CMLS, Cell. Mol. Life Sci. 54 (1998) 1203–1216
1420-682X/98/111203-14 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Review

The structure of human interferon-β: implications for activity

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Received 12 June 1998; received after revision 16 July 1998; accepted 16 July 1998

Abstract. Interferons (IFNs) are potent extracellular protein mediators of host defence and homoeostasis. This article reviews the structure of human IFN-β (HuIFN-β), in particular in relation to its activity. The recently determined crystal structure of HuIFN-β provides a framework for understanding of the mechanism of differentiation of type I IFNs by their common receptor. Insights are generated by comparison with the structures of other type I IFNs and from the interpretation of existing mutagenesis data. The details of the observed carbohydrate structure, together with biochemical data, implicate the glycosylation of HuIFN-β, which is uncommon among type I IFNs, as an important factor in the solubility, stability and, consequently, activity of the protein. Finally, these structural implications are discussed in the context of the clinical use of HuIFN-β.

Key words. Interferon; crystal structure; cytokine; aggregation; glycosylation; multiple sclerosis.

Introduction

The interferons (IFNs) comprise an evolutionarily conserved family of small, secreted proteins that participate as extracellular messengers in a wide range of host responses. Many chemical and biological agents such as viruses, bacteria, mycoplasma and protozoa can induce cells to synthesize and secrete IFNs [1]. Binding of IFNs to specific cell surface receptors triggers an intracellular signalling cascade ultimately resulting in the synthesis of proteins that mediate pleiotropic activities, including antiviral, antiproliferative and immunomodulatory responses [2] that act to maintain homoeostasis and in host defence. Interferons also have been shown to possess antifibrotic activity [3, 4] and to have a direct antiproliferative effect on cancer cells [5].

Interferons are classified as helical cytokines and are categorized as type I or type II according to their physical and functional properties. Type I IFNs, which are believed to act through a common receptor [6–8], are further divided into α, β, τ and ω subtypes that likely diverged from a common ancestral gene through mechanisms involving gene duplication [9]. The sole representative of type II is IFN-γ, which differs from type I IFNs by virtue of its homodimeric structure, expression profile and action through a unique receptor. Type I IFNs comprise more than 10 IFNs-α, together with the unique, naturally occurring, proteins IFN-β

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and IFN-ω (10). Interferon-τ has been found exclusively in ruminant ungulates, where it plays a role during pregnancy [8, 11]. The human IFNs-α share a high degree of sequence homology ($\sim$80%); their homology with IFN-β is lower but still is significant (30–50%). The only human type I IFNs known to be glycosylated are IFN-α2 [12] and IFN-β.

The type II IFN receptor is specific for the single ligand IFN-γ, and the crystal structure of its high-affinity chain in complex with IFN-γ has been solved [13]. The type I IFN receptor is not as well characterized at present. Two distinct chains of the type I IFN receptor, interferon-α receptor (IFNAR)-1 and IFNAR-2, have been identified and found to bind IFNs-α and IFN-β [14, 15]. IFNAR-1 and IFNAR-2 are members of the class 2 cytokine receptor family [16], along with the receptors for IFN-γ and interleukin (IL)-10 [17]. Despite the diversity of ligands and associated biological responses, type I IFNs are all believed to signal cells through the common IFNAR-1/IFNAR-2 cell surface receptor complex [7–9]. A major focus of research, therefore, is to elucidate the detailed mechanism by which the receptor distinguishes one IFN from another. Recently, three-dimensional structural information at the atomic level has become available through X-ray crystallographic and nuclear magnetic resonance (NMR) studies. Although the ultimate prize, a structure of a type I IFN complexed with a receptor protein, is yet to be attained, the evidence of three IFN crystal structures, in conjunction with biochemical data, allows for the formulation of an initial model for IFN receptor-ligand binding and signal transduction.

The broad therapeutic potential of IFN has been explored extensively over the last 2 decades, culminating in the approval of various type I IFNs for the treatment of numerous diseases [18, 19]. Questions of biochemical mechanism in determining clinical efficacy therefore may be considered in conjunction with pharmacological issues, especially in the case of a therapy that must be administered long-term. This article reviews recent structure-function research in the particular case of human IFN-β (HuIFN-β), which is commercially produced by recombinant methods and has been licensed for the treatment of relapsing forms of multiple sclerosis (MS). The article begins with a recapitulation of the recently solved X-ray crystal structure of HuIFN-β and a comparison with reported structures of other type I IFNs. Next, mutational studies with IFN-β and other IFNs that provide a model of the IFN-ligand/receptor complex are discussed, as are possible mechanisms for type I IFN differentiation by their common receptor. Finally, the article summarizes from a structural viewpoint recent research concerning issues relevant to the therapeutic use of IFN-β. As a unifying theme, we attempt to interpret biological activity in terms of structure and, conversely, to explore the potential for employing structural knowledge to improve our understanding of some clinically important issues.

Summary of HuIFN-β structure

To date, X-ray crystallographic structures have been published for three type I interferons: murine IFN-β [20], HuIFN-α-2b [21] and HuIFN-β [22]. In addition, the structure of HuIFN-α-2a was recently determined by NMR spectroscopy [23]. The structures show a high degree of homology with each other, as well as topological similarity with several less closely related helical cytokines such as IL-2, IL-4 and human growth hormone (HGH). Below, we consider the structure of HuIFN-β on its own and in relation to the two other structures. It should be noted that of the four type I IFNs mentioned above, only HuIFN-β and HuIFN-α2 are naturally glycosylated. The material used for the crystallographic study of this protein was prepared by recombinant methods, and securing crystals of HuIFN-β that were suitable for X-ray analysis depended on HuIFN-β being glycosylated as in the natural species. In an earlier attempt, Mitsui et al. failed to crystallize HuIFN-β that had been expressed without glycosylation, in *Escherichia coli*, citing as the cause the tendency of HuIFN-β to aggregate [24].

Human IFN-β consists of 166 residues. The crystallized material is recombinant HuIFN-β-1a (AVONEX®, Biogen, Inc., Cambridge, MA, USA), expressed and secreted from Chinese hamster ovary (CHO) cells, that is essentially identical to the natural protein. The secondary structure is dominated by five α helices (A–E), along with short stretches of 3_{10} helix in adjoining sequences and connecting loops (fig. 1). In describing the overall fold, following the systematization of Presnell and Cohen [25], IFN-β may be classified as a left-handed, type 2 helix bundle defined by helices A, B, C and E. Thus, helix A runs parallel with helix B and antiparallel with helices C and E; formally, helix D is part of the long loop connecting helices C and E. Another long loop joins the two parallel helices A and B, and may be structurally subdivided into three regions: AB1, AB2 and AB3.

There are two additional noteworthy elements in the covalent structure of HuIFN-β: a disulphide bridge extends between Cys 31 of loop AB and Cys 141 of loop DE. Both of these loops are believed to play important roles in receptor interactions (see below), and the disulphide linkage may act to coordinate their structure. Additionally, HuIFN-β is glycosylated at a single site, Asn 80 at the end of helix C. Compared with crystal structures of most other glycoproteins, relatively clear electron density could be seen for several of the sugar

residues. The sugar projects away from the IFN-β core into a large solvent-filled channel. At its proximal end, it interacts with Asn 86 (helix C) and Gln 23 (helix A) via hydrogen bonds. It has been shown that glycosylation diminishes the tendency of HuIFN-β to aggregate (see below); the presence of several exposed hydrophobic residues that are near Asn 80 in the HuIFN-β structure suggests that solvent shielding of these residues by the carbohydrate may be responsible for this effect.

Finally, HuIFN-β is observed to associate as a dimer in crystal lattice (fig. 2A). The asymmetric unit is defined by the two molecules in the dimer, identified as *A* and *B*. The dimer interface contains a zinc atom, which is coordinated by His 121 of molecule A and His 93 and His 97 of molecule *B*. A water molecule completes the tetrahedral zinc coordination geometry. There are additional contacts, both polar and hydrophobic, between helices A and C of molecule *B*, and helix D and the AB3 loop of molecule *A*. Thus, the contact surfaces are created from opposite faces of molecules *A* and *B*, and the molecules cannot be related by a symmetry operation. The D helix of molecule *A* is partially unfolded, and therefore is six residues shorter than that of molecule *B* presumably to avoid steric conflicts at the interface.

Dimerization of IFN is of interest because this behaviour has been observed for other helical cytokines and, in some cases, has been correlated with receptor activity [26]. Human IFN-α-2b also was found to crystallize as a zinc-mediated dimer [21]. Here, a zinc atom mediates contact between identical surface regions (consisting of the AB loop and helix D) of two adjacent molecules; therefore, the orientation of the two molecules could be described as face to face (fig. 2A), as opposed to face to back in the case of HuIFN-β. The zinc-chelating side chains are from Glu 41 and Glu 42 of each molecule. While it is tempting to postulate a functional role for these dimers, at least two facts argue otherwise: HuIFN-α-2b is active at concentrations well below the 50 μM required to induce association in solution [21]; moreover, the functional importance of the zinc-binding histidines in HuIFN-β has been tested in two mutants, H121A and H93A/H97A. Both showed

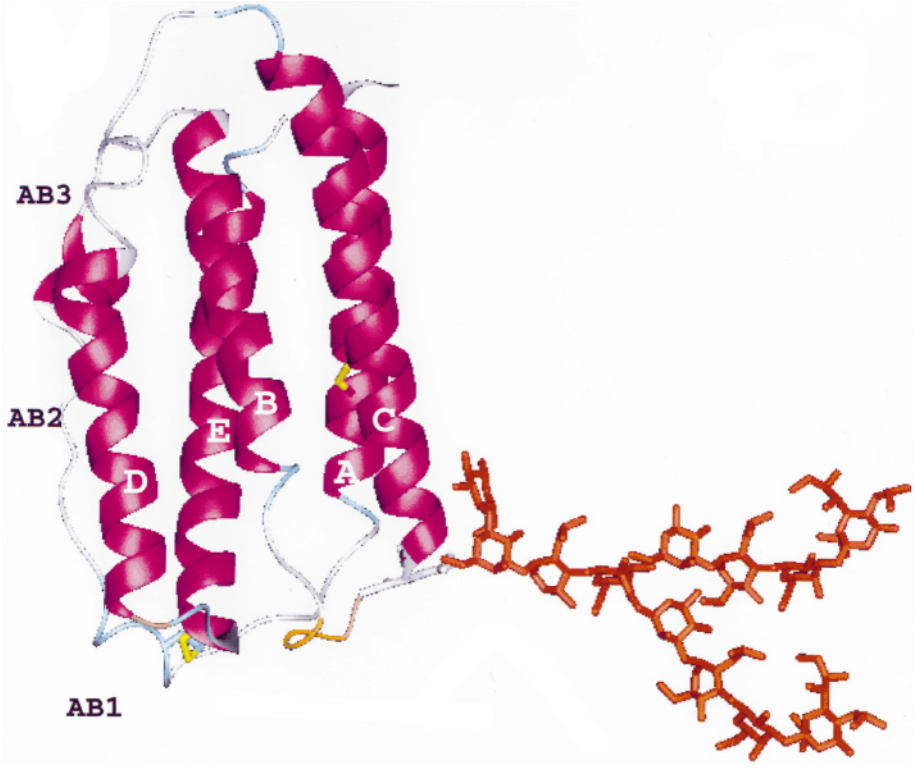

Figure 1. The structure of HuIFN-β. Ribbon representation of the polypeptide chain (molecule *A*), with full-size carbohydrate model based on observed portion of it shown in red. Helices are shown in magenta. The three cysteine residues also are shown in yellow. The figure was prepared with the program QUANTA (Molecular Simulations, Inc., Burlington, MA, USA).

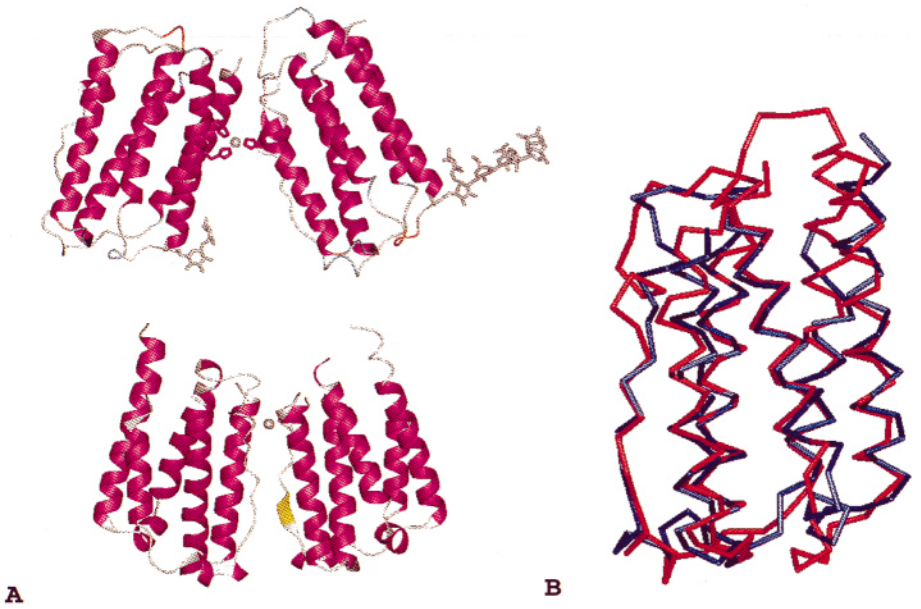

Figure 2. Comparison of HuIFN-β and IFN-α-2b crystal structures. (*A*) Crystallographic dimers of HuIFN-β (top) and HuIFN-α-2b (bottom). Zinc atoms are shown as gray spheres. The observed portion of the carbohydrates as well as the histidine residues of the zinc-binding site also are shown for HuIFN-β. (*B*) Superimposition of HuIFN-β (red) with HuIFN-α-2b (blue) Cα backbones. The figure was made with QUANTA.

wild-type activity in an antiviral assay, providing evidence against a functional role for zinc-mediated dimerization, at least in this instance (L. Runkel et al., unpublished observations). Nonetheless, dimer formation in the special environment of the cell surface receptor remains conceivable.

Comparison with other IFN structures

In agreement with the level of sequence homology, HuIFN-β shows slightly higher structural homology with murine IFN-β (2.49 Å rms deviation overall, 0.71 Å rms deviation for atoms that are structurally homologous) than with HuIFN-α-2b (4.95 Å overall, 1.25 for structurally homologous residues). Nevertheless, the overall folds of IFN-β and IFN-α-2b are highly similar, and with few exceptions, the elements of secondary structure are identical (fig. 2B). Hence, the major structural differences of the main chain reside in the exposed loops. Of course, there are differences in side-chain substitution (resulting from sequence diversity) within the helices as well as in the loops, and both structural elements are relevant to the functional differentiation of the various IFNs. Two important caveats are that, relative to the protein core, the loops tend to be less well defined crystallographically, and are conversely more susceptible to deformations resulting from intermolecular contacts within the crystal lattice.

Comparison of the three crystal structures highlights a network of buried hydrophobic and hydrogen-bonding interactions between a number of side chains that are probably essential to the stability of the tertiary fold. These interactions were first seen in the structure of murine IFN-β [20], and were shown to be highly conserved in a revised alignment of type I IFN sequences. Some examples of such interactions in HuIFN-β include the hydrogen bonds between Tyr 125, Tyr 126, Glu 149 and Asn 153 (IFN-α consensus sequence positions are 123, 124, 147 and 151), which help orient helix D with helix E. Another conserved network of hydrogen bonds is formed between residues on helix D (His 121 and Arg 128) and residues on the AB loop (Phe 38, Asp 39 and Glu 43), which appear to stabilize the extended geometry of the AB2 sequence. The AB1 portion of the loop, in turn, is stabilized by a hydrogen bond between the side chain Nε of Arg 147 and the backbone carbonyl of Leu 24, in both murine and human IFN-β.

Despite similarities, the structure of HuIFN-β reveals some clear divergences from those of HuIFN-α-2b and murine IFN-β. Murine IFN-β, unlike all human type I IFNs, lacks a disulphide bridge between the AB loop and helix E. Compared with the human protein, the

murine sequence also contains five deletions in the AB loop region. Correspondingly, the HuIFN-α AB loop is longer in the crystal structure, and contains two turns of 3_{10} helix that are not seen in murine IFN-β. All three IFNs possess highly dynamic CD loops, and, in fact, no electron density is observed for the CD loop in HuIFN-α-2b. The CD loop in the case of HuIFN-β appears to be more solvent-exposed than that of murine IFN-β. Two other notable differences with the structure of HuIFN-α-2b concern helix B, which is kinked in HuIFN-α-2b but not in HuIFN-β; and helix E, which is rotated around its axis in HuIFN-α-2b relative to its orientation in HuIFN-β. Both helices are very similar in HuIFN-β and murine IFN-β.

The structure of HuIFN-α-2a, determined by NMR spectroscopy, was found to correspond closely to that of HuIFN-α-2b [23]. These IFNs differ at only one residue (Lys/Arg 23). Given this minor variation at a solvent-exposed site, most differences between the two structures would be expected to arise from analysis in solution vs. the crystalline state. Notably, there was no evidence of dimerization in solution at sample concentrations up to 2 mM. The unique contribution of the NMR structure of HuIFN-α-2a lies in its quantitation of backbone dynamics. Heteronuclear Overhauser effect (heteronuclear NOE) measurements show a wide variation in loop mobility: the N-terminal part of the AB loop and the DE loop are relatively well ordered (NOE = 0.6), whereas the C-terminal part of the AB loop, and especially the CD loop from Ile 100 to Lys 112 (positions 102 and 114 in HuIFN-β), is highly flexible. These results are in good accord with what was previously inferred from X-ray crystallography. Comparison of the X-ray and NMR results confirms the prediction made from sequence alignment, namely that the three-dimensional structures of the four proteins are highly homologous, and that some of the major conformational differences (such as at the AB1 loop) are obviously associated with sequence variations (e.g. the presence of cysteines).

IFN receptor structure

The IFN receptor is understood in considerably less structural detail than are the IFNs. Here, we briefly summarize current knowledge regarding the structure and composition of the type I IFN receptor as a prelude to a discussion of the interactions of HuIFN-β with the receptor at the atomic level.

Two IFN receptor proteins, IFNAR-1 and IFNAR-2, have been identified. Like other cytokine receptors, they may be considered in terms of contiguous extracellular, transmembrane and cytoplasmic domains. The existence of these two distinct receptor chains was first made clear by cross-linking experiments with receptor-directed monoclonal antibodies [27–29]. IFNAR-1 was cloned and sequenced first [14], followed by IFNAR-2 [15, 30, 31]. Alignment of the amino acid sequences revealed that the extracellular domains of both IFNAR chains share significant homology with those of other cytokine receptors [16]. Both IFNAR-1 and IFNAR-2 contain duplicated modules of $\sim$100 residues each that are homologous to fibronectin type III (FNIII) motifs. Based on the location of four conserved cysteine residues, IFNAR-1 and IFNAR-2 were designated as class 2 cytokine receptors, along with the receptors for IFN-γ and IL-10 [16, 32].

Comparison of the sequences of the two IFNAR chains reveals significant differences in the structure of their extracellular and cytoplasmic domains. The 411-amino acid residue segment comprising the extracellular portion of IFNAR-1 was found to contain four FNIII repeats, double the number found in IFNAR-2, HGH-R, or other cytokine receptors [14]. The crystal structure of HGH in complex with its receptor suggests that two such FNIII-like repeats can be thought of as comprising a single cytokine-binding domain on a given cytokine receptor chain [33]. The duplication of this feature in the extracellular domain of IFNAR-1 raises the possibility that IFNAR-1 might simultaneously bind two molecules of ligand [14]. Of the remaining 122 residues of IFNAR-1, 100 comprise the cytoplasmic domain. The observed molecular mass of IFNAR-1 varies among different cell lines; this has been explained by differences in glycosylation [34]. IFNAR-2 has been shown to exist in three forms that result from alternative splicing of the same gene [30, 31]. These include a soluble form lacking transmembrane and cytoplasmic domains, a short form designated IFNAR-2-1 and a long form, IFNAR-2-2. The extracellular domains of all three are identical and contain two FNIII repeats, comprising a single putative IFN-binding domain. The cytoplasmic domain of IFNAR-2-2 consists of 251 residues, whereas that of IFNAR-2-1 consists of a unique sequence of only 67 amino acids.

Both IFNAR-1 and IFNAR-2-2 are required to form a fully functional receptor. IFNAR-1 was found to possess only weak binding affinity for IFNs on its own; stable binary complexes have been reported only with two HuIFNs, α2 and α8 [35]. However, anti-IFNAR-1 antibodies can block the action of a wide spectrum of IFNs, indicating that IFNAR-1 forms an integral part of the receptor complex [36, 37]. Unlike IFNAR-1, IFNAR-2-2 displays a relatively high affinity for binding IFNs [38]. It has been shown that coexpression of IFNAR-1 with IFNAR-2 both enhances the binding and alters the relative affinities towards different type-I IFNs [38].

Like other class 1 and class 2 cytokine receptors, IFNAR-1 and IFNAR-2 contain no intrinsic protein kinase activity. Instead, the cytoplasmic domains contain regions that serve as binding sites for the noncovalent association of cytoplasmic signalling kinases. IFNAR-1 interacts directly with the Janus kinase (JAK) tyk 2 [39], and IFNAR-2-2 with JAK1 [15]. Once activated by formation of an IFN/receptor complex, the JAK kinases catalyse phosphorylation of each other and of both receptor chains, further activating their kinase activity and creating a binding site for the SH2 domains of associated signal transducers and activators of transcription (STAT) proteins [32, 40]. Yang et al. demonstrated that STAT3 associates with IFNAR-1 [41]. IFNAR-2-2 was found to bind to STAT2, and also, in a STAT2-dependent manner, to STAT1 [42]. Phosphorylation of the STAT proteins leads to the formation of STAT homodimers and heterodimers that subsequently migrate to the nucleus, where they bind to specific promoter sites and activate the transcription of various type I IFN-responsive genes [40]. Unexpectedly, it has been shown that tyrosine phosphatase CD45, and tyrosine kinases Lck and ZAP-70, known for their role in T-cell-dependent gene expression, are involved in IFN-α transmission of growth-inhibitory signals [43]. Additional details of signalling pathways activated through the type I IFN receptor are beyond the scope of this article but are discussed elsewhere [44, 45].

The shortened cytoplasmic domain of IFNAR-2-1 lacks JAK and STAT binding sites, and failed to reconstitute a functional signalling pathway in combination with IFNAR-1 in murine L-929 cells [30] and mutant U5A cells [30]. No biological activity yet has been associated with the cytoplasmic tail of IFNAR-2-1. Interestingly, despite the sequence homology between their extracellular domains, the IFNAR-2 long form was found to bind HuIFN-α-2b > 10 times more strongly than the short form [31]. Conversely, whereas expression of HuIFNAR-1 in mouse cells renders the cells functionally sensitive to HuIFN-β and HuIFN-α8, this sensitivity is reduced upon coexpression of HuIFNAR-1 and HuIFNAR-2-1, suggesting that IFNAR-2-1 competes with murine IFNAR-2 to inhibit signal transduction [46]. Because both soluble IFNAR-2 and IFNAR-2-1 possess a significant binding affinity for type I IFNs, it has been proposed that these truncated forms act as antagonists of IFN signalling by sequestering the IFN ligand either in solution [15] or on the cell surface [46] in a form in which it cannot productively interact with a functional receptor. If so, then the subtype specific differences in IFN binding reported for IFNAR-2 alone compared with the intact receptor may represent an additional element regulating whether a given type I IFN will interact productively with the functional receptor under a given set of conditions. Despite these unresolved questions, the different forms of IFNAR-2 exemplify the complex relationship between cytokine binding and receptor activation that are involved in coordinating and regulating type I IFN signalling in vivo.

IFN-IFNR binding interactions

Among all helical cytokine receptors, the only X-ray structures of receptor-ligand complexes so far obtained are that of HGH in complex with a soluble, extracellular construct of its receptor [33], and with a prolactin receptor chain [47], and that of IFN-γ in a complex with its high-affinity receptor chain [13]. The HGH/HGH-R complex comprises a single HGH molecule cupped between two receptor molecules (each of which consists of two β-barrel domains) that make direct contact with each other via a fairly extended contact interface. The two receptor molecules are roughly symmetric by a rotational axis and present the same gross binding surface to opposite sides of the ligand. In contrast, the prolactin receptor complex pairs HGH with a single receptor molecule, showing a binary complex that represents a putative intermediate in the formation of the fully active ternary complex [48]. The IFN-γ ligand/receptor complex consists of the homodimeric IFN bound to two receptor molecules that do not directly interact [13]. While these examples provide potentially useful working models of the type I IFN receptor, they serve mainly to illustrate the multicomponent nature of the receptor and the variety and complexity of the interactions that can characterize the complex between receptor and ligand.

Prior to the recent availability of high-resolution structures of type I IFNs, models of the interaction between type I IFNs and their receptors were based primarily on structure-activity studies using IFN mutants. These studies fall into two broad categories: point mutations, reviewed in Mitsui et al. [24], and chimeras constructed by fusing coding sequences of different IFN subtypes, summarized in Uzé et al. [17]. In the case of the chimeras, functional domains were identified by comparing the activity of different IFN hybrids to those of parental IFNs that had distinguishable activities in selected assay systems. Here, we summarize data concerning the putatative sites of receptor interaction common to all type I IFNs, and in the next section return to the particular case of HuIFN-β in relation to how IFN subtype differentiation might occur.

Two mutational hot spots, identified for IFN-α corresponding to regions of the structure for which activity is particularly sensitive to mutation, have been found to map to approximately opposite faces of the IFN structure. One region comprises residues of the C-terminal

part of the AB loop (numbered 28–41 in the IFN-α consensus sequence), helix D and part of loop DE [20, 24]. Alignment of 45 IFN sequences reveals that part of this region, including the AB and DE loops, is especially highly conserved. These loops are linked by a disulphide bond in most IFNs, underscoring their functional interdependence. In murine IFN-β, where this disulphide is absent, a 10-fold enhancement of antiviral activity is achieved by engineering two cysteines into the sequence at the proper location [49]. Further evidence for this interdependence is seen in the importance of buried residues such as Tyr 123 (IFN-α consensus numbering; position Tyr 125 in HuIFN-β) that do not interact directly with the receptor but are nonetheless crucial for maintaining the proper spatial relationship of the D and E helices [22, 24].

A second IFN-α hot spot was proposed by Uzé et al. to involve residues in helices A and C [17, 50]. The key results cited as the basis for this hypothesis include data from the HuIFNα1/α2 hybrids of Weber et al. [51], which showed that residues 16–29 (helix A and the N-terminal part of the AB loop) play a dominant role in differentiating the behaviour of α1 and α2 on human cells. In a separate study, Mouchel-Vielh et al. found that transfection of bovine IFNAR-1 alone into human cells equalized the activity of α1 and α2 in antiviral assays. Human IFN-α1 is much more active in bovine cells than in human cells, while HuIFN-α2 is highly active in both. This result thus suggests that the IFNAR-1 interaction is largely responsible for the difference in specific activity between these subtypes [52]. The importance of helix C was inferred from crossover hybrids between IFN-α1 and -α8. [50]. Human IFN-α8 is inactive on mouse cells, whereas the hybrid α8(60)α1(92)α8 is partially active (the numbers specify the crossover points). The hybrid differs from α8 at four positions on helix C that are solvent-exposed (84, 86, 87 and 90, corresponding to the IFN-β positions 86, 88, 89 and 92, respectively). Mutation of any of these residues to the α8 sequence causes significant losses in activity on murine cells, and the 84/90 double mutation abolishes activity entirely. The double mutant can be compensated by transfection with HuIFNAR-1, and this complementation can be neutralized with antiserum against IFNAR-1.

A priori, the observation that mutating a certain residue or group of residues results in a reduction in IFN activity does not constitute proof that the mutated sites are directly involved in receptor binding. Analysis of the crystallographic data in parallel with the results of mutagenesis is therefore crucial to obtaining an accurate picture of receptor binding. For instance, the X-ray structure of HuIFN-β reveals that residues Tyr 125 and Glu 149 are buried and interact with each other via a hydrogen bond. Mutation of Tyr 125 resulted in an inactive, poorly expressed IFN-β protein [53]. Thus, the significant decrease in activity that is observed when these residues are mutated results not from the disruption of direct interactions with the receptor, but is instead an indirect effect of longer-range disruption of the overall tertiary structure. Conversely, the IFN-β crystallographic results confirm that a number of muta-

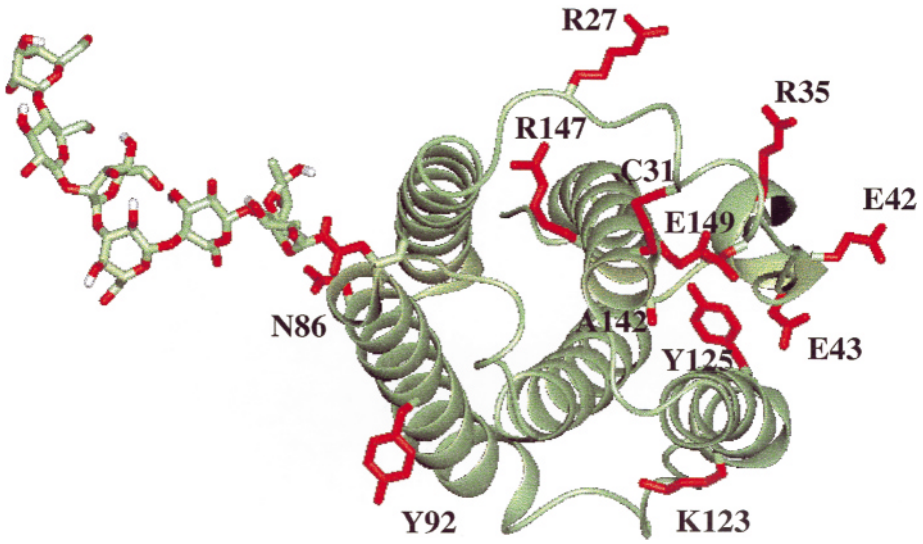

Figure 3. Ribbon diagram of HuIFN-β (molecule *A*), with side chains of residues known to be important for activity in red colour. The observed portion of the carbohydrate is also shown. The figure was made with QUANTA.

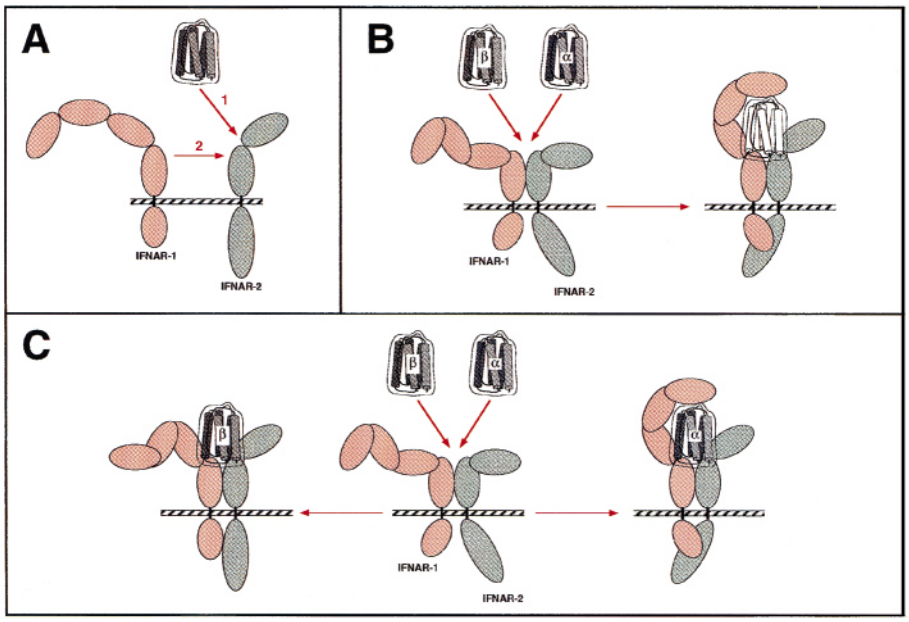

Figure 4. Cartoon illustrating hypothetical mechanisms for the activation and isotype selectivity of the type I IFN receptor. (*A*) Ligand-induced receptor dimerization. IFNs bind first to the high-affinity IFNAR-2 chain, and the resulting IFN/IFNAR-2 complex subsequently recruits IFNAR-1 to form the ternary, activated complex. (*B*) Allosteric mechanism. Binding of IFN to preassociated IFNAR-1/IFNAR-2 causes a conformational change that is allosterically transmitted to the cytoplasmic domains of the receptor chains, resulting in an activated state of the receptor. (*C*) IFN isotype selectivity considered in terms of an allosteric activation mechanism. Different type I IFNs interact differently with the extracellular portions of IFNAR-1 and IFNAR-2, thus giving rise to distinct activated states of the receptor.

genically sensitive residues are in fact exposed and available for receptor interaction, such as Arg 35 on the AB loop and Lys 123 on helix D (fig. 3). The X-ray structures are consistent with Uzé's hypothesis that helices A and C could bind IFNAR-1 directly [50]. These helices interdigitate to form a contiguous surface, and the important Asn 86 and Tyr 92 side chains are exposed. These residues correspond to the IFN-α positions 84 and 90, found to be important for subtype-specific activities [50]. Binding of helices A and C by IFNAR-1 would thus leave the AB loop, helix D and the DE loop on the other side of the molecule free to interact with IFNAR-2.

Mechanism of IFN receptor activation and subtype discrimination

Despite containing patches of highly conserved residues, the regions of type I IFNs that are implicated in receptor binding also include sites that are highly diverse among the different IFN sequences, thereby affording a potential basis for subtype differentiation [20]. As noted above, IFN-β possesses significantly lower sequence homology with the IFNs-α than the latter do with each other. IFN-β also diverges from the IFNs-α in certain functional ways, such as its unique ability to mediate coimmunoprecipitation of phosphorylated IFNAR-1 and -2 chains [54–57]. In addition, cells lacking the tyk-2 Janus kinase are insensitive to IFN-α, but retain some response to IFN-β [58]. IFN-β thus appears to present a clear example of the ability of the receptor to discriminate between different ligands. How this subtype discrimination might be achieved is intimately connected with the question of how binding of an IFN to the receptor brings about receptor activation at all. Some of the unanswered questions concerning how the type I IFN receptor is activated by the binding of IFNs are illustrated in figure 4.

Various members of the class 1 cytokine receptor family have been shown to be activated by ligand-induced receptor chain dimerization (fig. 4A) [48]. In this mechanism, the individual receptor chains are not preassociated in the absence of ligand; binding of the ligand to one receptor chain promotes the recruitment of one or more additional receptor chains into an activated receptor complex. The resulting proximity of the cytoplasmic domains of the receptor chains within the activated complex allows the receptor-associated kinases to interact with each other and with additional downstream

signalling molecules, and thus brings about signalling. Ligand-induced receptor chain dimerization has been proposed to occur for a large number of oligomeric receptors, including class 2 cytokine receptors such as the type I IFN receptor [33], though in many cases there has been no direct demonstration that this is the case. In fact, existing experimental evidence for the type I IFN receptor tends to argue against the operation of this mechanism: it is well documented that antibodies directed against the extracellular domain of IFNAR-1, which has little intrinsic affinity for binding most type I IFNs, can block the binding of ligands to the intact receptor [18, 38]. Since IFNAR-2 binds IFNs with high affinity even in the absence of IFNAR-1 [39], this result argues strongly that these receptor chains are preassociated in the absence of ligand. Allosteric activation of the receptor (fig. 4B) represents an alternative mechanism. In this mechanism, binding of ligand causes a conformational change in the receptor that is allosterically transmitted across the membrane to give a corresponding change in the conformation of the cytoplasmic domains. The observations that the affinity of IFNs-α8 and -α2 binding is affected by the absence of intracellular tyk 2 [59], and that IFNAR-2-1 and IFNAR-2-2 differ substantially in their affinities for binding IFN-α-2b despite having identical extracellular structures [31], lend significant support to the hypothesis that there is allosteric communication between cytoplasmic and extracellular domains of the receptor. Such an allosteric mechanism is also appealing because it easily can accommodate the concept of IFN subtype specificity if different type I IFNs are assumed to interact with the receptor differently to form distinct activated conformations. This hypothesis is illustrated in figure 4C, and is similar to explanations that have been advanced to account for agonist-dependent differences in the activation of certain G protein-coupled receptors [60].

One strategy for probing the basis of IFN-subtype specificity is to measure the effect of introducing mutations at likely receptor-binding sites, using assays that discriminate between IFN subtypes. As recently described by Runkel [53], assays that discriminate between IFNs-α and IFN-β include coimmunoprecipitation of phosphorylated IFNAR-1 and -2; activity in human tyk 2-deficient cells; and relative antiviral activities in human, equine, and bovine cells (where the IFN-α-2b:β potency ratio is 1:1, 2000:1 and 15:1, respectively). Mutation of IFN-β residues in the AB loop and the D helix had no significant effect on relative subtype-specific activities. In parallel with the data for IFN-α, some of the mutations did cause an absolute loss in antiviral activity (IFN-α consensus sequence position R33). These losses observed with IFN-β mutations were modest, however, compared with the effects of corresponding changes in IFNs-α, suggesting that the two IFN subtypes might be partly distinguished by differences in the relative contributions of individual residues to the overall binding interaction. On the other hand, marked differences in the subtype-specific assays were found for HuIFN-β double mutants at residues 86 and 92 in the C helix of IFN-β, the same sites that initially identified this region as crucial for IFNAR-1 binding of IFNs-α. Introduction of charge at these sites (N86E or K, Y 92D) abolished induction of phosphorylated IFNAR-1/2 coimmunoprecipitation and lowered the activity of the mutants on tyk 2-deficient cells. While these results need not imply the adoption of IFN-α properties per se, they strongly suggest that the helix C/IFNAR-1 interaction is important to the definition of an IFN-β-type signalling response.

The availability of high-resolution structures for HuIFN-β, HuIFN-α-2a and HuIFN-α-2b allow structure-activity studies of type I IFNs to be designed with increased insight into the likely structural consequences of any changes that are made to the molecules. This structural information improves our ability to interpret the resulting activity data in terms of receptor interaction properties of specific sites on the IFN molecules. Studies of this kind that make use of the new wealth of structural data and analysis are underway in many laboratories.

Role of glycosylation in IFN solubility, stability, and activity

IFN-β and IFN-α2 are the only human IFNs that are naturally glycosylated. In this section, we revisit the role of glycosylation in the overall structure and activity of HuIFN-β based on a comparison of recombinant commercial preparations of HuIFN-β that differ in their state of glycosylation according to their means of manufacture.

The major oligosaccharide chains in recombinant HuIFN-β secreted by CHO cells are of the biantennary complex type, containing an α1-6 linked fucose on the peptide proximal GlcNac residue and two α2-3 linked NeuAc on the terminal galactose residues [61]. Perhaps the most remarkable feature of the glycan in the HuIFN-β crystal structure [22] is its apparent high degree of order. Ordinarily, such groups are extensively solvated and conformationally flexible, so that their electron density is resolved poorly, if at all. The HuIFN-β glycan also projects away from the IFN core, occupying a large solvent channel between neighbouring molecules of protein. However, the carbohydrate possesses sufficient conformational bias that electron density is clearly visible for a significant portion of it. A total of seven hexose rings in a biantennary arrangement could be incorporated into the model for one

molecule of the asymmetric unit (molecule *A*; in molecule *B*, only two rings could be modelled with confidence). This finding suggests that the HuIFN-β glycan may be more closely integrated into the tertiary structure of the protein than is usually the case. Further support for this idea was found in the interactions of the proximal part of the glycan with the IFN polypeptide. Two hydrogen bonds were discernible between the α1-6 fucose and the side chains of Gln 23 (helix A) and Asn 86 (helix C). These residues comprise part of an exterior region of HuIFN-β that contains several neutral and/or hydrophobic side chains that may be shielded from exposure to solvent by interactions with the glycan (fig. 5). It is notable that in HuIFN-α-2b, which is not glycosylated at this position, Gln 23 and Asn 86 are replaced by charged residues (Lys and Arg), while Asn 80 is substituted by Asp (fig. 5). Taken together, these results suggest that the high degree of structural definition in the HuIFN-β glycan reflects an intrinsic role for the sugar in stabilizing the protein in its native, folded state.

An early clue to the structural importance of HuIFN-β glycosylation was the failure of recombinant, nonglycosylated material to yield diffractable crystals, as mentioned above [24]. Aggregation of the protein was cited as a probable cause. The relevance of glycosylation to functional activity also has become a focus of interest within the last decade as HuIFN-β has been licensed for the treatment of MS. Two recombinant forms of HuIFN-β are now commercially available. AVONEX® is expressed in mammalian CHO cells, and is identical to natural HuIFN-β in its primary sequence and also in its glycosylation at Asn 80. In contrast, BETASERON® (interferon β-1b, Berlex Laboratories, Richmond, CA, USA) is a nonglycosylated protein expressed in *E. coli*. In addition, IFN-β-1b also varies from the natural material in its sequence; Met 1 is deleted and Cys 17 is mutated to a serine.

A detailed study of the role of glycosylation in the functional activity of HuIFN-β was reported recently [62], and showed that AVONEX® has an $\sim$10-fold higher specific activity than BETASERON® in three separate functional assays based on the antiviral, antiproliferative and immunomodulatory activities of IFN-β. To systematically investigate the structural basis for the differences in activity, this study evaluated novel constructs in which the three established structural differences between HuIFN-β-1a and HuIFN-β-1b were introduced one at a time. The removal of Met 1 had no measurable effect on the activity of the protein, or on its secondary or tertiary structure. This result is consistent with the crystal structure of HuIFN-β-1a, which shows Met 1 to be relatively exposed to solvent. The glycosylated IFN-β, engineered to have a Cys17Ser mutation, also showed an activity in antiviral assays that was indistinguishable from that of wild-type IFN-β-1a.

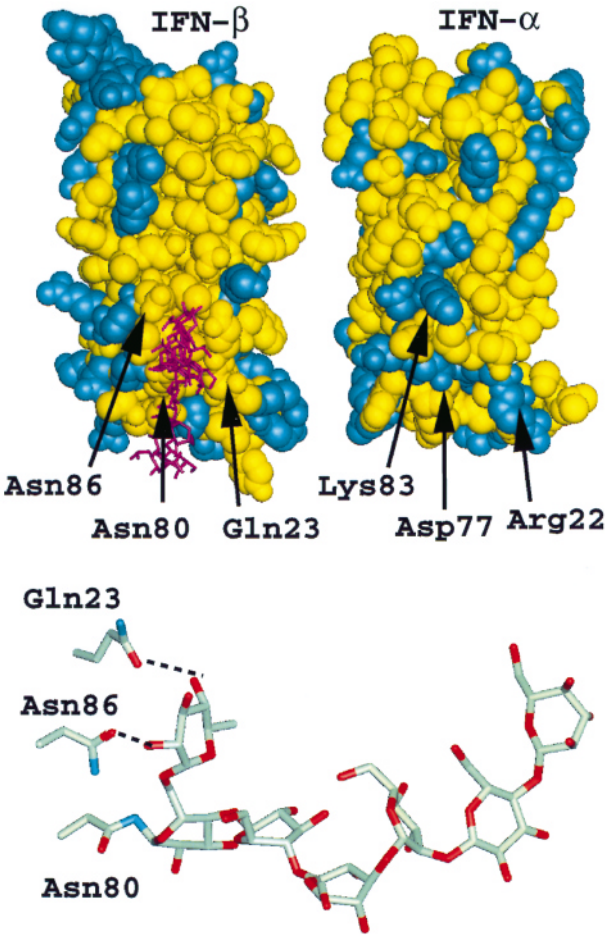

Figure 5. Space-filling representation of IFN-β showing the region of the surface close to the glycosylation site. The corresponding region of an IFN-α model also is shown. The carbohydrate is shown in a stick representation in magenta colour. The amino acid residues have been coloured as follows: charged (blue), noncharged (yellow). The interactions of the carbohydrate with protein side chains are shown with more detail at the bottom of the figure. Reprinted with permission from: Runkel L., Meier W., Pepinsky B., Karpusas M., Whitty A., Kimball K. et al. (1998) Structural and functional differences between glycosylated and nonglycosylated forms of human interferon-beta (IFN-beta). Pharm. Res. **15:** 641–649, © 1998 Plenum Publishing Co., New York.

In contrast, deglycosylated HuIFN-β-1a showed a marked increase in sample turbidity, and could be separated into soluble and precipitated fractions by centrifugation. Forty percent by mass of the protein remained in solution following centrifugation and filtration, and on SDS-polyacrylamide gel electrophoresis (SDS-

PAGE) migrated as a single band corresponding to monomeric deglycosylated HuIFN-β-1a under both reducing and nonreducing conditions. Circular dichroism spectroscopy suggested that the secondary and tertiary structures of this soluble fraction were similar to the glycosylated protein. However, thermal denaturation experiments showed that the deglycosylated HuIFN-β-1a underwent thermal unfolding at a temperature that was 4–5 °C lower than the glycosylated material. In the case of the precipitate, under reducing conditions SDS-PAGE resulted in a single band migrating at the predicted mass of deglycosylated HuIFN-β-1a. However, analysis under nonreducing conditions revealed the presence of high molecular weight species, suggesting that the precipitate consisted of denatured aggregates joined through scrambled disulphide bonds. Aggregates of this type have been observed previously in wild-type HuIFN-β expressed in *E. coli* [63]. These results suggest that removal of the sugar destabilized the native structure of the molecule. Deglycosylation of IFN-β-1a also affected activity; the soluble fraction possessed $\sim$50% lower antiviral activity, as well as $\sim$50 and $\sim$30% losses in antiproliferative and immunomodulatory activities, while the insoluble material displayed a much larger loss in activity.

The tendency of IFN-β-1b to aggregate was demonstrated independently using size exclusion chromatography (SEC). When BETASERON® was subjected to SEC under physiological conditions, $\sim$60% of the IFN-β-1b protein eluted in the excluded volume of the column, corresponding to an apparent molecular weight of $>$600 kDa, while the remaining $\sim$40% eluted at the position expected for a monomer. Under the same conditions, HuIFN-β-1a eluted $>$98% as a monomer. This tendency of IFN-β-1b to aggregate was demonstrated using a variety of buffers and column matrices, indicating that the observed aggregation was not due to any arbitrary feature of the experimental conditions. SDS-PAGE of the high molecular weight fraction showed that it was composed of noncovalent IFN-β-1b aggregates, presumably because the Cys17Ser mutation eliminates the possibility of disulphide scrambling and intermolecular disulphide bond formation. The aggregated IFN-β-1b had an activity in antiviral assays that was threefold lower than that of the soluble starting IFN-β-1b material (i.e. some 30-fold lower than IFN-β-1a). Thus, these data indicate that the greater biological activity of IFN-β-1a is due to a stabilizing effect of the carbohydrate on structure. The importance of the HuIFN-β glycosyl moiety is thereby established from the three perspectives of crystallographic, biophysical and functional assays.

Therapeutic challenges defined by IFN-β structural analysis

Human IFN-β is established as an effective treatment of relapsing-remitting MS. There are a number of issues that should be considered in light of the foregoing discussion of structure-activity relationships that concern transforming a naturally occurring protein into a viable drug product. Efficient production requires expression of IFN-β in an appropriate cell-based system, as opposed to its isolation from natural sources. Expression in *E. coli* is convenient and well established, but yields a nonglycosylated IFN-β protein retained in aggregated form in inclusion bodies within the cytoplasm of the bacterial cell. The bacterial cell must be physically disrupted to release the unglycosylated product. Following its separation from the cell debris, the protein is then solubilized and unfolded using detergents or denaturants, and must then be refolded to an active form. Expression in a mammalian system (CHO cells), as is done with HuIFN-β-1a, would appear preferable in that glycosylated protein is secreted from cells in its monomeric native state, thus facilitating its isolation; therefore, the mammalian host system has the double advantage of providing a glycosylated preparation of HuIFN-β in its most active, stable form, as opposed to a nonnative and nonglycosylated form that must be recovered from an inactive state.

Another therapeutic challenge is raised by the nature of MS itself. Treatment of this chronic disease aims to slow its progression over the long term, rather than to reverse preexisting conditions. Therefore, this strategy requires consideration of the physiological response to exposure to a drug product over a period of years. Despite the similarity of recombinant HuIFN-β to the natural material, its proteinaceous nature and the long course of treatment increase the potential for a patient to develop an antibody response to HuIFN-β. The generation of specific antibodies to IFN-β could alter its biodistribution and clearance. Also, within the spectrum of antibodies raised against IFN-β, a subset is defined as neutralizing (NABs), in that these prevent the binding of IFN-β to its receptor and thereby compromise its functional activity. In patients treated with AVONEX®, such NABs have been shown to be associated with a titre-dependent reduction in the in vivo induction of neopterin (a pharmacodynamic marker of interferon activity), as well as with a trend to reduce the drug's ability to suppress gadolinium-enhancing lesions [64]. The negative therapeutic effects of NABs have been documented for IFN-β-1b treatment of MS as well [65], and have been reviewed more generally for both IFN-α and -β therapies [66].

Immunogenicity may be related to a wide spectrum of factors including dosage, frequency and route of admin-

istration, and manufacturing/formaulation, in addition to the molecular nature of the active ingredient. Clinical studies of the three commercial preparations of HuIFN-β reveal differing levels of immunogenicity [64–68]. These studies show IFN-β-1b to be highly immunogenic [65]; in contrast, both AVONEX® and the commercial IFN-β-1a product, REBIF™ (Ares-Serono, Geneva, Switzerland) were significantly less immunogenic [64, 68, 69]. Moreover, a higher incidence of IFN-β-specific NABs was reported for patients receiving REBIF™ [68, 69] compared with those receiving AVONEX® [64]. Besides the formulation differences mentioned above, the greater level of immunogenicity of BETASERON® and REBIF™ relative to AVONEX® may be due to differences in frequency and route of administration [every other day subcutaneously (SC) vs. once per week intramuscularly (IM)]. Consistent with this is the report that SC administration of IFN-α-2a is more immunogenic than IM administration in animal studies, as is administration every other day vs. once per week [70]. As an additional advantage, IM injection of AVONEX® was reported to result in higher levels of serum IFN activity, as well as serum neopterin activity than SC or intravenous injection [71].

The much greater difference in immunogenicity between HuIFN-β-1a and HuIFN-β-1b might naively be ascribed to the need to deliver a larger amount of the latter protein, which has a 10-fold lower specific activity compared with AVONEX®, to achieve a sufficient dosage. However, the rate of NAB formation has been reported to be essentially independent of dosage [65]. Based on recently published studies of recombinant HuIFN-α-2a immunogenicity, the tendency of HuIFN-β-1b to aggregate also would appear to be a necessary consideration. A report of HuIFN-α-2a immunogenicity in mice as a function of storage conditions found a greater immunogenic effect at higher storage temperatures, correlating with the formation of HuIFN-α-2a aggregates in the vials [72]. Human IFN-α-2a that was deliberately aggregated via glutaraldehyde cross-links also exhibited much higher immunogenicity than native HuIFN-α-2a [70].

To summarize, the causes of immunogenicity are complex, with the potential for involving every detail of the therapeutic regimen. Nonetheless, it is possible to infer a connection with the stability and solubility of the IFN-β molecule, which emphasizes the importance of these issues in the design and production of the recombinant protein.

Conclusion

The high-resolution structure of HuIFN-β reviewed here provides detailed verification and corroboration of the results of previous structure-activity studies undertaken in the absence of detailed structural information. Determination of the HuIFN-β crystal structure, together with those of murine IFN-β and HuIFN-α-2, represents an important advance towards the structural delineation of a complete type I IFN ligand/receptor complex and an improved understanding of the workings of this intricate signalling system. In a broader sense, the use of structural information at the atomic level continues to show promise in meeting the challenges of drug design. Thus, as progress is made towards a detailed picture of the nature and mechanism of the interactions of IFNs with their receptor, it may be possible to engineer recombinant IFNs in order to achieve desired immunomodulatory outcomes. We also have sought in this review to show that structural considerations may facilitate the interpretation of clinical data, thus providing guideposts for design that optimize bioavailability and efficacy of the drug product.

Acknowledgements. The authors wish to express special appreciation and acknowledgement to Chris Arico-Muendel, their valued colleague in this review of IFN-β structure. We also wish to thank our colleagues and collaborators: Matthias Nolte, R. Blake Pepinsky, Werner Meier, Susan Goelz, William N. Lipscomb, Knud Mogensen, Gilles Uzé, Ling Ling Chen, Margot Brickelmaier, Celine Muldowney and Wendy Jones.

1 Stewart W. E. II (1981) The Interferon System, pp. 135–156, Springer, New York
2 Tyring S. K. (1995) Interferons: biochemistry and mechanisms of action. Am. J. Obstet. Gynecol. **172:** 1350–1353
3 Duncan M. R. and Berman B. (1985) Gamma interferon is the lymphokine and beta interferon the monokine responsible for inhibition of fibroblast collagen production and late but not early fibroblast proliferation. J. Exp. Med. **162:** 516–527
4 Gutterman J. (1994) Cytokine therapeutics: lessons from interferon-alpha. Proc. Natl. Acad. Sci. USA **91:** 1198–1205
5 Pestka S., Langer J. A. and Zoon K. C. (1987) Interferons and their actions. Annu. Rev. Biochem. **56:** 727–777
6 Branca A. A. and Baglioni C. (1981) Evidence that types I and II interferons have different receptors. Nature **294:** 768–770
7 Flores I., Mariano T. M. and Pestka S. (1991) Human interferon omega binds to the alpha/beta receptor. J. Biol. Chem. **266:** 19875–19877
8 Li J. and Roberts R. M. (1994) Interferon-tau and interferon-alpha interact with the same receptors in bovine endometrium. Use of a readily iodinatable form of recombinant interferon-tau for binding studies. J. Biol. Chem. **269:** 13544–13550
9 Weissman C. and Weber H. (1986) The interferon genes. Prog. Nucleic Acid Res. Mol. Biol. **33:** 251–300
10 Pestka S. (1997) The human interferon-alpha species and hybrid proteins. Semin Oncol **24** (3 Suppl. 9): S9-S4S917
11 Bazer F. W., Spencer T. E. and Ott T. L. (1997) Interferon tau: a novel pregnancy recognition signal. Am. J. Reprod. Immunol. **37:** 412–420
12 Adolf G. R., Kalsner I., Ahorn H., Maurer-Fogy I. and Cantell K. (1991) Natural human interferon-alpha 2 is O-glycosylated. Biochem. J. **276:** 511–518
13 Walter M. R., Windsor W. T., Nagabhushan T. L., Lundell D. J., Lunn C. A., Zauodny P. J. et al. (1995) Crystal structure of a complex between interferon-gamma and its soluble high-affinity receptor. Nature **376:** 230–235
14 Uzé G., Lutfalla G. and Gresser I. (1990) Genetic transfer of a functional human interferon alpha receptor into mouse cells: cloning and expression of its cDNA. Cell **60:** 225–234

15 Novick D., Cohen B. and Rubinstein M. (1994) The human interferon alpha/beta receptor: characterization and molecular cloning. Cell **77:** 391–400
16 Bazan J. F. (1990) Structural design and molecular evolution of a cytokine receptor superfamily. Proc. Natl. Acad. Sci. USA **87:** 6934–6938
17 Uzé G., Lutfalla G. and Mogensen K. E. (1995) Alpha and beta interferons and their receptor and their friends and relations. J. Interferon Cytokine Res. **15:** 3–26
18 Baron S., Tyring S. K., Fleischmann R., Coppenhaver D. H., Niesel D. W., Klimpel G. R., Stanton G. J. et al. (1991) The interferons. Mechanisms of action and medical applications. JAMA **266:** 1375–1383
19 Weinstock-Guttman B., Ransohoff R. M., Kinkel R. P. and Rudick R. A. (1995) The interferons: biological effects, mechanisms of action and use in multiple sclerosis. Ann. Neurol. **37:** 7–15
20 Senda T., Saitoh S.-I. and Mitsui Y. (1995) Refined crystal structure of recombinant murine interferon-beta at 2.15 Å resolution. J. Mol. Biol. **253:** 187–207
21 Radhakrishnan R., Walter L. J., Hruza A., Reichert P., Trotta P. P., Nagabhushan T. L. et al. (1996) Zinc mediated dimer of human interferon alpha 2b revealed by x-ray crystallography. Structure **4:** 1453–1463
22 Karpusas M., Nolte M., Benton C. B., Meier W., Lipscomb W. N. and Goelz S. (1997) The crystal structure of human interferon beta at 2.2-Å resolution. Proc. Natl. Acad. Sci. USA **94:** 11813–11818
23 Klaus W., Gsell B., Labhardt A. M., Wipf B. and Senn H. (1997) The three-dimensional high resolution structure of human interferon alpha-2a determined by heteronuclear NMR spectroscopy in solution. J. Mol. Biol. **274:** 661–675
24 Mitsui Y., Senda T., Shimazu T., Matsuda S. and Utsumi J. (1993) Structural, functional and evolutionary implications of the three-dimensional crystal structure of murine interferon-beta. Pharmacol. Ther. **58:** 93–132
25 Presnell S. R. and Cohen F. E. (1989) Topological distribution of four-α-helix bundles. Proc. Natl. Acad. Sci. USA **86:** 6592–6596
26 Sprang S. R. and Bazan J. F. (1993) Cytokine structural taxonomy and mechanisms of receptor engagement. Curr. Opin. Struct. Biol. **3:** 815–827
27 Colamonici O. R., D'Alessandro F., Diaz M. O., Gregory S. A., Neckers L. M. and Nordan R. (1990) Characterization of three monoclonal antibodies that recognize the interferon alpha 2 receptor. Proc. Natl. Acad. Sci. USA **87:** 7230–7234
28 Colamonici O. R., Pfeffer L. M., D'Alessandro F., Platanias L. C., Gregory S. A., Rosolen A. et al. (1992) Multichain structure of the IFN-alpha receptor on hematopoietic cells. J. Immunol. **148:** 2126–2132
29 Colamonici O. R. and Domanski P. (1993) Identification of a novel subunit of the type I interferon receptor localized to human chromosome 21. J. Biol. Chem. **268:** 10895–10899
30 Domanski P., Witte M., Kellum M., Rubinstein M., Hackett R., Pitha P. et al. (1995) Cloning and expression of a long form of the beta subunit of the interferon alpha beta receptor that is required for signaling. J. Biol. Chem. **270:** 21606–21611
31 Lutfalla G., Holland S. J., Cinato E., Monneron D., Reboul J., Rogers N. C. et al. (1995) Mutant U5A cells are complemented by an interferon-alpha beta receptor subunit generated by alternative processing of a new member of a cytokine receptor gene cluster. EMBO J. **14:** 5100–5108
32 Heldin C.-H. (1995) Dimerization of cell surface receptors in signal transduction. Cell **80:** 213–223
33 De Vos A. M., Ultsch M. and Kossiakoff A. A. (1992) Human growth hormone and extracellular domain of its receptor: crystal structure of the complex. Science **255:** 306–312
34 Ling L. E., Zafari M., Reardon D., Brickelmeier M., Goelz S. E. and Benjamin C. D. (1995) Human type I interferon receptor, IFNAR, is a heavily glycosylated 120–130 kD membrane protein. J. Interferon Cytokine Res. **15:** 55–61
35 Lim J. K., Xiong J., Carrasco N. and Langer J. A. (1994) Intrinsic ligand binding properties of the human and bovine alpha-interferon receptors. FEBS Lett. **350:** 281–286
36 Uzé G., Lutfalla G., Eid P., Maury C., Bandu M.-T. and Gresser I. (1991) Murine tumor cells expressing the gene for the human interferon alpha beta receptor elicit antibodies in syngeneic mice to the active form of the receptor. Eur. J. Immunol. **21:** 447–451
37 Benoit P., Maguire D., Plavec I., Kocher H., Tovey M. and Meyer F. (1993) A monoclonal antibody to recombinant human IFN-alpha receptor inhibits biologic activity of several species of human IFN-alpha, IFN-beta and IFN-omega. Detection of heterogeneity of the cellular type I IFN receptor. J. Immunol. **150:** 707–716
38 Cutrone E. C. and Langer J. A. (1997) Contributions of cloned type I interferon receptor subunits to differential ligand binding. FEBS Lett. **404:** 197–202
39 Colamonici O. R., Uyttendaele H., Domanski P., Yan H. and Krolewski J. J. (1994) p135tyk2, an interferon-alpha-activated tyrosine kinase, is physically associated with an interferon-alpha receptor. J. Biol. Chem. **269:** 3518–3522
40 Darnell J. E. (1997) STATs and gene regulation. Science **277:** 1630–1635
41 Yang C. H., Shi W., Basu L., Murti A., Constantinescu S. N., Blatt L. et al. (1996) Direct association of STAT3 with the IFNAR-1 chain of human type I interferon receptor. J. Biol. Chem. **271:** 8057–8061
42 Li X., Leung S., Kerr I. M. and Stark G. R. (1997) Functional subdomains of STAT2 required for preassociation with the alpha interferon receptor and for signaling. Mol. Cell. Biol. **17:** 2048–2056
43 Petricoin E. F. III, Ito S., Williams B. L., Audet S., Stancato L. F., Gamero A. et al. (1997) Antiproliferative action of interferon-alpha requires components of T-cell-receptor signalling. Nature **390:** 629–632
44 David M., Petricoin E. III, Benjamin C., Pine R., Weber M. J. and Larner A. C. (1995) Requirement for MAP Kinase (ERK2) activity in interferon alpha- and interferon beta-stimulated gene expression through STAT proteins. Science **269:** 1721–1723
45 Kalvakolanu D. V. and Borden E. C. (1996) An overview of the interferon system: signal transduction and mechanisms of action. Cancer Invest. **14:** 25–53
46 Pfeffer L. M., Basu L., Pfeffer S. R., Yang C. H., Murti A., Russell-Harde D. et al. (1997) The short form of the interferon alpha/beta receptor chain 2 acts as a dominant negative for type I interferon action. J. Biol. Chem. **272:** 11002–11005
47 Somers W., Ultsch M., De Vos A. M. and Kosslakoff A. A. (1994) The x-ray structure of a growth hormone-prolactin receptor complex. Nature **372:** 478–481
48 Wells J. A. (1994) Structural and functional basis for hormone binding and receptor oligomerization. Curr. Opin. Cell. Biol. **6:** 163–173
49 Day C., Schwartz B., Li B. L. and Pestka S. (1992) Engineered disulfide bond greatly increases specific activity of recombinant murine interferon-beta. J. Interferon Res. **12:** 139–143
50 Uzé G., Di Marco S., Mouchel-Vielh E., Monneron D., Bandu M.-T., Horisberger M. A. et al. (1994) Domains of interaction between alpha interferon and its receptor components. J. Mol. Biol. **243:** 245–257
51 Weber H., Valenzuela D., Lujber G., Gubler M. and Weissmann C. (1987) Single amino acid changes that render human IFN-alpha2 biologically active on mouse cells. EMBO J. **6:** 591–598
52 Mouchel-Vielh E., Lutfalla G., Mogensen K. E. and Uzé G. (1992) Specific antiviral activities of the human alpha interferons are determined at the level of receptor (IFNAR) structure. FEBS Lett. **313:** 255–259
53 Runkel L., Pfeffer L., Lewerenz M., Monneron D., Yang C. H., Murti A. et al. (1998) Differences in activity between alpha and beta type I interferons explored by mutational analysis. J. Biol. Chem. **273:** 8003–8008
54 Platanias L. C., Uddin S. and Colamonici O. R. (1994) Tyrosine phosphorylation of the alpha and beta subunits of the type I

interferon receptor. Interferon-beta selectively induces tyrosine phosphorylation of an alpha subunit-associated protein. J. Biol. Chem. **269:** 17761–17764
55 Abramovitch C., Shulman L., Ratovisky E., Harroch S., Tovey M., Eid P. et al. (1994) Differential tyrosine phosphorylation of the IFNAR chain of the type I interferon receptor and of an associated protein in response to IFN-α and IFN-β. EMBO J. **13:** 5817–5877
56 Constantinescu S. N., Croze E., Murti A., Wang C., Basu L., Hollander D. et al. (1995) Expression and signaling specificity of the IFNAR chain of the type I interferon receptor complex. Proc. Natl. Acad. Sci. USA **92:** 10487–10491
57 Croze E., Russell-Harde D., Wagner T. C., Pu H., Pfeffer L. M. and Perez H. D. (1996) The human type I interferon receptor. Identification of the interferon beta-specific receptor-associated phosphoprotein. J. Biol. Chem. **271:** 33165–33168
58 John J., McKendry R., Pelligrini S., Flavell D., Kerr I. M. and Stark G. R. (1991) Isolation and characterization of a new mutant human cell line unresponsive to alpha and beta interferons. Mol. Cell. Biol. **11:** 4189–4195
59 Velazquez L., Mogensen K. E., Barbieri G., Fellous M., Uzé G. and Pellegrini S. (1995) Distinct domains of the protein tyrosine kinase tyk2 required for binding of interferon-alpha/beta and for signal transduction J. Biol. Chem. **270:** 3327–3334
60 Jarpe M. B., Knall C., Mitchell F. M., Buhl A. M., Duzic E. and Johnson G. L. (1998) [D-Arg1,D-Phe5, D-Trp7,9,Leu11] substance P acts as a biased agonist toward neuropeptide and chemokine receptors. J. Biol. Chem. **273:** 3097–3104
61 Conradt H. S., Egge H., Peter-Katalinic J., Reiser W., Skilosi T. and Schaper K. (1987) Structure of the carbohydrate moiety of human interferon-beta secreted by a recombinant Chinese hamster ovary cell line. J. Biol. Chem. **262:** 14600–14605
62 Runkel L., Meier W., Pepinsky B., Karpusas M., Whitty A., Kimball K. et al. (1998) Structural and functional differences between glycosylated and nonglycosylated forms of human interferon-beta (IFN-beta). Pharm. Res. **15:** 641–649
63 Mark D. F., Lu S. D., Creasey A. A., Yamamoto R. and Lin L. S. (1984) Site-specific mutagenesis of the human fibroblast interferon gene. Proc. Natl. Acad. Sci. USA **81:** 5662–5666
64 Rudick R. A., Simonian N. A., Campion M., Scaramucci J. D., Jones W., Coats M. E. et al. (1998) Incidence and significance of neutralizing antibodies to interferon beta-1a in multiple sclerosis. Multiple Sclerosis Collaborative Research Group. Neurology **50:**1266–1272
65 The IFNβ Multiple Sclerosis Study Group and the University of British Columbia MS/MRI Analysis Group (1996) Neutralizing antibodies during treatment of multiple sclerosis with interferon beta-1b: experience during the first three years. Neurology **47:** 889–894
66 Antonelli G. (1997) In vivo development of antibody to interferons: an update to 1996. J. Interferon Cytokine Res. **17** (Suppl. 1)**:** S39–S46
67 The IFNβ Multiple Sclerosis Study Group and the Univerity of British Columbia MS/MRI Analysis Group (1995) Interferon beta 1b in the treatment of multiple sclerosis: final outcome of the randomized controlled trial. Neurology **45:** 1277–1285
68 Abdul-Ahad A. K., Galazka A. R., Revel M., Biddoni M. and Borden E. C. (1997) Incidence of antibodies to interferon-beta in patients treated with recombinant human interferon-β1a from mammalian cells. Cytokines Cell. Mol. Ther. **3:** 27–32
69 REBIF® Package Insert, Serono Laboratories, Inc., Norwell MA
70 Palleroni A. V., Aglione A., Labow M., Brunda M. J., Pestka S., Sinigaglia F. et al. (1997) Interferon immunogenicity: preclinical evaluation of interferon-alpha2a. J. Interferon Cytokine Res. **17** (Suppl. 1)**:** S23–S27
71 Alam J., McAllister A., Scaramucci J., Jones W. and Rogge M. (1997) Pharmacokinetics and pharmacodynamics of interferon beta-1a (IFNβ-1a) in healthy volunteers after intravenous, subcutaneous or intramuscular administration. Pharmacology **14:** 35–43
72 Hochuli E. (1997) Interferon immunogenicity: technical evaluation of interferon-alpha2a. J. Interferon Cytokine Res. **17** (Suppl. 1)**:** S15–S21