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Mini Review

Human Interferons Alpha, Beta and Omega

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Type I interferons (IFNs), IFN-α, IFN-β, IFN-ω, IFN-δ and IFN-τ are a family of structurally related, species-specific proteins found only in vertebrates. They exhibit a variety of biological functions, including antiviral, antiproliferative, immunomodulatory and developmental activities. Human Type I IFNs interact with the human IFN alpha receptor (IFNAR), which is composed of two identified subunits (IFNAR-1 and IFNAR-2). The interaction of IFN-α/β with its receptor components results in the activation of a number of signaling pathways. The regulation of specific genes and proteins contributes to the numerous biological functions of Type I IFNs.

Keywords: Type I interferons; Human IFN alpha receptor; Cytokines; Polypeptide

INTRODUCTION

In 1957, Alick Isaacs and Jean Lindenmann discovered interferon (IFN). Their experiment was a classic one in which they demonstrated that a supernatant derived from chick chorioallantoic membranes incubated with inactivated influenza virus was capable of protecting other membranes from infection by a live influenza virus challenge (Isaacs and Lindenmann, 1957). We now know that this supernatant contained soluble protein factors capable of inducing a variety of biological functions, including antiviral, antiproliferative, immunomodulatory and developmental activities. These factors were called IFNs and, to date, they have only been found in vertebrates. The original classification of IFNs was based on their cells of origin (e.g. IFN-α was known as leukocyte IFN and IFN-β as fibroblast interferon) and their antigenicity. Today, they are described by their gene and protein sequences.

There are two major categories of IFNs, designated as Type I and Type II. Type I IFNs primarily include IFNs-α, β and ω. IFN-ω diverged from IFN-α/β approximately 100 million years ago, yet has biological properties similar to IFN-α/β. In addition, IFN-δ and -τ have been reported to be Type I IFNs. IFN-τ is a 172 amino acid polypeptide that has been identified in ruminants and giraffes, but not in humans, although it is active on human cells. It is secreted by trophoblasts and plays a key role in the establishment of pregnancy (Martal *et al.*, 1998). IFN-δ is a 149 amino acid glycoprotein that is thus far only produced by pig trophoblasts. It has a high antiviral activity on porcine cells but not on human cells (Lefevre *et al.*, 1998). Type II IFN includes only IFN-γ. This article will focus on the human Type I IFNs, α, β and ω.

IFN-α consists of a family of structurally related proteins, while IFN-β and -ω exist as single protein subtypes (Table I). The induction, production and action of human Type I IFNs are shown in Fig. 1. These IFNs are induced by dsRNAs, viruses, other microorganisms, cytokines and growth factors. Interferon production is predominantly mediated by transcription factor complexes, (e.g. Nuclear factor kappa-B [NF-κB]), ATF (activating transcription factor)/JUN and interferon regulatory factors (IRFs), e.g. IRF-3 and IRF-7. It has been reported that upon induction, IFN-α4 and IFN-β are initially produced as a result of the action of IRF-3 and then are secreted by the cells. Subsequently, they interact with the Type I IFN receptor complex (IFNAR-1/ IFNAR-2) in an autocrine and/or paracrine manner. The interaction of Type I IFNs with their receptor complex results in the activation of IRF-3 and IRF-7. This event leads to the enhanced production of many Type I IFNs and then to the activation of multiple signal transduction pathways including the JAK/STAT pathway. These actions result in the transcription of numerous IFN-stimulated genes (ISGs) and ultimately to the biological functions of the Type I IFNs (see, Fig. 4 and Katze *et al.*, 2002).

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TABLE I   Human Interferons α, β and ω

Class	Alpha	Beta	Omega
Number of genes	14	1	1
Number of proteins	>22*	1	1
Glycosylation	≥3 species	Yes	Yes
Apparent molecular weight	17,500–27,000	20,000	25,000
Disulfide bonds	2	1	2
Number of amino acids	165–166	166	172
Chromosome coding for IFN	9	9	9
Chromosome coding for IFN receptor	21	21	21

*Thirteen functional proteins are expressed by the 13 functional genes. However, due to post translational modifications, more than 22 subtypes of IFN-α exist.

STRUCTURE AND FUNCTION

There are, at least, 14 human IFN-α non-allelic functional genes found on the short arm of chromosome 9 (Table I). They encode for 13 different human IFN-α proteins (α1, α2, α4, etc) with α1 and α13 resulting in identical mature proteins (minus the leader sequences) (Fig. 2) (Foster and Finter, 1998). All have 166 amino acids except IFN-α2, which contains 165 due to a deletion at position 44. In addition, some of these subtypes exist in variant forms e.g. IFNs-α2a (K23, H34), -α2b (R23, H34) and α2c (R23, R34). The various human IFN-α species have 75–99% amino acid sequence identity and are also highly species-specific with regard to biological properties. Of interest, the genes that encode for IFNs-β and -ω are also located on chromosome 9. The human IFN-β gene is present as a single copy. There is one human IFN-ω gene that results in a functional protein (Fig. 2), and this protein has six additional amino acids located at the carboxy-terminus (172 amino acid residues). With respect to the primary structures of the Type I IFNs, there is approximately 30% amino acid sequence identity between IFN-α and -β and about 75% identity between IFN-α and -ω.

X-ray crystallographic structures have been determined for HuIFN-α2b (Radhakrishnan *et al.*, 1996) and HuIFN-β (Karpusas *et al.*, 1997). In addition, a three dimensional, high resolution structure of IFN-α2a has been generated using heteronuclear NMR spectroscopy (Fig. 3) (Klaus *et al.*, 1997). Consistent among all Type I IFNs, is the tertiary structure containing multiple helices. However, unlike the four helices commonly found in other α-helical cytokines, IFN-α2a has been shown to contain five helices. The IFN-α2 helices are designated A (S11-M21), B and B' (T52-S68, K70-A75), C (E78-I100), D (L110-E132) and E (P137-L157) (Fig. 3), and are connected by loops AB, BC, CD and DE. IFN-β has also been shown to possess the same five helical structure. However, the helices consist of unique amino acid sequences.

The most conserved feature of Type I IFNs is the disulfide bond, with IFNs-α and -ω containing two disulfide bonds and IFN-β having one. The disulfide bonds in IFN-α are found between Cys1–Cys99 (98) and Cys29–Cys139(138). The residue numbers in parantheses refers to IFN-α2 which has a deletion at position 44. Three-dimensional modeling using human interferon-α consensus (IFN alfacon-1) has revealed three domains within the molecule (amino acid residues 29–35, 78–95 and 123–140). Domains 29–35 and 123–140 are in close proximity, given the disulfide bond between Cys-29 and Cys-139, and may comprise a single receptor recognition site. Replacement or modification of either cysteine connecting loop AB to helix E, results in a significant loss of biological activity. The third domain (78–95) may also play a role in receptor binding (Korn *et al.*, 1994). The one single disulfide bond found in IFN-β (Cys31–Cys141) is analogous to the Cys29–Cys139 (138) found in IFN-αs and is also thought to be fundamental in binding to the IFN receptor complex.

Production and Action of Human Type-1 Interferons
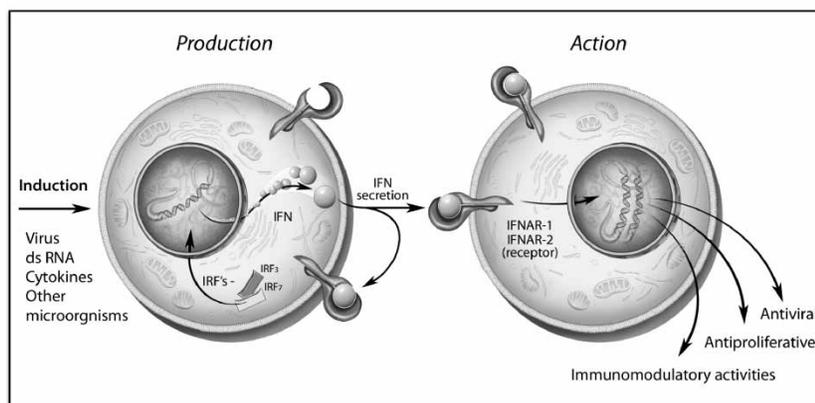
FIGURE 1   Production and Action of Human Type I Interferons. Interferon production can be induced by a number of agents (viruses, cytokines, etc.). Upon secretion, interferon binds to the Type I receptor complex (IFNAR-1/IFNAR-2), resulting in antiviral, antiproliferative and immunomodulatory effects. In addition, toll-like receptors have been implicated in induction via activation of interferon regulatory factors 3 and 7 (IRF-3 and IRF-7).
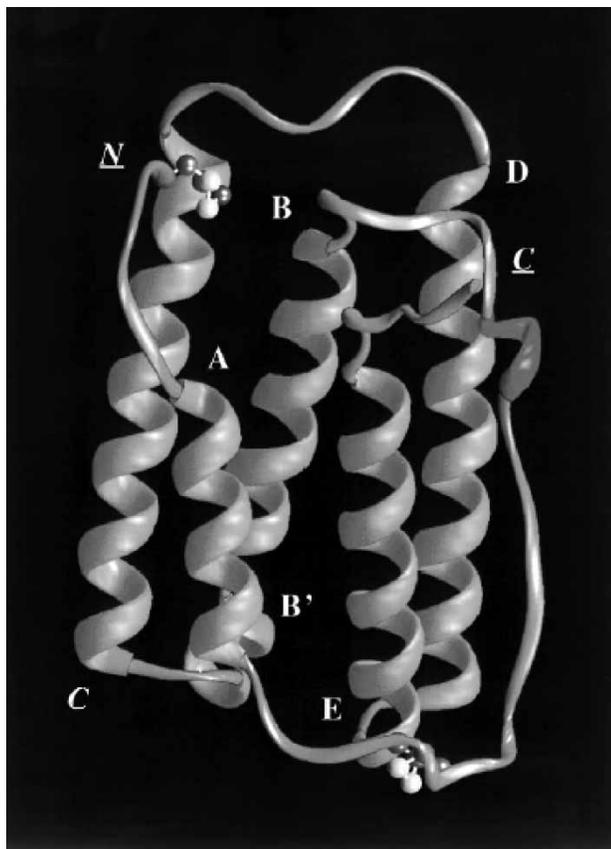
FIGURE 2 Amino acid sequences of 13 IFN-α species, IFN-β and IFN-ω. Of note are (1) IFNs- α1 and α13 are identical differing only by one amino acid in their leader sequences (not shown) and (2) there is a deletion at position 44 in IFN-α2.

FIGURE 3 Ribbon display of the structure of Interferon-α2a. The IFN-α2 helices are designated A (S11-M21), B and B′ (T52-S68, K70-A75), C (E78-I100), D (L110-E132) and E (P137-L157). The termini of the protein are indicated by N and C and the two disulfide bridges are shown in a ball-and-stick representation (Klaus et al., 1997).

Studies have shown that the region between amino acids 81–95 is important for IFN-α mediated antiproliferative activity on Daudi (Burkitt's lymphoma) cells (Hu et al., 2001). Construction of IFN-α mutants using site directed and cassette mutagenesis revealed that the Tyrosine Y-86 and Y90 have an important role in the antiproliferative activity. Four mutants were created in which Y86 was replaced with either aspartic acid (D), isoleucine (I), lysine (K) or alanine (A). It was observed that an isoleucine replacement had essentially no effect on the antiproliferative activity whereas substitution of D, K or A resulted in decreased antiproliferative activity by up to 100-fold. In contrast, replacing the asparagine (N) at position 90 with a Y significantly increased the antiproliferative activity. Circular dichroism (CD) studies showed that the secondary structure of the molecule did not change significantly as a result of the above mutations. Thus, a major conformational change does not appear to be responsible for the reduced antiproliferative activity. Advances in technology allowing for more information regarding the structures of the various IFNs (particularly the numerous IFN-αs), may help elucidate the diverse biological properties observed in these molecules that are so closely related.

TYPE I INTERFERON RECEPTORS

Although Type I IFNs induce multiple cellular effects, they appear to act through a common receptor complex, IFNAR (Type I IFN receptor complex), present in low numbers (100–5000 molecules/cell) on the surface of all vertebrate cells examined. The receptor complex consists of two known subunits, IFNAR-1 and IFNAR-2. Mature human IFNAR-1, resulting from removal of the peptide leader sequence is a 530 amino acid residue integral membrane protein. It is composed of an extracellular domain of 409 amino acid residues, a transmembrane domain of 21 residues and an intracellular domain of 100 residues. Mature human IFNAR-2 has been isolated as three forms. The full length receptor chain comprised of 487 amino acids, is referred to as IFNAR-2c and is 115 kDa in size. Two hundred and fifty-one of these amino acid residues reside in the cytoplasmic portion of the molecule. The two shorter forms are referred to as IFNAR-2b and IFNAR-2a. IFNAR-2b, consisting of 303 amino acids is 55 kDa of which only 67 residues are in the cytoplasmic portion (Novick et al., 1994; Pfeffer et al., 1997). IFNAR-2a is a soluble form lacking the transmembrane and cytoplasmic portions and is 40 kDa (Novick et al., 1994). Both HuIFNAR-1 (Ling et al., 1995) and HuIFNAR-2 (Novick et al., 1994) are glycosylated proteins.

On the basis of modular organization and also sequence alignment with other cytokine receptor chains, IFNAR-1 and IFNAR-2 are members of the class II helical cytokine receptor family (Uze et al., 1995). All share conserved structural fibronectin type III (FNIII) regions that form the extracellular ligand-binding domain. The extracellular portion of IFNAR-1 is composed of four fibronectin type III-like repeats, which make up two domains separated by a three-proline motif. There is 19% sequence identity and 50% sequence similarity (identity plus conserved amino acid changes) between these two domains. Each domain is composed of approximately 200 residues and can be further subdivided into two homologous subdomains of approximately 100 amino acids. The extracellular portion of IFNAR-2 has two FNIII domains (characteristically partitioned into two β-sheets), each containing 100 amino acids with seven β-strands and connecting loops. IFNAR-1 and -2 contribute to ligand binding to different extents, but both are necessary to form a functional binding site for IFNs. Transmembrane domains of IFNAR-1 and -2 do not have intrinsic enzyme activities, but their cytoplasmic domains associate noncovalently with the tyrosine kinases, TYK-2 and JAK-1, respectively (TYK-2 associates with IFNAR-1 and JAK-1 associates with IFNAR-2), which are also required for the productive presentation of the binding site (Uze et al., 1992; Novick et al., 1994; Stark et al., 1998). When IFNs bind to the extracellular domains of the receptor complex, the cytoplasmic domains activate specific tyrosine kinases (Stark et al., 1998; Sen, 2001). In addition to tyrosine kinases, tyrosine phosphatases also associate with

the functional Type I IFN receptor complex and regulate the engagement of TYK-2 and JAK-1 in IFN signaling (Platanias, 2003). The importance of both IFNAR-1 and -2 for IFN-mediated signaling is highlighted by the observation that in the absence of either chain there is neither high affinity binding of IFN, activation of the requisite tyrosine kinases or STAT proteins, nor a biological response (Uze *et al.*, 1992).

Human IFNAR-1 (HuIFNAR-1, 135 kDa) exhibits low intrinsic binding of human Type I IFNs (Kd $\sim 10^{-7}$ M) but strongly affects the affinity and differential ligand specificity of the IFNAR complex (Kd $\sim 10^{-11}$ M). Human IFNAR-2 (HuIFNAR-2, 115 kDa) binds all human Type I IFNs but with lower affinity (Kd $\sim 10^{-9}$ M) than the IFNAR complex (Novick *et al.*, 1994; Russell-Harde *et al.*, 1995; Cutrone *et al.*, 1997). When HuIFNAR-1 is co-transfected with HuIFNAR-2 into *Xenopus laevis* oocytes, the affinity of the receptor complex for most human Type I IFNs, including IFN-β increases by approximately 10-fold over IFNAR-2 alone (Lim *et al.*, 1994).

One of the most intriguing aspects of the function of the Type I IFN receptor complex is its ability to discriminate between the various types and subtypes of IFN, and to elicit different biological responses depending upon which ligand is bound. Although IFN-α and -β share a common receptor complex they interact differently with IFNAR-1 and -2. For example, IFN-β selectively induces the association of tyrosine-phosphorylated IFNAR-1 and -2 (as detected by immunoprecipitation with anti-IFNAR-1 antibodies), while IFN-α1, -α2, -α6, -α7, -α8 and IFN-ω do not (Platanias *et al.*, 1996). Previously, the anti-proliferative and competitive binding activities of 20 purified components of human lymphoblastoid interferon (IFN-α) were compared with that of recombinant human IFN-α2b on Daudi and AU937 cells. These data suggest that there may be more than one binding site or receptor for human IFN-α, and/or there may be additional protein components associated with the receptor complex which are involved in the varied biological actions of these molecules (Hu *et al.*, 1993).

From the point of view of the various Type I IFNs, despite their sequence differences, they most likely bind to extracellular portions of IFNAR-1 and -2 in a similar manner but mediate different biological responses (Lewerenz *et al.*, 1998; Lu *et al.*, 1998). The binding site for IFNAR-2 was primarily mapped on the AB-loop and D-helix of IFN-α2. Six amino acid residues in the interferon molecule have been designated as "hotspots" for binding to the IFNAR2 portion of the Type I IFNAR complex. They are L30 and R33 (both found in the AB loop) and R144, A145, M148 and R149 (all situated in helix E) (Deonarain *et al.*, 2002). Schreiber *et al.* carried this study further by performing mutational analyses of the binding interface. These mutations included R144A, A145G, M148A and R149A. Each mutation led to a significant decrease in binding affinity (>20-fold). Mapping studies and comparisons with structurally

similar cytokines (IFN-β, IL-19, IL-22) suggest the involvement of the E-helix of IFN-α2 in the binding of IFNAR-2 (Seto *et al.*, 1995). In addition, more recent findings showed that the E helix of IFN-α2 constitutes the center of the IFNAR-2 binding site (Piehler *et al.*, 2000). In contrast, the binding site for IFNAR-1 is probably on the C-helix of IFN-α2 (Mogensen *et al.*, 1999; Fickenscheer *et al.*, 2002).

The significance of the C-terminus of the IFN molecule in IFNAR-2 binding has been an area of contention, with some reporting that IFNs truncated at the C-terminus maintain antiviral activity and others finding the opposite result. Piehler *et al.* have shown, however, that the affinity and rate constants of the truncated and wild-type protein are similar, hence concluding that the C-terminus does not appear to be involved in IFNAR-2 binding (Piehler *et al.*, 2000). In spite of intensive studies, the interactions between IFNs and their receptor complex and the molecular mechanisms underlying the functional biological differences among the IFNs are still largely unknown.

SIGNAL TRANSDUCTION

Studies on the mechanism of signals induced by Type I IFN ligand binding to its receptor complex have elucidated multiple pathways resulting in the discovery of several key components (Kalvakolanu, 2003). These include association with Janus kinase (JAK) and Tyrosine kinase (TYK), subsequent activation of signal transducer and activator of transcription (STAT) proteins, RAS-related C3 botulinum substrate-1 (Rac1), p38/mitogen activated protein kinase (MAPK), insulin receptor substrate (IRS), the phosphotidylinositol-3 kinase (PI3K) cascade, and CrkL, a member of the Cdc2 related kinase family.

The classic pathway induced by Type I IFNs involves the interaction of the IFN with two-receptor subunits, IFNAR-1 and -2, which are associated with TYK-2 and JAK-1, respectively. TYK-2 and JAK-1 phosphorylate tyrosine residues on the receptor that provide docking sites for the src-homology-2 (SH2) domains of STATs in a cell-type specific manner (Fasler-Kan *et al.*, 1998) (see Fig. 4). Once phosphorylated, STATs are released from the receptor and form homodimers as well as heterodimers. In response to Type I IFNs, STAT2 is recruited to the IFNAR1 chain, where it is phosphorylated by TYK-2 and serves as a lure for STAT1 (Goodbourn *et al.*, 2000). Although this observation suggests that STAT1 phosphorylation is contingent upon the prior activation of STAT2, conflicting data indicates the activation of STAT1 in the absence of STAT2. Once released from the receptor, the resulting STAT1: STAT2 heterodimer associates with p48, a DNA binding protein (also called IRF-9), forming a complex called IFN-stimulated gene factor-3 (ISGF3). After formation, ISGF3 translocates to the nucleus where it binds to the IFN-stimulated response elements (ISRE) upstream of IFN response genes and initiates transcription. The ISRE regulates most genes that respond
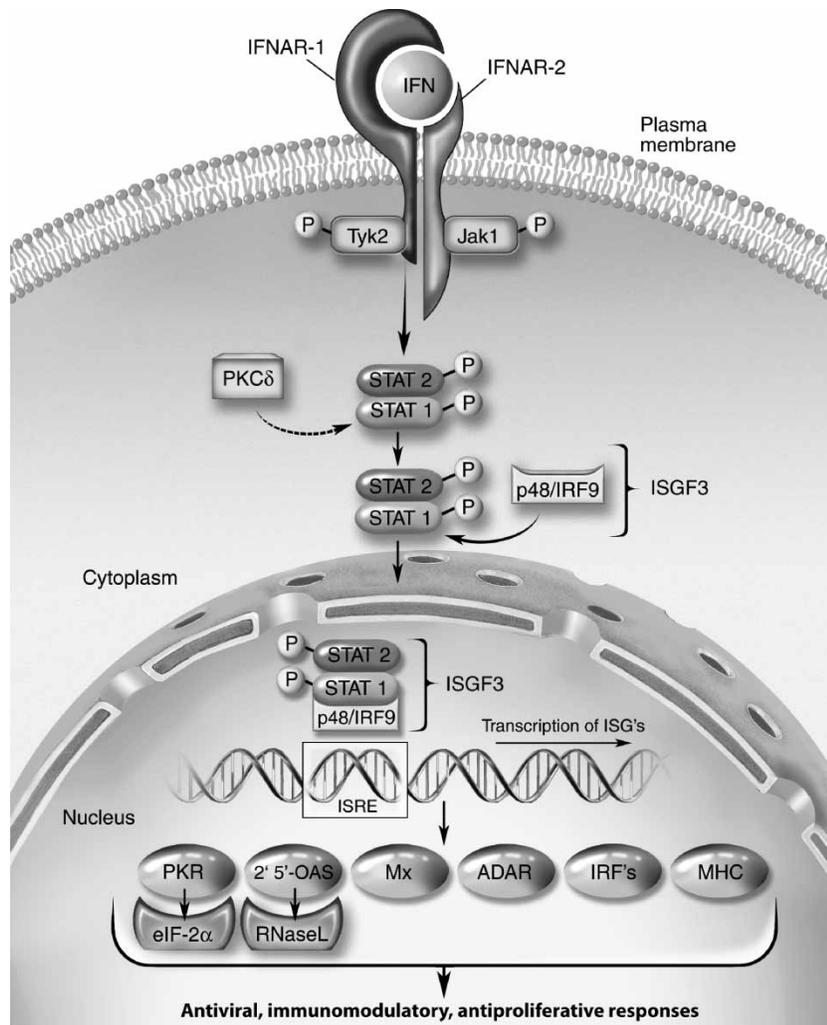
FIGURE 4   The signal transduction mechanism of Hu IFN-α/β. Interferon binding causes the two subunits of the receptor (IFNAR-1/IFNAR-2) to aggregate, which leads to activation and phosphorylation of TYK2 and JAK1. These then phosphorylate STAT1 and STAT2. The DNA-binding protein p48 (IRF-9) forms a complex with STAT1 and STAT2 that moves to the nucleus, where it stimulates transcription of genes bearing an interferon-response element (ISRE). Some of the Type I IFN mediated ISG's contributing to cellular antiviral responses, are serine/threonine protein kinase (PKR), $2',5'$-oligoadenylate synthetase (OAS), myxovirus-resistance proteins (Mx), RNA-specific adenosine deaminase (ADAR), IRFs and MHC (Katze, 2002).

to IFNα and generally is found within 200 base pairs of the transcription start site. Although phosphorylation of STAT1 at tyrosine 701 is required for ISGF3 DNA binding, an additional activation site at serine 727, mediated by protein kinase Cδ (PKC-δ), is essential for efficient transcriptional activity (Brierley *et al.*, 2002; David, 2002). Studies using STAT1 and STAT2 knockout mice have demonstrated an increased suscep-tibility to viral and other microbial infections due to impaired IFNα signaling further demonstrating their classic role in viral resistance.

In addition to STATs 1 and 2, STATs 3, 4, 5 and 6 have also been shown to have a role in the response to Type I IFNs. In addition to its interaction with IFNAR-1, a recent study observed STAT3 tyrosine phosphorylation mediated through the IFNAR-2 subunit. STAT3 also functions as an adapter protein for the p85 subunit of the serine kinase, phosphatidylinositol 3-kinase (PI3K). This interaction may function by coupling a serine kinase cascade to

the IFN receptor. IFNα/β mediated formation of STAT3 homodimers and STAT1: STAT3 heterodimers have also been found to bind IFN-γ activated sequences (GAS) located upstream of IFN-stimulated gene (ISG) promoters (Platanias and Fish, 1999). In addition, IFN-α mediated phosphorylation of STAT4 may be involved in the development of Th1 cells at the point of convergence of innate and adaptive immunity. Finally, STAT5 and STAT6 were also shown to be activated by IFNα treatment of Daudi cells; however, the mechanism by which this occurs remains unclear. STAT5 was also shown to interact with CrkL in response to Type I IFN. These two proteins form a complex that subsequently translocates to the nucleus where it participates in the regulation of the GAS elements (Lekmine *et al.*, 2002). In addition, CrkL also acts as an adaptor protein, binding upstream to CBL and down-stream to C3G. C3G is a guanine exchange factor for the small G-protein Rap1, which is associated with tumor suppressor activity (Platanias and Fish, 1999).

Furthermore, STAT6 has been shown to interact with STAT2 and p48 resulting in an ISGF3-like trimeric complex. Further studies are needed to reveal the role of the complex.

It has been suggested that the PI3K pathway plays a significant role in cell survival in response to Type I IFNs. The JAK-dependent PI3K serine kinase is composed of a p85 subunit and a catalytic subunit p110. The p85 subunit interacts with both IRS-1 and -2, resulting in the activation of the p110 subunit. Downstream of PI3K in the pathway, nuclear factor kappa-B(NF-κB) and PKC-δ, are associated with anti-apoptotic effects observed in neutrophils incubated with IFN-β (Wang *et al.*, 2003). Another protein located downstream in the IFN-dependent PI3K pathway and involved in cell survival is the extracellular regulated kinase (ERK). Interestingly, ERK has been found to coimmunoprecipitate with STAT1. The role of IFN-α-stimulated STAT1 activation and the effect on ERK binding needs to be examined further.

The antiviral response stimulated by Type I IFNs may be dependent on the p38 pathway also downstream of PI3K. The p38 MAPK signaling cascade is induced via two small G-proteins, Rac1 and Cdc42. Studies in hematopoietic cells have led to the speculation that JAK tyrosine kinases phosphorylate the *vav* proto-oncogene product, which may subsequently function in promoting GDP/GTP exchange for Rac1 (Platanias *et al.*, 2003). Although the intermediary between Rac1 and p38 has not yet been elucidated, studies have shown that PKCδ functions as an upstream regulator of p38. In addition, Type I IFN-treated p38α knockout cells exhibited defective gene transcription. Furthermore, the pharmacological inhibition of p38 appears to block IFN-α-mediated antiviral responses. Finally, a recent study suggests that two p38 downstream targets, Mitogen and Stress Activated Kinase 1 (MSK1) and MAP Kinase-Activated Protein Kinase 2 and 3 (MAP KAP-2 and MAP KAP-3) participate in the regulation of gene transcription (Li *et al.*, 2004).

Other factors are suggested to play a role in the production, regulation and biological action of Type I IFN. Two members of the interferon regulatory factor (IRF) family, IRF3 and IRF7 are necessary for transcription of Type I IFNs. Both IRF3 and IRF7 are phosphorylated in response to viral infection downstream of the toll-like receptors (TLR) (Fitzgerald *et al.*, 2003). IRF7 gene expression, in turn, is dependent on the Type I IFN mediated ISGF3 complex, thereby involved in a positive feedback loop (Taniguchi and Takaoka, 2002).

It has also been demonstrated that CD45 and Zap-70, in the T cell receptor (TCR) signaling pathway, contribute to the antiproliferative activity of Type I IFN when they are associated with IFNAR-1. Moreover, Type I IFN has been shown to induce nuclear translocation of nuclear factor of activated T-cells (NFAT), which is pre-associated with STAT1. Taken together, these findings provide extensive insight on the intracellular events involved in Type I IFN signaling and in the potential for cross-talk between the pathways.

CLINICAL USE OF HUMAN INTERFERON α AND β

Human IFN-α2a, recombinant (Roferon-A®, Hoffmann La Roche, Inc.) and human IFN- α2b, recombinant (Intron-A®, Schering Corporation) were the first two IFNs licensed by the Food and Drug Administration in 1986 (USA) for the treatment of hairy cell leukemia (Physicians' Desk Reference, 2004; www.fda.gov). Both of these IFNs were derived from genetically engineered *Escherichia coli* containing either the human IFN-α2a gene or the human IFN-α2b gene, respectively. Each of these IFNs has an antiviral specific activity of approximately 2×10^8 IU/mg protein, is >95% pure by SDS PAGE prior to formulation, and is comprised of 165 amino acids. Subsequently these IFNs were approved for clinical use for a variety of viral and cancer indications including treatment of chronic hepatitis B and chronic hepatitis C (with and without ribavirin USP), and AIDs-related Kaposi's Sarcoma (see Table II). Some of the side effects of these products include fever, flu-like symptoms, fatigue, myalgia, chills, headache, nausea, hepatic disorders, vomiting, depression, suicidal ideation, alopecia, cardiovascular effects and hematologic disorders (for a complete list of side effects see package insert of each IFN product). In general, the higher the dose of IFN administered, the greater the frequency and severity of the side effects that are observed.

Subsequently, pegylated forms of these two IFNs have been approved in the USA for the treatment of chronic hepatitis C either alone or in conjunction with ribavirin

TABLE II   Human Type I Interferons licensed in the USA

Product	Protein	Indications
Intron-A®	Interferon alfa-2b	Hairy cell leukemia, chronic hepatitis B, condylomata acuminata, follicular lymphoma, AIDS-related kaposi's sarcoma, chronic hepatitis C, malignant melanoma
Roferon-A®	Interferon alfa-2a	Chronic myelogenous leukemia, hairy cell leukemia, AIDS-related kaposi's sarcoma, Chronic hepatitis C
Infergen®	Interferon alfacon-1	Chronic hepatitis C
Alferon®	Interferon alfa-n3	Condylomata acuminata
PEG-Intron®*	Pegylated interferon alfa-2b	Chronic hepatitis C
PEGASYS®*	Pegylated interferon alfa-2a	Chronic hepatitis C
Betaseron®	Interferon β-1b	Multiple sclerosis
Avonex®	Interferon β-1a	Multiple sclerosis
Rebif®	Interferon β-1a	Multiple sclerosis

* Can be used with ribavirin for the treatment of chronic hepatitis C (see Physicians' Desk Reference (2004)).

(USP). Pegylated interferon-α2a (PEGASYS®, Hoffmann La Roche, Inc.) is a covalent conjugate of human IFN-α2a with a single branched bis-monomethoxy-polyethylene glycol (PEG) chain with an apparent molecular weight of 40,000 (total apparent molecular weight of 60,000). Pegylated interferon-α2b (PEG-INTRON®, Schering Corporation) has an approximate apparent molecular weight of 31,000 and a specific antiviral activity of 0.7×10^8 IU/mg protein. The side effects of these pegylated interferon products are similar to those of unpegylated IFN products. The advantage of the pegylated forms of these IFNs appears to be their longer half-lives. Thus, use of pegylated IFNs results in a reduction in the number of doses needed per week for the treatment of chronic hepatitis C with a concomitant decrease in some of the side effects.

A number of additional IFN-αs have been approved for human use over the past 18 years (see Table II, as of May 1, 2004). Interferon-α-n3 (ALFERON N Injection®, Interferon Sciences, Inc./Hemispherx Biopharma, Inc.) is currently the only natural human IFN-α licensed in the USA. IFN-α-n1 (lymphoblastoid derived; Wellferon®, GlaxoWellcome now GlaxoSmithKline, Inc.) was approved for use in the USA, but the license was subsequently voluntarily withdrawn. Interferon-α-n3 is produced from human leukocytes induced with Sendai virus and purified by immunoadsorbent affinity and size exclusion chromatography. It consists of approximately 10 IFN-α proteins with apparent molecular weights of 16,000–27,000. Its specific antiviral activity is approximately equal to or greater than, 2×10^8 IU/mg of protein and it is approved for the treatment of refractory or recurring external condylomata acuminata using intralesional injection. Some of the side effects include fever, headache, chills and fatigue. Finally, interferon alfacon-1 (INFERGEN®, InterMune Pharmaceuticals, Inc.) is a recombinant non-naturally occurring IFN-α with 166 amino acids. It was generated by assessing the most highly conserved amino acids in each corresponding region based on the known cloned IFN-α sequences. It has 88% sequence identity to IFN-α2b and a specific antiviral activity of approximately 10^9 IU/mg protein. Interferon alfacon-1 is approved for the treatment of chronic HCV infection in patients 18 years or older with compensated liver disease. The side effects are similar to those observed with human IFN-α2a or -2b.

The first human IFN-β to be licensed in the USA was interferon-β-1b (Betaseron®, Chiron Corporation) for the treatment of relapsing- remitting multiple sclerosis to reduce the frequency of clinical exacerbations (Table II) (Physicians' Desk Reference, 2004). Betaseron® is a 165 amino acid recombinant DNA-derived interferon produced in *E. coli* and unlike the native interferon-β, Betaseron is not glyscosylated and has a serine in lieu of a cysteine residue at position 17. It has a specific antiviral activity of approximately 3×10^7 IU/mg protein, which is about 10-fold less than native IFN-β. Subsequently two other human IFN- βs, interferon- β-1a

(Avonex®, Biogen, Inc.) and interferon-β-1a (Rebif®, Serono, Inc.) were licensed in the USA for the treatment of patients with relapsing forms of multiple sclerosis to decrease the frequency of clinical exacerbations and delay the accumulation of physical disability. Avonex® is composed of a purified 166 amino acid polypeptide produced by recombinant DNA technology using Chinese Hamster Ovary (CHO) cells that have the interferon-β-1a gene inserted. This IFN is glycosylated and has a specific antiviral activity of approximately 3×10^8 IU/mg protein, similar to the purified native IFN-β. Rebif® is also composed of a purified 166 amino acid glycosylated (one N-linked site) protein with an apparent molecular weight of 22,500. This protein was produced by recombinant DNA technology using CHO cells into which the interferon-β-1a gene has been introduced. It also has an antiviral specific activity of approximately 3×10^8 IU/mg protein. Human IFN-β products are generally administered intramuscularly or subcutaneously for most indications. The side effects of the human interferon-βs are similar to those observed with the human interferon-αs. Some adverse events include flu-like symptoms, fever, headache, myalgia, hematologic disorders, liver function disorders, injection site reaction, depression, and suicidal ideation (for the complete list, see the package insert of the designated product).

In addition, a number of patients develop both neutralizing and non-neutralizing antibodies in response to the IFN-α (both natural and recombinant forms) or IFN-β treatment. Patients may need to be monitored for the production of these antibodies, since high titers of neutralizing antibodies can abrogate the clinical effectiveness of these IFNs and their natural counterparts.

CONCLUSION

In conclusion, Type I IFNs are important components of our natural defenses against microbiological infections, cancer and many other disorders. While we now know much about their structure, function and clinical use, much more remains to be learned in terms of their interaction with their receptor(s), their signal transduction pathways, and their clinical applications.

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