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To provide health care professionals with a comprehensive continuing medical education program focused on evolving concepts in the management of hepatitis C treatment failures that results in improved patient care

INTERFERON ALFA: ACTIONS, DIVERSITY, AND CLINICAL IMPLICATIONS FOR PATIENTS WITH HEPATITIS C

MICHAEL GALE, JR, PhD

Interferons (IFNs) are the charter members of the cytokine superfamily. They were first identified in 1957 as multifunctional secreted proteins involved in antiviral defense, cell growth regulation, and immune activation.¹ They act through complex molecular and genetic pathways, and the medical community is just beginning to understand how these molecules work in the treatment of a variety of viral infections, including hepatitis C virus (HCV). In addition, IFNs are structurally and functionally diverse, with varying affinities for receptor binding, immune-cell induction, and viral suppression. Thus, as clinicians consider treatment options for their HCV-infected patients, particularly those in difficult-to-treat populations, relative biological potency makes logical sense as a starting point.

IFNs are produced by mammalian cells in response to viral infection and mediate cellular homeostatic responses through the inhibition of viral replication.² IFNs are classified into at least 2 distinct types, according to the type of cell surface receptors they bind to. Type 1 IFNs bind to the α/β receptor, and type 2 IFNs bind to the γ receptor. Type 1 IFNs are subdivided into 4 structurally related families: IFN α , IFN β , IFN ω , and IFN τ . There are approximately 12 subtypes of IFN α but only 1 subtype of IFN β . Unlike type 1 IFNs, which are produced by a wide range of cell types in direct response to viral infection, type 2 IFNs are only produced by immune cells (ie, activated T lymphocytes and natural killer cells) in response to the recognition of infected cells.²

The primary antiviral action of IFNs occurs via a complex cellular pathway that is stimulated by viral attachment and ultimately leads to the expression and synthesis of interferon stimulated gene (ISG) products. HCV infection triggers IFN-independent signaling events in the host cell, including transcription factors and cofactors that

promote gene expression. The convergence of these signaling pathways initiates the production and secretion of IFN β . Secreted IFN β and therapeutically administered IFN α bind to a common receptor expressed on the surface of the target cell. This leads to the activation of the Jak-STAT signaling pathway, which features receptor-associated protein kinases that catalyze phosphorylation events leading to the activation and heterodimerization of the signal transducer and activator of transcription (STAT) proteins. The STAT 1/2 complex then translocates to the cell nucleus, where it associates with regulatory factors to form interferon-stimulated gene factor 3 (ISGF3). ISGF3 then binds to the IFN-stimulated response element (ISRE) and initiates the expression of ISG products, many of which have antiviral or immunomodulatory functions.³

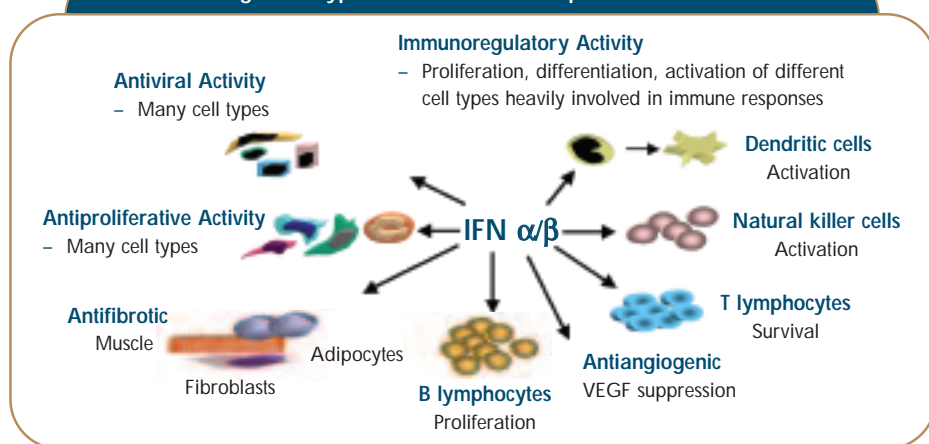
Type 1 IFNs have a large number of biological actions; most of these actions involve the immune system (see Figure 1). IFN induction, early in the innate immune response, provides a priming mechanism that influences many subsequent

innate and adaptive immune functions. For example, type 1 IFNs can activate natural killer (NK) cells and increase T lymphocyte survival.^{4,5,6} The primary action of type 1 IFNs on viral replication is their ability to suppress infection. IFNs can inhibit viral replication at several sites and by multiple mechanisms, including processes that disrupt viral entry and/or uncoating, alter viral RNA transcription and replication, alter viral and host RNA metabolism, influence processes of viral RNA translation, or effect viral maturation, assembly, or release. Type 1 IFNs also have antiproliferative, antifibrotic, and antiangiogenic biologic actions.

Viral decay in response to exogenous IFN displays a bimodal pattern. After a lag period of 4 to 12 hours, in which ISGs are synthesized and activated, a rapid decline in viral load is initiated.

A block of virion production or release characterizes the first phase of viral decay, with the effect being IFN-dose dependent. The rapidity of viral decline in the first phase is predictive for

Figure 1. Type 1 IFNs: Exhibit Multiple Activities^{4,5,6}



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achieving sustained virologic response. The slope of the second phase is less steep and is determined by the rate of infected cell clearance. In HCV, the second phase is prolonged, lasting weeks to months, and results from the elimination of infected cells in the liver, most likely by IFN-stimulated activated T-cells and natural killer cells.

Of note, when treating HCV patients, ribavirin works in synergy with IFN during the second phase of viral decline. This enhancement in the second phase means that the addition of ribavirin to patients with slower rates of viral decline (eg, difficult-to-treat patients such as African Americans, those with genotype 1, and certain nonresponders) may help to increase the rate of complete viral clearance.⁸

The type I IFN family of proteins is 166 amino acids in length. Approximately 85 of these amino acids are conserved in the known IFN α subtypes. The approved IFN for HCV treatment, IFN α -2a and IFN α -2b, are structurally very similar to each other, and both are substantially different from consensus interferon (CIFN). Specifically, there is only 1 amino acid difference between IFN α -2a and IFN α -2b. In contrast, there are 18 amino acid differences between IFN α -2a and CIFN, while there are 19 amino acid differences between IFN α -2b and CIFN.^{9,10}

The structural differences among known IFN α subtypes contribute significantly to the observed differences in biological activity of these agents. For example, Ozes and colleagues compared differences in antiviral activity between available therapeutic IFNs against vesicular stomatitis virus (VSV).¹¹ In these assays, HeLa (human epithelioid cervical carcinoma) and ME-180 (human cervical carcinoma) cells were incubated with IFN for 24 hours and then exposed to VSV. One unit of IFN was defined as the amount of IFN that inhibited the cytopathic effects of VSV by 50%. Notably, CIFN has approximately 10-fold greater antiviral activity (units per mg of protein) against VSV in HeLa cells, and approximately 100-fold greater activity against VSV in ME-180 cells compared with IFN α -2a and IFN α -2b. The activity of CIFN against VSV in HeLa cells was 625-fold greater than that of PEG-IFN α -2a and 31-fold greater than that of PEG-IFN α -2b. Of note, the pegylated interferons display reduced antiviral activity in vitro compared to their standard counterparts because the pegylation process alters the pharmacokinetic profile of the drug (they are cleared from the body more slowly) in exchange for a reduction in antiviral activity.

The relative antiproliferative effects of CIFN, IFN α -2a, and IFN α -2b appear to parallel the antiviral activity. These antiproliferative effects

were investigated in a human Burkitt's lymphoma cell line (Daudi cells). At equimolar concentrations, CIFN exhibited greater antiproliferative activity (inhibition of Daudi cell growth) compared with IFN α -2a and IFN α -2b at 72 and 96 hours after exposure. This difference is particularly pronounced at lower concentrations (in the range of maximum serum concentrations seen in human subjects).^{10,11}

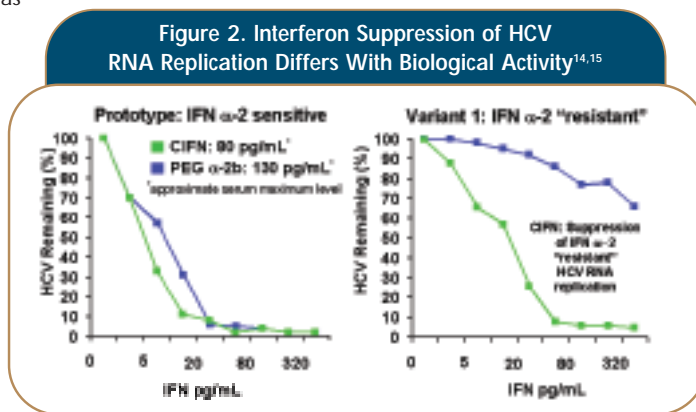
During the course of IFN therapy, IFN-mediated NK cell activation has been postulated to play a central role in the removal of HCV-infected cells during the second phase of viral decline. The ability of α -IFN subtypes to induce NK cell activity is also variable. When IFN-stimulated (CIFN, IFN α -2a, or IFN α -2b) NK cells are exposed to relatively NK sensitive chronic myelogenous leukemia cells (K-562), CIFN more effectively induces cytotoxic activity than either IFN α -2a or IFN α -2b ($P < 0.05$ in 6, 12, and 25 effector cell-to-target ratio, and $P < 0.01$ in 100 effector cell-to-target cell ratio). CIFN also has a significantly greater ability to induce NK activity against a relatively NK resistant target cell line (Eskol, hairy cell leukemia) than either IFN α -2a or IFN α -2b ($P < 0.05$).¹¹

Although the basis of the apparent enhanced efficiency seen with CIFN has not been completely elucidated, current theory holds that increased receptor binding affinity and possibly the induction of ISGs may account for these observed effects. Cell surface binding studies have demonstrated that CIFN had significantly greater receptor binding affinity compared with IFN α -2b and an approximately 10-fold greater biologic potency.¹³ Moreover, at any given level of biological activity, reduced molar concentrations of CIFN were needed to occupy similar receptor site numbers as IFN α -2a or IFN α -2b, indicating that differences in cell surface binding may have been sufficient to produce differences in biological activity.¹³ In addition, CIFN, when compared to IFN α -2b, was associated with an approximately 2-fold increase in PKR gene expression and a 5- to 6-fold increase in ISG56 expression (these and other ISGs were involved in controlling HCV replication).¹⁴

What do these interferon differences mean for the treatment of patients with HCV infection? Theory holds that part of the reason that nearly 50% of

patients with HCV infection fail treatment with PEG-IFN plus ribavirin involves IFN α resistance. In other words, host cell antiviral response directs selective pressure for viral sequence evolution, potentially leading to resistance.¹⁵ Specifically, targeted suppression of ISGs, such as ISG56 via mutations in NS5A and other viral proteins, may contribute to viral persistence and resistance to exogenous IFN α as well as host response evasion.¹⁶

The ability of treatments to be effective against resistant viral strains is critical when considering management options in HCV patients who have failed previous attempts at therapy. Emerging evidence suggests that the relative antiviral properties of CIFN and PEG-IFN α -2b in IFN α -2 resistant replicon variants differ significantly (see Figure 2).^{14,15} In IFN α -2 sensitive variants, CIFN and PEG-IFN α -2b produced similar reductions in HCV levels across a wide range of IFN concentrations. However, in IFN α -2 resistant variants, CIFN was associated with greater antiviral activity. At approximate maximum serum concentrations of each agent (80 pg/mL for CIFN and 130 pg/mL for PEG-IFN α -2b), CIFN produced an approximately 90% decrease in HCV levels, while PEG-IFN α -2b only produced about a 20% reduction in viral levels.¹⁴



Thus, the antiviral properties of all type 1 IFNs are not equal, and on a molecular level, virologic response and biologic potency may be influenced by selective pressures leading to direct viral resistance to IFN. These HCV genetic adaptations and resistance mechanisms pose problems in the management of HCV infection with IFN-based therapies. When HCV infected patients are exposed to a course of IFN α and develop resistance to treatment, evidenced clinically as treatment failure, retreatment with the same or similar IFN α generally has very little success. Consequently, to provide patients with improved chances of achieving a sustained virologic response, IFN potency must be considered in the approach to HCV therapy.

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CASE STUDY: RETREATMENT OF HCV NONRESPONDER

CARROLL B. LEEVY, MD

History of Present Illness

A 31-year-old Indian gentleman was referred to a hepatologist by his primary care physician after a local gastroenterologist unsuccessfully treated him with peginterferon α -2b plus ribavirin. The patient was diagnosed 18 years prior with non-A non-B chronic hepatitis, and in 1998, the diagnosis was confirmed to be chronic hepatitis C with a positive hepatitis C antibody test and an HCV RNA PCR greater than 850,000 IU/mL (the superior limit of detection at that time). His genotype is 1b.

The patient received interferon α -2b, 3 MU TIW plus ribavirin 1000 mg daily for 48 weeks between August 1999 and July 2000. He was also treated with peginterferon α -2b weekly and ribavirin 1000 mg daily for 48 weeks between March 2001 and February 2002. At the end of the second course of therapy, his HCV PCR RNA was 754,000 IU/mL, and his ALT was 59 IU/L. At the time of his first visit to the hepatologist, the patient complained of heartburn and right upper quadrant abdominal pain.

Past Medical/Surgical History

His past medical history revealed blood transfusions at birth in India due to erythroblastosis fetalis and chronic bronchitis diagnosed in 1997.

Social History

The patient is married with 1 daughter and denied ever having used illicit or intravenous drugs. He does not habitually drink alcohol.

Medications/Allergies

The patient takes no medications and has no known drug allergies.

Physical Exam

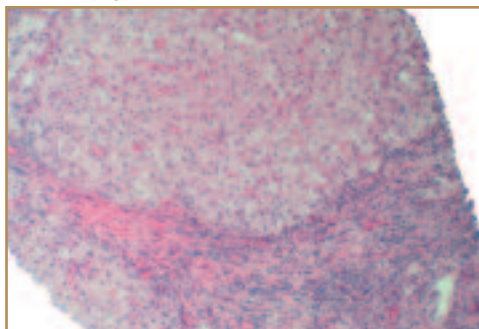
On physical examination, a mild hepatomegaly (14 cm in the midclavicular line and 11.6 cm in the midsternal line) was observed. A mild jaundice was noted on sclera examination. There was no ascites, pedal edema, or asterixis.

Vital Signs					
Height	Weight	Temperature	Pulse	Blood Pressure	Respiratory Rate
5 ft 8 in	178 lbs	36.7°C	80/min	110/70 mm Hg	20/min

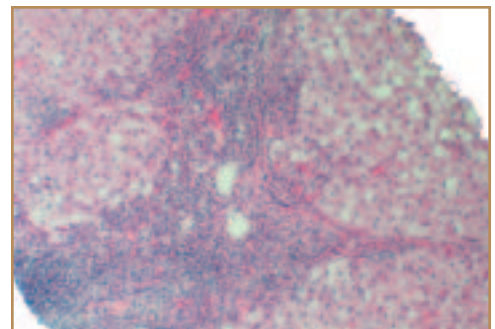
Laboratory Tests

Baseline Laboratory Values									
ALT IU/L	AST IU/L	Total Bilirubin mg/dL	HCV RNA IU/mL	WBC cells/ μ L	ANC cells/ μ L	Hemoglobin g/dL	Platelets cells/ μ L	TSH mIU/L	AFP ng/mL
273	270	1.49	1,469,000	4,900	2,562	15.7	161,000	0.79	6.2

Liver Biopsy



The liver biopsy showed stage III fibrosis with focal piecemeal necrosis involving most portal areas, mild parenchymal involvement, moderate chronic inflammation, and mild fatty change. A trichrome stain shows evidence of periportal fibrosis with focal evidence of bridging fibrosis.



Treatment/Clinical Course

He was started on daily interferon alfacon-1, 15 μ g subcutaneously plus ribavirin 1000 mg/day. The risks and benefits of this treatment as well as the potential teratogenic effects of treatment were discussed with the patient. In addition, the patient was informed that the proposed treatment regimen of daily interferon alfacon-1 plus ribavirin is off-label and not FDA approved.

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CASE STUDY

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The patient was seen on a monthly basis. During these visits he was assessed for depression and insomnia. Prior to each visit, blood was drawn for complete metabolic panel, CBC with differential, PT, PTT, and TSH. After the first month, the patient did complain of body aches, significant fatigue, and flu-like symptoms. According to the patient, these symptoms were similar in intensity to those experienced when he was on pegylated interferon.

Week 8: Temazepam 7.5 mg QD PRN was added to his regimen because of insomnia. The patient did not complain of depression.

Week 12: A triple phase CT scan of the abdomen was ordered because of an elevated AFP (24.7 ng/mL) and showed an enlarged spleen and liver with no enhancing masses. A follow-up MRI of the abdomen was suggested.

Week 20: A further drop in his neutrophil count (ANC 598 cells/ μ L) was noted and filgrastim (300 μ g sc QW) was added to his medication regimen. At the same visit, his AFP remained high at 22.3 ng/mL, and an MRI was done and the results confirmed the absence of any focal lesion in the liver.

Week 24: HCV RNA was undetectable for the

first time, and ALT and AST normalized to 47 IU/L and 42 IU/L, respectively.

Week 28: Further improvement in ANC (3,276 cells/ μ L, with the patient still on filgrastim) was noted. The patient denied any symptoms of depression or insomnia (he was still on temazepam 7.5 mg QD PRN).

Week 32: Filgrastim was discontinued due to an ANC of 7,200 cells/ μ L.

Week 36: HCV RNA was repeated and remained negative. Hemoglobin reached a low value of 11.1 g/dL.

Week 48: Daily interferon alfacon-1 plus ribavirin therapy was completed. At the end of this treatment, his HCV RNA PCR was negative (both qualitative and quantitative tests). An MRI of the liver did not show any focal lesion.

Six months after completing daily interferon alfacon-1 plus ribavirin therapy, his HCV RNA was still negative. An MRI of the liver did not show any focal lesions. The patient will be followed on a 6 month basis with repeat HCV RNA, alpha fetoprotein (AFP), complete metabolic panel, CBC with differential, PT, PTT, and INR. An MRI of the liver will be performed on an annual basis.

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