Hepatitis C virus versus innate and adaptive immune responses: a tale of coevolution and coexistence

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Since the identification of the hepatitis C virus (HCV) 20 years ago, much progress has been made in our understanding of its life cycle and interaction with the host immune system. Much has been learned from HCV itself, which, via decades of coevolution, gained an intricate knowledge of host innate and adaptive immune responses and developed sophisticated ways to preempt, subvert, and antagonize them. This review discusses the clinical, virological, and immunological features of acute and chronic hepatitis C and the role of the immune response in spontaneous and treatment-induced HCV clearance.

Historical perspective

HCV was identified by Choo et al. in 1989 using the then-novel approach of molecular cloning instead of classic virus purification (1). Assays to detect HCV antibodies were introduced less than 3 years later — a significant advance that virtually stopped the transmission of HCV via blood transfusions in the US and reduced the incidence of new cases to less than 40,000 per year, most resulting from injection drug use. Less frequent modes of infection include perinatal transmission (estimated to occur in 2%–8% of babies born to HCV-infected mothers) and sexual transmission, which is much less effective for HCV than for other viruses, such as HIV and HBV, and is rare among people in long-term monogamous relationships (2).

Despite advances in the prevention of new HCV infection, more than 4 million individuals infected in the US and more than 120 million worldwide are currently chronically infected. About half do not mount a sustained response to the currently available therapy, a combination of pegylated IFN and ribavirin. The incidence of complications from chronic HCV infection, such as cirrhosis and hepatocellular carcinoma, is therefore predicted to increase (3), possibly reaching the same incidence as in Japan, where widespread distribution of HCV occurred decades earlier than in Western countries (4).

From the beginning, HCV research has proven challenging. In the absence of tissue culture and small animal models of infection, the first functional HCV cDNA clones had to be tested in chimpanzees (5). Since then, several models have been developed to study the viral life cycle. The first milestone was the generation of selectable subgenomic HCV replicons that self amplified in transfected hepatoma cells (6). Long-term propagation of replicon-harboring cells resulted in selection for HCV adaptive mutations and increased replication efficiency. However, HCV sequences with in vitro selected, adaptive mutations were not infectious. This was overcome by the isolation of the HCV JFH1 strain from a patient with fulminant hepatitis (7). This strain does not require adaptive mutations to replicate efficiently in hepatoma cell lines with defective IFN responses and maintains its in vivo infectivity (8–10). Several models to study HCV binding and entry were developed in parallel. Virus-like particles produced in the baculovirus system (11) and retroviral pseudoparticles with HCV envelope glycoproteins (12, 13) were used as in vitro models, and immunodeficient mice transplanted with human hepatocytes (14) are now available to screen antibodies and antiviral agents in vivo.

The virus and its life cycle

HCV is an enveloped, positive-stranded RNA virus and represents the *Hepacivirus* genus in the Flaviviridae family (15). Six major HCV genotypes and more than 100 subtypes have been identified. In the blood of infected patients, HCV is physically associated with VLDL, LDL, and HDL. Entry into hepatocytes requires the tetraspanin CD81 (16), the scavenger receptor class B type I (17), and the tight junction proteins claudin (18, 19) and occludin (20, 21), which confer species specificity (21). HCV also binds to other molecules, such as glycosaminoglycans, the LDL receptor, and the lectins DC-SIGN and L-SIGN, but these are not essential entry factors and do not confer tissue specificity.

After clathrin-mediated endocytosis and pH-dependent release from early endosomes, HCV translation and replication start in the cytosol. Translation is initiated through an internal ribosomal entry site in the 5′ untranslated region (UTR) and generates a single polyprotein of approximately 3,000 amino acids that is cleaved by cellular and viral proteases into 10 structural and nonstructural proteins. An alternate open reading frame encodes a short protein of unknown function (Figure 1). Following synthesis and maturation, nonstructural proteins and viral RNA form membrane-associated replication complexes and catalyze the transcription of negative-strand RNA intermediates from which, in turn, progeny positive-strand RNA molecules are generated (15). Capsid proteins and genomic RNA assemble to form a nucleocapsid, which buds through intracellular membranes into cytoplasmic vesicles. Enveloped, mature virions leave the cell via the secretory pathway.

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Nonstandard abbreviations used: ALT, alanine aminotransferase; cDC, conventional DC; IPS-1, IFN-β promoter stimulator protein 1; IRF, IFN regulatory factor; ISG, IFN-stimulated gene; ISGF3, ISG factor 3; 2′-5′ OAS, 2′-5′ oligoadenylate synthetase; PD-1, programmed death–1; pDC, plasmacytoid DC; PKR, protein kinase R; RIG-I, retinoic acid–inducible gene I; TRIF, Toll–IL-1 receptor domain–containing adaptor inducing IFN-β; UTR, untranslated region.

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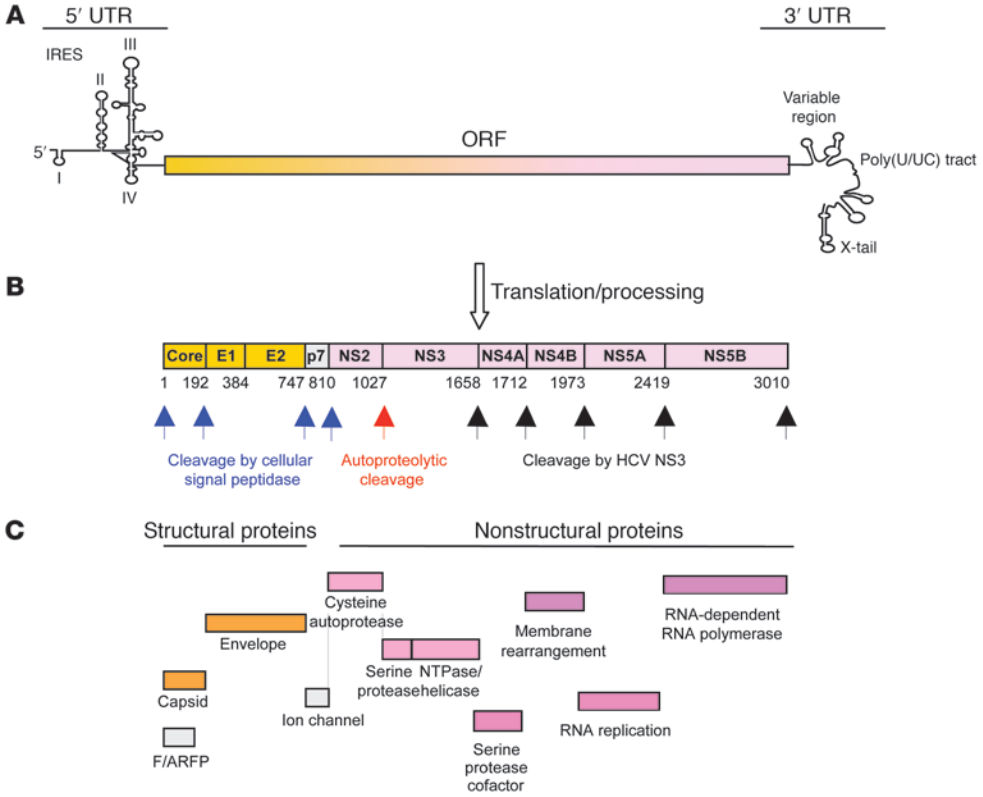

Figure 1
HCV genome organization. (**A**) The single-stranded RNA genome encodes a long open reading frame (ORF) flanked by 2 UTRs, which contain signals for viral protein and RNA synthesis and the coordination of both processes. Translation is initiated through an internal ribosomal entry site (IRES) in the 5′ UTR. U, uridine; C, cytidine. (**B**) The translated polyprotein is cotranslationally and posttranslationally processed by cellular and viral proteases. Numbers below the polyprotein indicate the amino acid positions of the cleavage sites. (**C**) Function of the resulting 10 structural and nonstructural proteins. A frameshift (F) protein is translated from a short alternate reading frame (ARF). Figure modified with permission from *Nature Reviews Immunology* (S23).

The disease

Hepatitis C is typically not diagnosed until alanine aminotransferase (ALT) levels rise, 8–12 weeks after initial infection. At this time, HCV-specific antibodies and T cells become detectable, and the appearance of HCV-specific T cells in the liver coincides with the first decrease in HCV titer. Of infected patients, 60%–80% develop chronic hepatitis, which is associated with an increase in the odds of membranoproliferative glomerulonephritis (7-fold increase), cryoglobulinemia (11-fold increase), and skin disease such as lichen planus (2-fold increase) and porphyria cutanea tarda (12-fold increase; ref. 22).

The incubation phase: viral attenuation of innate immune responses

Because of the asymptomatic onset of HCV infection, much of our knowledge of the early phase of infection is derived from the chimpanzee model. Within days of infection, HCV titers rise, with a mean doubling time half-life of 0.5 days (23). Concomitant with induction of intrahepatic type I IFN responses (24, 25), the mean half-life of the virus slows to 7.5 days (23), and viral titers plateau. Recent research identified multiple strategies that HCV employs to attenuate innate type I IFN responses.

Innate response of hepatocytes. Although all nucleated mammalian cells are able to secrete type I IFN, the first response is thought to be IFN-β production by infected hepatocytes. It is initiated by 2 pattern recognition receptors, TLR3 and retinoic acid–inducible gene I (RIG-I; Figure 2A). TLR3 senses dsRNA in endosomes, whereas RIG-I recognizes the polyuridine motif of the HCV 3′ UTR in the cytoplasm (26). Upon activation, TLR3 recruits the adapter molecule Toll–IL-1 receptor domain–containing adaptor inducing IFN-β (TRIF), and RIG-I recruits the adapter molecule IFN-β promoter stimulator protein 1 (IPS-1; also called CARD adaptor inducing IFN-β CARDIF], virus-induced signaling adapter [VISA], and mitochondrial antiviral signaling protein [MAVS]). Both processes result in downstream signaling, nuclear translocation of IFN regulatory factor 3 (IRF3), and synthesis of IFN-β (reviewed in ref. 27).

Secreted IFN-β induces an antiviral state that extends to not-yet-infected neighboring cells (Figure 2B). Binding of IFN-β to the IFN-α/β receptor activates the JAK/STAT pathway, which results in the induction of IFN-stimulated genes (ISGs) such as the *OAS1*/RNAse L system, which degrades viral and cellular RNA (28), and the RNA-specific *ADAR1*, which converts adenosine residues into inosine residues in dsRNA strands, thereby mutating and destabilizing secondary viral RNA structures (29). ISGs also include *P56* (30) and *PKR* (31), which inhibit translation of viral and host RNAs. Induction of ISGs amplifies the IFN response, because many pattern recognition and signaling molecules such as *RIG-I* are ISGs and because the ISG *IRF7* stimulates IFN-α subtype diversification.

However, HCV attenuates the IFN response at multiple levels (Figure 2). A key player is the HCV NS3/4A protein, which, when overexpressed in cell culture, cleaves the adapter molecules TRIF (32) and IPS-1 (33) and thereby blocks TLR3 and RIG-I signaling (Figure 2A). Overexpression of downstream signaling molecules circumvents this block and restores IFN-β production. A second key player is HCV core (Figure 2B), which, when overexpressed in cell culture, interferes with JAK/STAT signaling and ISG expression by (a) inhibiting STAT1 activation and inducing its degradation (34); (b) inducing SOCS3, an inhibitor of the JAK/STAT pathway (35), and protein phosphatase 2A (PP2A), which — via induction of other inhibitory molecules — reduces the transcriptional activity of ISG factor 3 (ISGF3; ref. 36); and (c) inhibiting ISGF3 binding to IFN-stimulated response elements. Several additional HCV proteins interfere directly with the function of ISGs: HCV NS5A inhibits 2′-5′ oligoadenylate synthetase (2′-5′ OAS) and induces IL-8,

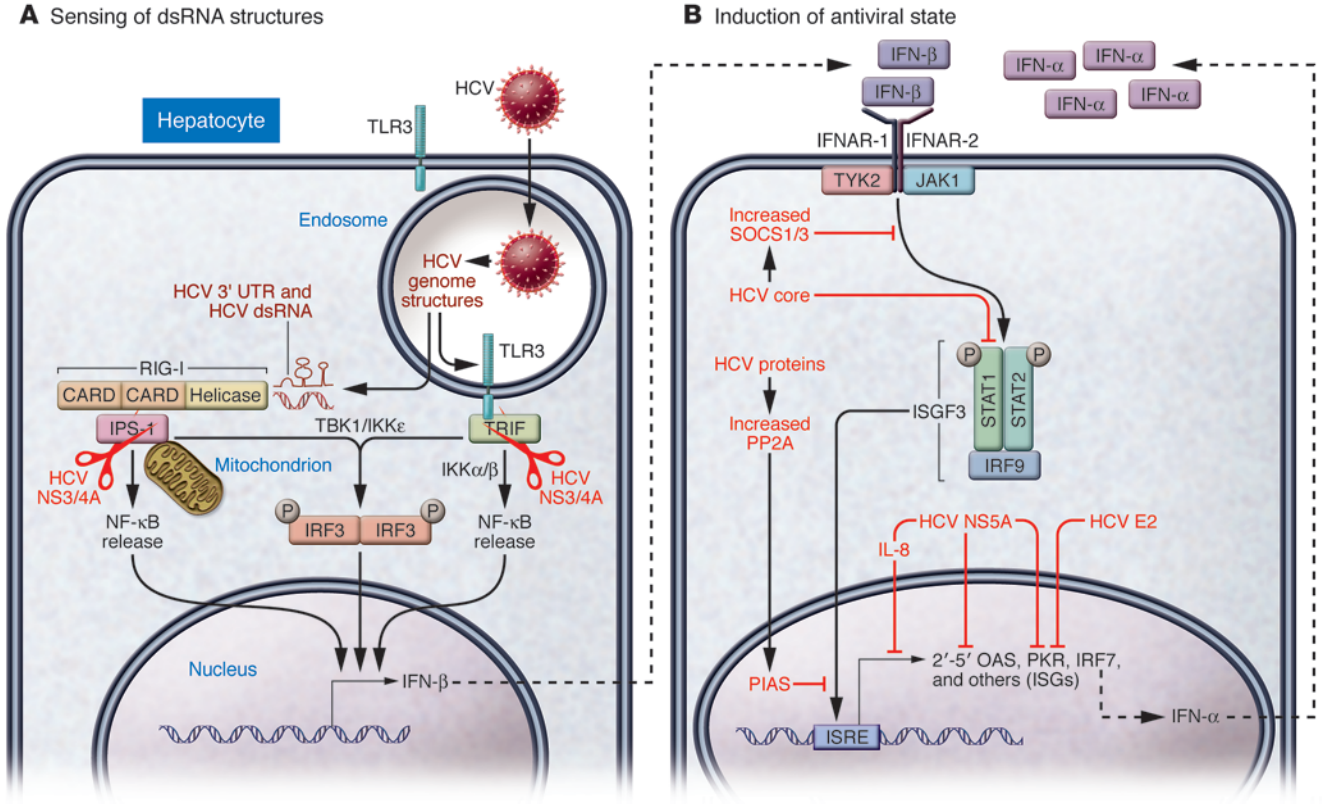

Figure 2

HCV attenuates innate immune responses. (**A**) Hepatocytes sense HCV dsRNA structures via pattern recognition receptors RIG-I and TLR3. These receptors activate via their adaptor molecules IPS-1 and TRIF, respectively, NF-κB, and the downstream kinases IKKε and TNF receptor–associated factor family member–associated NF-κB activator–binding kinase–1 (TBK1). IKKε and TBK1 phosphorylate the transcription factor IRF3, which dimerizes, translocates to the nucleus, and activates IFN-β gene transcription in synergy with NF-κB. HCV NS3/4A cleaves the adapter molecules TRIF and IPS-1, thereby blocking TLR3 and RIG-I signaling. (**B**) Binding of IFN-β to the IFN-α/β receptor (IFNAR-1 and -2) activates the JAK/STAT pathway. Specifically, TYK2 and JAK1 kinase activation results in the generation, phosphorylation, and assembly of the trimeric ISGF3 transcription factor complex, which consists of a STAT1–STAT2 heterodimer and IRF9. This complex translocates to the nucleus, binds to IFN-stimulated response elements (ISREs) within the promoter/enhancer region of ISGs and induces 2′-5′ OAS, PKR, and IRF7 production. HCV core interferes with the JAK/STAT pathway by inducing SOCS1/3 and by inhibiting STAT1 phosphorylation. The HCV polyprotein induces protein phosphatase 2A (PP2A), which interferes with STAT1 methylation, thereby increasing the binding of STAT1 to protein inhibitor of activated STAT1 (PIAS). STAT1/PIAS interaction impairs the binding of the ISGF3 complex to the IFN-stimulated response element and blocks the transcription of ISGs. HCV E2 and HCV NS5A inhibit the function of several ISGs (see text for details).

which inhibits overall ISG expression (37); HCV NS5A forms heterodimers with protein kinase R (PKR) and thereby inhibits its function (38); and HCV E2 acts as decoy target to PKR (Figure 2B and ref. 39). These findings are intriguing because E2 sequences of HCV genotype 1, which is relatively resistant to IFN therapy, inhibit PKR more efficiently than do E2 sequences of HCV genotypes 2 and 3, which respond better to IFN therapy (39).

Although these viral escape strategies, which have been identified biochemically or in transfected cell culture, still need to be demonstrated in vivo in the HCV-infected liver, the available data suggest that HCV has established redundant means to coexist with the host IFN response. They also raise the intriguing possibility that viral protease inhibitors, which are now in clinical testing, may not only inhibit viral polyprotein processing, but also restore innate immune signaling.

Innate responses of DCs. Type I IFNs are also produced by nonparenchymal cells, especially by plasmacytoid DCs (pDCs) in inflamed tissues and draining lymphoid nodes (40). In HCV infection, the frequency of pDCs in the blood (41), and their ability to produce IFN-α upon in vitro stimulation, are reduced (42). Two underlying mechanisms have been proposed. First, in vitro studies demonstrate that HCV core and NS3 activate monocytes via TLR2 to produce TNF-α, which in turn inhibits IFN-α production and induces pDC apoptosis (42). Second, HCV itself inhibits IFN-α production of pDCs in vitro (43). The inhibitory effect is exerted by infectious and by inactivated HCV and not abrogated by neutralizing antibodies (43), which suggests that it does not require infection of and replication in pDCs. This is consistent with the observations that pDCs express CD81, but not claudin-1, and that HCV cannot be propagated in these cells in vitro (44). Influenza virus restores DC function (43), consistent with the finding that HCV-infected patients are not impaired in their responses to other viruses such as influenza (45). Thus, HCV may attenuate IFN-α production by (a) hepatocytes, by a mechanism that requires infection, and (b) pDCs, by direct interaction independent of infection.

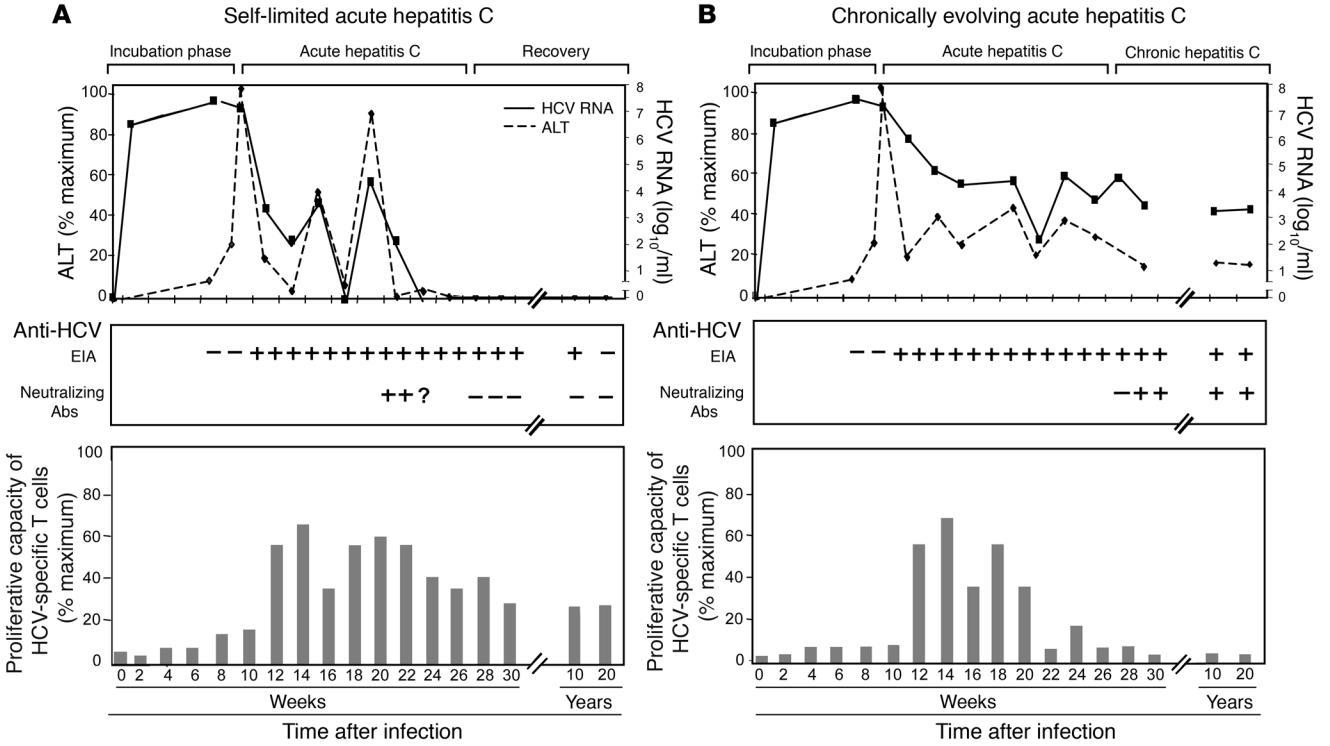

Figure 3
Schematic of the clinical, immunological, and virological course of acute HCV infection. (**A**) Acute hepatitis C followed by clinical recovery. The incubation phase occurs during the first 2–3 months after infection. The acute phase is marked by increased ALT levels in most patients, although only about 20%–30% develop clinical symptoms such as jaundice. ALT elevations coincide with the onset of HCV-specific T cell responses in the liver, and these responses are detectable in the blood by proliferation and cytokine assays. HCV-specific antibodies are detectable by enzyme immunoassay (EIA) at this time, but the presence of transient, strain-specific neutralizing antibodies is still controversial. After clearance of HCV, antibody titers decrease and may eventually become undetectable. HCV-specific T cells remain detectable in the blood, and in vitro recall responses to HCV antigens remain indicators of previous infection and recovery. (**B**) Chronically evolving acute hepatitis C. Chronic HCV infection is characterized by relatively stable HCV RNA levels, which are about 2–3 $\log_{10}$ lower than in the acute phase and do not vary by more than 1 $\log_{10}$ during the chronic phase. The titers of HCV-specific antibodies, including those of neutralizing antibodies against multiple HCV strains, increase during chronic hepatitis. HCV-specific T cell reactivity decreases over time, and in vitro recall responses to HCV antigens are typically weak to undetectable. Figure modified with permission from *Seminars in Liver Disease* (S24).

A second group of DCs, conventional DCs (cDCs), resides in tissues and transports antigen via afferent lymph to draining lymph nodes. Defective cDC function could possibly result in insufficient T cell priming and delayed HCV-specific T cell responses. Indeed, both maturation and functional differentiation of cDCs are altered in HCV infection, with decreased IL-12 and increased IL-10 production in vitro (46, 47). HCV core protein has been shown to bind to the globular domain of the complement receptor of macrophages and DCs and to downregulate IL-12 production (48). However, an impaired allostimulatory capacity of cDCs has been described in some (49, 50), but not all, patient studies (51, 52); in vitro studies with recombinant HCV proteins often do not reflect the in vivo situation in infected patients; and clinical signs of a globally impaired immune response, such as increased risk for opportunistic infections, have not been observed.

Innate responses of NK cells. NK cells are frequently found in the liver and are able to rapidly exert cytotoxicity and release cytokines. Genetic factors appear to contribute to the level of NK cell responsiveness, as shown in a large immunogenetic study in which the presence of individual killer cell Ig-like receptor/HLA (KIR/HLA) compound genotypes correlates with HCV clearance (53). Because the interaction between KIRs expressed on NK cells and HLA expressed on target cells plays a key role in NK cell activation, it has been suggested that the activation threshold of NK cells may be lower in patients with protective KIR/HLA compound genotypes, which is supported by faster NK cell degranulation and IFN-γ release in vitro (54).

It was also reported that NK cells from HCV-infected patients, but not from healthy controls, overexpress inhibitory receptors and produce cytokines that attenuate the adaptive immune response, such as TGF-β and IL-10, in vitro (55). Initial studies suggested that the HCV E2 protein inhibits NK cells directly by crosslinking CD81 (56, 57). However, HCV E2 does not efficiently crosslink CD81 on NK cells when it is part of infectious virions, and NK cell functions remain intact after in vitro exposure to high concentrations of cell culture–produced HCV (43). Likewise, NK cell function remains intact after in vitro exposure to HCV-antibody complexes (43). Thus, the results of in vitro studies should be regarded with caution when drawing conclusions regarding the immunopathogenesis of hepatitis C. The development of reliable

in vivo models is an essential future step in studying the function of immune cells in the context of the complete network of innate and adaptive immune responses in the affected organs.

Acute HCV infection: T cells attempt viral clearance

One of the key characteristics of HCV infection is the delayed immune responses despite the early increase in HCV titer and the induction of ISGs. HCV-specific T cells are typically detectable 5–9 weeks after infection (58, 59), and HCV-specific antibodies are detected 8–20 weeks after infection (Figure 3 and ref. 60). Defective T and B cell priming have been discussed as a possible mechanism to explain this delay. At present, however, it is not clear whether T cell priming occurs exclusively in draining lymph nodes or whether hepatocytes are able to directly prime T cells under inflammatory conditions (61), nor is it clear how the tolerogenic liver environment, mediated at least in part by liver-specific antigen-presenting cells such as liver sinusoidal endothelial cells and Kupffer cells, changes to an inflammatory environment. Any priming defect would have to be HCV specific, as there is no general immunosuppression and no increase in opportunistic infections.

Humoral immune responses. HCV can be cleared without humoral immune responses in immunocompromised (e.g., hypogammaglobulinemic) patients (62). In immunocompetent patients, neutralizing antibodies appear late and are isolate specific, which necessitates the generation of viral particles with patient-specific HCV sequences to detect these antibodies in in vitro neutralization assays (63, 64). The question of to what extent transient isolate-specific neutralizing antibody responses coincide with HCV clearance has not yet been answered and requires further analysis of large, prospectively followed cohorts with individualized, strain-specific reagents.

It is clear, however, that neutralizing antibodies increase in titer and breadth, typically exhibiting crossreactivity against multiple HCV genotypes once chronic HCV infection is established (60). Although they fail to clear the virus at this stage, they continue to exert selection pressure on viral variants and thereby contribute to the evolution of the HCV envelope sequences throughout the course of infection (65, 66). The overall concentration of IgG and the frequency of IgG-secreting B cells are also increased in chronic hepatitis C (67). However, most of these Igs, and the B cells that secrete them, are not HCV specific. It has therefore been proposed that HCV stimulates B cells in a B cell receptor–independent manner. This is consistent with the in vitro demonstration that $CD27^+$ B cells of HCV-infected patients exhibit enhanced conversion into Ig-secreting cells, along with decreased proliferation and rapid apoptosis (67). Mono- and polyclonal B cell expansion has also been observed and may evolve into type II mixed cryoglobulinemia and even into B cell malignancies such as non-Hodgkin lymphoma (68).

T cell responses. In contrast to antibodies, HCV-specific T cells are critical for HCV clearance (58, 69). The decrease of viral titer coincides precisely with the appearance of HCV-specific T cells and IFN-γ expression in the liver (70), which suggests that viral clearance is T cell mediated. Whether IFN-γ is directly involved in HCV clearance or whether it is just a marker for other T cell functions has not yet been determined. Direct antiviral functions would be consistent with the observation that IFN-γ–mediated inhibition of subgenomic and genomic HCV RNAs is about 100- to 1,000-fold more effective than cytotoxicity (71).

HCV-specific $CD4^+$ T cells are essential in the generation of a successful HCV-specific immune response. At the time of clinical presentation and ALT elevation, vigorous proliferation of HCV-specific $CD4^+$ T cells (72, 73) with concomitant IL-2 and IFN-γ production (74, 75) is readily detectable in the blood of patients who later recover and clear the infection. In contrast, HCV-specific $CD4^+$ T cell responses are absent or weak in those who subsequently develop chronic infection. Furthermore, loss of initially strong $CD4^+$ T cell responses has been associated with recurrent viremia even after several months of apparent viral control (76, 77).

In contrast to HCV-specific $CD4^+$ T cells, $CD8^+$ T cells are detectable in the blood of acutely infected patients regardless of virological outcome (74). During acute HCV infection, $CD8^+$ T cells appear stunned, with impaired proliferation, IFN-γ production, and cytotoxicity (58, 69, 78) and increased levels of programmed death–1 (PD-1; ref. 79). Whether the stunned phenotype is induced by a viral factor or whether it represents a natural step in the maturation and/or migration of HCV-specific $CD8^+$ T cells is still unknown. Antiviral therapy in this phase of the infection results in a rapid decay of $CD8^+$ T cell responses (80), which suggests that most HCV-specific $CD8^+$ T cells are short-lived, antigen-dependent effector cells rather than self-sustaining memory T cells. However, the dysfunction of HCV-specific $CD8^+$ T cells resolves, and IL-7 receptor α–positive (i.e., $CD127^+$) memory $CD8^+$ T cells become detectable, as soon as HCV-specific $CD4^+$ T cell responses develop and the HCV titer decreases (58, 69, 75). Consistent with this finding, in vivo depletion of $CD4^+$ T cells from HCV-recovered chimpanzees abrogates protective $CD8^+$ T cell–mediated immunity upon rechallenge (81), which suggests that $CD4^+$ T cell help is required for the generation and maintenance of protective $CD8^+$ T cells.

Chronic HCV infection: exhaustion of HCV-specific T cells

Chronic HCV infection is associated with continuous activation yet impaired function and reduced breadth of HCV-specific T cells (45, 82). Significant changes (>1 $\log_{10}$) in HCV titer, ALT spikes, and spontaneous HCV clearance are exceedingly rare, which suggests coevolution of virus and host immune responses.

Impaired function caused by chronic antigenic stimulation. As first described in mice persistently infected with lymphocytic choriomeningitis virus (LCMV), high levels of persisting viral antigen result in chronic T cell activation with sequential loss of T cell function. The capacity to produce IL-2 is lost first, followed by cytotoxicity, TNF-α production, and ultimately IFN-γ production (83). Likewise, HCV-specific $CD4^+$ and $CD8^+$ T cells are impaired in all effector functions, with weak IFN-γ production remaining as the only readout (45, 58, 69, 75). A correlation between reduced IL-2 production of $CD4^+$ T cells (84) and dysfunction of $CD8^+$ T cells has been observed, and reduced IL-2–activated killing by $CD3^+CD56^+$ T cells has recently been shown to precede the development of chronic hepatitis C (85). HCV core has been implicated in this process via binding to the receptor for the globular head domains of complement component C1q (gC1qR) and inhibition of Lck/Akt activation and T cell function (86). Dysfunctional HCV-specific T cells express the inhibitory receptor PD-1 (87, 88), which is a direct result of chronic antigenic stimulation and decreases when HCV mutates in T cell epitopes (89). Interaction of PD-1 on T cells with its ligand, PD-L1 (expressed on sinusoidal endothelial cells, Kupffer cells, stellate cells, and type I IFN–exposed hepatocytes in the liver), inhibits effector functions and induces T cell apoptosis (90). Based on in vivo data in LCMV-infected mice (91), it has been established that the function of HCV-specific T cells that are isolated from the blood of infected patients can be rescued by in vitro exposure to PD-1–blocking antibodies (92, 93). HCV-specific T cells

isolated from liver biopsies of infected patients appear to be more severely impaired, requiring CTLA4 blockade in addition to PD-1 blockade to restore their function (94).

Induction of Tregs. Tregs are derived from natural or induced T cell populations. Natural $CD4^+$ Tregs constitutively express the transcription factor forkhead box P3 (FoxP3), the IL-2Rα chain (CD25), and the glucocorticoid-induced TNF receptor family–related gene (GITR). They are generated during normal T cell development in the thymus, whereas induced Tregs are generated from mature T cells (95). HCV infection amplifies the induction and/or proliferation of Tregs, as evidenced by a relative decrease in T cell receptor excision circles (96) and increased proliferation in response to IL-2 signaling during ALT flares (97). Because in vitro depletion of $CD25^+$ cells results in increased responsiveness of the remaining HCV-specific effector cells (98–100), it has been suggested that induction of Tregs plays a causal role in the establishment of chronic HCV infection. However, FoxP3 expression levels and Treg-mediated suppression in the acute phase of HCV infection do not differ between patients who subsequently clear HCV and those who develop chronic infection (101), which suggests that Tregs are induced as a result of acute inflammation.

Inhibition by IL-10. IL-10 levels are typically increased in chronic HCV infection (102). IL-10–producing T cells are preferentially located in areas of liver sections with low hepatocellular apoptosis and low laminin expression, whereas IFN-γ–producing T cells are localized to areas with strong hepatocellular apoptosis and laminin expression (103). IL-10–producing HCV-specific $CD8^+$ T cells can be readily expanded from liver biopsies and suppress IFN-γ production and proliferation of virus-specific $CD4^+$ and $CD8^+$ T cells in vitro (104). IL-10 can also be produced by monocytes in response to HCV core–mediated TLR2 stimulation in vitro (42) and by NK cells. IL-10 inhibits IFN-α production (105), promotes apoptosis of pDCs (42), and downregulates effector T cell responses. Thus, IL-10 attenuates the inflammatory response in the liver, albeit at the cost of efficient antiviral immune responses.

HCV escape mutations. The high HCV replication rate and the lack of proofreading capacity of its polymerase allow for the virus's rapid escape from emerging humoral and cellular immune responses. Large-scale, full-length HCV sequencing studies suggest that HCV escape mutants are selected at the population level in the context of the prevailing HLA haplotypes. Upon transmission into individuals that do not share those respective HLA alleles, HCV spontaneously reverts to its original sequence (106, 107), which is indirect evidence of selection pressure exerted by HLA-restricted $CD8^+$ T cells.

Prospective analysis of the rates of nonsynonymous and synonymous substitution (substitution of one base for another in a gene, such that the amino acid sequence produced is modified or is not changed, respectively) during the course of infection revealed that the highest level of selective pressure occurs during the acute phase of infection and decreases as the infection continues (108). Selection pressure is exerted by both antibodies (65, 66) and T cells (66, 106, 109–111). At the T cell level, HCV escape affects epitope processing (106, 111), MHC binding (106, 109), and recognition by both $CD8^+$ (109) and $CD4^+$ T cells (66). The resulting altered peptide ligands may downregulate T cell responses against wild-type peptides (109) and fail to effectively prime new T cells (112). Not surprisingly, therefore, some of the most successful T cell responses target epitopes that do not allow sequence changes because of high viral fitness costs (113), which the virus may only compensate for with additional clustered mutations (114).

Disease progression

It has long been assumed that liver injury and disease progression in HCV infection are immune mediated. This assumption was mostly based on observations from the acute phase of HCV infection, in which the onset of liver injury is temporally correlated to T cell infiltration of the liver. However, rapid disease progression in patients with immunodeficiencies or therapeutic immunosuppression (115) implicates a role of additional, possibly viral factors. Indeed, HCV replication coincides with induction of cell death–related genes and apoptosis in cell culture (116), but this observation is limited to the sole HCV strain that can be grown in cell culture, JFH1.

In immunocompetent patients, the histologic pattern of HCV infection consists of moderate lymphocyte infiltration of the parenchyma, reactive bile duct changes, and the presence of lymphoid follicles in portal areas. Lymphoid follicles display a germinal center–like structure, with activated, clonally restricted B cells surrounded by follicular DCs and an outer T cell zone (117). The function of intrahepatic B cells is currently unknown, but recent in vitro studies have shown that B cells bind HCV and that B cell–associated HCV infects hepatoma cells more readily than extracellular virus (118).

HCV-specific T cells are present at a higher frequency in the liver than in the blood and can be readily cloned from liver biopsies (119). High expression of CD95, TNF receptor 1, and TNF-related apoptosis-inducing ligand and the absence of sufficient growth factors (90) contribute to a high turnover of HCV-specific T cells. Their continuous recruitment and death, the lysis of some — but not all — HCV-infected hepatocytes, and the secretion of inflammatory and profibrotic cytokines such as TGF-β activate stellate cells, the primary source of extracellular matrix. The portal area expands, with thin collagen fiber extensions between layers of hepatocytes. As the disease progresses, fibrous bridges form between adjacent portal areas, and cirrhosis develops. Hepatocellular carcinoma usually arises after 2–4 decades, typically on the basis of underlying cirrhosis and possibly aided by an inherent carcinogenic potential of HCV (120).

HCV-specific immunity and vaccination

HCV-specific antibodies may decline to undetectable levels within 1–2 decades of recovery from HCV infection (121) and do not protect against reinfection (122). In contrast, HCV-specific memory T cells remain detectable for decades in both blood (121) and liver (123) and have been shown to protect HCV-recovered chimpanzees upon homologous, heterologous, and cross-genotype HCV rechallenge (77, 124). While viremia is not prevented, it is of significantly lower titer and shorter duration than in the primary infection, and the rechallenge virus is cleared without ALT elevation. Clearance of the challenge virus coincides with strong cellular, but not humoral, recall responses (77, 125) and an increase of intrahepatic *IFNG* mRNA levels (77). Protection is lost when either $CD4^+$ or $CD8^+$ T cells are depleted prior to rechallenge (81, 123). Consistent with these findings in the chimpanzee model, the risk of developing persisting HCV viremia is significantly lower for injection drug users who have successfully cleared a previous HCV infection than for those who have never been infected (S1). This apparent immune protection is lost upon subsequent infection with HIV (S1), thus supporting a role of $CD4^+$ T cells in immune protection.

While these studies clearly show that spontaneous resolution of HCV infection can result in T cell–mediated immune protection, its

incidence needs to be further studied. Clearly, this level of immune protection is not observed in all subjects, as reinfection resulting in chronic HCV viremia has been observed in patients (S2) and chimpanzees (S3, S4). Apparently robust immune protection can also be lost if chimpanzees are rechallenged multiple times (S4), a finding that may provide an explanation for the high rate of HCV persistence in polytransfused thalassemic patients (S2).

Prospects for vaccination

A recent study demonstrated that a strong, multispecific and multifunctional T cell response can be successfully induced by vaccination of chimpanzees with a replication-deficient adenoviral vector encoding HCV NS3–NS5B and booster vaccination with intramuscular injection of a recombinant DNA plasmid (S5). Given that about 50% of chimpanzees are able to clear HCV spontaneously (125), it is not surprising that 4 of 5 vaccinated chimpanzees cleared HCV, compared with 3 of 5 mock-vaccinated chimpanzees. However, all vaccinated chimpanzees with strong HCV-specific T cell responses displayed a significantly shorter duration of viremia and lower HCV titer than the other chimpanzees, and HCV was cleared without ALT elevation, reminiscent of natural immune protection (77, 123, 125). Similar data were obtained in chimpanzees vaccinated with DNA plasmids expressing HCV E1, E2, core, and NS3, followed by a boost with recombinant protein (S6), or vaccinated with DNA plasmids encoding HCV NS3–NS5B and envelope proteins, followed by a boost with recombinant adenovirus (S7). Thus, although vaccine-induced, HCV-specific T cells cannot prevent HCV infection upon rechallenge, they appear to ameliorate disease, mediate rapid HCV clearance, and protect from development of chronic infection. It remains to be determined how long vaccine-induced immune responses last and whether they are effective against heterologous HCV genotypes.

In addition to T cells, neutralizing antibodies are a key feature of effective vaccines against many viruses and a means of passive postexposure prophylaxis. Although the humoral immune response appears to fail in the natural course of HCV infection (60), it remains possible that a panel of antibodies can be cloned and used as postexposure prophylaxis (S8). HCV infection of chimpanzees can be prevented by in vitro neutralization with a rabbit hyperimmune serum raised against the E2 protein (S9), and vaccine-induced antibodies have been shown to modulate HCV RNA levels (S10). Furthermore, prophylactic anti-CD81–antibody treatment of immunodeficient mice transplanted with human hepatocytes protects upon subsequent HCV challenge (S11). However, development of antibody-based vaccines remains exceedingly difficult in the face of HCV mutations and the recent reports on direct cell-to-cell transmission of HCV in vitro (S12).

Role of immune response in therapy-induced HCV clearance

Current treatment for HCV consists of pegylated IFN and ribavirin, a guanosine analog thought to increase IFN's effect and prevent late relapse by increasing the mutation rate of HCV toward error catastrophe. A sustained virological response, defined as undetectable HCV RNA at the end of treatment and 6 months later, is achieved in 42%–46% of cases infected with HCV genotype 1 with a 48-week course of treatment and in 75%–95% of cases infected with the less frequent HCV genotype 2/3 with a 24-week course of treatment (S13). A sustained virological response is durable in more than 97% of patients, even in cases with consecutive immunosuppression (S14), and is associated with resolution of intrahepatic inflammation and regression of liver fibrosis.

Which patients mount a virological response, and what is the role of the innate and adaptive immune response? A virological response is predicted by a rapid, several-log decrease in viral level within the first days of treatment. In addition to certain host factors (male gender, advanced age, increased body mass index, comorbidities, African-American) and viral factors (high viral titer, HCV genotype 1; ref. S13), chronic activation of innate immune responses (high levels of IFN-inducible protein 10 and other ISGs) predicts failure to respond to therapy (S13). These findings suggest that maximally upregulated, yet ineffective ISGs cannot be further improved by administration of exogenous IFN.

Likewise, chronic activation of the adaptive immune response, as evidenced by increased PD-1 expression on lymphocytes, predicts treatment failure (S15). Early restoration of HCV-specific T cell responses is not an essential requirement for a rapid viral decline (S16), and the overall vigor of the HCV-specific T cell response decreases during treatment, even if initiated early after infection (74, 80, S17). Early antiviral therapy, however, is able to rescue a very small subset of polyfunctional HCV-specific memory T cells (S18).

New therapies in clinical development are antiviral agents that specifically inhibit the HCV NS3/4A protease and the NS5B RNA-dependent polymerase (S19). Potent inhibition of HCV replication has been demonstrated in short-term monotherapy trials (S19), but selection of viral escape mutants, many of which are already present in treatment-naive patients (S20, S21), is a concern. Combination therapy and addition of polyethylene glycol–IFN or ribavirin needs to be evaluated for individual antivirals and patient populations. Antiviral agents may also allow for testing of the hypotheses that innate immune responses increase when TLR3 and RIG-I signaling is restored (S22) and that adaptive immune responses increase when viral load is reduced and rest from chronic antigen stimulation is provided. This strategy could potentially be combined with subsequent therapeutic vaccination in an effort to not only clear HCV, but also restore long-term immune protection.

Future directions

The host immune response plays a unique role in HCV infection because of its potential to contribute not only to viral clearance and, in some cases, protective immunity, but also to liver injury. HCV balances this equation by attenuating both innate and adaptive immune responses, thereby reducing the likelihood of viral clearance as well as the degree of immune-mediated liver injury and allowing coexistence of both virus and hosts. HCV continually optimizes this process in each individual host, as evidenced by the emergence of viral escape mutants that interfere with multiple aspects of effective T and B cell responses. Key questions for future studies remain for nearly every aspect of the host immune response. Why does therapy with exogenous IFN mediate HCV clearance, while production of endogenous IFNs does not? How does the modulation of innate immune responses by HCV affect the quality of the adaptive immune response? Does HCV affect and possibly delay the priming of T and B cells? Does HCV interfere with the recruitment of various immune cells, in particular with molecules such as integrins, chemokines, and relative receptors involved in rolling and migration of various immune cell populations? Why are only HCV-specific T cell responses impaired, whereas immune responses to other pathogens remain intact? It is hoped that recent advances in the development of in vitro infection

models and in our understanding of the HCV receptor complex will lead to the development of small animal models to allow evaluation of antivirals, neutralizing antibodies, and immunotherapies.

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