting reports have been described about the role of HCV-specific CD8⁺ T cell in the chronic phase of HCV infection. Classic adoptive transfer studies, as well as newer murine models in which HCV expression is induced after the period of neonatal tolerance, strongly suggest that CD8⁺ T cells mediate liver injury [183; 184]. Clinical studies have been inconsistent with respect to the relationship between the presence of CD8⁺ T cells and the HCV viral load [166; 185; 186; 187; 188; 189]; however, HCV viral load does not predict the extent of liver injury. Similarly, the presence of an HCV-specific CD8⁺ T cell response has an inconsistent relationship to the severity of liver injury on biopsy [102; 190]. The inflammatory infiltrate in chronic HCV appears to consist of a large number of T cells that are not specific for HCV [191; 192]; this “bystander activation” may be critical in liver injury [192; 193]. However, there are also novel CD8⁺ T cell populations within the liver that may inhibit inflammation through production of IL-10 [156; 192] or through TGF- β [194]. Further resolution of this issue awaits larger, prospective trials with cohorts with known duration of HCV infection and/or precise evaluation of the rate of liver injury using serial examinations.

Different NK subsets may have different roles in the pathogenesis of liver injury, but this too is an area that needs further development. Lin *et al.* found that activated CD56^{bright} correlated with inflammation but not fibrosis [38] and Morishima *et al.* found that CD56^{dim} correlated inversely with fibrosis stage [37]. Whether low levels of CD56^{dim} cells are the cause of increased fibrosis, by failing to protect the liver, or are simply associated with longstanding disease, as there may be apoptosis and loss of these cells over time, will require prospective studies.

Role of immune responses in outcome following IFN and ribavirin treatment

The current standard of treatment of HCV is a combination therapy with pegylated IFN β (peginterferon) and ribavirin. Overall sustained virologic response (SVR), defined as the absence of virus in the blood 24 weeks after therapy is ended, in about 50% of chronic infected patients treated with combination therapy [195]. However, SVR rates vary depending on viral (e.g., viral genotype and viral load) and host characteristics (e.g., gender, age, ethnic background and fibrosis stage) [196; 197]. For example, SVR rates are typically 42 to 52% among patients with HCV genotype 1 and 76 to 82% of those with genotype 2 or 3 [195; 198; 199; 200; 201]. Why certain genotypes are more responsive to treatment is not known, and is only beginning to be explored as culture systems for both genotype 1 and non-1 isolates become available. Similarly, it is clear that African Americans have lower

SVR rates than Caucasians [202; 203]; African Americans have lower baseline CD4+ responses against HCV than Caucasians but it is not clear whether this is the basis for the observed difference in SVR [204; 205].

In addition to direct antiviral effects, both IFN β and ribavirin may be immunomodulatory: IFN β enhances APC maturation and CD4+ T cell function, but with little effect on CTLs [206; 207] and ribavirin induces a switch from Th2 to Th1 profile [208] and inhibits IL10 production [209]. As new antiviral agents are developed that specifically target HCV proteins [210; 211], it is not known whether IFN and/or ribavirin or any immunomodulation will still need to be part of the regimen to lead to cure. Surprisingly, there is relatively little data on whether or not T cell responses at baseline or during treatment are associated with SVR. Cramp *et al.* demonstrated that higher CD4+ T cell proliferation in the early phase of therapy was correlated with SVR [209], with a more recent studies demonstrating that the baseline immune response is the critical determinant of SVR rather than enhancement of immune responses on treatment [205; 212]. In a study of individuals with HIV/HCV co-infection, HCV-specific production of IL-10 was a stronger predictor of response than other well known host factors such as race [213].

Since treatment of acute HCV typically leads to high SVR rates even in genotype 1 infection – at least 80% SVR compared to approximately 55% SVR in the setting of chronic HCV – the host factors associated with this have been studied. Kamal *et al.* demonstrated that the high rate of SVR in acute HCV is associated with strong T cell responses [214], although this has not been seen in every study [62]. A rapid drop in viral load on treatment is associated with more vigorous T cell responses, although whether this is cause or effect is not known [215]. Other host factors in the IFN-regulatory pathway might be associated with the responses to antiviral treatment [216; 217]. Interferon-sensitive gene 15 (ISG15) and ubiquitin-specific protease 18 (USP18), which are linked biochemically in IFN-regulatory pathway, modulate the IFN responses against HCV [216]. USP18 up-regulation is one of the factors predicting a lack of response to IFN treatment [217].

Conclusions and future directions

In recent years, a more complete understanding of the correlates of protective immunity in HCV has been achieved. There is broad consensus that induction of CD4+ T cells and probably CD8+ T cells targeting multiple viral epitopes will be critical to the development of an effective prophylactic vaccine; however, there are considerable technical challenges in formulating a vaccine that would be effective against the multiple circulating quasispecies. There is much less understanding of how HCV leads to persistent infection in most exposed persons, although it is likely that this virus uses multiple mechanisms to evade the immune system. The area in which there is least consensus is the role of T cells in resolution of HCV following during treatment and the mechanisms of liver injury. Further study of these areas is needed as we enter a new generation of HCV therapeutics that do not target the immune system, and to address the needs of the large number of patients with chronic HCV who face the threat of cirrhosis and liver cancer.

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Figure 1.

The HCV genome and gene products. HCV is positive-sense single-stranded RNA virus. This viral genome contains a single open reading frame (ORF) encoding a polyprotein, processed into at least 10 proteins; three structural proteins and seven non-structural proteins.

Figure 2.

HCV RNA induces activation of IRF-3 via both the RIG-I and TLR3 pathways. Activated IRF-3 leads to IFN β production and secretion from infected cell. HCV NS3/4A serine protease blocks these two pathways by cleavage of IPS-1 and TRIF.

Figure 3.

Newly synthesized IFN β binds to its cognate receptor and activates the expression of numerous IFN-stimulated genes (ISG) via JAK/STAT pathway. HCV core protein inhibits this pathway via several mechanisms. HCV core protein inhibits activation or translocation of STAT-1 and formulation of ISGF3 by inducing SOCS-3. HCV core protein also blocks DNA binding by ISGF3. NS5A inhibits activation of PKR and 2'-5' OAS. E2 inhibits activation of PKR.

Table 1

Functions of HCV structural and non-structural proteins (NS)

HCV protein	Function
Core	Nucleocapsid
E1 and E2	Envelope glycosylate membrane proteins Entry into cell; binding to CD81 Contain HVR **
p7	Viroporin Ion channel
NS2	NS2/3 protease
NS3	Serine protease RNA helicase NTPase **
NS4A	Cofactor for NS3 protease activity
NS4B	Hydrophobic protein Integral membrane protein Formation of the HCV RNA replication complex *
NS5A	Polyphosphorylated protein Formation of the HCV RNA replication complex * Responsible for IFN sensitivity of HCV *
NS5B	RdRp ** Key enzyme for viral transcription and replication

* A proposed function

** HVR; hyper variable region, NTPase; Nucleoside triphosphatase, RdRp; RNA-dependent RNA polymerase

Table 2

Interactions of HCV with components of innate host defense

HCV protein	Effect	References
Core	Inhibits activation or translocation of STAT-1	[113; 114; 115; 116]
	Increases degradation STAT-1	[115; 117]
	Induces expression SOCS-3	[113; 118; 119]
	Blocks DNA binding by ISGF3	[120]
	Inhibits SOCS-1	[218]
E2	Inhibits PKR activity	[128]
	Inhibits NK cell function	[44; 45]
NS3/4A	Inhibits TLR3 function	[110; 111]
	Block RIG-I signaling	[105; 107; 108; 109]
NS5	Represses activation of PKR	[121; 122; 123]
	Blocks 2'-5' OAS	[124]
	Increases IL8	[125]

STAT; signal transducer and activator of transcription, SOCS; suppressor of cytokine signaling, ISGF; interferon-stimulated gene factor, PKR; double-stranded RNA-dependent protein kinase, NK cell; natural killer cell, TLR; Toll-like receptor, RIG-I; retinoic-acid-inducible gene I, OAS; oligodeoxynucleotide synthetase, IL; interleukin