Evasion of intracellular host defence by hepatitis C virus

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Viral infection of mammalian cells rapidly triggers intracellular signalling events leading to interferon α/β production and a cellular antiviral state. This 'host response' is our first line of immune defence against infection as it imposes several barriers to viral replication and spread. Hepatitis C virus (HCV) evades the host response through a complex combination of processes that include signalling interference, effector modulation and continual viral genetic variation. These evasion strategies support persistent infection and the spread of HCV. Defining the molecular mechanisms by which HCV regulates the host response is of crucial importance and may reveal targets for novel therapeutic strategies.

Hepatitis C virus (HCV) is remarkably successful. It typically produces a persistent infection that, unless interrupted by interferon (IFN)-based therapy, will continue for the lifetime of the individual and present vast opportunities for further transmission within the human population. This success is linked to an ability of HCV to evade and antagonize the immune response of the host and to resist the antiviral actions of IFN therapy. Until recently, native HCV could not be adequately propagated in cultured cells to support molecular studies of the virus–host relationship. Insight into its evasion strategies has come from studies of model systems and human patients. These studies have revealed several levels of immune regulation and evasion conferred by HCV protein products.

This review will explore the mechanisms by which HCV triggers, controls and evades antiviral defences directly within the infected hepatocyte and hepatic tissue to support HCV replication and persistence.

The host response to infection

Virus infection initiates a series of intracellular events that culminate in the generation of an antiviral state directly within the infected cell and indirectly within the surrounding tissue. To replicate and spread successfully, viruses direct various strategies to evade host defences[1]. In recent years much has been learned about the molecular mechanisms by which viruses trigger and regulate these antiviral processes, referred to here as the 'host response'. It is the hepatic host response that imposes initial immune defences against HCV infection. The host response is triggered when a pathogen-associated molecular pattern (PAMP) presented by the infecting virus is recognized and engaged by specific PAMP receptor factors expressed in the host cell, initiating signals that ultimately induce the expression of antiviral effector genes[2]. For RNA viruses, protein and nucleic acid products of infection or replication, including single-stranded (ss) or double-stranded (ds) RNA and polyuridine signatures, have been identified as viral PAMPs and are each engaged by specific Toll-like receptors (TLRs) or nucleic-acid-binding proteins that serve as PAMP receptors (Fig. 1)[3,4]. The viral RNA of HCV contains each of these PAMP signatures and is sufficient to trigger the host response when introduced into naive cells[5,6]. In hepatocytes (the target cell of HCV infection), independent pathways of retinoic-acid-inducible gene I (RIG-I) and TLR3 signalling comprise two major pathways of host defence triggering by dsRNA[6–8].

The largest effect of PAMP receptor engagement is the activation of latent cellular transcription factors that mediate the rapid onset of gene expression, thus marking the immediate-early phase of the host response[9]. Interferon regulatory factor (IRF)-3 (refs 10, 11) and nuclear factor κB (NF-κB)[12] figure largely in this response. IRF-3 and NF-κB are activated through viral PAMP-responsive signalling cascades that culminate in their nuclear translocation and transcription effector actions. IRF-5 and IRF-7 have also been implicated as direct transcription effectors of viral PAMP signalling[13,14], although the events that confer their activation are incompletely defined and their role in HCV infection is not known. Parallel processes that activate ATF-2 and direct chromatin remodelling result in the assembly of an enhanceosome complex with IRF-3 and NF-κB on the IFN-β promoter, leading to a transcriptional response that produces secreted IFN-β from the infected cell[2]. NF-κB is also involved in inducing the expression of chemokines and proinflammatory cytokines that function in parallel with IFN to mediate the inflammatory response to HCV[15]. Secreted IFN-β engages the local tissue through autocrine and paracrine processes of binding the IFN-α/β receptors. This results in activation of the Jak–STAT pathway, in which the receptor-associated Jak and Tyk1 protein kinases catalyse the phosphorylation of signal transducer and activator of transcription (STAT) proteins on critical serine and tyrosine residues. This confers STAT activation and stable association with IRF-9. The resulting IFN-stimulated gene factor-3 (ISGF3) transcription factor complex localizes to the cell nucleus, where it binds to the IFN-stimulated response element (ISRE) within the promoter/enhancer region of IFN-stimulated genes (ISGs). Jak–STAT signalling leads to a second and later wave of transcriptional activity marking ISG expression in the infected cell.

ISGs are the genetic effectors of the host response to virus infection, and the human genome encodes hundreds of ISGs[16]. ISG products impart cell or viral-regulatory functions that limit HCV replication through processes that include disruption of viral RNA translation and inhibition of antigenomic strand RNA synthesis[17–20]. The paracrine effects of IFN-β induce ISG expression within the neighbouring uninfected cells of the local tissue, inducing an antiviral state that limits cell-to-cell virus spread. Many PAMP receptors and their constituent signalling partners are ISGs, and although expressed basally at a low level that facilitates surveillance, their levels increase markedly after

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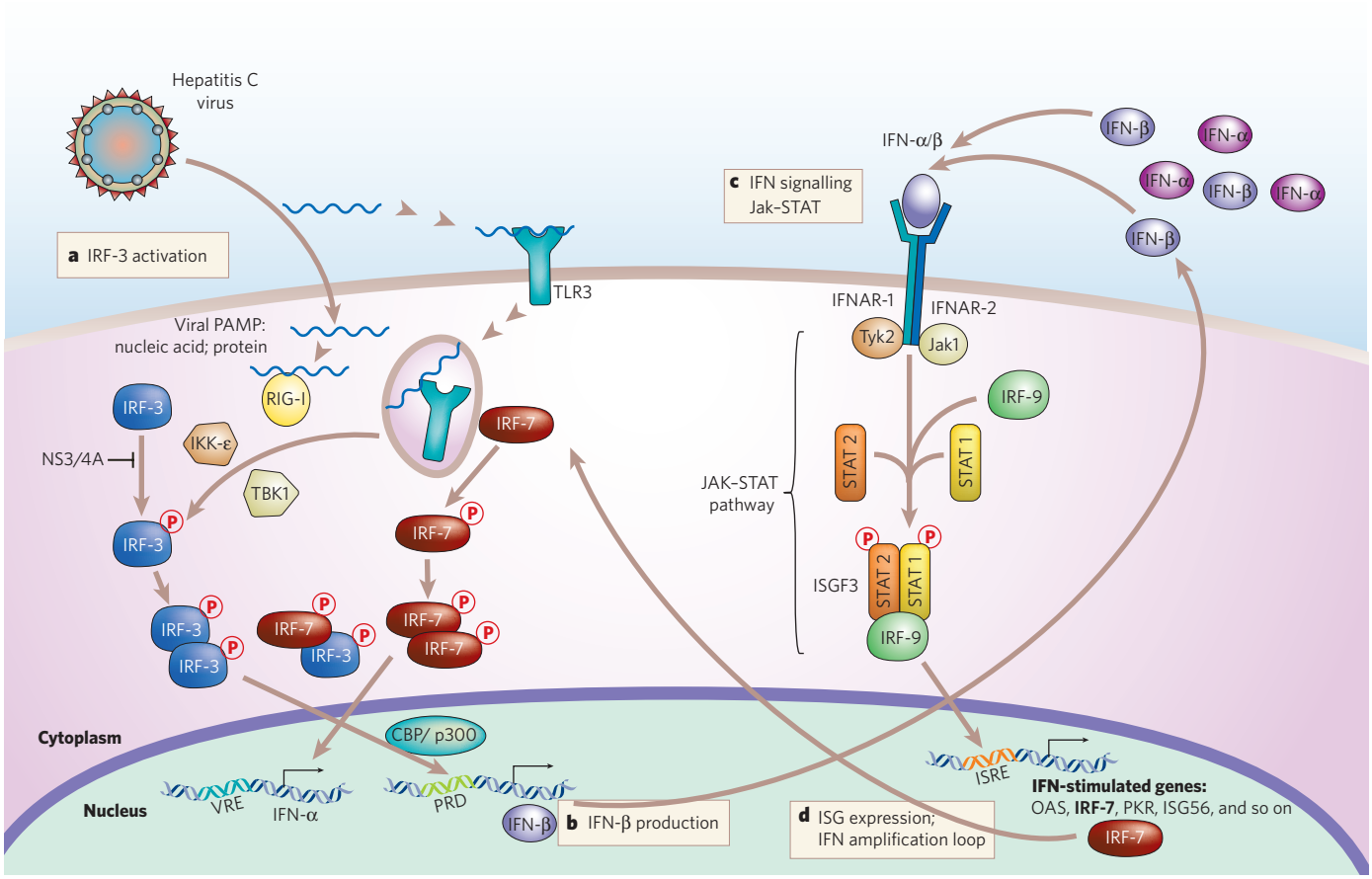

Figure 1 | Molecular processes that signal the host response to HCV infection. **a**, Viral PAMP (HCV RNA) binding to RIG-I or TLR3 results in the phosphorylation and activation of IRF-3 by the TBK1 or IKK-ϵ protein kinases[47,48,89]. The dimer of phospho-IRF-3 translocates to the cell nucleus, interacts with its transcription partners, including CBP/p300 (ref. 90) and binds to the cognate-DNA positive regulatory domain (PRD) in the promoter region of IRF-3 target genes, including IFN-β. **b**, IRF-3 activation results in IFN-β production and secretion from the infected cell. **c**, IFN-β binding to the IFN-α/β receptor signals the activation of the associated Tyk2 and Jak1 protein kinases to direct the phosphorylation and assembly of a STAT1–STAT2 heterodimer and trimeric ISGF3 complex containing IRF-9 (ref. 2). The ISGF3 complex locates to the cell nucleus, where it binds to the ISRE on target genes to direct ISG expression. **d**, ISGs are the genetic effectors of the host response. IRF-7 is a transcription factor and an ISG. It is activated after expression through viral PAMP signalling pathways that overlap with the pathways of IRF-3 activation. IRF-7 phosphorylation, dimerization and heterodimerization with IRF-3 allow it to bind its cognate virus-responsive element (VRE) in the promoter region of IFN-α genes, resulting in the production of various IFN-α subtypes that further signal ISG expression[91]. This increases the abundance of RIG-I and viral PAMP signalling components whose continued signalling serves to amplify IFN production and the host response. The therapeutic administration of IFN-α provides antiviral action against HCV by signalling ISG expression through the IFN-α/β receptor and the Jak–STAT pathway. RIG-I and TLR3 signalling ablation by the HCV NS3/4A protease blocks IRF-3 activation and attenuates the host response to infection.

IFN production. In human liver this most probably serves to enhance the sensitivity of signalling in infected tissue. *In vitro* studies have shown that this signalling provides an amplification loop to further promote IFN and ISG expression, limiting HCV RNA replication[21]. IRF-7 is an ISG and is expressed in many tissue types, including complex liver tissue, in response to IFN[22]. The transcription effector action of IRF-7 promotes IFN-α subtype expression and diversification of the ISG response, establishing a positive-feedback loop that amplifies IFN production and antiviral action[23]. It is the IFN-α component of the host response that is exploited by the current IFN-based therapy for HCV infection (Fig. 1)[24]. In addition to inducing ISG expression, IFN-α primes or induces the maturation of immune effector cells, and it potentiates the production of other proinflammatory cytokines by resident hepatic cells to indirectly modulate the cell-mediated defences and adaptive immunity to HCV[2]. Viral triggering and control of the host response may define cellular permissiveness for HCV RNA replication and influence the outcome of infection.

Hepatic defences triggered by HCV are not alwayssufficient

Hepatic defences to HCV infection have been studied *in vivo* through functional genomic and biochemical approaches to evaluate human liver from patients with chronic infection and chimpanzee liver from animals undergoing experimental HCV infection. Functional genomic analyses from cohorts of human subjects with chronic infection have shown that infection is associated with a hepatic gene expression profile marked by ISGs, whose levels vary widely among patients and possibly with different degrees of liver fibrosis and cirrhosis[22]. These observations suggest that HCV can both trigger and control the hepatic host response during infection. Similar studies of infected chimpanzees have revealed insights into how this host response is associated with infection outcome. Unlike humans, in which acute HCV infection progresses to chronic infection with high frequency that includes a wide-ranging disease course, infection of chimpanzees can progresses to chronic infection but usually with lower frequency and with only minor disease[25]. Gene expression profiling has demonstrated that acute resolving HCV infection in chimpanzees is associated with a robust host response characterized by high level hepatic ISG expression[26] and that the overall expression level of certain ISGs and virus-responsive genes identified them as 'outcome predictors' of infection. In the latter example, this gene set was defined as those virus-responsive genes whose high expression associated with low viraemia and viral clearance but whose low expression correlated with progression to chronic infection[27]. Like many virus-responsive genes and ISGs, the various products of this 'outcome predictor' gene set interact with components of T-cell immunity, impli-

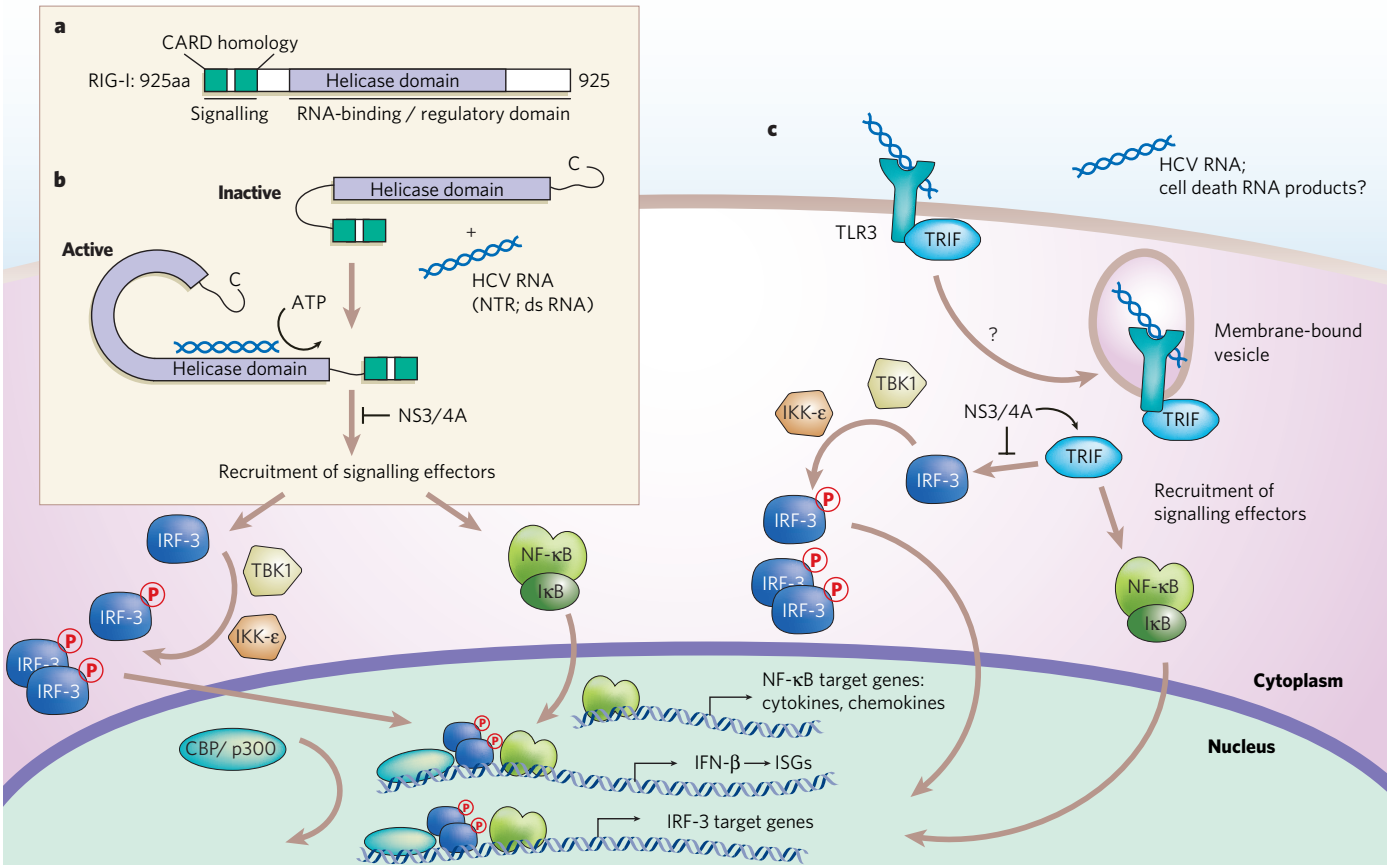

Figure 2 | Triggering IRF-3 activation by HCV through RIG-I or TLR3, and signalling control by NS3/4A. **a**, The domain structure of RIG-I includes N-terminal tandem caspase activation and recruitment domain (CARD) homology regions followed by a C-terminal DEx/D box RNA helicase domain[7]. The RIG-I CARD domains signal IRF-3 and NF-κB activation. The RIG-I helicase domain binds the HCV RNA PAMP[6]. **b**, In the absence of RNA PAMP binding, the RIG-I helicase domain suppresses the signalling actions of the CARD domains, most probably by mediating an autoinhibitory conformation. RNA PAMP binding during HCV RNA replication[21] is thought to result in an open conformation that permits CARD signalling and probably involves recruitment of signalling factors that direct the phosphorylation and activation of IRF-3. Other factors signal the parallel release of NF-κB from its inhibitor (IκB). CBP, CREB-binding protein. **c**, PAMP signalling through TLR3 is thought to initiate as a result of RNA PAMP binding to the ectodomain of TLR3 that is presented on the cell surface or within membrane-bound cytosolic vesicles. PAMP signalling through TLR3 requires the TRIF adaptor protein, which signals the downstream phosphorylation and activation of IRF-3 by IKK-ε or TBK1 protein kinases. TRIF also directs the MyD88-independent activation of NF-κB[33]. As a result of PAMP signalling through RIG-I or TLR3, the active, nuclear forms of IRF-3 and NF-κB promote the expression of specific target genes that have antiviral or immunomodulatory actions. The NS3/4A protease disrupts viral PAMP signalling[21,42,46,92]. NS3/4A protease activity cleaves or inactivates one or more signalling components of the RIG-I pathway that are essential for downstream IRF-3 and NF-κB activation (**a**). The shallow and 'featureless' protease substrate-binding cleft of NS3 may accommodate a variety of substrates, allowing NS3/4A to cleave multiple distinct cellular proteins[43]. Cleavage of TRIF between residue positions 372 and 373 by NS3/4A ablates viral PAMP signalling through the TLR3 pathway. The separate N-terminal and C-terminal proteolytic fragments of TRIF are unstable and signal neither the downstream phosphorylation and activation of IRF-3 nor the activation of NF-κB (**b**)[42].

cating a complex cross-talk within the host response between parameters of virus, IFN signalling, and the adaptive immune response to HCV infection. ISG expression profiles have also been observed in animals with chronic HCV infection[28]. These observations demonstrate, first, that the hepatic host response is triggered during HCV infection but is differentially regulated in association with disease course, and second, that in chronic infection HCV can successfully control or evade the host response to persist in the infected cell and hepatic tissue.

Triggering the host response to HCV infection

Although HCV is a ssRNA virus, its genome RNA encodes regions of extensive secondary dsRNA structure that impart potential PAMP signatures, thus presenting the possibility that HCV RNA is recognized and engaged by host-cell PAMP receptors during infection[29]. Various studies have shown that genome-length or specific subgenomic fragments of HCV RNA are sufficient to trigger IFN-β promoter activation and IFN production when introduced into cultured human hepatoma cells[5,30], indicating that during infection these HCV RNA motifs are recognized and engaged by PAMP receptor(s) that trigger the host response[6].

The nature of at least one HCV RNA PAMP receptor was revealed through complementation studies of cells with defective host-response signalling programmes[7], including cells that are highly permissive to HCV RNA replication[6,31]. This work identified RIG-I as a viral PAMP receptor that binds dsRNA[7], including dsRNA motifs of the HCV genome[6], to signal the downstream activation of IRF-3 and NF-κB, thereby inducing IFN-β expression and onset of the host response. RIG-I is a DEx/D-box RNA helicase belonging to a small family of helicases involved in host response signalling[7,32]. RIG-I contains amino-terminal regions of homology to the caspase activation and recruitment domain (CARD; Fig. 2a). Signalling is mediated by the CARD homology motifs, which direct the downstream activation of IRF-3 and NF-κB through processes independent of TLR3 (Fig. 2b)[7]. TLR3 is a dsRNA PAMP receptor that also signals a host response on engagement of a dsRNA ligand (Fig. 2c)[7]. TLR3 directs the activation of IRF-3 and NF-κB through processes that require the protein Toll–interleukin-1 receptor-domain-containing adaptor inducing IFN-β (TRIF)[33]. Virus signalling through RIG-I and TLR3 pathways confers a host response that regulates cellular permissiveness for viral replication.

Protein products of virus infection may also stimulate the host response or activate specific components of this response as they accu-

mulate in the cell. Expression of the HCV NS5A protein induces cellular stress and signalling pathways that activate STAT3 (ref. 34). STAT3 promotes gene expression through processes that involve the Jak–STAT pathway[35]. This results in a gene-expression profile that includes ISGs and proinflammatory cytokines that may influence the overall level of HCV RNA replication[36]. Moreover, the HCV core protein can activate protein kinase R (PKR), a cellular antiviral protein kinase and an effector component of the host response to virus infection[37]. PKR is an ISG and a dsRNA-binding protein whose RNA-dependent activation results in localized translational suppression and parallel stimulation of NF-κB and IRF-1 transcription-effector actions[38]. It is likely that PKR activation by the core protein is associated with the similar ability of the latter to bind RNA[39], thereby providing the PKR activator substrate and a possible mechanism of PKR activation during HCV infection. Cell interactions with virus particles may also trigger signalling events that induce IFN production. HCV pseudo-particle binding to dendritic cells has been shown to mediate particle uptake and dendritic cell activation[40]. Because dendritic cells represent a major source of IFN production during viral infection, modulation of their function may influence systemic and/or local IFN signalling and ISG expression[41].

Control and evasion of the host response

The success of HCV in persisting is linked to its overall ability to disrupt the host response and evade antiviral defences. Key sites of HCV control over the host response are found within the PAMP-responsive signalling pathways that impart IRF-3 activation, within the IFN-α/β receptor signalling pathway that confers ISG expression, and at the level of ISG effector protein products[1]. The HCV NS3/4A protease functions as an antagonist of virus-induced IRF-3 activation and IFN-β expression through its ability to block RIG-I signalling and to ablate TLR3 signalling by cleaving the TRIF adaptor protein[21,42,43] (Fig. 2). NS3 is a bifunctional enzyme. Its N-terminal domain encodes a serine protease, and its carboxy-terminal domain encodes an RNA helicase; the latter may support replication by unwinding the viral RNA[44]. The NS3/4A complex constitutes the essential viral protease, which liberates the non-structural proteins from the HCV polyprotein during virus replication[45]. The helicase activity of NS3 is dispensable for the control of IRF-3 activation, but NS3/4A protease activity is required for this regulation[46]. The proteolytic targeting of host factors by NS3/4A as an evasion strategy from host defence was affirmed through pharmacological studies with a peptidomimetic active-site NS3 protease inhibitor. Treatment of cells that express functional NS3/4A alone or in the context of active HCV RNA replication showed that the protease inhibitor effectively removed the blockade to RIG-I and TLR3 signalling imposed by NS3/4A, thereby restoring virus-induced IRF-3 phosphorylation/activation and the activation of NF-κB[21,42,46]. This provides pharmacological confirmation that the protease action of NS3/4A is a functional antagonist of the host response induced by dsRNA and viral PAMP signalling.

The viral disruption of RIG-I or TLR3 signalling has many implications. First, this control attenuates two major pathways of IFN production in hepatocytes[8]. Second, many of the components of these pathways, including RIG-I, TLR3, TRIF and the downstream IκB kinase (IKK)-ε kinase (one of the enzymes that can phosphorylate and activate IRF-3)[47,48], are responsive to IFN and although expressed at low basal levels their abundance is induced severalfold on exposure of cells to IFN-α/β. The IFN-responsiveness of these factors confers amplification of PAMP signalling action to further enhance the magnitude and duration of the host response. The signalling blockade imposed by NS3/4A breaks this IFN amplification loop (see Table 1)[21]. Third, the MHC components of antigen processing and presentation are themselves ISG products[16], and host-response regulation may effect alterations in antigen presentation, leading to inefficient activation of cytolytic T cells and an inability of the adaptive immune response to clear HCV-infected hepatocytes[27,49]. Fourth, in addition to its role in host defence, IRF-3 has been ascribed proapoptotic and tumour suppressor functions[50,51]. In this case, prolonged blocking of IRF-3 function could disrupt these actions, perhaps

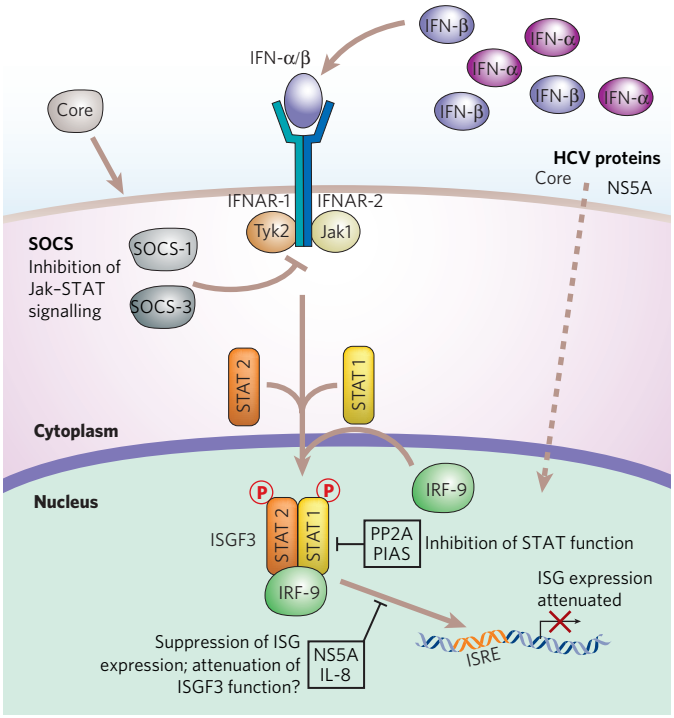

Figure 3 | HCV attenuates IFN signalling through multiple mechanisms. Receptor signalling by IFN from autocrine/paracrine and therapeutic sources is subject to feedback inhibition by suppressor of cytokine signalling (SOCS) proteins. The HCV core protein has been shown to induce the aberrant expression of SOCS-3, which can suppress Jak–STAT signalling events and block the IFN-induced formation of ISGF3 (ref. 58). HCV protein expression in liver cells is associated with induction of the protein inhibitor of activated STAT (PIAS) expression and concomitant inhibition of STAT function *in vivo*, possibly mediated by protein phosphatase 2A (PP2A) signalling events and STAT demethylation[56]. Patients with chronic HCV infection have exhibited high levels of serum IL-8 (ref. 64). The biological activity of IL-8 interferes with IFN signalling events that catalyse ISGF3 assembly and function[62]. HCV modulation of IFN signalling events attenuates ISG expression, allowing HCV to evade the antiviral actions of the host response and IFN therapy.

to render a tumorigenic potential to infected cells, thus providing a possible biochemical link between chronic HCV and hepatocellular carcinoma[52]. Last, the blockade of virus-induced NF-κB activity regulates the expression of a variety of chemokines and cytokine genes whose expression is dependent on NF-κB[21]. Among these is interleukin (IL)-1, which mediates antiviral actions against HCV[53]. Viral control of NF-κB may therefore contribute to the broader systemic immune defects and enhanced permissiveness for HCV infection.

Regulation of IFN signalling

Local IFN production in hepatic tissue is likely to influence HCV replication and may impart antiviral effects that contribute to the resolution of acute infection[26]. The overall low response rate of HCV (particularly genotype 1 HCV) to IFN therapy[24] indicates that HCV can evade or resist IFN actions *in vivo*, both locally in the context of a hepatic host response and more globally in the context of IFN therapy (Fig. 3). Assessment of IFN-α/β receptor signalling processes has revealed mechanisms by which HCV proteins can antagonize IFN signalling. HCV protein expression in general has been associated with the inhibition of STAT1 function independently of STAT tyrosine phosphorylation[54,55]. This has been attributed in part to the expression of high levels of protein phosphatase 2A within HCV-infected liver tissue, which may signal STAT1 hypomethylation and inactivation[56]. Expression of the HCV core protein has been associated with increased expression levels of suppressor of cytokine signalling (SOCS)-3 in cultured cells[57]. The SOCS proteins are best known for their role as negative regulators and inhibitors of Jak–STAT signalling, where they

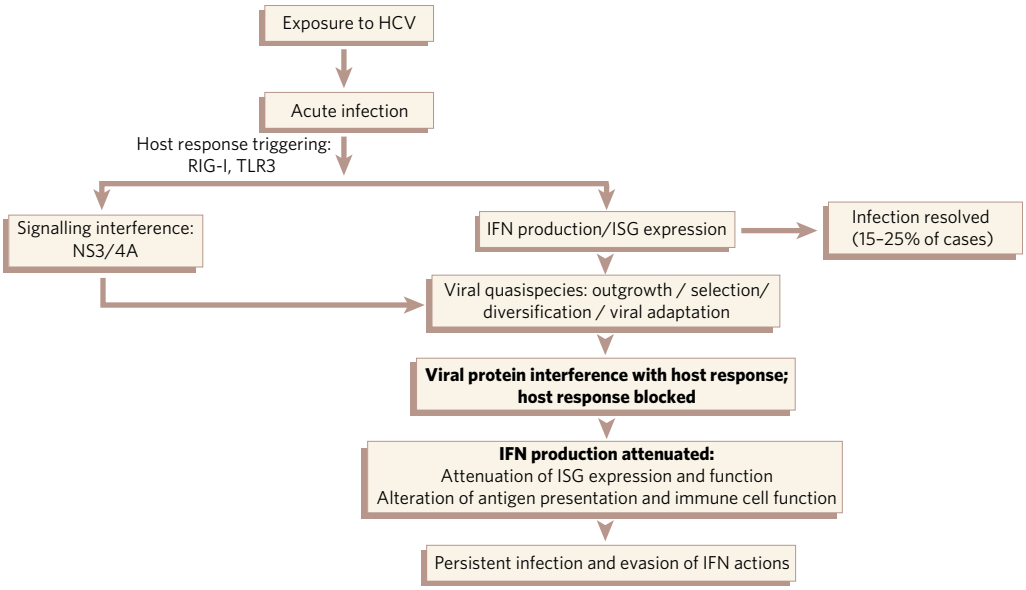

Figure 4 | HCV–host interactions regulate the host response and affect the outcome of HCV infection. A flow diagram (described in the text) is shown in which virus–host interactions within the host response to HCV infection define the outcome from acute exposure to HCV.

mediate a classic negative feedback loop on IFN-α/β receptor signalling events[58]. Induction of SOCS-3 expression by the HCV core protein could impart evasion from IFN actions, but the overall role of SOCS-3 in HCV infection is not known. The actions of IFN are pleiotropic, both at the signalling level and the ISG response, and it is most likely that HCV evades IFN effects through multiple strategies, including possibly disruption of non-canonical IFN signalling pathways[59].

Regulation of ISG expression or function

HCV evasion of the host response includes various strategies directed by viral proteins to control ISG expression or function (Table 1). The HCV NS5A protein has been identified as an IFN antagonist, and expression of NS5A alone can suppress IFN-α actions sufficiently to rescue the replication of an IFN-sensitive virus in cultured cells[60]. Functional genomics analyses have shown that NS5A expression confers a general attenuation of ISG expression[61]. A explanation for this comes from the observation that NS5A can induce IL-8 expression and secretion. IL-8 is a proinflammatory chemokine whose actions interfere with IFN[62]. NS5A stimulates IL-8 production through transactivation of the IL-8 promoter[63], and serum IL-8 levels have been found elevated in patients with chronic hepatitis C[64]. The mechanisms by which IL8 antagonizes IFN actions are not known but probably involve an end result of altering ISG expression.

The HCV NS5A and E2 proteins of HCV are both inhibitors of PKR[65–67]. Inhibition of PKR may allow HCV to evade in part the translational-suppressive actions of IFN and the PKR-dependent signalling processes that amplify the host response to infection[1]. However, this regulation is not universal and is subject to alteration through viral genetic variation (see below)[68], indicating that evasion of PKR-independent processes of ISG function contribute to HCV escape from IFN action. The ISG56 product, p56, imparts translational suppressive actions of IFN on HCV RNA replication[17], and reduced levels of ISG56 expression have been associated with IFN resistance of HCV RNA replication *in vitro*[69]. Further examination of HCV interactions with the IFN-induced 2′,5′-oligoadenylate synthetase (OAS)/RNase L pathway have revealed that HCV proteins also interact with this pathway[70], and that once activated the pathway directs the capacity of RNase L to cleave HCV genomic RNA into non-functional nucleolytic products[71]. Genetic studies have revealed that RNase L preferentially cleaves HCV RNA only at certain UU and UA dinucleotide sites[72]. Genotype 1 HCV sequences in general have fewer RNase L cleavage sites than HCV genotypes 2 or 3 (ref. 72). This may provide a genetic basis for HCV 1a and 1b resistance to IFN therapy (Table 1).

Viral genetic variation and the host response to infection

As with all RNA viruses, the viral polymerase of HCV lacks a proofreading function. In the course of persistent infection, error-prone

Table 1 | ISG regulation by HCV

Viral strategy	Mechanism of action	Implications	References
IL-8 induction	NS5A induces IL-8 production through processes involving NF-κB and AP-1 transcription factor activation	Attenuates ISG expression	63
Induction of SOCS expression	The HCV core protein can induce expression of SOCS1 and SOCS3	Blocks Jak–STAT signalling action through the IFN-α/β receptor	57
PKR inhibition	NS5A and E2 proteins bind PKR and inhibit its catalytic activity	Disruption of PKR-dependent translational control and signalling actions	65,66,68
IRF-1 regulation	NS5A blocks dsRNA-induced IRF-1 action through inhibition of PKR signalling	Relieves IRF-1 suppression of HCV RNA replication	93,94
Evasion of 2′,5′ OAS/RNase L pathway	HCV genome sequence	The HCV genome encodes a paucity of RNase L recognition sites, which allow protection from nucleolytic processing	71,72
Disruption of STAT1 function	HCV proteins	HCV proteins induce PP2A expression and STAT1 hypomethylation to attenuate ISG expression	54,56
Suppression of ISG56 expression	HCV non-structural proteins	*In vitro*: NS3/4A and non-structural proteins disrupt virus signalling to the ISG56 promoter. Removes the ISG56 block to viral RNA translation	17,69
Regulation of RIG-I signalling	NS3/4A protease blockade of signalling	Blockade of RIG-I signalling breaks an IFN amplification loop that otherwise enhances ISG expression	21
Regulation of TLR3 signalling	NS3/4A protease cleavage of TRIF	Disruption of a TLR3-pathway IFN amplification loop	42

virus replication generates a repertoire of highly related but genetically distinct viral variants or 'quasispecies'. This is most problematic for the infected patient because quasispecies variation affords remarkable adaptive potential to HCV and has been implicated in evasion and control of the host response to infection and differential sensitivity to IFN therapy[73]. The hostile antiviral host environment may drive the outgrowth of HCV 'evasion variants' from a pre-existing quasispecies pool or through viral genetic adaptation. Indeed, sequencing studies have shown that the resolution of acute HCV infection is associated with an overall reduction in viral quasispecies complexity within the E1 and E2 coding regions of HCV, whereas progression to chronic infection and resistance to IFN therapy is associated with increased viral genetic complexity[74,75]. This indicates that host immune pressure may drive the outgrowth or selection of viral evasion variants able to persist and resist IFN action. Sequence analysis of the HCV NS5A coding region has similarly identified specific domains that exhibit sequence variation in association with the outcome of IFN therapy. This association has been variable in different patient populations, but recent meta-analyses and long-term follow-up of these studies provide overall support for NS5A sequence variation within a 40-residue 'interferon sensitivity determining region' (ISDR) that is associated with IFN therapy outcome[76–78]. This region of NS5A encompasses a genetically flexible domain that is a key site of adaptations that influence HCV RNA replication fitness[79,80]. Thus, ISDR variation may affect the host response to infection indirectly by altering replication efficiency and the abundance of viral proteins available for interaction with and regulation of host response effectors.

Exogenous induction of antiviral hepatic defences

Multiple studies provide molecular evidence for a clear absence or only a low level of IFN-α/β gene expression in hepatocytes of patients with chronic HCV[81]. The expression of IFN-β and various IFN-α subtypes is dependent on virus activation of IRF-3 (ref. 2), and the lack of IFN-α/β gene expression within the HCV-infected liver provides indirect evidence that HCV imposes a blockade to IRF-3 activation *in vivo*. This may explain why some patients with chronic infection do not express significant levels of hepatic ISGs, but it fails to explain why others exhibit broad and abundant ISG expression despite a paucity of IFN-α/β expression in the infected liver. It is notable that hepatic ISG expression has been associated with liver pathology[22]. This raises the possibility that ISG expression can be induced indirectly as a result of cellular stress from fibrosis and/or cirrhosis, or is induced through TLR engagement exogenously by extracellular products of damaged tissue or viral replication. The former possibility is indicated by cell-culture studies in which stress-induced cytokines, including TNF-α and IL-1, triggered signalling crosstalk to activate IRF-1 and derive a level of IFN-β production[82]. In the context of chronic HCV, the latter possibility could occur through TLR3 engagement of viral or host RNA products by hepatocytes and surrounding cells that are not infected and remain competent to signal an ISG response. Exogenous/extrahepatic immune effector cells that infiltrate the liver, including IFN-producing macrophages and dendritic cells[83], may also contribute to hepatic ISG expression. By this model, hepatic ISG levels would vary with the composition and extent of immune cell infiltration, which has been observed[22,27]. Secretion of IFN-γ by hepatic effector T cells and NK cells also contributes a level of ISG expression partly redundant with the ISGs induced during the IFN-α/β response[16]. IFN-γ exerts antiviral effects on HCV RNA replication[84]. This response probably has a role in controlling HCV infection[27].

A current model

Studies defining the viral induction, evasion and control of the host response to HCV collectively provide a model of virus–host interactions and viral adaptation that form a foundation for chronic infection (Fig. 4). Transmission of HCV from a source individual and infection of a recipient host present enormous pressure for the virus to adapt to the new host environment and to control the host response to infection. The transmission event results in an acute infection that involves viral regulation of the host response though RIG-I, TLR3 and other virus-responsive signalling pathways within the infected hepatocyte[6,8]. Highly fit variants of HCV will mediate signalling interference, in which the NS3/4A protease will block RIG-I and TLR3 signalling pathways to evade the host response to infection and viral RNA replication. Genetic distinctions between virus strains and viral genotypes are likely to impart differential levels of control and activation of this response[46,68,72], and during acute infection their activation of the host response will lead to the production of IFN and ISG to mediate an antiviral state in the local hepatic tissue[26]. About 15–25% of exposures to HCV typically render an acute resolved infection[85]. Thus, if successful, the hepatic host response will provide protection against the replication and spread of HCV. The host response and the ensuing adaptive immune response present pressures that will select for the outgrowth of viral quasi-species that can evade and successfully control the host response and immune defences[69,75]. HCV–host interactions within RIG-I, TLR3, IFN signalling pathways and ISG pathways, and at other key sites of host defence, serve to control the host response and may attenuate the therapeutic actions of IFN, thus providing a foundation for persistent HCV replication and spread. This model invokes an important role for viral adaptation or quasi-species selection in the successful evasion and control of the host response, and projects a 'foot race' between the virus and the host for control of this response that in most cases the virus will win. The recent development of cell-culture models of HCV infection[86,87,88] now provides a foundation from which to define the molecular mechanisms and novel sites for therapeutic modulation of the host response controls that regulate the HCV infection and replication cycle. ■

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ERRATUM

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In situ multi-satellite detection of coherent vortices as a manifestation of Alfvénic turbulence

David Sundkvist, Vladimir Krasnoselskikh, Padma K. Shukla, Andris Vaivads, Mats André, Stephan Buchert & Henri Rème

Nature 436, 825–828 (2005)

In the print and PDF versions of this Letter, the colour scale bars in Fig. 2a, c and d should have been respectively labelled as follows: 'Proton number flux ($cm^{-2}\,s^{-1}\,sr^{-1}\,keV^{-1}$)'; '$B$ ($nT^2\,Hz^{-1}$)'; and 'DOP'. This labelling is correctly shown in the online HTML full text version.

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Evasion of intracellular host defence by hepatitis C virus

Michael Gale Jr & Eileen M. Foy

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In Table 1 of this Review Article, some reference citations are incorrect. Those in the fourth row from the top (IRF-1 regulation) should be 89,90 (and not 93,94); in the second-to-last row from top (regulation of RIG-I signalling), reference 94 should be included (to read 21,94).

CORRIGENDUM

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Action potential refractory period in ureter smooth muscle is set by Ca sparks and BK channels

T. Burdyga & Susan Wray

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The panels of Fig. 3 in this Letter are incorrectly cited in the text. In the first full paragraph on page 560, these should be: line 7, 'Fig. 3b, top'; line 11, 'Fig. 3b'; line 12, 'Fig, 3c, top'; and line 13, 'Fig. 3c, bottom'. Other citations of Fig. 3 are correct. In addition, the vertical voltage scale on the bottom trace in Fig. 3d should read from −70 to 10 mV.