Interferon-Induced Effector Proteins and Hepatitis C Virus Replication

Michael Frese* **and Eva Dazert**

Abstract *Hepatitis C virus* (HCV) is a small, enveloped RNA virus that is often capable of establishing a persistent infection, which may lead to chronic liver disease, cirrhosis, hepatocellular carcinoma, and eventually death. For more than 20 years, hepatitis C patients have been treated with interferon-alpha (IFN-α). Current treatment usually consists of polyethylene glycol-conjugated IFN-α that is combined with ribavirin, but even the most advanced IFN-based therapies are still ineffective in eliminating the virus from a large proportion of individuals. Therefore, a better understanding of the IFN-induced innate immune response is urgently needed. By using selectable self-replicating RNAs (replicons) and, more recently, recombinant full-length genomes, many groups have tried to elucidate the mechanism(s) by which IFNs inhibit HCV replication. This chapter attempts to summarize the current state of knowledge in this interesting field of HCV research.

Introduction

Interferons and the Antiviral State

Interferons (IFNs) are a diverse class of cytokines with key functions in the innate immune response to viruses (reviewed in Pestka et al., 2004; Goodbourn et al., 2000; Samuel, 2001). Three types of IFNs can be distinguished that have partially overlapping biological properties. Type I IFNs are secreted by most virus-infected cells and by a highly specialized leukocyte population, termed natural IFN-producing cells or plasmacytoid dendritic cells (Colonna et al., 2002). The human genome contains many type I IFN genes encoding 12 IFN-α subtypes, IFN-β, IFN-ε, IFN-κ, and IFN-ω. The reason why the human genome encodes so many IFN-α subtypes is

* Corresponding Author: Dr. Michael Frese, School of Health Sciences, University of Canberra, ACT 2601, Australia, Phone: +61 (0)2 6201 2243, Fax: +61 (0)2 6201 5727, E-mail: michael.frese@canberra.edu.au

not known but has been speculated that different subtypes elicit a slightly different antiviral response. Furthermore, it is tempting to speculate that the most recent multiplication of IFN-α genes is a consequence of an ongoing arms race between viruses that encode soluble IFN receptors and the innate immune defense system. Type I IFN genes differ from all other IFN genes by the fact that they lack introns.

Recently, three distantly related cytokines have been identified that share sequence similarities with type I IFNs and the interleukin-10 (IL-10) family. Accordingly, these cytokines have been named IFN-λ1, IFN-λ2, and IFN-λ3 (Kotenko et al., 2003) or IL-29, IL-28A, and IL-28B, respectively (Sheppard et al., 2003). Although it seems that many biological properties of this most recently discovered group of IFN-like cytokines resemble those of type I IFNs, they are referred to as type III IFNs.

In contrast to types I and III IFNs, of which numerous genes have been identified, the human genome contains only one type II IFN gene. The gene product, IFN-γ, is only expressed in specialized immune cells such as activated T lymphocytes and natural killer (NK) cells.

All types of IFNs bind to highly specific cell surface receptors that trigger the phosphorylation and nuclear translocation of a family of latent transcription factors, known as signal transducers and activators of transcription (STATs). Type I IFNs bind to the IFN-α receptor (IFNAR), which leads to the formation of the IFN-stimulated gene factor-3 (ISGF-3), a heterotrimer consisting of STAT1, STAT2, and IFN-response factor-9 (IRF-9/p48). ISGF-3 activates gene transcription via the IFN-stimulated response element (ISRE). Type III IFNs bind to a different receptor complex consisting of the IFNLR1/IL-28Rα subunit and the IL-10β subunit (Donnelly et al., 2004) but nevertheless trigger a signaling cascade that is very similar to that of type I IFNs (Doyle et al., 2006). A slightly different signaling pathway has been described for IFN-γ. The type II IFN binds to the IFN-γ receptor (IFNGR) which leads to the phosphorytation of the gamma activation factor (GAF), a phosphorylated STAT1 homodimer, is translocated to the nucleus, where it enhances gene expression by binding to the gamma activation site (GAS). Beside these well-established signaling pathways, alternative pathways have been described, but their contribution to the antiviral activity of IFN remains to be further elucidated (Pestka et al., 2004).

Types I and III IFNs are believed to execute their antiviral activities through the induction of proteins that accumulate inside an infected host cell. These effector proteins may interfere with distinct steps in viral replication or trigger the degradation of viral RNAs. By contrast, IFN-γ predominantly induces the expression of proteins with systemic functions, such as those involved in antigen processing and presentation. In addition, IFN-γ induces the expression and release of chemokines that activate and orchestrate the adaptive immune response (e.g., IP-10). However, at least in some virus infections, IFN-γ may also contribute to the establishment of an antiviral state by the induction of proteins with direct antiviral activities (reviewed in Guidotti & Chisari, 2001). Of note, all IFNs that have been tested so far inhibit hepatitis C virus (HCV) RNA replication in cultured cells, although differences have been noted in respect to the IC_{50} and the kinetics of inhibition.

HCV Replication

HCV is a member of the genus *Hepacivirus* that belongs to the family *Flaviviridae* (van Regenmortel et al., 2000). HCV isolates can be grouped into at least 6 genotypes that differ in their nucleotide sequence by 31% to 34%. Furthermore, within a given genotype, subtypes can be defined that differ in their nucleotide sequence by 20% to 23%. Different genotypes show a remarkable degree of heterogeneity with respect to antiviral treatment (McHutchison & Fried, 2003). For example, only 50% of patients infected with genotype 1 mount a sustained antiviral response, whereas 80% to 90% of those infected with genotype 2 and genotype 3 viruses do so. This is of immediate medical significance, and numerous attempts have been made to identify the viral factor(s) that determine the outcome of current IFN therapies. The underlying molecular mechanisms are, however, still controversial.

HCV has a ~9.6-kb single-stranded RNA genome of positive polarity (reviewed in Bartenschlager et al., 2004). The genome encodes a large polyprotein that is co- and post-transcriptionally cleaved by cellular and viral proteinases into 10 proteins (core, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B). The production of an additional protein (F) by ribosomal frame shifting has been reported, but its function remains to be defined. The coding sequence is flanked by nontranslated regions (NTRs) that are important for RNA translation (5′NTR) and replication (5′ and 3′NTR). Both NTRs are highly structured and contain numerous stem loops, most notably in the internal ribosome entry site (IRES) of the 5′NTR (Honda et al., 1999) but also in other regions such as the X-tail sequence of the 3′NTR (Blight & Rice, 1997). Furthermore, NTR sequences have been shown to interact with complementary coding sequences, which further increases the amount of intramolecular base pairing (Kim et al., 2003; Friebe et al., 2005). Figure 1 shows the organization of the HCV genome and depicts some of its key structural elements. The figure also summarizes major protein functions, including those that counteract the innate immune response.

HCV replication takes place in the cytoplasm of persistently infected hepatocytes, the principal host cells of the virus. Detailed information on the mode of RNA replication is not available for HCV, but by analogy to other flaviviruses (Westaway et al., 2002), it has been proposed that the incoming positive-stranded RNA genome is used as a template for the synthesis of a negative-stranded RNA molecule that remains base-paired with its template. The resulting double-stranded RNA (also called "replicative form") is then transcribed multiple times, which results in the generation of numerous full-length, positive-stranded progeny RNAs that may used for replication, translation, or packaging into newly formed virus particles (for details, see Bartenschlager et al., 2004).

Interferon Production in HCV-Infected Cells

Double-stranded RNA that is formed by intramolecular base pairing between complementary sequences of positive-stranded HCV RNAs or during viral replication should alert the double-stranded RNA detection system of the host cell. This would

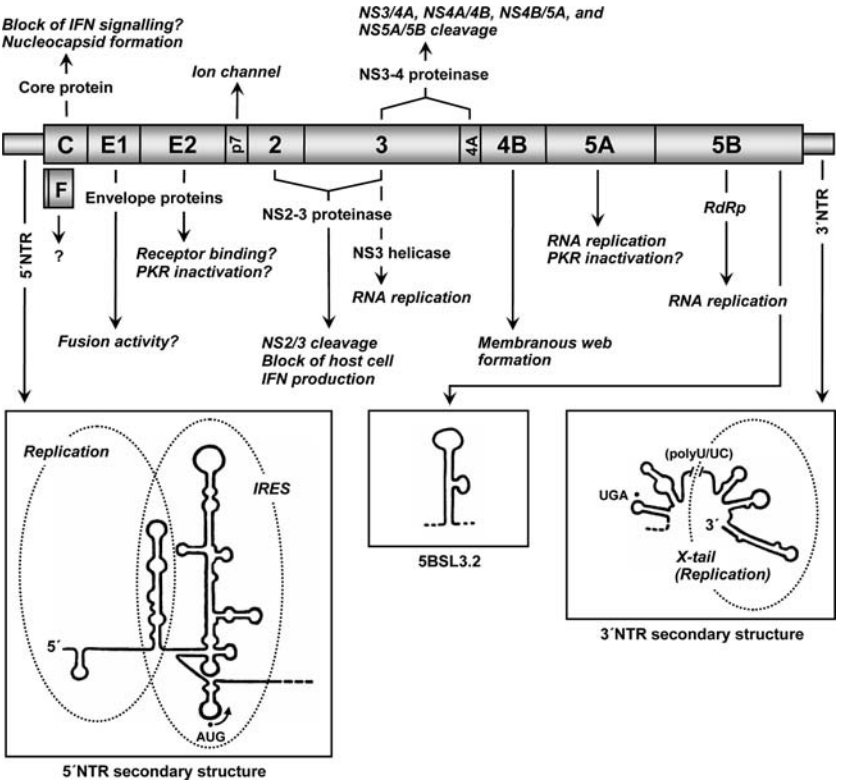

Fig. 1 HCV genome organization, presumed protein functions, and RNA secondary structures. The virus genome contains a large open reading frame (ORF) that encodes all major viral proteins and an alternative ORF that encodes the frame shift protein (F), which has an unknown function. The structural proteins C, E1, E2, and p7 are liberated from the polyprotein by cellular signal peptidases, and all other cleavages are performed by viral proteases. RNA secondary structures are drawn according to Blight & Rice (1997), Honda et al. (1999), You et al. (2004), and Friebe et al. (2005). Black dots indicate the position of the start and the stop codon of the large ORF. The minimum regions in the 5′ and 3′ nontranslated regions (NTRs) required for replication and initiation of translation are encircled with dotted lines. RdRp, RNA-dependent RNA polymerase; IRES, internal ribosome entry site; 5BSL3.2, stem loop 3.2 within the coding sequence of NS5B

normally result in the production of type I IFNs and the subsequent expression of IFN-induced effector proteins, which in turn would establish an antiviral state in the IFN-producing cell itself and in neighboring cells. HCV-infected hepatocytes, however, seem not to produce much IFN, as liver biopsy samples of most chronic hepatitis C patients lack detectable amounts of type I IFN mRNAs (Mihm et al., 2004). This finding is in line with the observation that cultured human hepatoma cells produce only small amounts of IFNs in response to viral infections or other stimuli such as poly(inosine[I])-poly(cytosine[C]), suggesting that human hepatocytes are generally rather poor IFN producers (Keskinen et al., 1999). However, primary chimpanzee and tamarin hepatocytes were found to be highly responsive to poly(I)-poly(C), which raises the question of why the liver of chronic hepatitis C

patients does not contain type I IFNs (Lanford et al., 2003). This enigma has recently been solved by Foy and co-workers, who reported that the HCV NS3/4A protease interferes with the ability of cells to sense double-stranded RNA (Foy et al., 2003). NS3/4A-mediated cleavage of the adaptor protein TRIF reduces its abundance and inhibits poly(I)-poly(C)-activated signaling through the Toll-like receptor 3 pathway before its bifurcation to IRF-3 and nuclear factor-κB (NFκB)-mediated gene activation pathways (Li et al., 2005). Furthermore, NS3/4A cleaves the adapter protein MAVS/IPS-1/VISA/Cardif, which interrupts the signaling between the double-stranded RNA binding protein RIG-I and kinases that phosphorylate the IFN regulatory factors IRF-3 and IRF-7 (Meylan et al., 2005). This act of sabotage efficiently prevents the nuclear import of the latent transcription factors IRF-3 and IRF-7, a crucial step in the activation of type I IFN gene transcription (reviewed by Hiscott et al., 2006). Taken together, these findings suggest that HCV-infected hepatocytes are prevented from producing the amount of type I IFN that is needed to assist virus clearance. Nevertheless, type I IFN-induced mRNAs/proteins are readily detectable in liver biopsies of hepatitis C patients, even in liver samples that do not contain detectable amounts of type I IFN mRNAs (Mihm et al., 2004). This begs the question as to where the IFN that is not locally produced comes from. According to Mihm and co-workers, natural IFN-producing cells or plasmacytoid dendritic cells may represent an important extrahepatic source of IFN in hepatitis C patients (Mihm et al., 2004). However, natural IFN-producing cells have the propensity to migrate to secondary lymphoid organs rather than to sites of inflammation (Penna et al., 2002). As a consequence, the expression of type I IFN-induced proteins may never reach levels required to eliminate the virus from already infected cells and/or to prevent the infection of new host cells.

Recombinant Interferon as an Antiviral Agent

All currently licensed HCV therapies rely on the antiviral activity of type I IFN (mostly polyethylene glycol-conjugated IFN-α2) that is given alone or in combination with ribavirin. The administration of recombinant IFN bypasses the block of IFN production in HCV-infected host cells and dramatically increases the expression of type I IFN-induced proteins throughout the body. This leads in most cases to a rapid decline of HCV RNA levels (first-phase response), which is believed to reflect an inhibition of virus replication. Later on, HCV RNA levels decline more gradually (second-phase response) as the liver is cleared of virus-infected cells (Neumann et al., 1998; Layden & Layden, 2002). Although IFN-α initially reduces the viral load in almost all patients, a sustained response (as defined by the loss of detectable HCV RNA during therapy and its continued absence for at least 6 months after the treatment has been ended) is not experienced by all patients. Especially those patients who suffer from an infection with genotype 1b viruses often fail to eradicate the virus (Manns et al., 2001; Fried et al., 2002). The correlation between therapy success and the infecting genotype suggests the involvement of viral factors, but the underlying molecular mechanisms are not yet understood.

With the development of HCV replicons (Lohmann et al., 1999), it became possible to analyze the role of individual cytokines in the innate immune response against HCV. Because most patients respond, at least initially, to a treatment with IFN-α, it was not unexpected that this IFN and other type I IFNs also block RNA replication of different HCV genotypes in human hepatoma cells (Blight et al., 2000; Frese et al., 2001; Guo et al., 2001; Cheney et al., 2002; Larkin et al., 2003; Okuse et al., 2005; Windisch et al., 2005; Miyamoto et al., 2006) and in cells of nonhepatic origin, e.g., HeLa cells (Guo et al., 2003) and 293 cells (Ali et al., 2004). Similar results were obtained by using type III IFNs (Robek et al., 2005; Marcello et al., 2006) and IFN-γ (Cheney et al., 2002; Frese et al., 2002) but not other antivirally active cytokines such as TNF-α (Frese et al., 2003). The idea that IFN-γ enforces the critical first line of defense in the HCV-infected liver was further elaborated by Li and co-workers, who demonstrated in a co-culture experiment that NK cells block HCV replication in Huh-7 cells through the secretion of IFN-γ (Li et al., 2004). Clinical data are limited and it is still controversial discussed whether hepatitis C patients benefit from IFN-γ administrations. Nevertheless, it is interesting to note that types I and II IFNs inhibit HCV RNA replication in Huh-7 cells in a highly synergistic manner (Larkin et al., 2003; Okuse et al., 2005). Given the power of combination therapies in the treatment of other persistent virus infections, it might be rewarding to elucidate the mechanism(s) responsible for the observed synergistic antiviral effects of different IFN types. For example, do IFN-α and IFN-γ enhance the expression level of one or more effector proteins in a synergistic manner as suggested by Tan et al. (2005), or do they induce the expression of different, IFN type-specific effector proteins that interfere with more than one step of the HCV life cycle as suggested by Windisch et al. (2005)? The answers to these questions may help physicians to predict the outcome of IFN therapies and lead to the improvement of IFN-based therapies.

IFN-Induced Proteins That May Inhibit HCV Replication

General Remarks

Several attempts have been made to analyze systematically the IFN-induced changes in the gene expression of HCV host cells. In one approach, liver biopsy samples were taken from expetimentally infected chimpanzees and the gene expression profile was monitored by using cDNA microarrays. The results revealed that the infection of the liver rapidly leads to the upregulation of numerous genes including those encoding well-known IFN-induced effector proteins such as the chimpanzee homologue of MxA (Bigger et al., 2001; Su et al., 2002). In both studies, the expression of MxA and that of other type I IFN-induced proteins correlated with the magnitude and duration of the infection. However, transient and sustained viral clearances were rather associated with the production of IFN-γ and the subsequent expression

of type II IFN-induced genes, suggesting a biphasic course of the innate immune response and a crucial role for IFN-γ in virus clearance.

In another approach, cDNA microarrays were used to analyze IFN-induced changes in the gene expression profile of cultured human cells containing HCV replicons (Zhu et al., 2003; Hayashi et al., 2005). Even if these and similar studies did not lead to the identification of the effector proteins that inhibit HCV replication in IFN-stimulated cells, they will guide present and future investigations by suggesting potential candidate genes. The contribution of some of the most prominent IFN-induced effector proteins (Figure 2) to the IFN-induced inhibition of HCV replication is discussed in the following paragraphs.

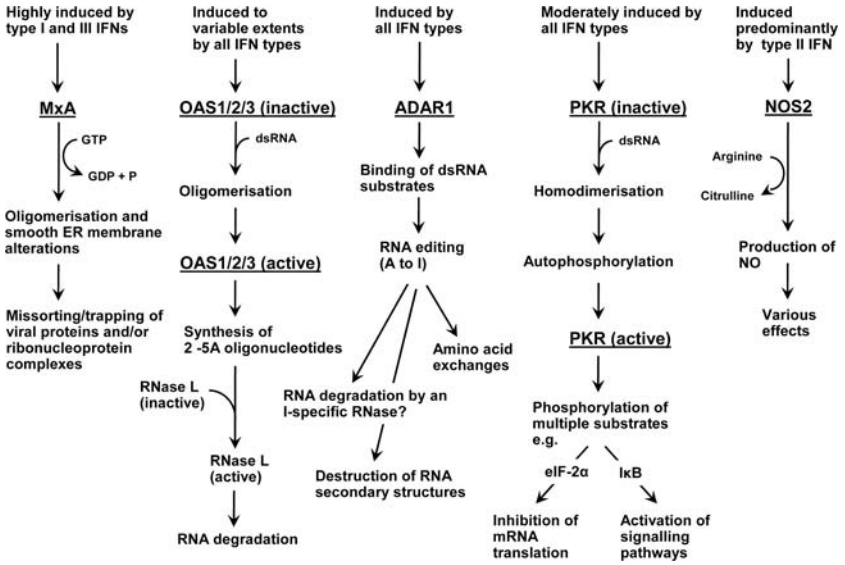

Fig. 2 Antiviral pathways that may contribute to the establishment of the so-called antiviral state in IFN-stimulated human cells. From left to right: The MxA GTPase inhibits viral replication by missorting and trapping of viral components into large membrane-associated complexes (the role of GTP hydrolysis in this process is not fully understood). Three different IFN-induced oligoadenylate synthetases (OAS1, OAS2, and OAS3) are encoded by the human genome. Binding to double-stranded RNA (dsRNA) leads to hetero- and/or homo-oligomerization and subsequently to the production of oligoadenylates with a 2′,5′-phosphodiester bond linkage. These 2-5A oligonucleotides activate the latent endoribo-nuclease RNase L, which leads to the degradation of viral and cellular RNAs (in some cell types, the expression of RNase L is also regulated by IFNs). The p150 isoform of the adenosine deaminase ADAR1 binds to double-stranded RNA and catalyzes the conversion of adenosine to inosine (A to I). Such editing may occur selectivity at one or a few positions, or more frequently, at a large number of sites. Editing of viral RNAs may change the coding sequence, activate an I-specific RNase, and/or destroy RNA secondary structures by disrupting adenosine/uracil base pairings. The double-stranded RNA-activated protein kinase PKR may block viral protein translation by the phosphorylation and thereby inactivation of the eukaryotic initiation factor eIF2a. Furthermore, PKR may activate intracellular signaling pathways that contribute to the establishment of a robust antiviral response. The inducible nitric oxide synthetase NOS2 produces large amounts of nitric oxide (NO), which is implicated in a variety of immune functions such as the activation of macrophages

MxA

Mx proteins belong to the superfamily of dynamin-like large GTPases and their expression is tightly regulated by type I and type III IFNs (Holzinger et al, 2007; reviewed in Haller & Kochs, 2002; Haller et al, 2007). Of the two human Mx proteins, MxA and MxB, only MxA has demonstrable antiviral activity. MxA is a cytoplasmic protein with a size of ~78 kDa that has been shown to inhibit the replication of a broad variety of RNA viruses. Cell culture experiments demonstrate that MxA inhibits orthomyxoviruses, bunyaviruses, rhabdoviruses, birnaviruses, reoviruses, and togaviruses (Mundt 2007 reviewed in Haller et al., 1999). In some cases, viral replication is almost completely blocked by MxA. For example, stably transfected Vero cells that constitutively express MxA produce up to 1,000,000-fold lower virus titers than control cells that did not express any Mx proteins (Frese et al., 1995). The antiviral effect of MxA has also been analyzed *in vivo* by using transgenic mice that constitutively express the human MxA protein but lack functional mouse Mx proteins and mice that constitutively express MxA but cannot mount a proper IFN-induced antiviral response due to a disruption in the gene for the β subunit of the IFN type I receptor. In both cases, MxA-expressing animals were found to be completely resistant to *Thogoto virus*, a tick-borne orthomyxovirus (Pavlovic et al., 1995; Hefti et al., 1999). Furthermore, MxA-expressing animals exhibited an enhanced resistance against ... *Influenza A virus* (family *Orthomyxoviridae*), *Vesicular stomatitis virus* (VSV; family *Rhabdoviridae*), *LaCrosse virus* (family *Bunyaviridae*), and *Semliki Forest virus* (family *Togaviridae*) (Pavlovic et al., 1995; Hefti et al., 1999). Other reports suggest that MxA has an even wider antiviral activity, but the supporting data are less convincing.

The *modus operandi* of MxA is not completely understood, but accumulating data indicate that cytoplasmic Mx proteins missort and immobilize viral components. In cells that had been infected with *LaCrosse virus*, MxA binds and translocates the viral nucleocapsid protein into membrane-associated perinuclear complexes (Kochs et al., 2002; Reichelt et al., 2004). A similar phenomenon was observed in cells that had been infected with *Thogoto virus*. In this case, however, MxA inhibited the nuclear transport of incoming viral nucleocapsids (Kochs & Haller, 1999), thereby preventing primary transcription and leading to an early and very efficient block of virus replication.

In healthy individuals, MxA expression is below the detection limit, but expression levels increase dramatically during many viral infections and as a consequence of IFN-α treatment (Roers et al., 1994; Chieux et al., 1998). MxA mRNA quantification in peripheral blood mononuclear cells has even been used to monitor the bioavailability of administered type I IFNs in hepatitis C patients (Gilli et al., 2002; Jorns et al., 2006). Not surprisingly, elevated MxA expression levels have also been found in the liver of chronic hepatitis C patients, indicating an ongoing struggle between the innate immune system and HCV (MacQuillan et al., 2002, 2003; Patzwahl et al., 2001).

A genetic study from Japan addressing a single nucleotide polymorphism at position -88 in the promoter sequence of the MxA gene revealed that a thymidine (T) in that position favors a sustained response of hepatitis C patients to treatment with

IFN-α, whereas a guanosine (G) is more frequently found among nonresponders (Hijikata et al., 2000). Interestingly, a T at that position increases the homology of the first ISRE in the MxA promoter to the ISRE consensus sequence (Hijikata et al., 2000). Furthermore, experiments with reporter constructs suggest that the T allele has a higher transcriptional activity than the G allele when stimulated with IFN-α (Hijikata et al., 2001). A similar association between the G/T single nucleotide polymorphism at position -88 of the MxA gene and the response of hepatitis C patients to IFN-α therapy was found in a European study, in which the T genotype was also found to be associated with the ability to clear HCV naturally without the help of recombinant IFN (Knapp et al., 2003).

Since MxA has the ability to efficiently inhibit a variety of different RNA viruses, MxA was the first IFN-induced effector protein to be analyzed for its antiviral activity in the HCV replicon system (Frese et al., 2001). However, no evidence was found for an involvement of MxA in the IFN-induced inhibition of HCV RNA replication. The constitutive expression of MxA did not inhibit subgenomic HCV replicons, and the expression of a dominant-negative mutant of MxA did not restore HCV RNA replication during IFN-α treatment (Frese et al., 2001). These earlier observations are in line with the more recent finding that IFN-α inhibits HCV RNA replication in Huh-7 cells and HuH6 cells with a similar IC_{50} (3 to 5 IU/ml and 5 to 10 IU/ml, respectively), although the former produce nearly 75-fold more MxA mRNAs than the latter (Windisch et al., 2005). Taken together, the data indicate that IFN-α inhibits HCV RNA replication by MxA-independent pathways.

Most recently, it has been noted that brefeldin A, a Golgi apparatus disrupting agent, renders the replication of *Kunjin virus* susceptible to MxA (Hoemen et al., 2007). Since *Kunjin virus* and HCV are both flaviviruses that use host cell-derived membranes to establish replication factories, it is tempting to speculate whether a disruption of the membranous web in HCV-infected cells would expose HCV RNA-protein complexes to antivirally active proteins such as MxA.

OAS/RNase L

The OAS/RNase L pathway (also known as IFN-inducible 2–5A response) requires two types of enzymes, an oligoadenylate synthetase and a ribonuclease (reviewed in Samuel, 2001). The human genome contains four gene loci that encode IFN-induced oligoadenylate synthetases (OAS1, OAS2, and OAS3) and an OAS-like protein. This and alternative splicing leads to the expression of numerous isoforms with sizes ranging from 40 to 100 kDa (Rebouillat & Hovanessian, 1999). OAS protein expression is enhanced in response to most, if not all, IFNs, but the magnitude of induction can vary dramatically with different IFNs and the type of the producing cell. Newly produced OAS proteins are believed to be inactive, but binding to double-stranded RNA leads to their oligomerization and starts the production of oligoadenylates with a 2′,5′-phosphodiester bond linkage (2-5A oligonucleotides). These oligonucleotides bind to and activate the latent ribonuclease RNase L, a

process that is associated with the formation of stable RNase L homodimers. Once activated, RNase L can degrade single-stranded RNAs of viral and cellular origin. Cleaving of target RNAs occurs preferentially on the 3′ side of uracil-adenosine (UA) and UU dinucleotides (Floyd-Smith et al., 1981; Wreschner et al., 1981). The cleavage of mRNA and rRNA may trigger a general protein shut-off in virus-infected cells, thereby limiting virus replication and spread. Different OAS proteins are associated with different cellular compartments, vary with respect to the amount of double-stranded RNA needed for activation, and produce 2-5A oligonucleotides of different sizes (Samuel, 2001). It is therefore tempting to speculate that the diversity of OAS proteins and isoforms evolved to fight a rather wide spectrum of DNA and RNA viruses including poxviruses, reoviruses, and picornaviruses. Members of the family *Picornaviridae* seem to be especially sensitive to the OAS/RNase L pathway. For example, overexpression of the 40-kDa form of the human OAS1 protein confers resistance to *Mengovirus* but not VSV (Chebath et al., 1987), and the constitutive expression of the 69-kDa form of the OAS2 protein inhibited the replication of *Encephalomyocarditis virus* but not that of VSV, *Sendai virus*, and a reovirus (Ghosh et al., 2000). In this context it is interesting to note that one of the *Oas* gene loci has recently been identified to confer increased resistance to the *West Nile virus* (family *Flaviviridae*) in laboratory mice (Perelygin et al., 2002) and that the transcript of the *Oas1b* allele in susceptible mice contains a premature stop codon, which results in a truncated protein (Mashimo et al., 2002). Experiments with congenic mice and cells derived from those mice revealed that expression of the full-length OAS1 protein limited virus production *in vivo* and in cell culture. Surprisingly, however, RNase L activity was highest in susceptible cells, and downregulation of RNase L activity in resistant cells did not restore virus titers to levels observed in susceptible cells (Scherbik et al., 2006).

As with many other IFN-induced proteins, OAS protein expression is slightly upregulated in hepatitis C patients (MacQuillan et al., 2003) and further enhanced in response to the administration of recombinant IFN-α (Murashima et al., 2000). Rather indirect evidence that the OAS/RNase L pathway may indeed target HCV replication/translation was recently provided by Taguchi and co-workers, who reported that the N-terminal portion of NS5A (amino acids 1 to 148), which lacks the so-called PKR-binding domain, binds to OAS proteins and there by counteract the antiviral activity of IFN-α (Taguchi et al., 2004). Furthermore, it has been demonstrated that purified recombinant RNAse L and that from HeLa cell extracts efficiently cleaves HCV RNA *in vitro* (Han et al., 2004). However, further investigations are needed to determine whether RNase L also cleaves HCV RNAs in infected HCV host cells and to what extent an OAS-induced block of HCV protein translation contributes to the IFN-induced inhibition of HCV RNA replication.

ADAR1

ADAR1 forms together with ADAR2 and the less extensively studied ADAR3 protein a small family of constitutively expressed adenosine deaminases that act on

RNA (reviewed in Valente & Nishikura, 2005; Toth et al., 2006). ADAR1 and ADAR2 bind highly structured RNAs and catalyze the hydrolytic C6 deamination of adenosine, a reaction that converts adenosine to inosine (A to I editing). ADAR-mediated editing may occur selectively at one or a few positions or, more frequently, at a larger number of sites (hyperediting or hypermutation). A to I exchanges may have severe consequences: (1) editing of coding sequences may lead to amino acid exchanges because I is recognized as G by the translational machinery (of note, A to I editing does not create stop codons); (2) editing of noncoding regions may affect RNA splicing, stability, or translational efficiency (e.g., by disrupting AU base pairs); (3) editing may regulate gene silencing (e.g., by disrupting AU base pairs); and (4) hyperedited RNA may be recognized and cleaved by an I-specific RNase (Scadden & Smith, 1997, 2001).

A prominent example of a cellular RNA that is edited by ADAR proteins is the mRNA of the alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptor subunit GluR-2. ADAR2 edits a codon in exon 11, which results in an amino acid exchange that changes the Ca^{2+} permeability of the receptor. This highly specific editing event has far-reaching consequences. ADAR2 knockout mice are prone to seizures and die young. The impaired phenotype appears to result entirely from a single underedited position in the GluR-2 mRNA, as it reverted to normal when both alleles for the underedited transcript were substituted with alleles encoding the edited version exonically (Higuchi et al., 2000). Likewise, genetic targeting of the *Adar1* locus revealed an essential requirement for this ADAR protein in the embryogenesis of mice (Wang et al., 2000, 2004; Hartner et al., 2004), and it has been suggested that its expression protects against stress-induced apoptosis (Wang et al., 2004).

A closer look at the *Adar1* gene locus revealed that protein expression is controlled by three promoters and alternative splicing (reviewed in Toth et al., 2006). Two constitutively active promoters drive the expression of a ~110-kDa protein (p110), whereas an IFN-regulated promoter with an ISRE controls the expression of a larger isoform (p150) that is expressed in response to inflammation or IFN treatment (Patterson & Samuel, 1995; George & Samuel, 1999; Yang et al., 2003a, 2003b). Both isoforms contain multiple nuclear localization signals, but only the IFN-induced p150 isoform has a nuclear export signal. Accordingly, p110 is a nuclear protein and p150 has been detected in both nuclear and cytoplasmic compartments.

Hypermutation of viral RNAs has been observed for several RNA viruses, including *Measles virus*, *Parainfluenza virus 3* (both family *Paramyxoviridae*), and VSV (O'Hara et al., 1984; Cattaneo et al., 1988; Murphy et al., 1991). It has been speculated that subacute sclerosing panencephalitis (SSPE), a fatal necropathic response in patients with a persistent measles virus infection of the brain, is associated with extensive editing of the matrix protein mRNA. This prevents virion assembly and release because these steps in the viral life cycle require a functional matrix protein. Other transcripts, however, are less frequently edited, which is thought to result in a persistent virus replication (Cattaneo et al., 1989; Baczko et al., 1993). Thus, an incomplete ADAR-mediated innate immune response might contribute to the pathology of SSPE.

Interestingly, certain viruses abuse ADAR proteins to control important checkpoints in replication and particle formation. A well-known example is the *Hepatitis D virus* (HDV), a subviral human pathogen that depends on *Hepatitis B virus* as a helper virus (reviewed in Casey, 2006). HDV has a small, circular RNA genome that encodes only a single protein, the hepatitis delta antigen (HDAg). Without editing, a 195-amino acid version of HDAg is made that is essential for virus replication (Kuo et al., 1989). Later on, in the viral life cycle a highly specific A to I editing event changes a UAG amber stop codon to an UIG tryptophan codon, and a 214-amino acid HDAg-L is produced that mediates genome packaging (Chang et al., 1991).

HCV RNAs may also be subject to ADAR-mediated modifications, but in this case, editing seems to be less specific and to inhibit virus replication. It has recently been reported that the silencing of ADAR1 expression in HCV replicon cells increases the amount of HCV RNA about 40-fold (Taylor et al., 2005). Moreover, Taylor and co-workers noted that IFN-α increases the frequency of A to G mutations in subgenomic replicon RNAs and that the transfection of ADAR-specific siRNAs rescues HCV RNA replication in the presence of moderate IFN-α concentrations. Based on these findings, Taylor et al. concluded that IFN-α inhibits HCV replication through ADAR-mediated hyperediting of viral RNA. In our laboratory, we have used specific antibodies to determine the intracellular localization of p150 and — despite its nuclear export signal —, we observed that p150 accumulates predominantly in the nucleus of IFN-treated Huh-7 cells. In the presence of subgenomic or full-length HCV RNAs, however, we observed that p150 localizes to distinct cytoplasmic structures (E. Dazert, R. Bartenschlager, and M. Frese, unpublished results). We also analyzed the antiviral effect of constitutively expressed p150 on HCV replication and found that the overexpression of p150 in Huh-7 cells did not block HCV RNA replication. Taken together, our findings support the idea of Taylor et al. that p150 interacts with HCV RNAs, but we argue that p150 does that only in the context off other IFN-induced proteins. Further studies are under way to fully elucidate the role of p150 in the IFN-induced inhibition of HCV replication.

PKR

Another prominent protein of the innate immune defense that has long been suspected of interfering with HCV replication is the double-stranded RNA-activated protein kinase PKR. This serine/threonine kinase is constitutively expressed and has multiple functions in the control of host cell transcription and translation (reviewed in Garcia et al., 2006). Upon stimulation with IFNs, most cells respond by increasing the expression of PKR. IFN-induced PKR accumulates in the cytoplasm and was found in association with ribosomes (Thomis et al., 1992). PKR may exert its antiviral activity through different pathways (reviewed in Toth et al., 2006). First, PKR is able to control the cellular translation machinery through phosphorylation of the α subunit of the eukaryotic translation initiation factor eIF-2α, which would affect the production of both host and virus proteins. Second, PKR-mediated phosphorylation is implicated in several signaling pathways that contribute to the establishment

of a robust antiviral response. For example, PKR has been shown to activate the latent transcription factor NFκB, which may lead to the enhanced expression of pro-inflammatory genes (Gil et al., 2004). In addition, PKR may activate other kinases such as the p38 mitogen-activated protein (MAP) kinase, which further intensifies and diversifies the innate immune response (Goh et al., 2000).

The concept that PKR-mediated phosphorylation events play an important role in the innate immune response against viral infections is largely based on the fact that many RNA and DNA viruses try to inhibit PKR by (1) overexpressing small RNAs that bind to but do not activate PKR, (2) producing eIF-2α decoys, and (3) enhancing PKR degradation (reviewed in Langland et al., 2006). If PKR is a key player in IFN-induced antiviral defense, genetically targeted knockout mice that lack functional PKR proteins should be extremely sensitive to viral infections. Two lines of $PKR^{-/-}$ mice have been generated in which the coding sequences of either the N-terminal or the C-terminal part of the protein have been disrupted (Yang et al., 1995; Abraham et al., 1999, respectively). $PKR^{-/-}$ mice are indeed more susceptible to certain virus infections than wild-type animals (Balachandran et al., 2000; Stojdl et al., 2000; Carr et al., 2006; Samuel et al., 2006), but at least in some cases, this seems to depend on the mouse strain used, and other experimental conditions (Murphy et al., 2003). Additional experiments have been conducted by using MEFs from $PKR^{-/-}$ mice, but a direct antiviral activity of PKR (e.g., the inhibition of virus multiplication by blocking protein translation) is still controversial. Interestingly, priming of $PKR^{-/-}$ mice with poly(I)-poly(C) or IFNs before the virus challenge points to a rather indirect mode of PKR action, such as the enhancement of double-stranded RNA-induced signaling events (Yang et al., 1995). However, it should be noted that most of these experiments have been performed with viruses that encode PKR inhibitors. It would be interesting to re-evaluate the phenotype of $PKR^{-/-}$ mice with genetically modified viruses that cannot express functional PKR inhibitors. Another problem in the characterization of $PKR^{-/-}$ mice is the presence of related kinases that also phosphorylate eIF-2α (Toth et al., 2006). Even if these kinases differ from PKR in their response to double-stranded RNA and/or other physiological stress signals, they may partially substitute for the lack of PKR in $PKR^{-/-}$ mice, thereby making it difficult to quantify the contribution of PKR to the innate immune response (as exemplified in Smith et al., 2005).

Two HCV proteins have been described as interacting with the kinase. By analyzing HCV sequences from Japanese hepatitis C patients, mutations within a discrete region of NS5A, the so-called IFN sensitivity determining region (ISDR), were proposed to confer resistance to IFN-α (Enomoto et al., 1995, 1996). Since the original reports by Enomoto and co-workers, numerous studies have been conducted in Japan as well as in other countries to determine the predictive value of NS5A sequences in the outcome of IFN-based therapies, but the existence of an ISDR is still controversial (reviewed in Tan & Katze, 2001; reinvestigated by Pascu et al., 2004; Brillet et al., 2007). Whether or not an ISDR really exists, the description of such a sequence put NS5A in the focus of HCV research. The subsequent finding that mutations in the ISDR affect the ability of NS5A to bind to and inhibit PKR (Gale et al., 1998) led to the hypothesis that PKR blocks HCV replication and that NS5A is able to counteract the antiviral activity of PKR. However, experiments

with HCV replicons provided no further evidence for an involvement of NS5A in IFN resistance. On the contrary, point mutations within the ISDR or a deletion of 47 amino acids encompassing the entire ISDR enhanced viral replication without affecting the IFN sensitivity of HCV replicons (Blight et al., 2000; Guo et al., 2001). These findings were extended by A. Kaul and R. Bartenschlager, who analyzed the function of NS5A by using two subgenomic genotype 1b replicons that differ only in the NS5A coding sequence. In one replicon, the ISDR was identical to that of IFN-susceptible strains, whereas the ISDR sequence of the other replicon contained mutations that have been suspected to confer PKR binding and IFN resistance (Gale et al., 1998). Despite these differences, both replicons were found to be equally sensitive to IFN-α (unpublished results). This result argues against the hypothesis that NS5A counteracts an IFN-induced and PKR-mediated block of viral protein translation. However, the result does not contradict the idea that NS5A inhibits other activities of PKR (e.g., a PKR-mediated priming of intracellular signaling pathways). Of note, several reports suggest that NS5A may sabotage the innate immune response through PKR-independent activities (discussed in MacDonald & Harris, 2004). For example, it has been reported that NS5A increases the production of interleukin (IL)-8, thereby attenuating the antiviral properties of IFNs (Polyak et al., 2001a, 2001b). It would be interesting to study the immunomodulatory activities of NS5A in an immunocompetent small animal model, which might finally put an end to the discussion about the role of PKR in HCV pathology.

A second HCV protein has been reported to interact with PKR. It was found that E2 binds to PKR through its PKR-eIF2α homology domain (PePHD) (Taylor et al., 1999, 2001; Pavio et al., 2002). However, the significance of this observation has been questioned because an increasing number of clinical studies demonstrate that the PePHD is a highly conserved region with no conspicuous mutations accumulating during IFN-α therapy (reviewed in Tan & Katze, 2001). Furthermore, E2 expression does not increase the resistance of HCV genotype 1b replicons toward IFNs. A genomic replicon that encodes an E2 protein with the PePHD sequence of a resistant HCV isolate had a similar degree of susceptibility as a subgenomic replicon lacking E2 (Frese et al., 2002; A. Kaul and R. Bartenschlager, unpublished results).

The adenovirus-associated RNA I (VA_I), a small, highly structured RNA that binds to PKR and but does not trigger its dimerization and activation, has recently been found to stimulate HCV RNA replication in the replicon system (Taylor et al., 2005). It was also reported that recombinant VA_I RNA efficiently rescues HCV RNA replication in the presence of as much as 500 IU/ml of IFN-α (Taylor et al., 2005). Since VA_I RNAs may also bind to other proteins of the innate immune response such as ADAR1, more research is needed to define the role of PKR in limiting HCV protein translation.

A more direct approach to the question of PKR interference with HCV RNA replication/translation has recently been undertaken by using RNA silencing. A. Kaul and R. Bartenschlager transfected cells containing subgenomic HCV replicons with siRNAs that target PKR mRNAs for degradation and subsequently treated the cells with different concentrations of IFN-α. In no case did they observe that a downregulation of PKR expression levels results in a restoration of HCV replication in the presence of IFN (unpublished results).

With the establishment of a new generation of HCV replicons that contain the consensus sequence from a Japanese genotype 2a isolate and replicate efficiently without the need for adaptive mutations, it became possible to study HCV RNA replication in a variety of new host cells including those of nonhepatic and non-human origin (Kato et al., 2005; Uprichard et al., 2006). Most recently, genotype 2a replicons were employed by Chang and co-workers, who set out to analyze the antiviral effect of type I IFNs on HCV replication in MEFs from $PKR^{-/-}$ mice and congenic wild-type mice. Interestingly, IFN-α as well as IFN-β inhibited HCV RNA replication in $PKR^{-/-}$ MEFs as efficiently as in $PKR^{+/+}$ MEFs (Chang et al., 2006), suggesting that PKR-mediated translational control plays only a minor role in the IFN-induced inhibition of HCV RNA replication.

NOS2 and Other Effector Proteins

The inducible nitric oxide (NO) synthetase, originally named iNOS but also abbreviated as NOS2, belongs to a small family of NO-producing enzymes. In unstimulated cells, NOS2 is virtually absent, but expression levels increase rapidly in response to pro-inflammatory cytokines, especially IFN-γ. The expression of NOS2 results in a long-lasting production of NO (Karupiah et al., 2000). The NO free radical has been recognized for its strong antimicrobial activity against various protozoa, bacteria, and viruses. For example, the replication of a coxsackievirus is suppressed by NO through inactivation of the viral cysteine protease by S-nitrosylation (Saura et al., 1999). Furthermore, NO production is essential for the T cell-mediated noncytopathic inhibition of *Hepatitis B virus* replication in virus-transgenic mice (Guidotti et al., 2000). Other viruses that have been reported as sensitive to NO include *Severe acute respiratory coronavirus* (Akerstrom et al., 2005), *Respiratory syncytial virus* (Stark et al., 2005), *Mouse hepatitis virus* (Pope et al., 1998), and *Herpes simplex virus type 1* (Adler et al., 1997). However, the use of NO by the infected host as an antimicrobial substance is a double-edged sword. NO-induced oxidative stress may cause severe cellular and organ dysfunction. Influenza A virus-infected wild-type mice, for example, suffer from an excessive production of NO in the lungs, which often leads to respiratory failure and death, whereas knockout mice that cannot express functional NOS2 survive the infection with little evidence of pneumonitis (Akaike et al., 1996; Karupiah et al., 1998).

In the liver of most HCV-infected individuals, NOS2 is easily detectable (Mihm et al., 1997; Majano et al., 1998; Schweyer et al., 2000). The enhanced expression of NOS2 in the liver of hepatitis C patients has largely been attributed to IFN-γ that is released by resident and infiltrating immune cells. In addition, it has been speculated that the HCV replication itself may stimulate the expression of NOS2 in infected hepatocytes (Machida et al., 2004). Based on genetic studies, it has been suggested that the production of NO is involved in HCV clearance, as certain NOS2 haplotypes were more frequently found among HCV-infected individuals who spontaneously cleared the infection and in IFN-treated hepatitis C patients who could mount a sustained antiviral response (Yee et al., 2004). This hypothesis,

however, lacks supporting evidence from cell culture experiments. The treatment of Huh-7 cells with the NO donor (Z)-1-[2-(aminoethyl)-N-(2-ammonioethyl)-amino]diazen-1-ium-1,2-diolate (DETA NONOate) or the arginase inhibitor NG-hydroxy-L-arginine (NOHA) did not result in an inhibition of HCV RNA replication (Frese et al., 2002). Furthermore, the NOS inhibitor L-N6-(1-iminoethyl)-lysine (L-NIL) did not even partially restore HCV replication in the presence of IFN-γ (Frese et al., 2002). One should, however, not overinterpret these results. The *in vivo* production of NO may have more complex consequences than those that could be investigated in cell culture. NO may act as a messenger rather than as an effector molecule, or NO may induce DNA damage and apoptosis (Jaiswal et al., 2000). Thus, further studies are needed to fully elucidate the role of NO in hepatitis C pathology.

So far, only a few further IFN-induced effector proteins have been investigated with respect to their potential to inhibit HCV RNA replication, most notably indoleamine 2,3-dioxygenase (IDO). The IDO-mediated depletion of tryptophan is well known as a defense mechanism against certain intracellular parasites (Carlin et al., 1989). Rather recently, this pathway has also been recognized as an IFN-γ-induced antiviral defense mechanism against herpesviruses (Bodaghi et al., 1999) and poxviruses (Terajima & Leporati, 2005). If IDO inhibits HCV replication as well, inhibition of the effector protein or addition of tryptophan to the cell culture medium should restore viral protein synthesis. However, the IDO inhibitor α-methyl-DL-tryptophan did not restore the replication of subgenomic HCV replicons in the presence of IFN-γ (Frese et al., 2002). Likewise, increased concentrations of L-tryptophan could not rescue the viral protein synthesis (Frese et al., 2002), suggesting that the depletion of tryptophan is not—or is not the only—mechanism by which IFN-γ inhibits the replication of HCV RNAs.

Beside the induction of direct antiviral activities in infected host cells, IFNs may enhance and direct the activities of NK cells and T cells. Such indirect effects are usually ascribed to IFN-γ, but Shin and co-workers noted that type I IFNs also stimulate the generation of immunoproteasomes in the liver of hepatitis C patients (Shin et al., 2006). It would be interesting to determine the extent to which these indirect effects contribute to the antiviral activity of type I IFNs.

Acknowledgments We are indebted to Kerry Mills, Sandra Thomas, Ali Zaid, Friedemann Weber, Brett Lidbury and Ralf Bartenschlager for helpful suggestions and careful reading of the manuscript; and Artur Kaul and R. Bartenschlager for the communication of unpublished results.

References

Abraham, N., Stojdl, D.F., Duncan, P.I., Methot, N., Ishii, T., Dube, M., Vanderhyden, B.C., Atkins, H.L., Gray, D.A., McBurney, M.W., Koromilas, A.E., Brown, E.G., Sonenberg, N., Bell, J.C. (1999). Characterization of transgenic mice with targeted disruption of the catalytic domain of the double-stranded RNA-dependent protein kinase, PKR. *Journal of Biological Chemistry,* 274: 5953–5962.

Adler, H., Beland, J.L., Del-Pan, N.C., Kobzik, L., Brewer, J.P., Martin, T.R., Rimm, I.J. (1997). Suppression of herpes simplex virus type 1 (HSV-1)-induced pneumonia in mice by inhibition of inducible nitric oxide synthase (iNOS, NOS2). *Journal of Experimental Medicine,* 185: 1533–1540.

Akaike, T., Noguchi, Y., Ijiri, S., Setoguchi, K., Suga, M., Zheng, Y.M., Dietzschold, B., Maeda, H. (1996). Pathogenesis of influenza virus-induced pneumonia: involvement of both nitric oxide and oxygen radicals. *Proceedings of the National Academy of Sciences of the United States of America,* 93: 2448–2453.

Akerstrom, S., Mousavi-Jazi, M., Klingstrom, J., Leijon, M., Lundkvist, A., Mirazimi, A. (2005). Nitric oxide inhibits the replication cycle of severe acute respiratory syndrome coronavirus. *Journal of Virology,* 79: 1966–1969.

Ali, S., Pellerin, C., Lamarre, D., Kukolj, G. (2004). Hepatitis C virus subgenomic replicons in the human embryonic kidney 293 cell line. *Journal of Virology,* 78: 491–501.

Balachandran, S., Roberts, P.C., Brown, L.E., Truong, H., Pattnaik, A.K., Archer, D.R., Barber, G.N. (2000). Essential role for the dsRNA-dependent protein kinase PKR in innate immunity to viral infection. *Immunity,* 13: 129–141.

Bartenschlager, R., Frese, M., Pietschmann, T. (2004). Novel insights into hepatitis C virus replication and persistence. *Advances in Virus Research,* 63: 71–180.

Baczko, K., Lampe, J., Liebert, U.G., Brinckmann, U., ter Meulen, V., Pardowitz, I., Budka, H., Cosby, S.L., Isserte, S., Rima, B.K. (1993). Clonal expansion of hypermutated measles virus in an SSPE brain. *Virology*, 197: 188–195.

Bigger, C.B., Brasky, K.M., Lanford, R.E. (2001). DNA microarray analysis of chimpanzee liver during acute resolving hepatitis C virus infection. *Journal of Virology*, 75: 7059–7066.

Blight, K.J., Rice, C.M. (1997). Secondary structure determination of the conserved 98-base sequence at the 3′ terminus of hepatitis C virus genome RNA. *Journal of Virology*, 71: 7345–7352.

Blight, K.J., Kolykhalov, A.A., Rice, C.M. (2000). Efficient initiation of HCV RNA replication in cell culture. *Science,* 290: 1972–1974.

Bodaghi, B., Goureau, O., Zipeto, D., Laurent, L., Virelizier, J.-L., Michelson, S. (1999). Role of IFN-induced indoleamine 2,3 dioxygenase and inducible nitric oxide synthase in the replication of human cytomegalovirus in retinal pigment epithelial cells. *Journal of Immunology,* 162: 957–964.

Brillet, R., Penin, F., Hezode, C., Chouteau, P., Dhumeaux, D., Pawlotsky, J.M. (2007). The nonstructural 5A protein of hepatitis C virus genotype 1b does not contain an interferon sensitivity-determining region. *Journal of Infectious Diseases,* 195: 432–441.

Carlin, J.M., Ozaki, Y., Byrne, G.I., Brown, R.R., Borden, E.C. (1989). Interferons and indoleamine 2,3-dioxygenase: role in antimicrobial and antitumor effects. *Experientia,* 45: 535–541.

Carr, D.J., Wuest, T., Tomanek, L., Silverman, R.H., Williams, B.R. (2006). The lack of RNA-dependent protein kinase enhances susceptibility of mice to genital herpes simplex virus type 2 infection. *Immunology,* 118: 520–526.

Casey, J.L. (2006). RNA editing in hepatitis delta virus. *Current Topics in Microbiology and Immunology,* 307: 67–89.

Cattaneo, R., Schmid, A., Eschle, D., Baczko, K., ter Meulen, V., Billeter, M.A. (1988). Biased hypermutation and other genetic changes in defective measles viruses in human brain infections. *Cell,* 55: 255–265.

Cattaneo, R., Schmid, A., Spielhofer, P., Kaelin, K., Baczko, K., ter Meulen, V., Pardowitz, J., Flanagan, S., Rima, B.K., Udem, S.A., Billeter, B.A. (1989). Mutated and hypermutated genes of persistent measles viruses which caused lethal human brain diseases. *Virology,* 173: 415–425.

Chang, F.L., Chen, P.J., Tu, S.J., Wang, C.J., Chen, D.S. (1991). The large form of hepatitis delta antigen is crucial for assembly of hepatitis delta virus. *Proceedings of the National Academy of Sciences of the United States of America,* 88: 8490–8494.

Chang, K.S., Cai, Z., Zhang, C., Sen, G.C., Williams, B.R., Luo, G. (2006). Replication of hepatitis C virus (HCV) RNA in mouse embryonic fibroblasts: protein kinase R (PKR)-dependent and

PKR-independent mechanisms for controlling HCV RNA replication and mediating interferon activities. *Journal of Virology,* 80: 7364–7374.

Chebath, J., Benech, P., Revel, M., Vigneron, M. (1987). Constitutive expression of (2′-5′) oligo A synthetase confers resistance to picornavirus infection. *Nature*, 330: 587–588.

Cheney, I.W., Lai, V.C., Zhong, W., Brodhag, T., Dempsey, S., Lim, C., Hong, Z, Lau, J.Y., Tam, R.C. (2002). Comparative analysis of anti-hepatitis C virus activity and gene expression mediated by alpha, beta, and gamma interferons. *Journal of Virology,* 76: 11148–11154.

Chieux, V., Hober, D., Harvey, J., Lion, G., Lucidarme, D., Forzy, G., Duhamel, M., Cousin, J., Ducoulombier, H., Wattre, P. (1998). The MxA protein levels in whole blood lysates of patients with various viral infections. *Journal of Virological Methods,* 70: 183–191.

Colonna, M., Krug, A., Cella, M. (2002). Interferon-producing cells: on the front line in immune responses against pathogens. *Current Opinion in Immunology,* 14: 373–379.

Donnelly, R.P., Sheikh, F., Kotenko, S.V., Dickensheets, H. (2004). The expanded family of class II cytokines that share the IL-10 receptor-2 (IL-10R2) chain. *Journal of Leukocyte Biology,* 76: 314–321.

Doyle, S.E., Schreckhise, H., Khuu-Duong, K., Henderson, K., Rosler, R., Storey, H., Yao, L., Liu, H., Barahmandpour, F., Sivakumar, P., Chan, C., Birks, C., Foster, D., Clegg, C.H., Wietzke-Braun, P., Mihm, S., Klucher, K.M. (2006). Interleukin-29 uses a type 1 interferon-like program to promote antiviral responses in human hepatocytes. *Hepatology*, 44: 896–906.

Enomoto, N., Sakuma, I., Asahina, Y., Kurosaki, M., Murakami, T., Yamamoto, C., Izumi, N., Marumo, F., Sato, C. (1995). Comparison of full-length sequences of interferon-sensitive and resistant hepatitis C virus 1b. Sensitivity to interferon is conferred by amino acid substitutions in the NS5A region. *Journal of Clinical Investigation,* 96: 224–230.

Enomoto, N., Sakuma, I., Asahina, Y., Kurosaki, M., Murakami, T., Yamamoto, C., Ogura, Y., Izumi, N., Marumo, F., Sato, C. (1996). Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. *New England Journal of Medicine,* 334: 77–81.

Floyd-Smith, G., Slattery, E., Lengyel, P. (1981). Interferon action: RNA cleavage pattern of a (2′-5′)oligoadenylate-dependent endonuclease. *Science,* 212: 1030–1032.

Foy, E., Li, K., Wang, C., Sumpter, R., Jr., Ikeda, M., Lemon, S.M., Gale, M., Jr. (2003). Regulation of interferon regulatory factor-3 by the hepatitis C virus serine protease. *Science,* 300: 1145–1148.

Frese, M., Kochs, G., Meier-Dieter, U., Siebler, J., Haller, O. (1995). Human MxA protein inhibits tick-borne Thogoto virus but not Dhori virus. *Journal of Virology,* 69: 3904–3909.

Frese, M., Pietschmann, T., Moradpour, D., Haller, O., Bartenschlager, R. (2001). Interferon-alpha inhibits hepatitis C virus subgenomic RNA replication by an MxA-independent pathway. *Journal of General of Virology,* 82: 723–733.

Frese, M., Schwärzle, V., Barth, K., Krieger, N., Lohmann, V., Mihm, S., Haller, O., Bartenschlager, R. (2002). Interferon-gamma inhibits replication of subgenomic and genomic hepatitis C virus RNAs. *Hepatology,* 35: 694–703.

Frese, M., Barth, K., Kaul, A., Lohmann, V., Schwärzle, V., Bartenschlager, R. (2003). Hepatitis C virus RNA replication is resistant to tumour necrosis factor-alpha. *Journal of General of Virology,* 84: 1253–1259.

Friebe, P., Boudet, J., Simorre, J.P., Bartenschlager, R. (2005). Kissing-loop interaction in the 3′ end of the hepatitis C virus genome essential for RNA replication. *Journal of Virology,* 79: 380–392.

Fried, M.W., Shiffman, M.L., Reddy, K.R., Smith, C., Marinos, G., Goncales, F.L., Jr., Haussinger, D., Diago, M., Carosi, G., Dhumeaux, D., Craxi, A., Lin, A., Hoffman, J., Yu, J. (2002). Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *New England Journal of Medicine,* 347: 975–982.

Gale, M., Jr., Blakely, C.M., Kwieciszewski, B., Tan, S.L., Dossett, M., Tang, N.M., Korth, M.J., Polyak, S.J., Gretch, D.R., Katze, M.G. (1998). Control of PKR protein kinase by hepatitis C virus nonstructural 5A protein: molecular mechanisms of kinase regulation. *Molecular and Cellular Biology,* 18: 5208–5218.

Garcia, M.A., Gil, J., Ventoso, I., Guerra, S., Domingo, E., Rivas, C., Esteban, M. (2006). Impact of protein kinase PKR in cell biology: from antiviral to antiproliferative action. *Microbiology and Molecular Biology Reviews,* 70: 1032–1060.

George, C.X., Samuel, C.E. (1999). Human RNA-specific adenosine deaminase ADAR1 transcripts possess alternative exon 1 structures that initiate from different promoters, one constitutively active and the other interferon inducible. *Proceedings of the National Academy of Sciences of the United States of America,* 96: 4621–4626.

Ghosh, A., Sarkar, S.N., Sen, G.C. (2000). Cell growth regulatory and antiviral effects of the P69 isozyme of 2-5 (A) synthetase. *Virology,* 266: 319–328.

Gil, J., Garcia, M.A., Gomez-Puertas, P., Guerra, S., Rullas, J., Nakano, H., Alcami, J., Esteban, M. (2004). TRAF family proteins link PKR with NF-kappa B activation. *Molecular and Cellular Biology,* 24: 4502–4012.

Gilli, F., Sala, A., Bancone, C., Salacone, P., Gallo, M., Gaia, E., Bertolotto, A. (2002). Evaluation of IFN-alpha bioavailability by MxA mRNA in HCV patients. *Journal of Immunological Methods,* 262: 187–190.

Goh, K.C., deVeer, M.J., Williams, B.R. (2000). The protein kinase PKR is required for p38 MAPK activation and the innate immune response to bacterial endotoxin. *EMBO Journal,* 19: 4292–4297.

Goodbourn, S., Didcock, L., Randall, R.E. (2000). Interferons: cell signalling, immune modulation, antiviral response and virus countermeasures. *Journal of General Virology*, 81: 2341–2364.

Guidotti, L.G., Chisari, F.V. (2001). Noncytolytic control of viral infections by the innate and adaptive immune response. *Annual Review of Immunology,* 19: 65–91.

Guidotti, L.G., McClary, H., Loudis, J.M., Chisari, F.V. (2000). Nitric oxide inhibits hepatitis B virus replication in the livers of transgenic mice. *Journal of Experimental Medicine,* 191: 1247–1252.

Guo, J.T., Bichko, V.V., Seeger, C. (2001). Effect of alpha interferon on the hepatitis C virus replicon. *Journal of Virology,* 75: 8516–8523.

Guo, J.T., Zhu, Q., Seeger, C. (2003). Cytopathic and noncytopathic interferon responses in cells expressing hepatitis C virus subgenomic replicons. *Journal of Virology,* **77:** 10769–10779.

Haller, O., Frese, M., Kochs, G. (1999). Mx proteins: mediators of innate resistance to RNA viruses. *Revue Scientific et Technique,* 17: 220–230.

Haller, O., Staeheli, P., Kochs, G. (2007). Interferon-induced Mx proteins in antiviral host defense. Biochimie (in press).

Haller, O., Kochs, G. (2002). Interferon-induced Mx proteins: dynamin-like GTPases with antiviral activity. *Traffic,* 3: 710–717.

Han, J.Q., Wroblewski, G., Xu, Z., Silverman, R.H., Barton, D.J. (2004). Sensitivity of hepatitis C virus RNA to the antiviral enzyme ribonuclease L is determined by a subset of efficient cleavage sites. *Journal of Interferon and Cytokine Research,* 24: 664–676.

Hartner, J.C., Schmittwolf, C., Kispert, A., Muller, A.M., Higuchi, M., Seeburg, P.H. (2004). Liver disintegration in the mouse embryo caused by deficiency in the RNA-editing enzyme ADAR1. *Journal of Biological Chemistry,* 279: 4894–4902.

Hayashi, J., Stoyanova, R., Seeger, C. (2005). The transcriptome of HCV replicon expressing cell lines in the presence of alpha interferon. *Virology,* 335: 264–275.

Hefti, H.P., Frese, M., Landis, H., Di Paolo, C., Aguzzi, A., Haller, O., Pavlovic, J. (1999). Human MxA protein protects mice lacking a functional alpha/beta interferon system against La Crosse virus and other lethal viral infections. *Journal of Virology,* 73: 6984–6991.

Higuchi, M., Maas, S., Single, F.N., Hartner, J., Rozov, A., Burnashev, N., Feldmeyer, D., Sprengel, R., Seeburg, P.H. (2000). Point mutation in an AMPA receptor gene rescues lethality in mice deficient in the RNA-editing enzyme ADAR2. *Nature,* 406: 78–81.

Hijikata, M., Ohta, Y., Mishiro, S. (2000). Identification of a single nucleotide polymorphism in the *MxA* gene promoter (G/T at nt -88) correlated with the response of hepatitis C patients to interferon. *Intervirology,* 43: 124–127.

Hijikata, M., Mishiro, S., Miyamoto, C., Furuichi, Y., Hashimoto, M., Ohta, Y. (2001). Genetic polymorphism of the *MxA* gene promoter and interferon responsiveness of hepatitis C patients: revisited by analyzing two SNP sites (-123 and -88) *in vivo* and *in vitro*. *Intervirology,* 44: 379–382.

Hiscott, J., Lin, R., Nakhaei, P., Paz, S. (2006). MasterCARD: a priceless link to innate immunity. *Trends in Molecular Medicine,* 12: 53–56.

Hoenen, A., Liu, W., Kochs, G., Khromykh, A.A., Mackenzie, J.M. (2007). West Nile virus-induced cytoplasmic membrane structures provide partial protection aginst the interferon-induced antiviral MxA protein. *Journal of General Virology,* (in press).

Holzinger, D., Jorns, C., Sterz, S. Boisson-Dupuis, S. Thimme, R., Weidmann, M., Casanova, J.L., Haller, O., Kochs, G. (2007). Induction of MxA gene expression by influenza A virus requires type I or type II interferon signaling. Journal of Virology, 81: 7776–7785.

Honda, M., Beard, M.R., Ping, L.H., Lemon, S.M. (1999). A phylogenetically conserved stem-loop structure at the 5′ border of the internal ribosome entry site of hepatitis C virus is required for cap-independent viral translation. *Journal of Virology,* 73: 1165–1174.

Jaiswal, M., LaRusso, N.F., Burgart, L.J., Gores, G.J. (2000). Inflammatory cytokines induce DNA damage and inhibit DNA repair in cholangiocarcinoma cells by a nitric oxide-dependent mechanism. *Cancer Research,* 60: 184–190.

Jorns, C., Holzinger, D., Thimme, R., Spangenberg, H.C., Weidmann, M., Rasenack, J., Blum, H.E., Haller, O., Kochs, G. (2006). Rapid and simple detection of IFN-neutralizing antibodies in chronic hepatitis C non-responsive to IFN-alpha. *Journal of Medical Virology,* 78: 74–82.

Karupiah, G., Chen, J.H., Mahalingam, S., Nathan, C.F., MacMicking, J.D. (1998). Rapid interferon gamma-dependent clearance of influenza A virus and protection from consolidating pneumonitis in nitric oxide synthase 2-deficient mice. *Journal of Experimental Medicine,* 188: 1541–1546.

Karupiah, G., Hunt, N.H., King, N.J.C., Chaudhri, G. (2000). NADPH oxidase, Nramp1 and nitric oxide in the host microbiol response. *Reviews in Immunogenetics,* 2: 387–415.

Kato, T., Date, T., Miyamoto, M., Zhao, Z., Mizokami, M., Wakita, T. (2005). Nonhepatic cell lines HeLa and 293 support efficient replication of the hepatitis C virus genotype 2a subgenomic replicon. *Journal of Virology,* 79: 592–659.

Keskinen, P., Nyqvist, M., Sareneva, T., Pirhonen, J., Melen, K., Julkunen, I. (1999). Impaired antiviral response in human hepatoma cells. *Virology,* 263: 364–375.

Kim, Y.K., Lee, S.H., Kim, C.S., Seol, S.K., Jang, S.K. (2003). Long-range RNA-RNA interaction between the 5′ nontranslated region and the core-coding sequences of hepatitis C virus modulates the IRES-dependent translation. *RNA,* 9: 599–606.

Knapp, S., Yee, L.J., Frodsham, A.J., Hennig, B.J., Hellier, S., Zhang, L., Wright, M., Chiaramonte, M., Graves, M., Thomas, H.C., Hill, A.V., Thursz, M.R. (2003). Polymorphisms in interferon-induced genes and the outcome of hepatitis C virus infection: roles of MxA, OAS-1 and PKR. *Genes and Immunity*, 4: 411–419.

Kochs, G., Haller, O. (1999). Interferon-induced human MxA GTPase blocks nuclear import of Thogoto virus nucleocapsids. *Proceedings of the National Academy of Sciences of the United States of America,* 96: 2082–2086.

Kochs, G., Janzen, C., Hohenberg, H., Haller, O. (2002). Antivirally active MxA protein sequesters La Crosse virus nucleocapsid protein into perinuclear complexes. *Proceedings of the National Academy of Sciences of the United States of America,* 99: 3153–3158.

Kotenko, S.V., Gallagher, G., Baurin, V.V., Lewis-Antes, A., Shen, M., Shah, N.K., Langer, J.A., Sheikh, F., Dickensheets, H., Donnelly, R.P. (2003). IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. *Nature Immunology,* 4: 69–77.

Kuo, M.Y., Chao, M., Taylor, J. (1989). Initiation of replication of the human hepatitis delta virus genome from cloned DNA: role of delta antigen. *Journal of Virology,* 63: 1945–1950.

Lanford, R.E., Guerra, B., Lee, H., Averett, D.R., Pfeiffer, B., Chavez, D., Notvall, L., Bigger, C. (2003). Antiviral effect and virus-host interactions in response to alpha interferon, gamma interferon, poly(I)-poly(C), tumor necrosis factor alpha, and ribavirin in hepatitis C virus subgenomic replicons. *Journal of Virology,* 77: 1092–1104.

Langland, J.O., Cameron, J.M., Heck, M.C., Jancovich, J.K., Jacobs, B.L. (2006). Inhibition of PKR by RNA and DNA viruses. *Virus Research,* 119: 100–110.

Larkin, J., Jin, L., Farmen, M., Venable, D., Huang, Y., Tan, S.L., Glass, J.I. (2003). Synergistic antiviral activity of human interferon combinations in the hepatitis C virus replicon system. *Journal of Interferon and Cytokine Research,* 23: 247–257.

Layden, J.E., Layden, T.J. (2002). Viral kinetics of hepatitis C: new insights and remaining limitations. *Hepatology,* 35: 967–970.

Li, K., Foy, E., Ferreon, J.C., Nakamura, M., Ferreon, A.C., Ikeda, M., Ray, S.C., Gale, M., Jr., Lemon, S.M. (2005). Immune evasion by hepatitis C virus NS3/4A protease-mediated cleavage of the Toll-like receptor 3 adaptor protein TRIF. *Proceedings of the National Academy of Sciences of the United States of America,* 102: 2992–2997.

Li, Y., Zhang, T., Ho, C., Orange, J.S., Douglas, S.D., Ho, W.Z. (2004). Natural killer cells inhibit hepatitis C virus expression. *Journal of Leukocyte Biology,* 76: 1171–1179.

Lohmann, V., Körner, F., Koch, J.O., Herian, U., Theilmann, L., Bartenschlager, R. (1999). Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. *Science,* 285: 110–113.

MacDonald, A., Harris, M. (2004). Hepatitis C virus NS5A: tales of a promiscuous protein. *Journal of General Virology,* 85: 2485–2502.

Machida, K., Cheng, K.T., Sung, V.M., Lee, K.J., Levine, A.M., Lai, M.M. (2004). Hepatitis C virus infection activates the immunologic (type II) isoform of nitric oxide synthase and thereby enhances DNA damage and mutations of cellular genes. *Journal of Virology,* 78: 8835–8843.

MacQuillan, G.C., de Boer, W.B., Platten, M.A., McCaul, K.A., Reed, W.D., Jeffrey, G.P., Allan, J.E. (2002). Intrahepatic MxA and PKR protein expression in chronic hepatitis C virus infection. *Journal of Medical Virology,* 68: 197–205.

MacQuillan, G.C., Mamotte, C., Reed, W.D., Jeffrey, G.P., Allan, J.E. (2003). Upregulation of endogenous intrahepatic interferon stimulated genes during chronic hepatitis C virus infection. *Journal of Medical Virology,* 70: 219–227.

Majano, P.L., Garcia-Monzon, C., Lopez-Cabrera, M., Lara-Pezzi, E., Fernandez-Ruiz, E., Garcia-Iglesias, C., Borque, M.J., Moreno-Otero, R. (1998). Inducible nitric oxide synthase expression in chronic viral hepatitis. Evidence for a virus-induced gene upregulation. *Journal of Clinical Investigation,* 101: 1343–1352.

Manns, M.P., McHutchison, J.G., Gordon, S.C., Rustgi, V.K., Shiffman, M., Reindollar, R., Goodman, Z.D., Koury, K., Ling, M., Albrecht, J.K. (2001). Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet*, 358: 958–965.

Marcello, T., Grakoui, A., Barba-Spaeth, G., Machlin, E.S., Kotenko, S.V., Macdonald, M.R., Rice, C.M. (2006). Interferons alpha and lambda inhibit hepatitis C virus replication with distinct signal transduction and gene regulation kinetics. *Gastroenterology,* 131: 1887–1898.

Mashimo, T., Lucas, M., Simon-Chazottes, D., Frenkiel, M.P., Montagutelli, X., Ceccaldi, P.E., Deubel, V., Guenet, J.L., Despres, P. (2002). A nonsense mutation in the gene encoding 2′-5′-oligoadenylate synthetase/L1 isoform is associated with West Nile virus susceptibility in laboratory mice. *Proceedings of the National Academy of Sciences of the United States of America,* 99: 11311–11316.

McHutchison, J.G., Fried, M.W. (2003). Current therapy for hepatitis C: pegylated interferon and ribavirin. *Clinics in Liver Disease,* 7: 149–161.

Meylan, E., Curran, J., Hofmann, K., Moradpour, D., Binder, M., Bartenschlager, R., Tschopp, J. (2005). Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. *Nature,* 437: 1167–1172.

Mihm, S., Fayyazi, A., Ramadori, G. (1997). Hepatic expression of inducible nitric oxide synthase transcripts in chronic hepatitis C virus infection: relation to hepatic viral load and liver injury. *Hepatology,* 26: 451–458.

Mihm, S., Frese, M., Meier, V., Wietzke-Braun, P., Scharf, J.-G., Bartenschlager, R., Ramadori, G. (2004). Interferon type I gene expression in chronic hepatitis C. *Laboratory Investigation,* 84: 1148–1159.

Miyamoto, M., Kato, T., Date, T., Mizokami, M., Wakita, T. (2006). Comparison between subgenomic replicons of hepatitis C virus genotypes 2a (JFH-1) and 1b (Con1 NK5.1). *Intervirology,* 49: 37–43.

Mundt, E. (2007). Human MxA confers resistance to double-standed RNA viruses of two virus families. *Journal of General Virology,* 88: 1319–1323.

Murashima, S., Kumashiro, R., Ide, T., Miyajima, I., Hino, T., Koga, Y., Ishii, K., Ueno, T., Sakisaka, S., Sata, M. (2000). Effect of interferon treatment on serum 2′,5′-oligoadenylate synthetase levels in hepatitis C-infected patients. *Journal of Medical Virology,* 62: 185–190.

Murphy, D.G., Dimock, K., Kang, C.Y. (1991). Numerous transitions in human parainfluenza virus 3 RNA recovered from persistently infected cells. *Virology,* 181: 760–763.

Murphy, J.A., Duerst, R.J., Smith, T.J., Morrison, L.A. (2003). Herpes simplex virus type 2 virion host shutoff protein regulates alpha/beta interferon but not adaptive immune responses during primary infection in vivo. *Journal of Virology,* 77: 9337–9345.

Neumann, A.U., Lam, N.P., Dahari, H., Gretch, D.R., Wiley, T.E., Layden, T.J., Perelson, A.S. (1998). Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon-alpha therapy. *Science,* 282: 103–107.

O'Hara, P.J., Nichol, S.T., Horodyski, F.M., Holland, J.J. (1984). Vesicular stomatitis virus defective interfering particles can contain extensive genomic sequence rearrangements and base substitutions. *Cell,* 36: 915–924.

Okuse, C., Rinaudo, J.A., Farrar, K., Wells, F., Korba, B.E. (2005). Enhancement of antiviral activity against hepatitis C virus in vitro by interferon combination therapy. *Antiviral Research,* 65: 23–34.

Pascu, M., Martus, P., Hohne, M., Wiedenmann, B., Hopf, U., Schreier, E., Berg, T. (2004). Sustained virological response in hepatitis C virus type 1b infected patients is predicted by the number of mutations within the NS5A-ISDR: a meta-analysis focused on geographical differences. *Gut,* 53: 1345–1351.

Patterson, J.B., Samuel, C.E. (1995). Expression and regulation by interferon of a double-stranded-RNA-specific adenosine deaminase from human cells: evidence for two forms of the deaminase. *Molecular and Cellular Biology,* 15: 5376–5388.

Patzwahl, R., Meier, V., Ramadori, G., Mihm, S. (2001). Enhanced expression of interferon-regulated genes in the liver of patients with chronic hepatitis C virus infection: detection by suppression-subtractive hybridization. *Journal of Virology,* 75: 1332–1338.

Pavio, N., Taylor, D.R., Lai, M.M. (2002). Detection of a novel unglycosylated form of hepatitis C virus E2 envelope protein that is located in the cytosol and interacts with PKR. *Journal of Virology,* 76: 1265–1272.

Pavlovic, J., Arzet, H.A., Hefti, H.P., Frese, M., Rost, D., Ernst, B., Kolb, E., Staeheli, P., Haller, O. (1995). Enhanced virus resistance of transgenic mice expressing the human MxA protein. *Journal of Virology,* 69: 4506–4510.

Penna, G., Vulcano, M., Sozzani, S., Adorini, L. (2002). Differential migration behavior and chemokine production by myeloid and plasmacytoid dendritic cells. *Human Immunology,* 63: 1164–1171.

Perelygin, A.A., Scherbik, S.V., Zhulin, I.B., Stockman, B.M., Li, Y., Brinton, M.A. (2002). Positional cloning of the murine flavivirus resistance gene. *Proceedings of the National Academy of Sciences of the United States of America,* 99: 9322–9327.

Pestka, S., Krause, C.D., Walter, M.R. (2004). Interferons, interferon-like cytokines, and their receptors. *Immunological Reviews,* 202: 8–32.

Polyak, S.J., Khabar, K.S., Paschal, D.M., Ezelle, H.J., Duverlie, G., Barber, G.N., Levy, D.E., Mukaida, N., Gretch, D.R. (2001a). Hepatitis C virus nonstructural 5A protein induces interleukin-8, leading to partial inhibition of the interferon-induced antiviral response. *Journal of Virology,* 75: 6095–6106.

Polyak, S.J., Khabar, K.S., Rezeiq, M., Gretch, D.R. (2001b). Elevated levels of interleukin-8 in serum are associated with hepatitis C virus infection and resistance to interferon therapy. *Journal of Virology,* 75: 6209–6211.

Pope, M., Marsden, P.A., Cole, E., Sloan, S., Fung, L.S., Ning, Q., Ding, J.W., Leibowitz, J.L., Phillips, M.J., Levy, G.A. (1998). Resistance to murine hepatitis virus strain 3 is dependent on production of nitric oxide. *Journal of Virology,* 72: 7084–7090.

Rebouillat, D., Hovanessian, A.G. (1999). The human 2′,5′-oligoadenylate synthetase family: interferon-induced proteins with unique enzymatic properties. *Journal of Interferon and Cytokine Research,* 19: 295–308.

Reichelt, M., Stertz, S., Krijnse-Locker, J., Haller, O., Kochs, G. (2004). Missorting of LaCrosse virus nucleocapsid protein by the interferon-induced MxA GTPase involves smooth ER membranes. *Traffic,* 5: 772–784.

Robek, M.D., Boyd, B.S., Chisari, F.V. (2005). Lambda interferon inhibits hepatitis B and C virus replication. *Journal of Virology,* 79: 3851–3854.

Roers, A., Hochkeppel, H.K., Horisberger, M.A., Hovanessian, A., Haller, O. (1994). MxA gene expression after live virus vaccination: a sensitive marker for endogenous type I interferon. *Journal of Infectious Diseases,* 169: 807–813.

Samuel, C.E. (2001). Antiviral actions of interferons. *Clinical Microbiological Reviews,* 14: 778–809.

Samuel, M.A., Whitby, K., Keller, B.C., Marri, A., Barchet, W., Williams, B.R., Silverman, R.H., Gale, M., Jr., Diamond, M.S. (2006). PKR and RNase L contribute to protection against lethal West Nile Virus infection by controlling early viral spread in the periphery and replication in neurons. *Journal of Virology,* 80: 7009–7019.

Saura, M., Zaragoza, C., McMillan, A., Quick, R.A., Hohenadl, C., Lowenstein, J.M., Lowenstein, C.J. (1999). An antiviral mechanism of nitric oxide: inhibition of a viral protease. *Immunity,* 10: 21–28.

Scadden, A.D.J., Smith, C.W.J. (1997). A ribonuclease specific for inosine-containing RNA: a potential role in antiviral defense? *EMBO Journal,* 16: 2140–2149.

Scadden, A.D.J., Smith, C.W.J. (2001). Specific cleavage of hyper-edited dsRNAs. *EMBO Journal*, 20: 4243–4252.

Scherbik, S.V., Paranjape, J.M., Stockman, B.M., Silverman, R.H., Brinton, M.A. (2006). RNase L plays a role in the antiviral response to West Nile virus. *Journal of Virology,* 80: 2987–2999.

Schweyer, S., Mihm, S., Radzun, H.J., Hartmann, H., Fayyazi, A. (2000). Liver infiltrating T lymphocytes express interferon-gamma and inducible nitric oxide synthase in chronic hepatitis C virus infection. *Gut,* 46: 255–259.

Sheppard, P., Kindsvogel, W., Xu, W., Henderson, K., Schlutsmeyer, S., Whitmore, T.E., Kuestner, R., Garrigues, U., Birks, C., Roraback, J., Ostrander, C., Dong, D., Shin, J., Presnell, S., Fox, B., Haldeman, B., Cooper, E., Taft, D., Gilbert, T., Grant, F.J., Tackett, M., Krivan, W., McKnight, G., Clegg, C., Foster, D., Klucher, K.M. (2003). IL-28, IL-29, and their class II cytokine receptor IL-28R. *Nature Immunology,* 4: 63–68.

Shin, E.C., Seifert, U., Kato, T., Rice, C.M., Feinstone, S.M., Kloetzel, P.M., Rehermann, B. (2006). Virus-induced type I IFN stimulates generation of immunoproteasomes at the site of infection. *Journal of Clinical Investigation,* 116: 3006–3014.

Smith, J.A., Schmechel, S.C., Williams, B.R., Silverman, R.H., Schiff, L.A. (2005). Involvement of the interferon-regulated antiviral proteins PKR and RNase L in reovirus-induced shutoff of cellular translation. *Journal of Virology,* 79: 2240–2250.

Stark, J.M., Khan, A.M., Chiappetta, C.L., Xue, H., Alcorn, J.L., Colasurdo, G.N. (2005). Immune and functional role of nitric oxide in a mouse model of respiratory syncytial virus infection. *Journal of Infectious Diseases,* 191: 387–395.

Stojdl, D.F., Abraham, N., Knowles, S., Marius, R., Brasey, A., Lichty, B.D., Brown, E.G., Sonenberg, N., Bell, J.C. (2000). The murine double-stranded RNA-dependent protein kinase PKR is required for resistance to vesicular stomatitis virus. *Journal of Virology,* 74: 9580–9585.

Su, A.I., Pezacki, J.P., Wodicka, L., Brideau, A.D., Supekova, L., Thimme, R., Wieland, S., Bukh, J., Purcell, R.H., Schultz, P.G., Chisari, F.V. (2002). Genomic analysis of the host response to hepatitis C virus infection. *Proceedings of the National Academy of Sciences of the United States of America,* 99: 15669–15674.

Taguchi, T., Nagano-Fujii, M., Akutsu, M., Kadoya, H., Ohgimoto, S., Ishido, S., Hotta, H. (2004). Hepatitis C virus NS5A protein interacts with 2′,5′-oligoadenylate synthetase and inhibits antiviral activity of IFN in an IFN sensitivity-determining region-independent manner. *Journal of General Virology,* 85: 959–969.

Tan, H., Derrick, J., Hong, J., Sanda, C., Grosse, W.M., Edenberg, H.J., Taylor, M., Seiwert, S., Blatt, L.M. (2005). Global transcriptional profiling demonstrates the combination of type I and type II interferon enhances antiviral and immune responses at clinically relevant doses. *Journal of Interferon and Cytokine Research,* 25: 632–649.

Tan, S.-L., Katze, M.G. (2001). How hepatitis C virus counteracts the interferon response: the jury is still out on NS5A. *Virology,* 284: 1–12.

Taylor, D.R., Shi, S.T., Romano, P.R., Barber, G.N., Lai, M.C. (1999). Inhibition of the interferon-inducible protein kinase PKR by HCV E2 protein. *Science,* 285: 107–110.

Taylor, D.R., Tian, B., Romano, P.R., Hinnebusch, A.G., Lai, M.M., Mathews, M.B. (2001). Hepatitis C virus envelope protein E2 does not inhibit PKR by simple competition with autophosphorylation sites in the RNA-binding domain. *Journal of Virology,* 75: 1265–1273.

Taylor, D.R., Puig, M., Darnell, M.E., Mihalik, K., Feinstone, S.M. (2005). New antiviral pathway that mediates hepatitis C virus replicon interferon sensitivity through ADAR1. *Journal of Virology,* 79: 6291–6298.

Terajima, M., Leporati, A.M. (2005). Role of indoleamine 2,3-dioxygenase in antiviral activity of interferon-gamma against vaccinia virus. *Viral Immunology,* 18: 722–729.

Thomis, D.C., Doohan, J.P., Samuel, C.E. (1992). Mechanism of interferon action: cDNA structure, expression, and regulation of the interferon-induced, RNA-dependent P1/eIF-2 alpha protein kinase from human cells. *Virology,* 188: 33–46.

Toth, A.M., Zhang, P., Das, S., George, C.X., Samuel, C.E. (2006). Interferon action and the double-stranded RNA-dependent enzymes ADAR1 adenosine deaminase and PKR protein kinase. *Progress in Nucleic Acid Research and Molecular Biology,* 81: 369–434.

Uprichard, S.L., Chung, J., Chisari, F.V., Wakita, T. (2006). Replication of a hepatitis C virus replicon clone in mouse cells. *Virology Journal,* 3: 89.

Valente, L., Nishikura, K. (2005). ADAR gene family and A-to-I RNA editing: diverse roles in posttranscriptional gene regulation. *Progress in Nucleic Acid Research and Molecular Biology,* 79: 299–338.

Van Regenmortel, M.H.V., Fauquet, C.M., Bishop, D.H.L., Carstens, E.B., Estes, M.K., Lemon, S.M., Maniloff, J., Mayo, M.A., McGeoch, D.J., Pringle, C.R., Wickner, R.B. (2000). Virus Taxonomy: The VIIth Report of the International Committee on Taxonomy of Viruses. Academic Press, San Diego.

Wang, Q., Khillan, J., Gadue, P., Nishikura, K. (2000). Requirement of the RNA editing deaminase ADAR1 gene for embryonic erythropoiesis. *Science,* 290: 1765–1768.

Wang, Q., Miyakoda, M., Yang, W., Khillan, J., Stachura, D.L., Weiss, M.J., Nishikura, K. (2004). Stress-induced apoptosis associated with null mutation of ADAR1 RNA editing deaminase gene. *Journal of Biological Chemistry,* 279: 4952–4961.

Westaway, E.G., Mackenzie, J.M., Khromykh, A.A. (2002). Replication and gene function in Kunjin virus. *Current Topics in Microbiology and Immunology,* 267: 323–351.

Windisch, M.P., Frese, M., Kaul, A., Trippler, M., Lohmann, V., Bartenschlager, R. (2005). Dissecting the interferon-induced inhibition of hepatitis C virus replication by using a novel host cell line. *Journal of Virology,* 79: 13778–13793.

Wreschner, D.H., McCauley, J.W., Skehel, J.J., Kerr, I.M. (1981). Interferon action -sequence specificity of the ppp(A2′p)nA-dependent ribonuclease. *Nature,* 289: 414–417.

Yang, J.H., Luo, X., Nie, Y., Su, Y., Zhao, Q., Kabir, K., Zhang, D., Rabinovici, R. (2003a). Widespread inosine-containing mRNA in lymphocytes regulated by ADAR1 in response to inflammation. *Immunology,* 109: 15–23.

Yang, J.H., Nie, Y., Zhao, Q., Su, Y., Pypaert, M., Su, H., Rabinovici, R. (2003b). Intracellular localization of differentially regulated RNA-specific adenosine deaminase isoforms in inflammation. *Journal of Biological Chemistry,* 278: 45833–45842.

Yang, Y.L., Reis, L.F., Pavlovic, J., Aguzzi, A., Schäfer, R., Kumar, A., Williams, B.R., Aguet, M., Weissmann, C. (1995). Deficient signaling in mice devoid of double-stranded RNA-dependent protein kinase. *EMBO Journal,* 14: 6095–6106.

Yee, L.J., Knapp, S., Burgner, D., Hennig, B.J., Frodsham, A.J., Wright, M., Thomas, H.C., Hill, A.V., Thursz, M.R. (2004). Inducible nitric oxide synthase gene (NOS2A) haplotypes and the outcome of hepatitis C virus infection. *Genes and Immunity,* 5: 183–187.

You, S., Stump, D.D., Branch, A.D., Rice, C.M. (2004). A cis-acting replication element in the sequence encoding the NS5B RNA-dependent RNA polymerase is required for hepatitis C virus RNA replication. *Journal of Virology,* 78: 1352–1366.

Zhu, H., Zhao, H., Collins, C.D., Eckenrode, S.E., Run, Q., McIndoe, R.A., Crawford, J.M., Nelson, D.R., She, J.X., Liu, C. (2003). Gene expression associated with interferon alfa antiviral activity in an HCV replicon cell line. *Hepatology,* 37: 1180–1188.