

# Mechanisms of HCV survival in the host

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**Abstract** | HCV infection is an important cause of liver disease worldwide—nearly 80% of infected patients develop chronic liver disease, which leads to the development of liver cirrhosis and hepatocellular carcinoma. The ability of HCV to persist within a host is believed to be related to the numerous mechanisms by which it evades the immune response of the host. These mechanisms can be divided into defensive and offensive strategies. Examples of defensive mechanisms include replication within enclosed structures, which provides protection from the host's antiviral defenses, genetic diversity created by inaccurate replication, which yields mutants resistant to the cell's antiviral strategies, and association of the virion with protective lipoproteins. Offensive mechanisms include virally encoded proteins and other factors that disrupt the ability of the host cells to detect the virus and downregulate its ability to respond to interferon, impair innate immune defense mechanisms and alter T-cell responses, and prevent the development of an effective B-cell-mediated humoral response. Greater understanding of these viral survival strategies will ultimately translate into more effective antiviral therapies and better prognosis for patients.

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## Introduction

HCV infection is a considerable public-health problem and an important cause of liver disease. Current interferon (IFN)-based therapies do not achieve a sustained virologic response (SVR) in many patients. Over 130 million people are estimated to be chronically infected with HCV worldwide.<sup>1</sup> Successful eradication of the virus is achieved in only 15–20% of newly infected individuals, while the remainder develop chronic infection.<sup>2</sup> The ability of the virus to persist within a host is astonishing, and is attributed to its efficient ability to evade the adaptive and innate components of the host's immune system. At present, the outcome of the conflict between host defense mechanisms and countermeasures of HCV favors the virus. As our understanding of the life cycle and survival mechanisms of HCV increases and this information is translated into the development of novel antiviral therapies, we hope that treatment will change this situation to favor the host's defense mechanisms and, therefore, benefit the patient. This Review provides a brief discussion of the HCV life cycle and describes the interaction of HCV with innate and adaptive immune responses of the host. We also highlight the features of the immune system that HCV uses to thwart a host's antiviral responses.

## The HCV life cycle

Key steps in the life cycle of HCV include entry into the host cell, uncoating of the viral genome, translation of viral proteins, viral genome replication, and the assembly

and release of virions. All these events occur outside the nucleus of the host cell.

## Viral entry

Various factors attributed to the host cell seem necessary to enable HCV entry. The first factor that was identified as necessary for HCV entry was the tetraspanin, CD81. Although CD81 is expressed on many cell types and cannot explain HCV's liver tropism, HCV entry is strongly reduced in the presence of antibodies to CD81, or when CD81 expression is downregulated in hepatoma cells.<sup>3</sup> The human scavenger receptor class B type I (SR-BI) is thought to be an additional factor that might mediate entry of HCV into cells.<sup>4</sup> SR-BI is a physiological HDL receptor that mediates selective HDL-cholesterol uptake,<sup>5</sup> but can also bind to other ligands,<sup>6</sup> some of which affect HCV infectivity.<sup>7,8</sup> Plasma-purified HDL enhances the entry of HCV pseudoparticles (HCVpp). This enhancement of HCVpp entry into host cells probably depends on HDL binding to SR-BI, because silencing of SR-BI expression by RNA interference (RNAi) markedly reduces HDL-mediated enhancement of HCVpp entry.<sup>9,10</sup> In 2008, Murao *et al.* showed that the endogenous expression of SR-BI by hepatocytes is suppressed by exposure to IFN- $\alpha$ ,<sup>11</sup> which suggests a link between the antiviral actions of IFN- $\alpha$ , inhibition of HCV cell entry and SR-BI expression. Coexpression of only CD81 and SR-BI is insufficient for HCV entry;<sup>12,13</sup> however, a unique screening approach has now identified additional factors that mediate HCV entry—the tight-junction proteins, claudin 1 (CLDN1) and occludin.<sup>14,15</sup> In addition, the HCV E2 envelope glycoprotein also binds to dendritic-cell-specific and liver-cell-specific intercellular adhesion molecule-3-grabbing nonintegrins (CD209 [DC-SIGN])

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## Competing interests

JS Glenn has declared associations with the following companies: Eiger BioPharmaceuticals, Epiphany Biosciences, Genentech, Presidio Pharmaceuticals, and Romark Laboratories. See the article online for full details of the relationships. EH Sklan, P Charuworn and PS Pang declared no competing interests.

**Key points**

- At each stage of the life cycle of the virus, HCV interfaces with antiviral mechanisms of the host
- Multiple, individual viral proteins inhibit key, cellular, antiviral defense pathways
- HCV infection is associated with impairment of virtually all parts of the adaptive immune system, although our understanding of the mechanistic details remains incomplete
- New therapeutic strategies are emerging that seek to translate this knowledge into improved outcomes

and CD209L [L-SIGN], respectively).<sup>16</sup> These receptors are calcium-dependent lectins, which are not expressed on hepatocytes and cannot, therefore, be the receptors that directly mediate HCV entry into hepatocytes. CD209L and CD209 might, however, be involved in the binding and transfer of HCV to hepatocytes.<sup>17</sup>

The components of HCV that are presumed to act as ligands for the receptors described above include the HCV envelope glycoproteins, E1 and E2, which have an essential role in entry of HCV into host cells. Two hypervariable regions (HVR) have been identified in the E2 envelope glycoprotein sequence,<sup>18</sup> and their role is reminiscent of the well-characterized mutational strategy used by many organisms to evade a host's immune response. The first 27 amino acids of the E2 ectodomain form the first hypervariable region (HVR1). Experimental deletion of the E2 HVR1 results in persistent (albeit low-level) viremia, which suggests that this region is not essential for viral replication, but that its disruption might lead to attenuation of the viral infection.<sup>19</sup> The second HVR (HVR2), has also been described within the E2 glycoprotein,<sup>19</sup> and has been proposed to modulate binding of E2 to CD81.<sup>20</sup> An association between specific amino-acid variations in the E2-HVR2 domain and HCV infection outcome has, however, not been demonstrated.<sup>21</sup> E1 and E2 are exposed on the surface of the HCV; therefore, these envelope proteins are potential targets for neutralizing antibodies.

**Uncoating and translation**

HCV is a positive, single-stranded RNA virus that contains a 9.6 kb genome. After entry of the viral genome into the host-cell cytoplasm, the virus undergoes an uncoating process to expose the viral genome to host-cell machinery. The viral genome is then translated in preparation for viral replication (Figure 1). The 5', non-translated region of the HCV genome contains an internal ribosome entry site (IRES) that permits ready access of the viral genome to the host translation machinery for viral-protein synthesis. IRES-mediated translation is a common mechanism used by many viruses to enable ongoing viral translation. This modality of translation is cap-independent and enables viral translation to continue even after host cap-dependent translation has been shut down in response to viral infection.<sup>22</sup> Host cells have developed a number of mechanisms to inhibit use of their own protein-translation machinery as an

antiviral strategy. For example, during viral RNA replication, the presence of double-stranded RNA can induce phosphorylation of the eukaryotic translation-initiation factor 2 $\alpha$  (eIF2 $\alpha$ ) by double-stranded, RNA-activated protein kinase (PKR), which prevents the initiation of further translation.<sup>23</sup>

Translation of the HCV genome produces a single ~3,000 amino-acid polyprotein, which is processed by cellular and viral proteases into at least 10 different protein products. These products include the structural proteins, which form the viral particle (the virus core and the envelope proteins E1 and E2), and the nonstructural proteins P7, NS3, NS4A, NS4B, NS5A and NS5B.<sup>24</sup>

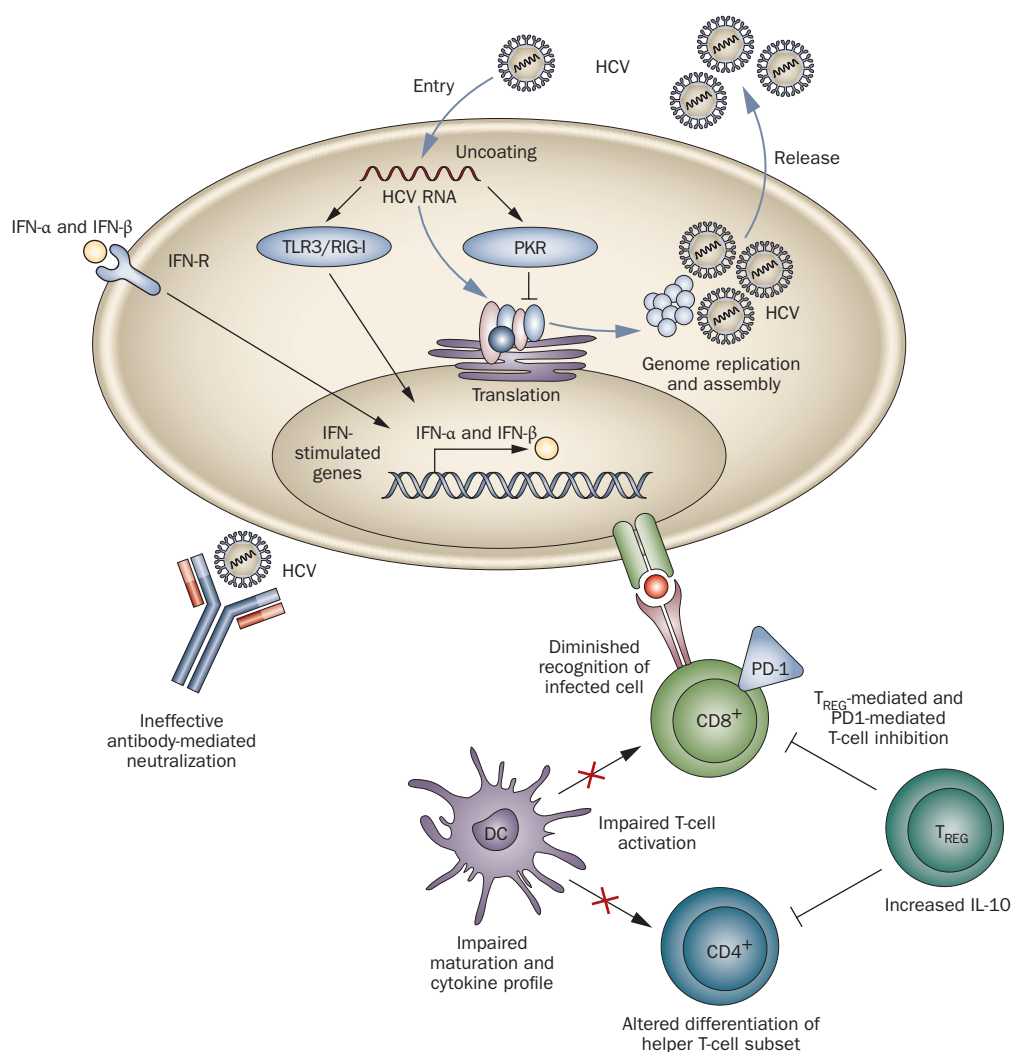
**Viral replication**

After translation of the viral proteins that are necessary to establish the viral-replication machinery, viral RNA replication begins (Figure 1). Similar to other positive-strand RNA viruses, HCV is believed to replicate in association with intracellular membranes, although the details of the replication-complex assembly and the role of the intracellular membranes in viral RNA synthesis are not understood completely. Proposed roles for the intracellular membranes include providing physical support to the virus, enabling a sufficiently high local concentration of viral factors for viral replication, and facilitating the structural organization of the replication complex.<sup>24</sup>

Compartmentalization of the viral-replication complex might also serve to protect the viral RNA from double-stranded RNA-mediated host defenses or RNAi.<sup>24</sup> HCV-replication complexes are partially protected from exogenously administered nucleases and proteases, which provides support for the notion that compartmentalization protects the viral RNA from host defense mechanisms.<sup>25</sup>

The details of the HCV-RNA replication process are still unclear, but investigation of the replication process of other flaviviruses suggest that the positive-strand, viral RNA genome serves as a template for the synthesis of a single, negative-strand RNA.<sup>26</sup> These two RNA strands remain base-paired, which results in the formation of a double-stranded RNA molecule that is copied multiple times by semiconservative replication to generate multiple progeny, positive-strand, viral RNA genomes.<sup>27–29</sup> The double-stranded RNA intermediate is one of the pathogen-associated molecular patterns that is recognized by the innate immune system and is discussed in further detail below.

A key protein responsible for viral RNA synthesis is the HCV NS5B—the catalytic subunit of the replication complex, which has RNA-dependent RNA polymerase (RdRp) activity. Importantly, the HCV RdRp lacks a proofreading function and is, therefore, highly error-prone. The lack of proofreading function results in the facile generation of genetic diversity, such that the virus population within an infected individual is best viewed as many different, but closely related, genomes, referred to as a quasispecies.<sup>30</sup> This genetic diversity provides an



**Figure 1** | Viral life cycle and major host cell defense pathways. The HCV life cycle (large blue arrows) begins with entry into the host cell, followed by uncoating of the HCV particle's nucleocapsid. The presence of viral, double-stranded RNA in the cytoplasm triggers several innate antiviral mechanisms including activation of PKR, TLR3 and RIG-I, which eventually leads to the release of IFN- $\alpha$  and IFN- $\beta$  (black arrows). These key intracellular events are demonstrated in further detail in Figure 2. Translation of the positive-strand viral genome generates nonstructural and structural viral proteins, which are critical for viral replication and assembly of new virus particles, respectively. Also illustrated in this figure are the key components of the host's adaptive immune response and the cellular impairment that is associated with HCV infection. HCV impairs the function of dendritic cells, and limits their ability to stimulate a robust, antigen-specific immune response in CD4<sup>+</sup> and CD8<sup>+</sup> T cells. Upregulation of PD-1 and increased IL-10 production further impair T-cell function, which facilitates HCV immune evasion. Abbreviations: DC, dendritic cell; IFN, interferon; IL-10, interleukin 10; PKR, double stranded RNA-activated protein kinase; T<sub>REG</sub>, regulatory T cells.

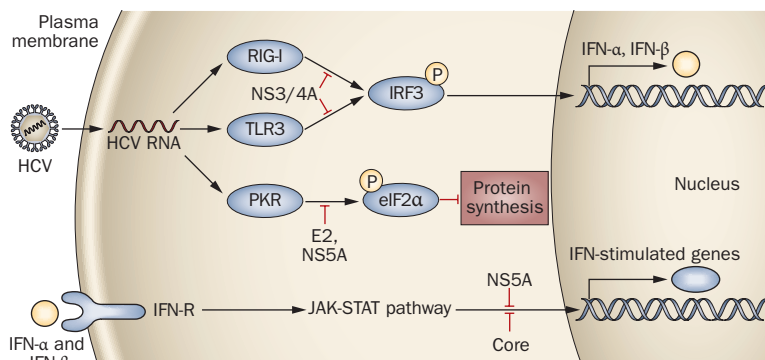
ideal pool from among which the viral genome that is best adapted to a given antiviral intervention is selected—a concept that is clinically manifested as resistance to treatment.

#### Viral assembly and release

Only a limited amount of data are available on the later stages of the HCV life cycle (Figure 1); however, with the emergence of *in vitro* systems that are able to produce infectious HCV,<sup>31–33</sup> important new insights are anticipated.

Secreted HCV particles have a characteristic low density, which suggests that the virus associates with lipoproteins for viral release.<sup>34</sup> The association of HCV with lipoproteins might also protect the secreted virus from the host's immune response.

Throughout its life cycle, HCV interacts with a variety of host-cell factors, including some involved in intracellular trafficking and RNA metabolism. For example, the inhibition of host trafficking machinery by the virus helps it control the host's cytokine secretion, and can prevent the presentation of major histocompatibility



**Figure 2** | Innate immune signaling pathways interdicted by HCV. HCV interferes with numerous defense mechanisms, including virus detection, IFN signaling, and antiviral effectors. TLR3 and RIG-I are pathogen-recognition receptors whose downstream signaling pathways are blocked by the HCV protease NS3/4A. HCV proteins NS5A and Core block IFN signaling via the JAK-STAT pathway. HCV proteins E2 and NS5A inhibit PKR, an antiviral effector that leads to inhibition of protein synthesis in infected cells. Abbreviations: IFN, interferon; IRF3, Interferon response factor 3; PKR, double-stranded, RNA-activated protein kinase; T<sub>REG</sub>, regulatory T cells.

complex (MHC) class I molecules on the infected cell's surface, which attenuates the host's immune response. Indeed, the HCV NS4A/B precursor inhibits the transport of MHC class I molecules to the cell surface.<sup>35</sup> HCV also hijacks other aspects of the host trafficking machinery. For example, HCV NS5A interacts with TBC1D20, a Rab GTPase activating protein of Rab1, which mediates endoplasmic reticulum to Golgi apparatus trafficking. This interaction of NS5A and TBC1D20 is essential for viral replication and is thought to redirect host machinery components from the endoplasmic reticulum to the viral-replication complex.<sup>36,37</sup>

MicroRNAs (miRNA) are small RNAs that inhibit the translation of RNA molecules.<sup>38</sup> A study in 2007 showed that IFN- $\beta$  rapidly modulates the expression of numerous cellular miRNAs.<sup>39</sup> Of note, several IFN- $\beta$ -induced miRNAs target the HCV RNA genome,<sup>39</sup> which suggests a role for miRNAs as endogenously produced antiviral effectors. By contrast with the notion that miRNAs might act as effectors against HCV, however, the liver-specific miRNA miR-122, the most abundant miRNA in liver cells, actually seems to be used by HCV to enhance its own replication.<sup>40</sup> Interestingly, IFN- $\beta$  administration to a human hepatoma-cell line significantly reduces expression levels of miR-122,<sup>39</sup> although such clear correlations between miR-122 and HCV RNA levels have, to date, been difficult to demonstrate clearly *in vivo*.<sup>41</sup>

RNAi was first discovered to be an important part of the host immune response to viruses in plants and invertebrates. Whether RNAi has a role in the mammalian immune response to viruses is still controversial.<sup>42</sup> A study by Randall *et al.* in 2007 showed that HCV replication was reduced by inhibiting components of the RNAi machinery that are also important for miR-122 biogenesis.<sup>43</sup> The authors of this study propose that these findings indicate that functional RNAi is required for

HCV replication, and that this requirement outweighs any RNAi-mediated antiviral effects.

## Factors that affect clinical outcome

### Viral factors

Numerous viral factors that inhibit aspects of the innate immune response and promote viral survival are described in this Review (Figure 2 and Table 1) and elsewhere.<sup>44,45</sup> The clinical relevance of many factors, however, remains unclear. The potential gap between observations made in model cell-culture systems and the clinical relevance of these observations in the human host is noteworthy and exemplified by the following points. First, robust HCV virion production in cell culture is still largely limited to one cell line, or derivatives of it, and the replication machinery of one HCV genotype, genotype 2.<sup>46</sup> This virus and cell-culture system is a clear breakthrough with regard to the study of the HCV life cycle. Nevertheless, many important details of how and why this model system actually produces virions (and in such an efficient manner) remain unknown, as does the definitive relevance of this culture system to clinical virus–host interactions. A second example of the current limitations of our model systems is the observation that the genotype 2 replicon is more resistant to IFN than the genotype 1 replicon in cell culture—the opposite of what is observed clinically.<sup>47</sup> Despite these limitations, these *in vitro* replication systems have been immensely useful for the advancement of HCV research in general. Notably, some innate immune-system evasion mechanisms that were discovered using *in vitro* approaches have been confirmed to be clinically relevant; three of these mechanisms are discussed in further detail below.

### Avoiding detection

Among the cellular sensors that enable a cell to detect pathogens are the pathogen-recognition receptors (PRRs). The components of the invader that are recognized by host PRRs are called pathogen-associated molecular patterns (PAMPs).<sup>48</sup> A classic example of a PAMP is double-stranded RNA. The best-described PRRs in hepatocytes are RIG-I, an ATP-dependent RNA helicase, and TLR3, a Toll-like receptor. When these PRRs detect viral invaders, such as HCV, they trigger signaling cascades that result in the transcription of IFNs and key messenger cytokines that activate host defenses (Figure 2).

RIG-I is activated by the binding of viral RNA, which enables RIG-I to bind to IFN promoter stimulator 1 (IPS-1, also called VISA, CARDIF and MAVS) and trigger a signaling cascade that results in IFN transcription.<sup>49,50</sup> IPS-1 is normally localized to the membranes of mitochondria. The HCV NS3–4A protease has been demonstrated to cleave IPS-1, which causes it to delocalize from the mitochondrial membrane and prevents RIG-I signaling.<sup>49,50</sup> Importantly, liver-tissue samples from patients infected with HCV demonstrate IPS-1 delocalization, which suggests that this mechanism is clinically relevant.<sup>51</sup> NS3–4A has also been demonstrated

**Table 1** | Viral factors and their involvement in viral evasion strategies

Viral factor(s)	Evasion strategy
HCV genomic sequence	2',5' OAS/RNase L pathway: RNase L digests viral RNAs. The genomic sequence of HCV has a paucity of RNase L cleavage sites <sup>100</sup>
HCV proteins	Induces an ER stress response and increase PP2A expression, which inhibits the JAK-STAT pathway. <sup>58</sup> Suppress ISG56, which normally antagonizes viral RNA translation <sup>101</sup>
HCV IRES	Inhibits PKR, which normally antagonizes viral RNA translation <sup>92</sup>
Core	Reduces the number of PDCs and decreases their ability to produce IFN- $\alpha$ <sup>64</sup> ; induces expression of SOCS, which downregulates the JAK-STAT pathway <sup>102</sup>
E2	Inhibits PKR, which normally antagonizes viral RNA translation <sup>59</sup>
NS3-4A	Disrupts two independent viral recognition pathways, RIG-I and TLR3 <sup>49,50,52</sup>
NS5A	Inhibits PKR, which normally antagonizes viral RNA translation, and activates the transcription factor IRF1, <sup>45,103</sup> induces IL-8 production, which attenuates the activity of IFN- $\alpha$ <sup>104,105</sup>

Abbreviations: ER, endoplasmic reticulum; IFN, interferon; IL-8, interleukin 8; IRES, internal ribosome entry site; IRF1, interferon response factor 1; OAS, oligoadenylate synthetases; PDC, plasmacytoid dendritic cell; PKR, double-stranded RNA-activated protein kinase; SOCS, suppressors of cytokine signaling.

to inactivate the cellular protein Toll-interleukin-1 receptor domain-containing adaptor inducing IFN (TRIF).<sup>52</sup> TRIF is an adaptor protein that is a critical component of the TLR3 signaling pathway. By cleaving IPS-1 and inactivating TRIF, HCV disrupts the ability of a cell to detect its presence. As a consequence, IFN production is diminished and host defenses are impaired.

This ability of the virus to evade detection is of particular clinical relevance because it suggests that the protease inhibitors currently in clinical trials may not only block viral replication, but might also boost immunity. Although such a hypothesis is appealing, we re-emphasize that the extrapolation of data from cell-culture studies to the complex human host could be misleading. Notably, microarray studies of liver biopsy samples from patients infected with HCV have repeatedly demonstrated that IFN-stimulated genes are transcriptionally active, even before IFN therapy.<sup>53-55</sup> Although how many of these upregulated transcripts are actually translated into protein products is unknown—particularly because PKR activation is expected to result in the translational repression of many genes, via eIF2 $\alpha$  phosphorylation—the upregulated expression of IFN-stimulated genes in HCV-infected patients has been shown to predict nonresponsiveness to exogenous IFN therapy.<sup>53</sup> This resistance to therapy is thought to be partly related to an already saturated JAK-STAT pathway that cannot be further activated.<sup>53</sup> Thus, while NS3-4A protease inhibitors might have the potential to boost innate immunity within infected hepatocytes, the hope that this will alter clinical outcomes could be founded on a reductionist view of complex pathophysiology, in which an already active and potentially ineffective innate immune system may be present, and numerous other cells, such as macrophages, natural killer cells, and dendritic cells, probably have an active role.

#### Blocking IFN signaling and response

The binding of IFNs to their common receptor results in activation of a JAK-STAT signaling cascade. This cascade eventually results in the transcription of hundreds of genes,

called IFN-stimulated genes, which include the genes that encode PKR, 2',5' oligoadenylate synthetases-RNaseL, and the Mx proteins (Table 1).

The cellular protein PP2A belongs to a family of phosphatases that have roles in cell-cycle regulation and signaling.<sup>56</sup> In the context of HCV infection and the innate immune response, viral proteins induce PPA2, which in turn inhibits JAK-STAT signaling.<sup>57</sup> Notably, the expression of PP2A is increased in liver-biopsy samples from chronically infected HCV patients.<sup>56</sup> Findings from cell-culture studies suggest that HCV causes PP2A upregulation by inducing an endoplasmic reticulum stress response,<sup>58</sup> which consequentially reduces the ability of a cell to respond to IFN.

HCV is also able to interfere with specific host defenses that are induced by IFNs. As mentioned earlier, the cellular factor PKR shuts down the production of proteins in infected cells. This strategy is a cellular mechanism that prevents cells from being used as factories for virus production. Our unpublished results suggests that overcoming this cellular brake on viral replication seems to have been a major driving force in HCV evolution.

Two HCV viral proteins, E2 and NS5A, inhibit PKR activation in cell culture.<sup>59,60</sup> Interestingly, the ability of NS5A to inhibit PKR seems to be HCV-genotype-specific and could be one reason for the greater SVR rate observed in patients infected with genotype 2 than in those with other HCV genotypes.<sup>61</sup> Of note, mutations in the IFN sensitivity-determining region (ISDR), which falls within the PKR binding domain of NS5A, correlate with treatment outcome<sup>62</sup> in some populations of patients, but this statistical correlation is of limited practical use for a number of reasons that have been reviewed elsewhere.<sup>44</sup>

#### Relative immunosuppression

Plasmacytoid dendritic cells (PDCs) are one of the main producers of IFN, and consequently have an important role in the activation of cellular defenses against all viral infections. Interestingly, patients who are

chronically infected with HCV have decreased numbers of PDCs compared with healthy controls.<sup>63</sup> Furthermore, PDCs from HCV-infected patients produce less IFN when stimulated compared with PDCs from healthy individuals.<sup>64</sup> Findings from *in vitro* studies suggest that the HCV core protein induces cytokines that are directly responsible for this PDC phenotype and, importantly, increased blood levels of HCV core protein correlate with decreased levels of PDCs in HCV-infected patients.<sup>64</sup> Together, these observations suggest that the HCV core protein induces a number of systemic changes that result in an attenuated immune response by the host.

Despite the advances in this field, many critical questions in relation to the mechanisms by which HCV evades the innate immune response remain unanswered. For example, we do not know when and how viral evasion mechanisms develop, whether a relationship exists between the development of chronic HCV infection and viral immune evasion, and the precise relationship between these evasion mechanisms and resistance of HCV to IFN therapy. Why microarray studies demonstrate that some patients clinically classed as nonresponders have a preactivated immune system also remains an observation without a molecular explanation. Finally, we also do not yet understand why different HCV genotypes have different SVR rates to IFN.<sup>53</sup> Given that IFN therapy acts in part by enhancing the innate immune response, an understanding of the mechanisms that underlie genotype-specific outcomes are likely to deepen our understanding of the innate immune system.

#### Host factors

The relative roles of viral factors and host genetics in the context of an infection are, perhaps not surprisingly, intertwined. The administration of IFN as a treatment for HCV infection has brought to light a number of host factors that influence clinical outcome. In addition, a small number of specific host polymorphisms seem to be correlated with spontaneous HCV clearance.

#### *Ethnicity and sex*

African American and white patients are the best-studied ethnic groups with respect to the effects of the treatment of chronic HCV infection. In one study, African Americans infected with HCV genotype 1 had a SVR rate of 26%, which was much lower than the corresponding rate of 39% achieved in white patients.<sup>65</sup> A potential reason for this difference was revealed in a molecular study by He *et al.*, who compared the IFN response of peripheral blood mononuclear cells (PBMC) in HCV-infected African American and white patients. The authors of this study used microarray technology to demonstrate that fewer genes were upregulated in response to IFN in African American patients than in white patients,<sup>66</sup> which suggests that African Americans may have an attenuated response to IFN. The exact role of PBMCs in the clinical response to IFN treatment is

still not entirely clear, however, and the accuracy of their use as a surrogate with which to measure hepatocyte responsiveness has not been demonstrated. Not surprisingly, microarray studies have shown that PBMCs and hepatocytes do not exhibit an identical gene-expression pattern in response to an IFN stimulus.<sup>53,67</sup>

Female patients with chronic HCV infection respond slightly, but significantly better, to IFN therapy than male patients do. Researchers have speculated that this difference is related to a potentiating effect of estrogen on IFN.<sup>68</sup> Interestingly, a serologic study from Egypt that was performed to assess HCV prevalence found that women were 10% more likely than men to be antibody-positive and HCV RNA-negative, which suggests that women with HCV infection might have improved viral clearance compared with HCV-infected men.<sup>69</sup> Other studies have suggested that innate immune activation is enhanced in patients with autoimmune diseases such as lupus,<sup>70</sup> which leads to interesting speculation that specific host populations might have different innate immune 'set-points', which could potentially balance autoimmunity with immune responsiveness.

#### *Genetic polymorphisms and other factors*

Certain human leukocyte antigen (HLA) allelic variants of DRB1 and DQB1 are associated with spontaneous HCV clearance,<sup>71</sup> as are polymorphisms in the interleukin (IL)-12B gene.<sup>72</sup> Khakoo *et al.* identified specific natural-killer cell receptor-HLA combinations that were associated with the clearance of HCV infection, but only in patients exposed to low infective doses of HCV.<sup>73</sup> Limited evidence suggests that polymorphisms in the *CCR5* gene and the *IL10* promoter could be associated with the patient's response to therapy; however, inconsistent data that relates to these findings also exists. The influence of genetic polymorphisms as well as the role of age, weight and cirrhosis on HCV survival and HCV response to IFN treatment is extensively reviewed elsewhere.<sup>74</sup>

#### **The adaptive immune response in HCV evasion**

In addition to evading the innate immune system, HCV has evolved effective means of thwarting the adaptive immune system. HCV evades the adaptive immune system by two methods: the first is related to the propensity of HCV to establish chronic infection, and the second is related to its ability to limit the host's capacity to develop protective immunity.<sup>75</sup> Although the exact mechanism by which HCV evades the adaptive immune response is not well defined, current research points towards defects at multiple levels of the immune response and the involvement of various cell types, from antigen-presenting cells to mature effector cells.

#### **Role of impaired dendritic cells**

Antigen-presenting cells, such as dendritic cells, have a critical role in alerting the immune system to the presence of ongoing viral infection. Impairment at various stages

of the interaction between antigen-presenting cells and T cells can severely impair the ability of the host to clear an infection, and promotes persistence of the infection. Cell-culture experiments that investigated the contribution of defective host dendritic cells to HCV survival produced conflicting results.<sup>76–79</sup> However, these *in vitro* studies vary widely in design, the dendritic cell populations that they studied and the methods used for dendritic cell stimulation. Differences in experimental design may account for some of the discrepancies between findings from various groups.

Despite these discrepancies, several lines of evidence suggest that HCV impedes the early stages of adaptive immune activation by altering dendritic cell function. For example, dendritic cells cultured in the presence of HCV peptides have a limited ability to stimulate an antigen-specific response by the main components of the adaptive immune response: functional helper (CD4<sup>+</sup>) T cells and effector (CD8<sup>+</sup>) T cells.<sup>79</sup> Part of this dysfunction probably lies in the altered cytokine-secretion profile that has been observed in dendritic cells isolated from the blood of HCV-infected patients, which probably leads to defective T-cell activation.<sup>78,79</sup> Cytokines broadly shape the differentiation and maturation of the immune system and any alteration in their production, therefore, has additional consequences, as is discussed in further detail below. The defective expansion of CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells (T<sub>REG</sub>) by dendritic cells in HCV-infected patients leads to the altered production of humoral factors, and might have a role in negatively modulating the activation of other T-cell populations.<sup>80</sup> Our understanding of the mechanisms by which HCV alters the function of dendritic cells, however, is still incomplete, and greater insight is needed to identify triggers that can be modified to restore appropriate immune activation in patients with HCV infection.

### HCV evasion of the humoral immune response

HCV can also prevent the development of an effective, B-cell-mediated humoral response, which enables evasion of another major arm of the adaptive immune system. In contrast to other diseased states, in which antibodies have a key role in clearing pathogens through virion neutralization and targeting foreign antigens for opsonization and degradation, antibodies do not seem to clear HCV effectively. The inability of the host to mount an effective humoral response against HCV might be partly explained by findings from studies that have highlighted interactions between viral proteins and putative cellular receptors. These studies have identified critical residues on the HCV envelope proteins, in particular on E2, which are important for the initial steps of viral binding to hepatocytes. A high rate of mutation within the E2 HVRs<sup>81</sup> in effect results in the production of HCV variants that impair the recognition and binding of potentially neutralizing antibodies. In addition, extensive glycosylation on E2 in the region

close to the CD81 binding site impairs antibody-mediated inhibition of viral attachment to HCV putative surface receptors.<sup>82</sup> Although antibodies to other HCV proteins, such as the core and nonstructural proteins, are produced by the host, they do not contribute to viral neutralization, as these proteins are not found on the surface of the virion.

### Multiple T-cell defects underlie HCV evasion

CD8<sup>+</sup> and CD4<sup>+</sup> T cells have a critical role in controlling most viral infections. Chimpanzee models have demonstrated that early HCV infection outpaces the initial T-cell responses and that an early and robust intrahepatic activation of CD4<sup>+</sup> and CD8<sup>+</sup> T cells is required to achieve effective control of the acute infection.<sup>83</sup> CD4<sup>+</sup> helper T cells, in particular, are the principal coordinators of the adaptive immune response. Experimental depletion of these cells resulted in a blunted CD8<sup>+</sup> effector T-cell response, which led to prolonged viremia.<sup>84</sup> Moreover, suboptimal T-cell-mediated control of viral replication allowed for the emergence of genetic mutations of key MHC class I epitopes, which promoted the likelihood of further immune evasion.<sup>84</sup> Clearly, the presence of activated, functional CD4<sup>+</sup> and CD8<sup>+</sup> T cells and their coordination of the adaptive immune response are vital to the host's ability to control the acute and chronic phases of HCV infection.

CD4<sup>+</sup> helper T-cell subsets, T<sub>H</sub>1 and T<sub>H</sub>2, have distinct roles, and their differential activation can have important clinical consequences. The T<sub>H</sub>1 subtype is characterized by a cell-mediated immune response that is evoked by T<sub>H</sub>1-associated cytokines (IL-2, IL-12 and IFN), and the T<sub>H</sub>2 subtype promotes a strong humoral response under the control of T<sub>H</sub>2-associated cytokines (that is, IL-4, IL-6 and IL-10). Given the apparent greater importance of a strong, targeted cellular response to HCV infection relative to that of an antibody-focused repertoire, the differentiation fate of these CD4<sup>+</sup> T cells might have an important role in determination of the clinical course of the infection and could represent a potential target for viral exploitation. For example, binding of the HCV core protein to the gC1q receptor on dendritic cells leads to decreased production of IL-12—a cytokine that is important for T<sub>H</sub>1 differentiation.<sup>85</sup> Reduced IL-12 secretion by dendritic cells skews the differentiation of CD4<sup>+</sup> T cells towards a T<sub>H</sub>2 phenotype, a mechanism by which the HCV protein can indirectly affect CD4<sup>+</sup> T-cell functionality.<sup>85</sup> In addition, certain immunodominant viral epitopes preferentially bias the CD4<sup>+</sup> T-cell response towards either a T<sub>H</sub>1 or T<sub>H</sub>2 phenotype—a possible mechanism by which selection of particular viral mutations can manipulate the fate of CD4<sup>+</sup> T-cell differentiation.<sup>86</sup> The importance of this bias in T<sub>H</sub>1 versus T<sub>H</sub>2 differentiation in HCV survival is reflected by the fact that an NS3 epitope of HCV that promotes a T<sub>H</sub>1-like immune response is, in effect, negatively selected for by the immune response during chronic infection.<sup>86</sup> As the virus

mutates, amino-acid substitutions in the viral epitope result in a diminished or absent CD4<sup>+</sup> T-cell response. Specifically, these mutated viral-peptide sequences alter interactions between MHC-peptide complexes and the CD4<sup>+</sup> T-cell receptors, which subsequently modifies the secreted cytokine profiles of responding T cells from one that was originally T<sub>H</sub>1, to a T<sub>H</sub>2 profile.<sup>87</sup> Targeting CD4<sup>+</sup> T<sub>H</sub>1 and T<sub>H</sub>2 differentiation, therefore, represents yet another example of the mechanisms by which HCV manipulates the immune system to its advantage.

While CD4<sup>+</sup> T cells facilitate the coordination of the immune response, CD8<sup>+</sup> T cells are key effector cells that mediate direct and indirect, antigen-specific cytotoxicity. HCV infection seems to impair the functionality of these CD8<sup>+</sup> T cells by disrupting their maturation and altering their effector function. Indeed, analysis of HCV-specific CD8<sup>+</sup> T cells in the liver and peripheral blood has revealed various defects in this effector-cell population. These defects include an altered maturation phenotype that confers a diminished capacity for proliferation, poor peptide-specific cell-killing ability, decreased secretion of IFN- $\gamma$  and tumor necrosis factor, reduced perforin and granzyme A production, and low expression levels of Fas (CD95).<sup>88–90</sup> Some of these CD8<sup>+</sup> T-cell defects seem to be HCV-specific and do not represent a generalized deficit in CD8<sup>+</sup> T-cell responses to other viral infections.<sup>91</sup> Ultimately, these alterations translate into a reduced capacity of the T cells to trigger key intracellular events that are essential for viral clearance and disposal of infected cells.

A further mechanism by which HCV evades host CD8<sup>+</sup> T cells involves genetic mutation. An extensive study of intrahepatic and extrahepatic CD8<sup>+</sup> T cells in patients infected with HCV revealed that, although these HCV-specific T cells targeted many different HCV epitopes, the presence of additional viral mutations within the viral genome would render many of the HCV-specific CD8<sup>+</sup> T cells ineffective.

This method of viral escape is estimated to contribute to at least half of the failed HCV-specific, CD8<sup>+</sup> T-cell-mediated responses<sup>92</sup> by weakening T-cell recognition of an epitope, altering the binding of viral peptide to MHC molecules or by impairing antigen processing.<sup>93</sup> Studies that investigated the T-cell-mediated responses to HCV infection have shed light on specific methods by which HCV deregulates immune function, but the exact mechanisms by which the virus mediates these cellular defects and the relative contribution of each defect to the host's response are poorly understood and remain areas of active investigation.

### Counter-regulatory mechanisms

Counter-regulatory measures of the immune system that are intended to avert a destructive, hyperactive immune response can unintentionally promote a persistent state of infection. One well-characterized counter-regulatory mechanism employed by the immune system in response

to chronic HCV infection involves upregulation of PD-1 expression on CD4<sup>+</sup> and CD8<sup>+</sup> cells. Upon binding of PD-1 to its ligands, PD-L1 and PD-L2, activated T cells enter an induced anergic, or exhausted phenotype that is characterized by decreased T-cell proliferation and cytokine release. This immune-mediated downregulation of T-cell activation in the presence of ongoing infection benefits viral evasion. The significance of this interaction has been demonstrated in experiments where the PD-1–PD-L1 interaction is experimentally disrupted.<sup>90</sup> Remarkably, blockade of the monocyte PD-L1 receptor enhances proliferation of IFN-producing, HCV-specific CD8<sup>+</sup> and CD4<sup>+</sup> T cells and induces a preferential increase in the production of T<sub>H</sub>1 cytokines,<sup>94</sup> highlighting a potential therapeutic avenue by which to enhance immune function.

In addition to the actions of immune-system-inhibiting receptors, T<sub>REG</sub> have a critical role in the regulation of peripheral tolerance in many disease processes. For example, T<sub>REG</sub> are a major secretor of IL-10, an anti-inflammatory cytokine with diverse effects that influences antigen processing, MHC expression and IL-12 production by antigen-presenting cells. A 2008 study demonstrated an inverse relationship between the clinical course of acute hepatitis C infection and plasma levels of IL-10, with high IL-10 levels associated with the development of chronic HCV infection.<sup>95</sup> *In vitro* blockade of the IL-10 receptor improved the antiviral function of peripheral blood monocytes.<sup>95</sup> HCV-specific T<sub>REG</sub> are increased in HCV-infected patients, and can inhibit the proliferation of HCV-specific CD8<sup>+</sup> T cells in a dose-dependent manner.<sup>96</sup> By impairing the function of other cellular components of the adaptive immune response, therefore, these host inhibitory cells appear to assist HCV immune evasion.

### Extrahepatic HCV replication

Many studies have now demonstrated the apparent presence of HCV viral genomes in extrahepatic sites of patients infected with HCV. Studies that use HCV-specific detection assays have demonstrated the infection of leukocytes by HCV and the potential of these cells to act as a reservoir of virus after treatment. Intriguingly, the pool of HCV quasispecies differs between plasma and peripheral blood monocytes, which demonstrates an independent perpetuation of HCV within different cell types.<sup>97,98</sup> HCV infection of a B-cell non-Hodgkin's lymphoma has also been demonstrated. Generation of a cell line derived from this lymphoma revealed the ability of these nonhepatocyte cells to not only support HCV replication, but to enable production of viral particles that were capable of infecting peripheral blood B cells.<sup>99</sup> The significance of these extrahepatic HCV reservoirs is not well understood, although one could speculate that the leukocyte compartments might represent an additional route by which HCV can directly manipulate the immune system—yet another means by which this virus eludes eradication.

## Conclusions

HCV has evolved an extensive array of mechanisms to evade innate and adaptive immune defenses. These strategies include viral factors that directly target mediators of intracellular antiviral defenses, as well as disruptions of multiple key nodes of cellular and humoral immunity.

Although our understanding of the molecular details by which HCV seems to evade detection and elimination by the immune system has increased, the relative contribution of these potential mechanisms to the clinical persistence of infection remains to be defined. The lack of appropriate model systems remains a significant obstacle; however, identification of evasion mechanisms of HCV is leading to an increasing array of potential therapeutic strategies with which to shift the balance back towards host control of HCV. In addition to providing new insights into cell biology and immunology

of the host, we hope that this research will ultimately be translated into improved outcomes for patients who suffer from an important cause of liver disease.

### Review criteria

PubMed was searched in June 2008 for English-language publications that contained the search terms “hepatitis C virus”, “chronic hepatitis C”, “HCV entry”, “HCV translation”, “HCV replication”, “HCV assembly”, “HCV miRNA”, “interferon”, “sustained virological response”, “HCV T cells”, “HCV dendritic cells”, “HCV B cells” and “genotype”. Additional publications were identified from the reference lists of papers identified by this search. No exclusion criteria were used and no date restrictions were placed on the search. The full text of identified articles of interest was obtained and relevant data abstracted. An extra reference was added in February 2009.

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