# Interferons Play a Central Role in the Natural Defense and Therapeutic Management of Hepatitis C: A Review

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#### Abstract

Hepatitis C infects millions of people globally. Interferons (IFNs) are central to the body's normal response to the virus; these cytokines stimulate infected and non-infected cells to produce chemicals that inhibit viral function and replication. IFNs mobilize cellular defenses, primarily T-cells, to fight the infection, and have mild direct cytotoxic effects. IFNs also contribute to the adverse effects of the immune response. Therapeutic modalities for treatment of hepatitis C are centered on the use of IFNs in conjunction with other antivirals. Questions remain related to appropriate timing for initiation and duration of treatment.

# INTRODUCTION

As far as diseases go, Hepatitis C is a relatively new kid on the block. Although investigators had been aware of a Non-A, Non-B hepatitis, and sera dating from the 1950s have been found to be infected with the hepatitis C virus (HCV) [1], the virus was not isolated and identified until 1989 [2]. While there remains no definitive cure for HCV, in this short period of time investigators have started to uncover the pathophysiology of the disease, identify the body's normal defensive mechanisms against this invader, and devise a treatment regimen to effectively control its progression. Interferons (IFNs) are central to these natural defenses and the treatment regimen.

# THE VIRUS

The hepatitis C virus (HCV) is newly designated as genus Hepacivirus of family Flaviviridae, a family composed of 73 known viruses [1]. (Other Flaviviridae include West Nile encephalitis, yellow fever, dengue, and Japanese encephalitis.) HCV is a 'distant relation' [3], and shares some characteristics of most flaviviruses, while differing in others. While most flaviviruses are genetically stable [4], HCV is highly variable, with high mutation frequencies related to error-prone RNA polymerase activity without a proofreading function [1]. In addition, there are up to six genotypes and multiple subtypes, with 30% variability in its nucleotide sequence [5]. These factors play a role in determining the infectivity and pathogenicity of the virus, as well as having "important implications for escape from immune surveillance, generation of drug resistance and vaccine failure" [1].

### **EPIDEMIOLOGY**

Although precise numbers are impossible to obtain, it is estimated that HCV infects 170-200 million people globally, or about 3% of the world's population, with 3-4 million new cases annually [ $_{1,5,6}$ ]. Approximately 1% of the population of developed countries (e.g. Australia and the US) is infected, with a much higher prevalence in developing countries. Transmission of the virus occurs by direct blood-to-blood contact, most often through shared intravenous needles or transfusion of contaminated blood products, and less often through maternal-fetus placental exchange. HCV is not generally found in significant levels in secretions other than blood, though transmission via breast feeding occurs rarely [ $_{1,77}$ ].

Given the usual mode of transmission, it comes as no surprise that HCV is frequently coinfective with other diseases. Many hemophiliacs, including nearly all who received blood transfusions prior to 1986, are infected with HCV; for these HCV is a major cause of morbidity and mortality [ $_8$ ]. Coinfection of hepatitis B (HBV) and HCV is common, and is associated with a significantly higher risk of developing hepatic failure, liver cirrhosis, and hepatocellular carcinoma (HCC) [ $_{910}$ ]. HCV is also common among those individuals who have human immunodeficiency virus (HIV). An estimated 25% (over 75% in some high risk populations) of HIV-infected individuals also carry HCV [ $_{11}$ ]. As treatment regimens for HIV improve, HCV has become the leading cause of death for that population  $[_{12}]$ .

Humans are the only natural host for HCV  $[_{13}]$ . In this, HCV differs from the rest of Flaviviridae, most (over two-thirds) of which are arboviruses; HCV is not carried or transmitted by vectors such as mosquitoes or ticks.

#### PATHOLOGY

HCV is almost exclusively hepatotropic  $[_1]$ . Hepatic steatosis occurs in roughly 50% of infected individuals  $[_{14}]$ . In industrialized countries, chronic hepatitis C accounts for 40% of end-stage cirrhosis and 60% of HCC  $[_{15}]$ . Among those with established cirrhosis, HCC develops at a 1-4% annual rate  $[_{5,6}]$ . The primary causes of morbidity and mortality in HCV (though in less than 10% of the cases) are cirrhosis and HCC. Slow progressors may show continued viremia without ever developing adverse sequelae  $[_{16}]$ .

Like all Flaviviridae, HCV is pleiotropic. As many as 36% of HCV patients experience extrahepatic pathologies related to HCV infection [17]. Most significant, perhaps, is neural tissue involvement [4,18]; Flaviviridae are notorious for causing a variety of encephalopathies. Additionally, HCV is associated with sarcoidosis [19] (implying viral effects on the immune system), glomerulonephritis and rheumatologic complications [1720], diabetes mellitus [21], vascular disturbances [1722], and possibly splenic lymphoma [17].

# **MOLECULAR BIOLOGY**

The molecular biology of HCV in the host cell is still largely hypothetical [ $_{23}$ ], due in part to lack of a small animal for in vivo studies (currently, the only suitable laboratory animal for HCV studies is the chimpanzee). Flaviviridae are small spherical ssRNA viruses with lipophilic envelopes and a glycoprotein coat completely covering the surface of the virus [ $_{3,24}$ ]. Envelope glycoproteins E1 and E2 are known to be critical for attachment to the target cell membrane [ $_{25,26,27}$ ]. Less clear is the identity of the cellular component. While CD81 has commonly been viewed as the receptor [ $_{113}$ ], new evidence suggests that other, unidentified, cell membrane molecules may take the primary receptor roles in conjunction with CD81 [ $_{26}$ ]. Alternatively, HCV may form complexes with LDL [ $_{1,13}$ ] or cryoglobulins [ $_{23}$ ] and bind with receptors specific to those molecules.

HCV infiltrates the cell through receptor-mediated endocytosis [3,13], probably via clathrin coated pits [28]. The virus is uncoated by an unknown mechanism, and viral proteins and RNA are released. Expressions of major histocompatibility complex (MHC) and other cell membrane molecules (ICAM, VCAM, e-selectin) are up-regulated in response to viral proteins [ $_{29,30}$ ]. E1 glycoprotein may alter cell membrane permeability, thus inducing cell lysis [ $_{31}$ ]. The core protein translocates to the endoplasmic reticulum (ER) and/or mitochondria, while viral RNA attaches to the ribosomes. At the ER, the core protein self-replicates [ $_{32}$ ], inducing ER stress and calcium depletion, possibly promoting apoptosis [ $_{33}$ ]. The expression of the protein in the mitochondria inhibits electron transport and promotes the generation of reactive oxygen species. It induces a state of oxidative stress and increased mitochondrial permeability, and leads to the accumulation of oxidative DNA damage [ $_{34,355}$ ]. Viral ssRNA serves as a template for viral replication and protein synthesis [ $_{13}$ ] at the ribosomes.

New viral particles are thought to be exported by the host cell's secretory mechanism after re-encapsulation at the ER [13]. The presence of HCV RNA (as well as anti-HCV antibodies) in the serum is diagnostic for infection.

Cellular changes include modulation of lipid metabolism and protein and genetic transcription, with subsequent steatosis and effects on cell signaling, proliferation, and death [ $_{13}$ ]. Histological changes include lobular inflammation and piecemeal necrosis of the limiting plates of hepatocytes around the portal tracts, resulting in expanded portal tracts and progressive fibrosis. This damage may extend to the bile ducts [ $_1$ ]. Ultimately, cirrhosis, HCC, and failure ensue.

Ironically, for all the deleterious effects of HCV on the normal hepatocyte, evidence indicates that HCV proteins inhibit apoptosis in hepatoma cells [<sub>36</sub>], contributing to the development of HCC.

#### **NATURAL DEFENSE – INTERFERONS**

Interferons, cytokines naturally secreted by various cell types in response to viral infection, play a central role in the normal immunological response. Two major types of interferons have been identified. Type I includes interferon alpha (IFN-I) and interferon beta (IFN-I). Type II includes interferon gamma (IFN-I). (Other IFNs with less significance in this context are not discussed here.) Type I IFNs seem to block viral translation and replication, while Type II IFNs induce antiviral chemical and cellular responses [3].

Although the exact mechanisms are still unknown [29], detailed hypotheses regarding IFN actions (based on in vitro studies) have been suggested [37,38]. The applicability of

these hypotheses to in vivo processes is still uncertain; tissue studies have largely been conducted with lymphoid and splenic tissues, rather than hepatic, and with viruses other than HCV. Moreover, the precise mechanisms, and cellular response to those mechanisms, vary from one virus to the next and even between different subtypes of the same virus. What is certain is that the induction and regulation of IFNs flows through a complicated series of positive feedback loops and interactions with multiple intra- and extra-cellular factors.

The body's initial reaction to any viral infection is the nonspecific inflammatory response, including secretion of cytokines that attract and activate macrophages, natural killer (NK) cells, and neutrophils [37]. These cells, along with fibroblasts, DC, leukocytes, and possibly other cell types, secrete IFNs. This response by itself fails to control viral replication, possibly due to the viral E2 glycoprotein binding to CD81 on the NK surface and inhibiting the actions of that cell (including the secretion of IFN-[]) [13].

Secondary (and more effective) INF activity begins when the virus attaches to the target cell, usually at a toll like receptor (TLR) or through binding of the capsular glycoprotein with a mannose receptor (though other pathways have been suggested as well) [<sub>37</sub>]. Interferon regulatory factors (IRFs), nuclear factor kappa-B (NF-kB) and other transcription factors are then produced intracellularly, leading to the production of Type I IFNs; Type I IFNs are among the first antiviral mediators released from the infected cell. INF then acts in both an autocrine and a paracrine manner, binding with IFN receptors (INFARs) on the surface of originating and neighboring cells. INFARs are present on the surface of all vertebrate cells [<sub>38</sub>], thus explaining the widespread effects of IFNs.

The binding of Type I IFNs to their receptors initiates a cascade of intracellular chemical reactions that may follow multiple pathways. The major pathway would seem to be through association with Janus kinase (JAK) and several signal transducer and activator of transcription (STAT) proteins. Signal transduction via the JAK/STAT pathway in the cytoplasm, which may also involve a variety of transcription factors such as IRFs and NF-kB, ultimately results in the production of IFN-stimulated gene factor 3 (ISGF-3). (Evidence suggests other pathways are stimulated by different stimuli [37].) ISGF-3 translocates to the nucleus where it binds with receptor genes and promotes the production of a variety of antiviral chemicals [38]. This

pathway is also speculated to mediate the development of Th-1 cells.

Similar to INFARs, IRFs are found in varying levels in most cell types. Their expression is enhanced by viral stimulus or by exposure to IFNs (endogenous or therapeutic) [<sub>37</sub>]. Thus, one IRF induces Type I IFNs to signal via IFNAR, which stimulates another IRF to promote production of more IFN in a positive feedback loop. IFN also acts as a survival factor in some cells that produce that cytokine, thus promoting its own expression [<sub>37</sub>].

Type I IFNs upregulate MHC and ICAM expression, thus making the cell more liable to recognition by and adhesion with T-cells and promoting viral clearance through induced apoptosis of infected cells  $[_{1,4,29}]$ . NK cell activity is increased by up to 100 times after exposure to IFNs  $[_{29}]$ . This is perhaps the most important role of the IFNs, as recognition of (and response to) MHC and/or antigen is the sine qua non of the immune response  $[_4]$ .

Type II interferon, IFN-I, is not induced directly by exposure to the virus, but rather is part of the 'second wave'. NK cells and Th-1 lymphocytes (activated by Type I IFNs) secrete IFN-I (and other cytokines) which act to support CTL generation [1,29,37,39]. NK and T-cells are then stimulated to produce more IFN-I [37]. In fact, IFN-I produced by stimulated T-cells is more effective at up-regulating ICAMs than that which is produced directly by infected cells [4]. To continue the positive feedback loop, IFN-I is essential for further recruitment of leukocytes [1,4].

Primary IFN effects on the cell are mediated by other cells. IFN attracts T-cells which promote viral clearance through apoptosis. IFNs induce expression of TNF-related apoptosis inducing ligand (TRAIL) on the surface T-lymphocytes and upregulate TRAIL receptors on the surface of infected cells; the binding of the ligand with the receptor then induces cell death [ $_{29:40}$ ]. Therapeutic clearance thus results from induction of Th-1 response; vigorous CD4+ response is predictive of disease resolution and lower grade of hepatocellular inflammation [ $_{113}$ ]. IFNs are more directly cytotoxic via induction of PKR to inhibit protein synthesis [ $_{40}$ ].

# **IMMUNE-MEDIATED TISSUE DAMAGE**

Immune mechanisms can have detrimental effects if left unregulated  $[_{37,41}]$ . Cellular changes resulting from HCV may relate to the immune response  $[_{1,13}]$ ; in this HCV is far from unique. While some viruses (e.g. HIV) are directly cytotoxic, in many others cases disease symptoms are related more to the immune response than to the virus itself. A classic example is the influenza pandemic of 1918 [ $_{42}$ ]. This disease killed primarily not the young, old, and weak, but rather the strong, vigorous individuals in the prime of life. In millions of cases death resulted from the devastating effects of an uncontrolled immune system response to the influenza virus. Those who for one reason or another had weaker immune response were far less likely to die.

IFNs contribute to tissue damage from the immune response, primarily from two central IFN effects. First, as noted above Type I IFNs act in both autocrine and paracrine manners. MHC expression is thus upregulated, not only in the infected cell, but in neighboring cells as well, leading to destruction of potentially uninfected tissue by CD8 cells [4]. As a result, IFN's may be responsible for over-vigorous immune response and pathogenesis of disease leading to tissue destruction. Second, myelosuppression and suppression of hemotopoeisis occurs in the context of IFN-I secretion, possibly causing fatal immunopathology [4,4]. IFN-I promotes anemia related to a decreased number of active erythroid progenitors; the presence of anti-inflammatory cytokines results in increased erythroid apoptosis  $[_{43}]$ . This myelosuppressive effect is the basis of therapeutic administration of IFN for polycythemia vera [41].

# VIRAL RESISTANCE

The persistence of HCV infection is due in large part to several modes of viral resistance. Most of these (e.g. genetic variation, downregulation of MHC, antibody-viral complexes) are independent of IFN activity. IFNs do contribute modestly to viral resistance. The stimulation of the JAK/STAT pathway (by IFNs) can have anti-apoptotic effects and lead to development of drug resistance [41]. More significant is the varying effect of IFNs on cells at different points in the cell cycle. While IFNs result in MHC upregulation of all cells, upregulation occurs to a much greater degree in the quiescent Go phase than in the active G1 phase. Non-offensive G0 cells are then statistically preferential targets of the immune response, while the G1 cells evade that response and continue the process of viral replication [4].

HCV has evolved various mechanisms to inhibit IFN activity [3]. Proteins from other (non-HCV) viruses have been found to impede IFNs by binding with STAT proteins. These complexes block IFN signaling, prevent passage of STAT/ISGF complexes into the nucleus, and inhibit antiviral transcription [44]. Whether this process applies to HCV is unknown.

#### TREATMENT GUIDELINES

As early as 1989, RCTs were being conducted to evaluate the use of recombinant IFN- $\mathbb{I}$  for treatment of HCV [45,46]. (Earlier investigators had tested IFNs against hepatitis in less well controlled settings.) These early trials indicated that IFN was effective, but only modestly and transiently. In the 17 years since then, numerous trials have been conducted with variants IFN- $\mathbb{I}$ 2a, IFN- $\mathbb{I}$ 2b, and IFN- $\mathbb{I}$ , as well as both combination and monotherapy. Continuing attempts to create a vaccine for HCV have thus far met with little success, due largely to the variety of genotypes and a plethora of constantly evolving quasispecies [1,47]

The current 'gold standard' for HCV therapy is the combination of pegylated IFN alpha-2b (pIFNI-2b) and ribavirin [8,48], which appear to act through host antiviral mechanisms [49]. Ribavirin is a nucleoside with an unknown mechanism of action. It appears to exert a weak antiviral effect; its greatest effect may be on prevention of relapse [8]. Pegylation is the process of binding the IFN molecule with a molecule of polyethylene glycol; this binding delays renal clearance of the drug, thus optimizing pharmacokinetics and pharmacodynamics, ensuring sustained concentrations with weekly dosing [5,49,50]. Pegylated IFN is more effective achieving a sustained viral response (SVR) and improving liver histology while maintaining a similar toxicity profile and reducing failure rates [8,15,48,49,50]. Numerous RCTs have shown this combination therapy to be more effective than monotherapy [<sub>8,15,50,51</sub>].

The effectiveness of this plan of therapy is largely related to HCV genotype. Genotypes 1 (the most common type in the US) and 4 are less responsive to treatment, whereas genotypes 2 and 3 have significantly higher rates of viral response [ $_{8,49,50}$ ]. SVR is obtained in approximately 50% of those treated, and perhaps as high as 90% with genotypes 2 and 3 [ $_{5,8,51,52}$ ].

Primary clinical questions currently are related to the onset and duration of treatment. While typical practice is to begin treatment as soon as possible, there is evidence that later initiation of IFN therapy is as effective in obtaining SVR [48]; delaying the onset of therapy allows for potential spontaneous clearance of the virus without exposing the patient to the risks and rigors of the treatment regimen. Avoidable delays in initiating treatment, however, may increase the risk of permanent tissue damage. Once initiated, it is unnecessary in many cases to continue therapy for as long as has been done in the past. Patients with genotypes 2 and 3 who have an early viral response (EVR) (undetectable viremia after 4 weeks) may be able to stop therapy after 12-16 weeks, while slow responders or those with genotype 1 may need to continue for up to 24 weeks [53]. SVR is most likely to be attained in those who have an EVR [8,52]. Conversely, continued viremia at 12 weeks implies virtually no chance that SVR will ever be attained, and continued therapy may be unwarranted [49,51].

# **ADVERSE EFFECTS**

Therapeutic application of antiviral chemicals in general and IFNs in particular is often limited by a wide variety of adverse effects (AEs). In some trials, most participants experienced AEs [ $_{54}$ ]. Investigators were required to reduce dosages for 12% to 40% of participants, while 7% to 22% were required to discontinue treatment altogether [ $_{15,55,56,57}$ ]. Combination therapy (IFN plus ribavirin) was noted by some to result in more AE's [ $_{15}$ ], while others noted fewer [ $_{8}$ ] or that the rate of AEs was related to the dose or duration of treatment [ $_{58}$ ]. Pegylation also reduced the rate of AEs [ $_{8}$ ].

The most commonly reported AE was a collection of vague flu-like symptoms [ $_{58,59}$ ]. Psychological disturbances, primarily depression, were noted by many investigators [ $_{59,60,61,62}$ ]. Up to 15% of the participants developed thyroid disorders [ $_{63,764}$ ]. Myelosuppression was common, with resultant hematological disorders such as anemia, leucopenia, and thrombocytopenia [ $_{8,15,41,58,59,65,66}$ ]. Nonetheless, investigators noted the absence of additional coagulopathy in hemophiliacs [ $_{67}$ ] or opportunistic infections [ $_{59}$ ]. Other significant AEs that have been noted by investigators include Bells Palsy [ $_{68}$ ], pericarditis [ $_{69}$ ], sarcoidosis [ $_{70,71}$ ], ophthalmologic disorders [ $_{72}$ ] and a deterioration of pre-existing rheumatological disease [ $_{10}$ ].

While glucocorticoids have been shown to be ineffective in clearing the virus, they may ameliorate AEs  $[_2]$ . Methotrexate eases rheumatological symptoms  $[_{20}]$ . Growth factors added to the regimen may counteract some of the myelosuppressive effects  $[_{66}]$ .

# CONCLUSION

Hepatitis C infects millions of people worldwide, and its

incidence is likely to increase in years to come. Current evidence is sufficient to recommend IFN treatment for all patients with acute hepatitis, but the long-term effectiveness of that treatment remains to be seen. HCV is still 'young' enough that extended longitudinal studies of disease progression and relapse rates are not yet available. While medical therapy with pIFN and ribavirin seems to be effective in attaining SVR in many patients, only time (and further studies) will tell how sustained that viral response actually is. Even in the absence of viremia over time, two issues cloud true estimates of effectiveness. The first is the inability to say how many people actually have (or have had) HCV. Most carriers are asymptomatic and unaware of their infected status [5], and the infection may clear spontaneously without ever being recognized [1]. Hence, an entire cohort of HCV patients escapes investigators' purview.

Second, even when the infection is diagnosed and treated, we cannot assume that viral clearance was due to the treatment. As noted above, the long term results are not in. Moreover, the 25% of HCV patients who are symptomatic, and hence most likely to seek care, are also the patients who are most likely to clear the virus spontaneously [16]. While they attained SVR with treatment, a longer period of observation might show that they would have attained that same SVR even without treatment.

In the 17 years since it was first identified, investigators have made great strides in coming to understand HCV and learning to control its deleterious effects on cells, tissues, and systems. Enhanced quality of life for HCV patients mandates continued study to unravel its remaining mysteries.

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