Published in final edited form as:

Gastroenterol Clin North Am. 2015 December ; 44(4): 735–760. doi:10.1016/j.gtc.2015.07.004.

Immunopathogenesis of Hepatitis C Virus Infection

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Summary

Despite advances in therapy, hepatitis C virus (HCV) infection remains a major global health issue with 3–4 million incident cases and 170 million prevalent chronic infections. Complex, partially understood, host-virus interactions determine whether an acute infection with hepatitis C resolves, as occurs in approximately 30% of cases, or generates a persistent hepatic infection as occurs in the remainder. Once chronic infection is established, the velocity of hepatocyte injury and resultant fibrosis is significantly modulated by immunological as well as environmental factors. Immunomodulation has been the backbone of antiviral therapy for most of the past fifteen years despite very poor understanding of its mechanism of action. More recent data regarding the role of T-cell exhaustion and antigen-presenting cell defects has encouraged early phase clinical trials of several novel immunomodulators with modest initial effects. An appreciation of the complexity, redundancy, and interdependence of the regulatory mechanisms involved in maintaining chronic infection may inform future approaches to restore immunoreactivity to HCV as feasible antiviral therapy.

Keywords

Hepatitis C; Human; innate immunity; adaptive immunity; regulatory T-cells

Introduction

Despite advances in therapy, hepatitis C virus (HCV) infection remains a major global health issue with 3–4 million incident cases and 170 million prevalent chronic infections (1). Complex, partially understood, host-virus interactions determine whether an acute infection with hepatitis C resolves, as occurs in approximately 30% of cases, or generates a persistent hepatic infection as occurs in the remainder. Once chronic infection is established, the velocity of hepatocyte injury and resultant fibrosis is significantly modulated by immunological as well as environmental factors. While the backbone of antiviral therapy for

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most of the past fifteen years has consisted of innate immune stimulation with interferon-alpha, more recent data regarding of the role of T-cell exhaustion and antigen-presenting cell defects has encouraged early phase clinical trials of several novel immunomodulators with modest initial effects. An appreciation of the complexity, redundancy, and interdependence of the regulatory mechanisms involved in maintaining chronic infection may inform future approaches to restore immunoreactivity to HCV as feasible antiviral therapy. This review will focus both on virus-induced immunological dysfunction that allows the establishment of persistent infection as well as the impact of these immunological defects on disease progression during chronic infection.

Intrahepatocytic Sensing and Signaling

The hepatitis C virus (HCV) is a highly mutable, hepatotropic, enveloped single-stranded RNA virus of the Flaviviridae family (2). The 9.5 kB genome encodes for a single polyprotein of 3011 amino acids flanked by 5' and 3' non-coding regions essential for viral replication (3). The viral polyprotein is translated *in toto* then processed by host cell- and virus-encoded proteases into structural (Core, E1, E2) and nonstructural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A, NS5B). The Core protein forms the viral nucleocapsid (4). E1 and E2 are the viral envelope glycoproteins that interact with various surface receptors including claudin-1, CD81, DC-SIGN, scavenger receptor B I (SR-BI), and the LDL receptor to mediate viral entry into target cells (5–11). The remainder of the nonstructural proteins create a membrane-associated viral replication complex critical for establishment for persistent, and along with structural proteins modulate host cell function to disrupt intra- and extra-cellular antiviral pathways.

The induction of type I (IFN α and IFN β), II (IFN γ) and III (IL-28A/IFN λ 2, IL-28B/IFN λ 3, IL-29/IFN λ 1, and IFN λ 4) interferons is critical for host-defense against intracellular pathogens. Type I, II and III IFNs signals in autocrine and paracrine manners through their respective heterodimeric receptors (IFNAR, IFNGR, IFNLR) to phosphorylate STAT-1 and STAT-2 leading to the formation of the transcription factor ISGF3 (consisting of pSTAT-1, pSTAT-2 and Interferon Response Factor 9 (IRF9)) that translocates to the nucleus to modulate the production of hundreds of IFN-stimulated antiviral genes (ISGs) (12). Specific ISGs suppress viral replication and sensitize infected cells to apoptosis (13). Critical to the development of adaptive immune responses, type I interferons also stimulate immunoproteasome formation critical for presentation of antigen by hepatocytes to CD8 T-cells (14). While IFNAR and IFNGR expression is ubiquitous, the heterodimeric IFNLR (consisting of IFN λ R1 and IL-10R2 chains) is restricted to epithelial cells, hepatocytes and dendritic cells (DCs) (15). While there is significant overlap in the number and types of genes induced by type I, II and III interferons, IFN α and IFN λ do have subtle differences in gene expression profiles and kinetics (16–18). Notably, the permissiveness of hepatocyte cell lines to support HCV infection *in vitro* appears critically dependent on defects in type I and III interferon signaling (19, 20).

After viral entry and uncoating, hepatocytes sense intracellular HCV infection to generate type I and III interferons both by toll-like receptors (TLRs) and by retinoic-acid-inducible-gene like receptors (RLRs), a family of proteins that includes retinoic acid-inducible gene-I

(RIG-I), melanoma differentiation-associated gene 5 (MDA-5), and laboratory of genetics and physiology gene 2 (Lgp2), in the cytoplasm. TLR3 recognizes double-stranded RNA replication intermediates on the cell membrane or within endosomes to activate TIR-domain-containing adapter-inducing interferon- β (TRIF), which in turn phosphorylates interferon response factor 3 (IRF3), the transcription factor critical for generating type I and III interferon production. Binding of specific viral dsRNA and/or ssRNA motifs by RIG-I and MDA-5 (19, 21–23) triggers a signaling cascade that similarly includes TRIF but also stimulates Mitochondrial AntiViral Signaling protein (MAVS, also known as Cardif, IPS-1 or VISA) to activate IKK-related kinases (IKK ϵ and TBK-1) which also phosphorylate IRF3 and IRF7 to trigger the production of type I and III interferons. Intracellular sensing mechanisms are initially preserved in HCV-infected hepatocytes. Type I IFN and ISGs are rapidly induced *in vivo* in experimentally infected chimpanzees (24). In humans, HCV predominantly produces a type III IFN response, predominantly with IFN λ 4 transcripts (25–27), that induces multiple ISGs that potentially could inhibit HCV viral replication by suppressing primary translation of viral RNA (28).

Therefore, disruption of these antiviral pathways is critical for establishment of viral persistence. Early after infection, the viral E2 and NS5A proteins both induce phosphorylation of protein kinase R (PKR) and elongation initiation factor 2 α which suppress general cellular mRNA translation without negatively impacting translation of HCV proteins (29–31). Subsequently, the NS3/4A protease cleaves MAVS preventing its dimerization, resulting in its disassociation from mitochondrial membranes and disruption of its signaling through IKK ϵ to activate IRF3 (32–39). The NS3/4A protease may also degrade TRIF (40), blocking TLR3 signaling, as well as directly interact with TBK1 and IKK ϵ (41). In addition to suppressing type I and III interferon production, MAVS inactivation reduces the production of the chemokines CXCL8 and CXCL10 critical for recruitment of inflammatory cells to the liver (42). HCV Core also interferes with TLR3-mediated secretion of interferons (43) which may particularly impair TLR3-dependent type III IFN production (44, 45). Furthermore, to block cellular responses to type I and III interferons that are produced, HCV Core protein upregulates SOCS3, a negative regulator of STAT phosphorylation (46), and also directly inhibits ISGF3 activity (47–49). Thus, HCV has evolved multiple strategies to disruption not only of the generation of antiviral type I and III interferons but also to interrupt interferon-induced gene expression to maintain viral replication.

Genome-wide association studies (GWAS) have identified three polymorphisms on chromosome 19 near the IFN λ 3 gene (rs12979860, rs8099917, and ss469415590/rs368234815) that are associated with spontaneous resolution of acute infection (50–52) and sensitivity to interferon-based antiviral therapy (27, 50, 51, 53–59). The rs8099917 TT or rs12979860 CC polymorphisms are associated with a stronger induction of IFN λ 2 upon stimulation (15). The ss469415590 polymorphism (TT $\rightarrow$ G) causes a frameshift mutation that creates IFN λ 4, protein that binds to IFNLR to induce STAT1 and STAT2 phosphorylation, ISGF3 and ISG expression (56). The TT polymorphism abrogates IFN λ 4 production which, somewhat counterintuitively, improves spontaneous resolution and antiviral treatment outcome (56, 60). Paradoxically, IFN λ 4 exerts strong antiviral activity *in*

vitro (61, 62) and it remains unexplained how the presence of this highly functional antiviral protein impairs antiviral responses in HCV (Table 1). In human liver, the apparent paradox appears to be relevant because intrahepatic expression of IFN λ 4 is positively rather than negatively associated with intrahepatic HCV RNA and ISG induction (26, 27). Other genetic polymorphisms that impact viral sensing and ISG expression, such as the rs3747517 MDA5 H843/T946 variant, also appear to impact the likelihood of spontaneous resolution of acute infection (63). IL-28B polymorphisms associated with greater clearance rates have variably been associated with more active necroinflammation and rapid fibrosis progression in chronic infection (64–67) suggesting impact of these genetic markers throughout the natural history of HCV disease.

In chronic infection, IFN λ polymorphisms associated with viral persistence are associated with chronic upregulation of ISGs made ineffective by concurrent upregulation of inhibitory ISGs such as USP18 (68, 69). Due to maximal upregulation, interferon-alpha therapy cannot upregulate ISGs further (69–76). Type III interferon induction of STAT1 phosphorylation does not appear to be impaired by type I-interferon induced USP18 (70) leading to interest in the use of interferon-lambda as an alternative therapeutic approach (77) but while better tolerated pegylated IFN λ to date has not shown superior antiviral efficacy. *In vitro*, NS3/4A protease inhibitors used as antiviral therapy can restore IFN β secretion but at concentrations greater than 100-fold higher than the EC50 (78) suggesting that restoration of type I interferon production constitutes a minor effect of protease inhibitor-based DAA regimens.

Induction of Cellular and Adaptive Immune Responses

Once hepatocytes are productively infected with HCV, cellular defenses become activated. Type I and III interferons and stress signals from hepatocytes trigger resident dendritic cell, hepatic stellate cells and Kupffer cells to produce cytokines such as MIP-1 α , IL-12, IL-15 and IL-18 to recruit IFN γ -producing NK cells to the liver. Type I and III interferons activate liver sinusoidal endothelial cells to produce chemokines such as CXCL10 and MIG to attract T-cells (reviewed in (79)). HCV has evolved mechanisms to disrupt several of these steps critical efficient induction of cellular immune responses.

Dendritic Cell dysfunction

Dendritic cells resident in tissues survey for pathogen infections, and upon detection activate, mature and migrate to lymphoid tissue to present antigens and induce B- and T-cell responses. Human dendritic cells are subdivided into three main subtypes: CD11c+ myeloid DC (mDC1), BDCA3+ (CD141+) myeloid DC (mDC2), and CD11c– CD123+ plasmacytoid DC (pDC) (80). While pDC typically traffic in lymphoid organs, mDC preferentially home to peripheral tissues. Upon recognition of pathogens through pattern-recognition receptor (e.g. TLRs) (81–83), DCs increase class I and II HLA expression, upregulate costimulation ligands, and produce immunostimulatory cytokines such as IL-12 (mDC1), IFN λ (mDC2), and IFN α plus IFN λ (pDC) (15, 45, 84–87). Circulating numbers of mDC and pDC are reduced in chronic HCV infection (reviewed in (85, 88)), possibly due to enhanced homing of activated mDC1 and mDC2 to the liver (15, 80).

That DC function is impaired in HCV is fairly well established, but the critical mechanisms remain controversial. Some studies indicate that HCV infection is associated with impaired DC maturation and antigen-presenting function (88–93), possibly mediated by the HCV core protein (90–92) and possibly due to upregulation of indoleamine-2,3,-deoxygenase (93). A few studies suggest the impairment may preferentially affect pDC subset (94) possibly by inducing pDC apoptosis (95). Other studies suggest that DC phagocytic function, expression of costimulation ligands, expression of class I and II HLA molecules, and cytokine function are preserved but that antigen processing by proteasomal subunits is dysregulated (85). DC migration to lymphoid tissue may also be impaired due to unresponsiveness to the chemokine CCL21 (96). Downregulation of HCV-sensing TLRs or critical adaptor molecules such as TRIF and TRAF6 have been implicated in the reduced activation of pDC (97) and mDC (98) *in vitro*. Two key effects of impaired DC function in early HCV infection include reduced cytokine-dependent NK cell maturation (44, 99) and defective priming of CD4 and CD8 T-cells with a resultant IL-10-secreting regulatory T-cell phenotype (88, 90–92, 100).

Antiviral Effect of Non-Parenchymal Liver Cells

Hepatic stellate cells (HSC) are pericytes that reside in the space of Disse between liver sinusoidal endothelial cells (LSEC) and hepatocytes, making up 5–8% of total human liver cells. During normal physiology, HSC store vitamin A but once activated HSC deposit extracellular matrix resulting in liver. HSC themselves do not appear to be permissive to HCV infection or replication (101). HSC express TLR3 and may exert an antiviral effect by producing IFN λ which could suppress HCV replication in infected neighboring hepatocytes (102 Ye L J Viral Hepat 2013). However, to date this effect has been demonstrated only *in vitro*. LSEC constitute approximately half of nonparenchymal liver cells. Human liver sinusoidal endothelial cells endocytose HCV but do not support replication. However, translated HCV RNA induces TLR7 and RIG-I dependent production of IFN α , IFN β and IFN λ (103) with potential antiviral effects. LSEC also may present antigen to T-cells (104). The importance of this effect *in vivo* remains poorly characterized. Kupffer Cells (KC) are a heterogeneous group of liver-resident macrophages that line hepatic sinusoids that are critical for phagocytosing translocated bacterial products and iron reutilization. *In vitro*, HCV Core protein, a ligand for TLR2 present in KC, blocks TLR3-mediated production of IL-1 β by macrophages (43), a cytokine that is thought to enhance the antiviral effects of IFN α (105), suggesting that inhibition of KC activation may be critical to viral persistence. However, in chronic HCV infections, KC retain normal activation, producing high levels of IL-1 β in a TLR7- and NLRP3 inflammasome-dependent manner (106) particularly in individuals with chronic infection and advanced fibrosis (106). KC also produce galectin-9 which has potent regulatory effects on HCV-specific T-cells (107).

Innate Cellular Responses to HCV infection

NK cells are critical early antiviral effectors that kill infected target cells by secretion of perforin or through death receptor ligands such as FasL or TRAIL, and also potentially produce IFN γ and chemokines such as MIP1 α , MIP1 β , RANTES, and GM-CSF needed to recruit T- and B-cells to infected tissues. NK cells are typically activated by stress-induced molecules such as MHC I-like proteins (MICA, MICB, ULBP) as well as by

cytokines such as IL-12, IL-15, IL-18, IL-21. NK cell activation is intricately controlled by the balance of activating and inhibitory signals including Killer Inhibitory Receptors (KIR) (which may be activating or inhibitory despite the nomenclature) that bind HLA-C, NKG2 family receptors which bind non-classical HLA ligands such as HLA-E, MICA and MICB, and activating receptors such as NKp30, NKp44 and NKp46. Two classic subsets can be defined based on the expression of CD16 (Fc γ RIII which binds IgG) and CD56; CD16⁺CD56^{dim} (90% of NK cells) have a cytotoxic phenotype and CD16⁻CD56^{bright} produce greater cytokine responses. There is critical cross-talk between DCs and NK cells. DC activate NK cells by binding to NKp30 and secreting IL-12. In turn, NK cells secrete IFN γ and TNF α to foster DC maturation and antigen-presenting function (108).

Certain aspects of NK cell function in acute hepatitis C infection are genetically determined. Specifically, genetic homozygosity for a weakly inhibitor KIR gene (KIR2DL3) when coexisting with homozygosity for the strongly activating HLA-C1 gene is strongly associated with early viral clearance (109, 110). The strong impact of NK cell activation on viral clearance creates context for the evolutionary priority for HCV to dysregulate NK cell function. Highly active cytotoxic NK cells are associated with absence of infection in highly exposed injection drug users (111). However, studies of acute hepatitis C patients don't universally indicate that the magnitude of NK cell cytotoxicity and IFN γ production clearly are associated with virological outcome, with both CD16⁺CD56^{dim} and CD16⁻CD56^{bright} subsets showing activation (112–114). Controversy exists about the impact of chronic HCV-infection of NK cell cytotoxicity (115, 116). While the frequency of peripheral NK cells appears to be decreased in chronic HCV, particularly among CD16⁺CD56^{dim} NK cells, no difference in NK cell cytotoxicity appears to be present between chronic HCV patients and healthy donors or resolved patients (117–120).

Possible mechanisms of NK cell inhibition in chronic HCV infection in the literature include but are not limited to: HCV E2 interaction with CD81, which has largely been disproven; HCV core-induced stabilization of HLA-E which would inhibit NK cells; altered NK cell activation receptor expression; and altered NK cell cytokine production leading to impaired activation of DCs (121). In 2002, two groups showed that plate-bound HCV E2 protein interacting with CD81 reduces NK cell function *in vitro* (122, 123). These findings have been since contradicted by studies using intact viral particles in which no inhibition occurred (118, 120). *In vitro*, an HCV Core_{35–44} peptide sequence has been shown to bind to HLA-E and stabilizes it on cell surfaces, inhibiting NK activation (124). The expression of HLA-E on multiple intrahepatic cell subsets including expression on HCV-infected hepatocytes was demonstrated (124) indicating possible *in vivo* relevance. Furthermore, hepatocyte HLA-E expression might skew NK cells to produce the immunosuppressive cytokines IL-10 and TGF β , which inhibit DC maturation (121). Monocyte-derived IL-10- and TGF β also downregulate NK cell expression of the NKG2D receptor that might reduce NK cell sensitivity to MICA and MICB on infected hepatocytes (125, 126). Controversy exists about the expression of other activating receptors such as NKp30 and NKp46 on NK cells in chronic HCV infection with some studies showing decreases (127) and others showing increases (118).

In established chronic infection, the balance of cytolytic and cytokine producing NK cells in the liver may be associated with fibrosis progression. Intrahepatic compartmentalization of activated cytotoxic NK cells (NKG2D⁺, inhibitory KIR^{lo}, CD16⁺CD56^{dim}, NKp44⁺ or TRAIL⁺ NK cells) with reduced IFN γ secretion correlates with liver injury (128–130). By contrast, NK cell IFN γ production may attenuate fibrosis by HSC (131). Recent work suggests that contact with HCV-infected hepatocytes reduces NK cell expression of microRNA-155 which upregulates the transcription factor T-bet and the inhibitory receptor Tim-3, and was associated with reversible inhibition of NK cell IFN γ production (132). Modulation of NK cell function also seems to impact response to interferon-alpha-based antiviral therapy with higher expression levels of inhibitory NK receptors in treatment non-responders (133) and increased expression of activating receptors in responders (134, 135).

In addition to NK cells, NKT cells are also overrepresented in liver. NKT cells are a heterogeneous population of lymphocytes including invariant NKT cells that express NK cell markers such as CD56, CD161, KIR and NKG2 receptors, as well as a T-cell receptor. Subpopulations include invariant NKT that express a specific CD-1d-reactive T-cell receptor (most commonly V α 24J α 18/V β 11), variant NKT cells, V δ 3 γ δ T-cells, and general cytolytic NKT cells (reviewed in (136)). The relative frequencies of NKT cells are variable in HCV-infected livers (137–139) and blood (140). Peripheral CD56⁺ NKT levels are reduced early in acute HCV infection (141) but whether or not this is due to compartmentalization to the liver is not known, an effect suggested by the association of intrahepatic activated NKT cells and spontaneous recovery (141). In chronic infection, there appears to be a regulatory effect of NKT cells that prime naive antigen-specific CD8⁺ T cells to produce IL-10 (142).

Adaptive Cellular Response to HCV infection

After activation mDC and pDC migrate to lymphoid tissue to stimulate the generation of antigen-specific B- and T-cell responses. Early adaptive immune responses are critical in the outcome of viral infections. CD8⁺ T cells are the immune effectors that directly eliminate virus-infected cells by both cytolytic and noncytolytic mechanisms (143) whereas CD4⁺ T cells play a key regulatory role, providing help for CD8⁺ T-cells and B-cells

The strength and scope of HCV-specific T-cell response are strongly associated with the outcome of acute HCV infection. In humans with acute hepatitis C, sustained vigorous and multispecific CD4⁺ and CD8⁺ IFN γ ⁺ T-cell responses to HCV (particularly to nonstructural antigens) in peripheral blood are associated with resolution of acute infection whereas weak, focused or transient responses are associated with viral persistence (144–151). In experimentally-infected chimpanzees, HCV-specific T-cell responses in the liver correlate with virological outcome, validating the observations made with peripheral blood in patients (152–154). Cell depletion experiments in chimpanzees confirm that both CD4⁺ and CD8⁺ T-cells are needed for efficient viral clearance (155–157). During very early phases of acute hepatitis infection, the capacity of CD4⁺ T-cells to proliferate in response to viral antigens (150, 158–164) is associated with conversion of hyporeactive “stunned” HCV-specific CD8⁺ T cells into strong antiviral effectors (150, 161) that can be cytotoxic to infected hepatocytes (165, 166).

The role of HCV-specific effector T-cells in the long-term outcome of chronic infection is less well defined. The detection of, albeit weak, peripheral HCV-specific CD4⁺ T-cell IFN γ responses has been associated with a more benign clinical course (167, 168). No consistent link between intrahepatic HCV-specific CD4⁺ and CD8⁺ T-cell IFN γ frequencies and long-term clinical outcomes has been shown (167, 169–172). However, circumstantial evidence for the importance of effector T-cells in control of HCV-related liver disease can be found in the setting of generalized T-cell defects (e.g. HIV/HCV coinfection, chronic steroid use, post-transplant immunosuppression) in which immune dysregulation leads to accelerated liver disease progression and high viral titers (173–175).

Failure of antiviral T-cells to control initial HCV infection is multifactorial, likely resulting from impaired priming of T-cells by DC (discussed previously), aberrant T-cell priming by intrahepatic antigen-presenting cells (LSEC, hepatocytes, NKT) (90, 104, 142, 176–178), viral escape mutation (164, 169, 179, 180 2003), induction of various regulatory T-cell subsets (181, 182), and T-cell anergy (183–185). Specific viral sequences in founder viruses may have varying degrees of immunogenicity (186), and certain HLA alleles are more or less likely to induce sterilizing immune responses (110, 187, 188). Presentation of epitopes from viral escape variants may antagonize HCV-specific T-cell responses (169, 189). Furthermore, HCV core may suppress T-cell activation through the C1q complement receptor (190, 191). The induction of multiple subtypes of regulatory T-cells and antigen-induced expression of inhibitory co-stimulation receptors are critical additional steps required for viral persistence.

Regulatory T-cells (Treg), typically defined by expression of the transcription factor foxp3 and high expression of the IL-2 receptor α chain (CD25), relevant in hepatitis C infection include natural CD304⁺ Tregs (nTreg) specific for self-epitopes (192, 193) and induced Tregs (iTregs) derived from virus-specific CD4⁺CD25[−] effector T-cells. Activated Tregs suppress effector T-cells in a contact-dependent manner (182, 194, 195) within target tissues. The generation of nTregs sequence homology for self-epitopes present in the HCV Core and p7 antigens (196, 197). *In vitro*, CD4⁺ T-cells co-cultured with HCV-infected hepatocyte cell lines develop a Treg phenotype with increased expression of foxp3, CD25, CTLA-4 and TGF β (198). High-level expression of a viral antigen in hepatocytes may also be a critical factor predisposing to the development of Tregs (199). The overall frequency of Tregs does not differ in acute resolving or persisting HCV infection, but the suppressive capacity of Tregs increases over time in persistent acute infection while decreasing in resolving infection (200). In chronic infection, there is not only increased circulating CD4⁺CD25⁺ Tregs (181, 201, 202) but also significant CD4⁺foxp3⁺ Treg infiltration into the liver (203) with associated suppression of necroinflammation and fibrosis (193, 204). Several mouse models of acute and chronic hepatitis have provided strong evidence that Tregs play a fundamental role in dampening the potentially deleterious impact of activated effector T-cells in the liver (205).

HCV-specific IL-10-producing Tr1 T-cells, typically not expressing foxp3 or high-level CD25, comprise an additional regulatory cell population important for HCV pathogenesis (206–215). Although IL-10 is produced by many cell types, including dendritic cells, macrophages, monocytes, NK and NKT-cells (216), IL-10⁺foxp3[−] CD4⁺ T-cells appear to

be an important source of IL-10 and TGF β , directly inhibiting effector T-cell function in both IL-10-dependent and IL-10-independent fashions (212, 214). *In vitro*, HCV proteins directly induce virus-specific T-cell production of IL-10 (217–219) in both CD4⁺ (220, 221) and CD8⁺ T-cell (222, 223) subsets. In acute infection, early skewing of antiviral T-cells to produce IL-10, possibly influenced by polymorphisms in the IL-10 promoter (224–228), is associated with chronic evolution (221, 229, 230). Viral mutation under immune selection pressure may also reprogram T-cells to produce IL-10 (180 2003). Due to its anti-inflammatory effects, exogenous administration of IL-10 in humans reduces hepatic fibrosis (231). The frequency CD8⁺IL-10⁺ T-cells in the liver also inversely correlates with inflammation (223) suggesting that T-cell IL-10 production may be a critical negative feedback to prevent rapid liver injury in chronic infection.

In chronic infection, prolonged antigenic stimulation leads to the upregulation of several inhibitory co-receptors on antigen-specific CD8⁺ T-cells that individually or in combination generate a state of functional hyporeactivity termed anergy. The inhibitory receptors best characterized for this effect include programmed death-1 (PD-1), cytotoxic T-lymphocyte associated protein-4 (CTLA-4), T-Cell Immunoglobulin and Mucin 3 Engagement of High-Mobility Group Box 1 (TIM-3), NK cell receptor 2B4 (CD244), lymphocyte associated gene-3 (LAG-3), Killer cell lectin-like receptor subfamily G member 1 (KLRG1) and CD160.

PD-1 is 55kDa glycoprotein member of the CD28 superfamily that contains an immunoreceptor tyrosine-based inhibitory motif (ITIM) and immunoreceptor switch motif (ITSM) that phosphorylates the kinase SHP-2, which then dephosphorylates various signal transduction kinases involved in T-cell receptor induction of IL-2 and proliferation (232). The ligand for PD-1, PD-L1 is expressed on intrahepatic myeloid DC, LSEC and Kupffer cells (233, 234). CTLA-4 is an immunoglobulin-like receptor that competes with CD28 for binding of CD80 and CD86 thereby blocking T-cell co-stimulation. PD-1 and CTLA-4 become highly expressed on HCV-specific CD8⁺ T-cells and CD4⁺ T-cells early in acute HCV infection, remaining elevated in persistent infection but normalizing after viral clearance (235–238). In chronic infection, HCV-specific memory CD8⁺ T-cells particularly within the liver express high levels of PD-1, impaired effector cytokines production, and low levels of cytolytic granules (233, 239, 240). Early studies indicated that single blockade of PD-1/PD-L1 interactions *ex vivo* augments the expansion of peripheral CD8⁺ T-cell expansion (233, 235, 239). However, intrahepatic HCV-specific CD8⁺ T-cells from patients with cirrhosis express high levels of other co-stimulation inhibitory receptors such as CTLA-4 and require multiple pathway blockade to restore antigen-specific responses in some patients (241, 242). More recent studies suggest that among peripheral HCV-specific CD8⁺ T-cells in individual patients, various combinations inhibitory receptors may be expressed and that responses to blockade of any single inhibitor is highly variable (243–246); indeed, four pathway blockade was required to improve HCV-specific CD8⁺ T-cell function in greater than half of patient samples in one study (246). Other studies suggest that the anergic state is irreversible, even after curing infection through antiviral therapy (247) and that residual functional CD8⁺ T-cells detected *ex vivo* are specific for viral sequence not expressed by circulating virus (243, 247, 248). Therapeutically, the use of anti-PD-1

antibody appeared to cause significant reduction of viral loads in one-third of treated chimpanzees (249) and a similar number of humans (250); in light of the relative inefficacy of single blockade in *ex vivo* experiments and the rapid evolution of direct acting antivirals, the further development of this treatment strategy is unknown. Nonetheless, induction of T-cell anergy is thought to play a critical role in preventing sterilizing antiviral CD8⁺ T-cell responses both in acute and chronic HCV infection.

The B-cell mediated antibody (Ab) response to HCV can be detected within 6–8 weeks of inoculation (158, 251–253). Neutralizing antibodies, which interfere with viral envelope binding to targets such as LDLR, SRBI, CD81 and claudin-1 (254, 255), in early acute infection are associated with resolution in some (256, 257) but not all studies (158, 253). However, chimpanzee cross-challenge experiments (152, 258–266) and human series (267) show that HCV-specific antibodies do not universally mediate protection. Possible reasons for the lack of antibody-mediated protection include: 1) the mutable quasispecies nature of HCV with rapid selection of antibody escape variants (253, 268); 2) intrinsic sequence specific variability of the sensitivity of E1E2 proteins to neutralization (269, 270); and 3) paradoxical facilitation of viral entry by “neutralizing” antibodies (269, 271). In up to 40% of patients with spontaneous viral clearance, HCV antibody titers may wane after 2–3 decades (272). In persistent infection, novel B-cell clones are continuously stimulated to respond to evolving viral mutations (257, 269, 273–275). Additionally, interactions between the HCV E2 envelope protein and B-cell CD81, an activating tetraspannin co-receptor, drive antigen-independent polyclonal B-cell stimulation (276) predisposing to B-cell lymphoproliferative disorders. Despite chronic activation of virus-specific and non-virus-specific B cells, memory B cells do not accumulate in chronically infected patients (277–281) for reasons yet to be clearly defined. Like seen with T-cells, chronic viral infection creates an anergic state in some HCV-specific B-cells, phenotypically defined as CD27⁺CD27^{-/lo} “tissue-like memory” B-cells with impaired proliferation (282–284) with unclear clinical significance. A clear pathogenic role for B-cells in liver disease progression in chronic HCV infection has not as yet been defined.

Summary/Discussion

The establishment of persistent infection, resistance to interferon-based therapy, and progression of fibrosis are tightly linked with immune dysregulation induced by specific hepatitis C viral proteins or by rapid viral mutation. Genetic polymorphisms that alter either cytokine responses, antigen-independent stimulation of innate immune cells, or antigen presentation in part determine the susceptibility of an individual human host to initial infection, and the impact on the liver of a persistent infection. Other host factors, some quantifiable such as age, gender, alcohol use and pre-existing immunological defects, and others poorly quantifiable, shape the complex dynamic host-virus interactions that determine permissiveness to chronic infection and disease progression rates. Critical events for the establishment of persistent infection include the disruption of type I and III interferon induction and signaling, interference with innate cellular activation, impairment and skewing of antigen-presentation to B- and T-cells, induction of various regulatory T-cell subsets, and functional inhibition of antigen-specific T- and B-cell responses. After initial failure to control infection, these processes control the magnitude of necroinflammation and

resultant fibrosis progression. The complexity of interactions makes it unlikely that any therapeutic modality aimed at any single component will have universal efficacy, and thus defining the critical nodes in these networks suitable for intervention remains an important endeavor despite the development of highly effective, oral therapy for chronic hepatitis C.

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Key Points

- Disruption of the generation of type I and III interferons (IFN), such as IFN α , IFN β and IFN λ is critical for establishment of persistent infection of hepatocytes. As such, the specific viral particles specifically inhibit several pathways required for generation of and response to secreted interferons
- The efficacy of dendritic cell antigen-presentation to adaptive B- and T-cell effectors determines whether or not brisk and effective response resolve acute infection or delayed and weak responses allow chronic infection.
- Multiple non-parenchymal cells in the liver can either contribute to antiviral effects or induce tolerance by T-cells.
- Regulatory T-cells play a fundamental role in suppressing early antiviral responses by T- and B-cells but may play a critical protective role against rapid liver injury in chronic infection
- Neutralizing antibodies produced by B-cells do not universally resolve early infection nor protect against reinfection, and can even facilitate viral entry into hepatocytes

Table 1**Selected Immunoregulatory Effects of Viral Proteins**

Core	Activation of protein kinase R (PKR) (285) Inhibition of TLR3-mediated secretion of type I and III interferon {Tu 2010} Inhibition of STAT1 activation (46, 49) Inhibition of ISGFR3 nuclear translocation (47) Impairment of dendritic cell maturation and antigen presentation (89, 90, 92, 93, 286, 289) Blockade of IL-1 β production in macrophages (43) Stabilization of HLA-E (124) C1q-mediated T-cell inhibition (190, 191)
E2	Activation of protein kinase R (PKR) and elongation initiation factor 2a (eIF2a) (30) Stimulation of CD81 on B-cells (276)
NS2	Inhibition of TBK1 and IKKe (287)
NS3/4A	Cleavage of MAVS (32–38) Inhibition of TBK1 and IKKe (41) Cleavage of TRIF (40) Suppression of CXCL8 and CXCL10 chemokines (42, 288)
NS5A	Activation of protein kinase R (PKR) (29)
NS5B	Viral mutations leading to escape or inhibition of T-cell function (169, 189)