Interferons as Immune Regulators: A Rivalry between HCV and Interferons

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Abstract

Interferons (IFNs) are an integral part of the immune system, which upon stimulation results in recruitment of cytokines for viral clearance. IFNs have been characterized as potent antiviral agents that can reduce viral titer and have been found to act as critical mediators for tumor regression in few cases. During the course of time Hepatitis C virus (HCV) has evolved and influence IFN efficiency through various pathways. Rapidly occurring amino acid substitutions in HCV’s core protein, sequence homology with protein kinase (PKR), increased numbers of quasi-species and wild-type Interferon sensitivity determining region (ISDR) strains are linked with an inefficient response to IFN therapy. This article describes the pharmacodynamics of IFNs with an aim to decipher the possible involvement of HCV proteins in subverting these responses. We hereby discuss IFN-based therapies targeting the host and viral genetic factors, since they have a strong impact in determining the efficacy of an IFN in HCV infected host.

Keywords: Interferon; HCV proteins; Interferon resistance; Interferon therapy; Hepatitis C virus

Introduction

Hepatitis C virus (HCV) is an RNA virus which was first discovered in year 1989 [1,2]. Since its discovery, interferons (IFNs) have been used against HCV as a cornerstone of the anti-HCV therapy. Isaacs and Lindenmann in 1957 discovered this family of cytokines and named them inter-ferons because of their ability to “interfere” with the viral replication, conferring resistance to viral infection transferred from infected chick cells into uninfected cells [3]. IFNs are one of the key components of the innate immune system, considered as the first cytokines to be cloned, sequenced, purified to recombinant forms and have therefore been utilized in a wide range of applications [4]. Some of the much emphasized functions performed by this class of cytokines includes; inhibition of cell proliferation [5,6], up-regulation of Major histocompatibility complex (MHC) class I [7,8], induction of maturity in Dendritic cells (DC), promotion of B-cell differentiation to plasmablasts [9], promotion of T-cell responses and induction of expression of pro-inflammatory cytokines [10-14]. With the discovery of its isoforms, IFNs have been categorized into three distinct groups (Type 1-3) based on their amino acid sequences and specific receptor recognition [15]. The therapeutic potential of Type-1 IFNs in viral infection was first discovered through its inhibitory action against respiratory viral infections. Since then IFNs have been acknowledged clinically as effective antiviral and anti-neoplastic therapeutic agents. Various functions performed by this group of cytokines have been highlighted in figure 1.

On the other hand, viruses have evolved many mechanisms to block IFN synthesis and alter their actions by interfering at various stages of IFN signaling pathway to evade the IFN mediated host responses. Viruses such as Influenza virus, Ebola virus, Papilloma viruses and the Human Herpes Kaposi’s Sarcoma-associated virus (KSHV) encode proteins that interfere with interferon regulatory factors (IRF) activation or induction [16]. Chemical modulators which may either selectively activate IFN synthesis or block the synthesis of inflammatory cytokines can have a broad therapeutic potential in autoimmune and are yet to be developed [17]. It can therefore reasonably be argued that complex organisms like mammals can only survive as long as their immune defenses are able to adjust with the strategies of invading pathogens. Hence, an adaptable IFN system is essential for mammals to make them capable of evading viral infections [18].

Some of the other related subjects widely discussed over the years include, the identification of viral mechanisms that resist the actions of IFN proteins and IFN-stimulated genes (ISGs). Amino acid substitutions in HCV’s core protein, sequence homology, higher numbers of quasi-species and wild-type Interferon sensitivity determining region (ISDR) strains are also linked to an inefficient response with IFN therapy. This article elucidates the pharmacodynamics of IFNs with an emphasis on the possible involvement of HCV proteins in subverting these responses [19]. The effects of mutations and suppressions of gene products which are initiated by the IFN system and leads to the progression of cancers have also been explained in this article [20,21].

IFN Family of Proteins

IFNs are categorized into three distinct groups, named as type 1, type II and type III IFNs [10]. In humans 17 non-allelic functional genes have been identified that encode type I IFNs [22,23]. All of them are clustered on chromosome 9 and lack introns [23].

The complex evolutionary history demonstrated by type I IFNs predict the fact that it may be the consequence of various viral combats resulting in its divergence to at least eight distinct subfamilies: IFN-
alpha (IFN-α), IFN-beta (IFN-β), IFN-epsilon (IFN-ε), IFN-kappa (IFN-κ), IFN-omega (IFN-ω), IFN-delta (IFN-δ), IFN-zeta (IFN-ζ) (limitin) and IFN-tau (IFN-τ) [22]. The first five are found in humans, of which there is only one IFN β but 13 IFN α subtypes [22]. All of them have a relatively higher specific potencies whereas most of them are non-glycosylated proteins of 165–200-plus amino acids as well, sharing homologies that range between 30–85% within a specie [24]. IFN τ is produced in trophectoderm of ruminants and appears to be important in early period of pregnancy [25]. IFN σ is expressed by trophoblasts of pigs [26]. IFN ζ (limitin) is expressed only in mice having a significantly greater homology to human IFNs [27,28].

**Role of IFNs and Immune Responses**

Interaction of IFN with its receptors activates intracellular signaling cascades rapidly induce the expression of a variety of overlapping and unique genes involved in inflammatory immune responses. The advent of novel cytokines is changing our approach towards pathogenesis and hence treatment of infectious diseases, allergies and autoimmunity [32]. Production of IFNs requires stimulation by viruses, microbial products or chemical inducers [20]. Various DNA and RNA viruses, bacteria and protozoa have also been reported to induce IFNs through activation of toll like receptors (TLRs) [31,33].

Retinoic acid–inducible gene (RIG)-1–like receptors (RLRs) are cytosolic RNA helicases that sense viral RNA and trigger signaling pathways which induce the production of IFNs and proinflammatory cytokines [34]. Immunohistochemical analysis has shown that RLRs are present in virus induced stress granules, accompanied by viral RNA and other antiviral proteins; altogether which is now termed as antiviral stress granules (aSGs) [34]. Whereas for type III IFNs; a heterodimer of IFNLR1 and IL10R2 is necessary to form a functional receptor, to initiate the defensive cascade of activated factors [31]. IFN genes are induced by the binding of TLR-activated transcription factors to their promoters. The most important transcription factors for induction are IFN regulatory factors (IRF), specifically IRF3, IRF7, ATF-2/c-Jun, and NFκB families [35-37]. Mammalian NFκB/Rel family comprises of five members; NFκB-1 (p50), NFκB-2 (p52), RelA (p65), RelB, and c-Rel. All of them play critical roles in the regulation of innate immune system by activating various immune responsive genes, such as cell adhesion molecules, proinflammatory cytokines and chemokines [38-40]. NFκB RelA is required during early phase of viral infections, whereas NFκB RelB with CCL19 plays a role in forming heterodimers with p50 and p52 in various genes transactivation [37,41-43]. IFNs also induce some GTPases in the activation of its pathway and Mx proteins are one of those GTPases, which belongs to the dynamin superfamily of Large GTPases. The antiviral activity showed by MxGTPases against a wide range of RNA viruses is a unique property belonging to this group member [44]. Direct and indirect mechanisms exploited by IFNs to counter viral attacks are outlined in table 1.

After recognizing the antiviral efficacy of IFNs against RNA and DNA viruses, they were included in the regimen against HCV and HBV. IFN-α and IFN-β have also shown reduced viral titers and

**Table 1:** The direct and indirect mechanisms followed by interferons to counter viral attack and enhance host immunity.

<table>
<thead>
<tr>
<th>Direct</th>
<th>Indirect</th>
</tr>
</thead>
<tbody>
<tr>
<td>PKR (Protein Kinase-R)</td>
<td>Up-regulation of human leukocyte antigen (HLA) class 1 expression. Which leads to Enhancing terminal differentiation of DCs</td>
</tr>
<tr>
<td>2'-5' oligoadenylatesynthetase (2'-5' OAS/ Rnase system)</td>
<td>T-Cell antiproliferative effects</td>
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<tr>
<td>Mx Protein</td>
<td>Up regulation of the expression of HCV antigens (modification of immunoproteosomes) required for presentation of antigens</td>
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**Figure 2:** Genomic and proteomic organization of Hepatitis C Virus (HCV).

**Table 2:** Various host and viral factors determining the effectiveness of interferon therapy in clearing the viral infection.

<table>
<thead>
<tr>
<th>Host Factors</th>
<th>Viral Factors</th>
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<tbody>
<tr>
<td>Age Less than 40 years</td>
<td>Genotypes 2 and 3</td>
</tr>
<tr>
<td>Gender; female</td>
<td>Viral Load &lt;2 million IU</td>
</tr>
<tr>
<td>Ethnicity; nonblack</td>
<td>Lack of mutations in Interferon-sensitive determining region (ISDR)</td>
</tr>
<tr>
<td>Lack of liver fibrosis</td>
<td>Decrease in E2 sequence homology with Protein Kinase R (PKR)</td>
</tr>
<tr>
<td>Anemic conditions</td>
<td>Viral Kinetics; Rapid Decline with therapy</td>
</tr>
<tr>
<td>Immune response</td>
<td>Increased duration of therapy</td>
</tr>
<tr>
<td>Interleukin-28 genetic polymorphism</td>
<td>Co-infections</td>
</tr>
<tr>
<td>Sex hormones and menopause</td>
<td>Non-alcoholic</td>
</tr>
<tr>
<td>Non-alcoholic</td>
<td>Organ transplant</td>
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<td>Organ transplant</td>
<td>Insulin resistance</td>
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<td>Insulin resistance</td>
<td>Obesity</td>
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therapy which is quantified through detection of HCV’s RNA in patient’s blood serum, at least six months after completion of antiviral therapy against chronic HCV infection [68]. After attaining a viremia during this period, the incidence of late relapses are minimal (<1%) [69].

Different proteins of HCV have been reported to regulate or inhibit the production or working of IFNs, which has been discussed as follows:

**Core protein of HCV**

Role of HCV proteins in reversing the actions of IFNs in host defense mechanisms are being identified with the passage of time through various experiments. Both viral and human polymorphisms have been correlated with the outcomes of IFN therapies. IL-28B polymorphism is under thorough studies now days, as it is believed to predict the efficacy of IFN therapy in different groups. Moreover, in a study conducted in Japan, it was concluded that host polymorphism (IL-28B) and viral polymorphism (HCV Core protein) contribute independently to a successful IFN therapy [51]. It was concluded that HCV core protein with mutations at position 70 and 91 is known to be very critical in non-responders of IFN therapy with genotype 1b in Japan [70]. The overall role for these positions is not much clear but they are believed to have an inhibiting activity in Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway however no correlation has yet been made in any other genotype of HCV [57,71]. The core protein also plays a role in generation of the suppressor of cytokine signaling 3 (SOCS3), which inhibits the function of interferon-stimulated gene factor 3 (ISGF3) [72]. SOCS-3 is a member of STAT-induced STAT inhibitor (SSI), which is cytokine-inducible negative regulators of cytokine signaling and their expression can also be induced by various cytokines, including IL-6, IL-10, and interferon-gamma [73-76]. SOCS proteins can bind to JAK2 kinase and inhibits its activity [77]. HCV core protein is also known to perform some other tasks; it help in inhibition of SOCS1, it accelerates kinase and inhibits its activity [77]. HCV core protein with mutations at position 70 and 91 is known to be very critical in non-responders of IFN therapy with genotype 1b in Japan [70]. The overall role for these positions is not much clear but they are believed to have an inhibiting activity in Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway however no correlation has yet been made in any other genotype of HCV [57,71]. The core protein also plays a role in generation of the suppressor of cytokine signaling 3 (SOCS3), which inhibits the function of interferon-stimulated gene factor 3 (ISGF3) [72]. SOCS-3 is a member of STAT-induced STAT inhibitor (SSI), which is cytokine-inducible negative regulators of cytokine signaling and their expression can also be induced by various cytokines, including IL-6, IL-10, and interferon-gamma [73-76]. SOCS proteins can bind to JAK2 kinase and inhibits its activity [77]. HCV core protein is also known to perform some other tasks; it help in inhibition of SOCS1, it accelerates the degradation of STAT1 and lastly it blocks DNA binding by ISGF3 (Figure 3) [19,78].

**Envelope protein (E2) of HCV (Genotypes and IFN resistance)**

HCV reportedly undermines the effectiveness of IFNs through sequence homology between a small region of an endoplasmic reticulum (ER) – bound E2 protein with the phosphorylation sites of double-stranded RNA-activated protein kinase PKR and its substrate, the eukaryotic translation initiation factor 2a (eIF2a) (Figure 4) [79].
E2 plays two important roles by modulating global translation through inhibition of the interferon-induced antiviral protein PKR via its PKR-eIF2α phosphorylation site homology domain (PePHD) and binding with the PKR-like ER-resident kinase (PERK), to inhibit its function [79]. This inhibition and binding can be related with the inherent resistance of chronic HCV genotype 1 patients to IFN therapy, but weaker links may be found in account of those with genotypes 2 or 3, in accordance with the clinical data [80]. PKR is basically an antiviral protein, which blocks protein synthesis by phosphorylation of eIF2α [81]. Although the inhibition of PKR is correlated with the similarity in sequences of PePHD sequence of E2, eIF2α and PKR, but it still stays controversial because it can only explain the resistances shown by genotype 1 of HCV, whereas research have proved that the PePHD protein, which blocks protein synthesis by phosphorylation of eIF2α, inhibits SOCS-3 (Suppressor of Cytokine signaling 3) (Suppressor of STAT pathway) and SOCS-1 [82]. Different viral and host factors, which play a vital role in the outcomes of IFN therapies are shown in table 2.

PEGylation is a process whereby one or more molecules of polyethylene glycol (PEG) covalently attaches to a biological molecule or drug and therefore transforms it into a improved drug with better pharmacokinetic and pharmacodynamic parameters [83]. The process of PEGylation involves incubation of a reactive derivative of PEG with the targeted protein and this attachment can mask the therapeutic protein from the host’s immune system, reducing antigenicity and immunogenicity. Furthermore it also increases the hydrodynamic size of the protein, which prolongs its half-life inside the body and decreases its renal clearance [83,84]. PEG is therefore added with therapeutic IFN to make it long lasting and to reduce dose frequencies, hence improves patient compliance with the treatment. SVR rates of combined pegylated interferon (PEG-IFN) therapy for genotypes 1, 2, 3 and 4 are approximately 50%, 80%, 70%, and 50% respectively (Figure 5).

Combined and mono therapies have strong effect on the SVR rates as 20% of HCV genome is not conserved at amino acid level in different genotypes, moreover HCV genotypes can be further subdivided into subtypes denoted by lower case alphabets (1a,1b,1c,etc) [19,61,84]. In common circumstances an individual is reported to be infected with a single subtype, however infection with multiple genotypes and subtypes have also been documented. Within each host, HCV is capable of multiplying number of directly related but discrete viral strains called quasi-species [84].

Hepatologists and scientists are facing many problems in the treatment of HCV all over the world because the genome of HCV is 10 times more diverse than human immunodeficiency virus (HIV) since the RNA-dependent RNA polymerase in HCV lacks the proof reading capacity [85]. Poor responses to IFN therapy have been reported with an increase in number of quasi species visible before therapy [84].

The varieties of genotypes differ with the differences in geographic locations and five genotypes have been mainly identified. Genotype 1 is predominant in United States of America (USA) and Western Europe accounting for 60-65% infected HCV individuals, genotype 3 is mostly prevalent in Pakistan [86], genotype 4 is widespread in Middle East,
Egypt and Central Asia whereas genotypes 5 and 6 are commonly found in South America and South East Asia respectively [87].

During the last century, there have been sudden outbreaks in the USA and Western Europe. A small number of subtypes including subtypes 1a, 1b, and 3a have been found prevalent, but genotype distribution has changed and diversified, which can be associated with intravenous drug use, blood transfusions and immigration to Europe and USA from endemic areas [88,89]. However efficacy of IFN therapy in different genotypes of HCV is found to be irregular. The reason behind this irregularity is a broad discussion but one of them is believed to be the outcome of sequence homology of envelope proteins of HCV genotype 1 with IFN (as discussed earlier) and this makes HCV capable of subverting the antiviral effects of IFN [90].

**NS3/4A protein of HCV**

The nonstructural proteins of HCV also possess a capacity of subverting the IFN activity. HCV NS3/4A serine protease prevents the phosphorylation of IRF3 and thus inhibits IFN induction [91], it also performs another duty with similar result by cleavage of the “Toll-IL-1 receptor domain-containing adaptor inducing IFN-β” (TRIF) protein that plays a key role in linkage of TLR3 to kinases responsible for the activation of IRF3 which has also been proved within an in vivo study that IRF3 is activated in the livers of patients infected with HCV (Figure 6) [92,93]. IFN induction is also interfered and downregulated through the disruption of RIG1 signaling by NS3/4A [94].

**NS5A protein of HCV**

The exact function of HCV NS5A is still unknown but it is possibly involved in the induction of proinflammatory chemokine interleukin-8 (IL-8), which leads to the fractional inhibition of IFN antiviral response [91]. Studies have shown that the increase in levels of IL-8 in HCV patients under IFN treatment is directly related to the failure of IFN therapy [61,95]. Recently, a study has reported from Taiwan that IRF3 is activated in the livers of patients infected with HCV (Figure 6) [92,93]. IFN induction is also interfered and downregulated through the disruption of RIG1 signaling by NS3/4A [94].

**NS5A that may bind and inhibit PKR**

**IFN induction is also interfered and downregulated through the disruption of RIG1 signaling by NS3/4A [94].**

**Mutation in ISDR increases SVR as in Asian population**

**NS5A contains ISDR (Interferon sensitivity determining region) that may bind and inhibit PKR**

**It induces IL-8 (Partial IFN response inhibitor)**

**HCV NSSA contains ISDR (Interferon sensitivity determining region) that may bind and inhibit PKR**

**INHIBITION OF INTERFERON MEDIATED IMMUNE RESPONSE BY HCV NON-STRUCTURAL PROTEIN**

**INTERFERENCE WITH INTERFERON-INDUCED GENE EXPRESSIONS**

**Conclusion**

Interferon therapy is a major treatment option in HCV infections but with the passage of time HCV has evolved mechanisms to cope with IFN therapy. Since HCV is a RNA virus having higher rates of mutations, these mutations play a pivotal role in virus survival [48,49]. Over the years, these mutations have helped HCV to evade host immune responses leading towards the failures of antiviral therapies. As a result HCV continues to be a challenging target being the foremost cause of demise and cancers related with livers. This article emphasizes the possible role of HCV proteins contributing towards reduced efficacy of IFN therapy. HCV core protein can hamper IFN mediated therapy by substitution of its amino acids resulting in development of IFN resistance and failure of treatment [51,57]. PEG-IFN and ribavirin therapies have varying effects on various genotypes of HCV; many
HCV also evades antiviral effects of IFN on account of homology between different host factors and viral proteins; NS5A protein has an important role in inhibition of IFN [62-64]. The ISDR region in HCV genome also contributes towards resistance against IFN therapy. These factors contribute to virus survival, and as a result, the host immune system fails to curb viral infection which may later damage the liver in case of HCV infection. HCV is a global threat effecting millions of people each year. Although IFN, PEG-IFN and other antiviral approaches have reduced the mortality rate, the HCV still remains a serious pathogen through its ability to persist by manipulating various host factors. Therefore, the need for modification of existing anti-HCV therapeutics as well as production of novel anti-HCV therapeutic agents is necessary to reduce the rate of HCV associated annual deaths.

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References


