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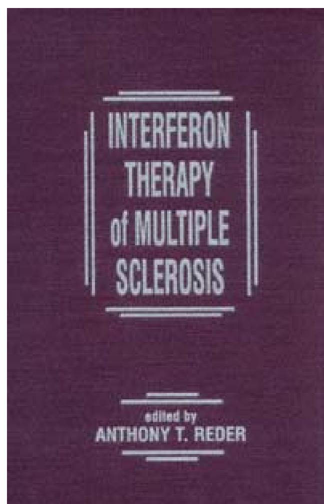

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INTERFERON THERAPY of MULTIPLE SCLEROSIS

INTERFERON THERAPY of MULTIPLE SCLEROSIS

edited by

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Preface

Multiple sclerosis (MS) affects 300,000 people in the United States and 1 million worldwide. It typically strikes an intelligent, educated woman just as her career and family life begin to flower.

Interferon-β (IFN-β) is the first and only FDA-approved therapy to change the course of MS. Its mechanism of action in MS is unknown. IFN- β has been approved for treatment of relapsing/remitting and relapsing/ progressive MS, but this is only one part of a spectrum of demyelinating diseases. Arguably, “MS” includes monosymptomatic demyelination, optic neuritis, transverse myelitis, primary progressive MS, Devic's disease, postinfectious encephalomyelitis, and possibly Leber's optic atrophy, and adrenoleukodystrophy. Would interferons treat any or all of these?

Drug trials in MS are particularly difficult. Large numbers of patients must be studied over years—the time between exacerbations averages two years, and progression is typically slow (at least from the examiner's viewpoint). The Kurtzke disability rating scale is certainly nonlinear—the change from a Kurtzke rating of 4 to 5 is four times as fast as the change from 6 to 7. Experienced neurologists are required—disease symptoms encompass every function of the central nervous system, and attacks range from inconsequential to devastating. The natural history also changes over time—attack frequency declines, and relapsing disease becomes progressive. Finally, medical care and patient motivation and outlook affect the results; study patients taking a placebo drug do better than untreated patients.

The rationale for using IFNs to treat MS has evolved side by side with the most popular views of the cause of the disorder. In the early 1980s, IFN-α, IFN-β, and IFN-γ were all advanced as therapies for MS because of their antiviral effects, since many investigators hypothesized that MS was caused by a virus. Unfortunately, IFN-γ caused exacerbations, and the "virus" remains elusive. On the basis of relatively thin evidence, MS is now widely assumed to be caused by an immune reaction to brain antigens. All forms of IFN could alter this immune response, but do so in different directions. Regardless of their effects on immunity, the benefit from type I IFN therapy in MS does suggest that IFNs are somehow involved in the etiology of MS.

The type I IFN family contains four gene families (IFN-α, β, ω, and τ). In addition, there are multiple natural preparations and recombinant subtypes. IFNα-n3, IFNα-2a, and IFNα-2b are approved in the United States for various indications; IFNβ-1a and IFNβ-1b are approved for MS. The spectrum of indications for use of IFNs is even broader in Europe. Understanding IFN-receptor interactions, signaling, pharmacokinetics, and clinical effects should optimize treatment of MS and other diseases.

MS is the first inflammatory/autoimmune disease to be successfully treated with IFNS. Therapy with recombinant cytokines is in its infancy, yet much has been learned from our experience with IFNS. Although type I IFNs are not a cure for MS, they offer a building block. Interferons will potentially synergize with other treatments under study in MS, such as cytotoxic drugs/chemotherapy, glucocorticoids, cyclosporine, cAMP agonists, specific immunomodulators, anti-macrophage agents, copolymer-l, T-cell receptor/HLA/adhesion/costimulatory molecule blockade or elimination, oral tolerance to central nervous system antigens, and other cytokines or cytokine antagonists.

This book covers the role of interferons in the treatment of MS. It begins with the molecular biology of IFN binding to its receptors, the signal cascade within the cell, gene regulation, and the induction of IFN- stimulated genes. This is followed by a description of IFN pharmacokinetics. Next, response to IFNs is discussed at the cellular level in neurons, glia, and immune cells. Experimental allergic encephalomyelitis (EAE) is used as a model of MS for reviews of therapy with oral and systemic IFN- α, IFN-β, and IFN-γ, and the newly discovered IFN-τ. Finally, there are clinically oriented descriptions of the benefits and also the side effects of IFN-α, IFNβ-la, and IFNβ-1b in MS, and the role of current and future magnetic resonance techniques for imaging MS lesions.

The target audience for this book is wide—basic scientists in biotechnology and academia, neurologists, other clinicians, and health care professionals who treat MS patients, and also pharmaceutical sales repre-

sentatives, MS clinic personnel, and patient groups, such as MS Society chapters. Efforts were made to ensure clarity; each chapter was written in depth and should serve as reference source for its area.

Authors were selected because they are doing cutting-edge basic or clinical work in relevant areas. This was done to infuse each chapter with ideas from people actively investigating IFNs and MS rather than simply review the existing literature. The authors have analyzed the literature and have expanded on their own published and unpublished research. They have also addressed several common threads within their general topic. These include (1) comparisons of all forms of IFN, (2) the implications for treatment of MS, (3) synergy or interference with other agents, based on clinical experience or on basic/theoretical mechanisms, and (4) hypotheses on how IFNs prevent disease activity in MS. These ideas will suggest new and better ways to treat MS, and possibly lead us to the cause of the disease.

Anthony T. Reder

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1 The Molecular Biology of Interferon-β from Receptor Binding to Transmembrane Signaling

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I. INTRODUCTION

Interferons (IFNs), which were independently discovered by two groups in the 1950s (1,2), are proteins capable of interfering with the viral infection of cells. Their discovery culminated many years of study on the basis for viral interference. Besides antiviral activity, the diverse biological actions of these cytokines also include inhibition of the proliferation of normal and transformed cells, regulation of differentiation, host responses to various pathogens, and modulation of the immune system (including activation of natural killer cells and macrophages). The human type I IFNs include 15 IFN-α subtypes, one IFN-β subtype, and two IFN-ω subtypes. Type I IFNs are acid stable, have similar protein structure and biological activities, bind and transduce signals through a common multiprotein cell surface receptor, are induced in response to viral and other inducers, and share a common gene locus on human chromosome 9. In contrast, type II IFN (or IFN-γ) is acid-labile and differs from type I IFNs in many of the above respects except that it shares similar biological actions. However, there are differences in biological potency of type I and type II IFNS. For example, type II IFN is considered more active in immunomodulation than type I IFN. In addition, the biological specific activities for the different type I IFN subtypes can vary by as much as three orders of magnitude on a log10 scale.

Several lines of evidence suggest that some of IFN's biological activities, such as the augmentation of natural killer cell activity, antiproliferative, and antiviral activities, can be elicited through different molecular pathways. IFNs elicit their effects by first binding to specific multiprotein receptors on the surface of target cells and then transducing a signal to the nucleus that results in selective gene expression (3–6). A family of early genes, the IFN-stimulated genes (ISGs), is transcriptionally activated within minutes by type I IFN. ISG transcriptional activation is mediated by the protein tyrosine kinase (PTK)-dependent phosphorylation of latent cytoplasmic transcriptional activators, termed the STAT proteins (for Signal Transducers and Activators of Transcription) (7,8). Type I IFNs activate STAT113 (Mr 113,000) and STAT91 (Mr 91,000), which then bind to the p48-DNA binding protein, forming the ISGF3 complex. This complex then moves into the nucleus and recognizes the highly conserved IFN stimulus-response element (ISRE) promoter element in ISGs directly to activate these genes (9,10). Immunologically related STAT proteins apparently function in the gene-activation pathway induced by other cytokines (11,12). Central to the IFN-α-activated PTK pathway are two Janus (JAK) PTKs, JAK1 and TYK2 (13,14), which apparently mediate the tyrosine phosphorylation of STATs, as well as other type I IFN receptor subunits (13–15).

Recent studies reveal that many cytokine receptors (including those for IFNs, erythropoietin [EPO], growth hormone [GH], colony-stimulating factors [CSFs], interleukins [ILs]) can associate with and activate members of the JAK family of cytoplasmic PTKs (13–15). These kinases are rapidly phosphorylated after receptor activation and share the unusual feature of having two kinase domains. EPO, GH, and IL-3 activate only JAK2. The ciliary neurotrophic factor (CNTF)/oncostatin M (OSM)/leukemia inhibitory factor (LIF)/IL-6 family of cytokines can activate JAK1, JAK2. and TYK2 in a cell type-dependent manner. IFN-γ responses involve JAK1 and JAK2, whereas IFN-α requires JAK1 and TYK2. Thus, both type I and type II IFNs activate JAK1 and the tyrosine phosphorylation of STAT91, whereas type I IFNs selectively activate TYK2 and the phosphorylation of STAT 113. Furthermore, although both STAT proteins are involved in ISG activation, STAT113 and STAT91 are phosphorylated independently of one another (16). These data suggest that JAK1 is responsible for the phosphorylation of STAT91, whereas TYK2 is responsible for STAT113 phosphorylation. The JAK kinases appear to be the most proximal kinases activated in response to ligand, playing a critical and common role in mediating responses to all of these disparate but distantly related cytokines. A major question that arises from these findings is where is the specificity of cytokine signaling maintained if different cyto-

kines with divergent biological effects all work through the JAK kinases. One possibility is that each member of the JAK family may have discrete substrate specificity. However, it is more plausible that specificity is dictated by the specific association of substrates with receptor subunits.

The role of the JAK/STAT pathway in ISG activation is described in detail in Chapter 2. The type I IFN-activated JAK/STAT pathway serves as a paradigm for cytokine signal transduction in general. IFNs are highly effective molecules; the occupancy of only a few receptors per cell triggers a biological response in IFN-responsive cells. Besides the activation of JAK PTKs, the type I IFNs also rapidly activate Ca^{2+} -independent protein kinase C (PKC) subspecies through the production of the lipid second- messenger DAG (17). Both PTK and serine/threonine kinases are involved in the regulation of IFN-α/β-induced ISG mRNA levels and in the establishment of antiviral activity in various human cells (17–20). It is unknown how these varied biological signals are integrated at the level of the type I IFN receptor (IFNIR).

This chapter describes the molecular basis of IFN-β action with a focus on the roles of IFNIR subunits at the levels of receptor structure and the components of the signaling pathway rapidly activated on ligand-receptor interaction. Since human IFNs (huIFNs) are clinically useful in the treatment of various human diseases (multiple sclerosis, hairy cell leukemia, laryngeal and genital papillomas, acquired immunodeficiency syndrome (AIDS), Kaposi's sarcoma, and chronic viral hepatitis), it is essential to understand how IFNs interact with cells. Knowledge of these interactions should expedite the therapeutic use of huIFN-β and provide a basis for developing new strategies in the treatment of multiple sclerosis (MS) with IFN-β alone or in combination with other agents.

II. DIFFERENCES IN BIOLOGICAL EFFECTS BETWEEN IFN-α AND IFN-β

Type I IFNs share a common ligand binding site and induce common biological effects. However, the intrinsic properties of all type I IFNs are not identical. For the purpose of this chapter we assume that all type I IFNs act on the IFNIR in a similar manner, but we will emphasize any IFN-β-specific events that have been identified. For example, we and others have recently identified the tyrosine phosphorylation of a IFNIR- associated protein that is induced by IFN-β but not by IFN-α2 or IFN- α8 (21–23). In addition, IFN-β may exert type I IFN actions at specific sites after autocrine secretion, and thus these effects may also be considered as IFN-β specific.

IFN-α and IFN-β differ markedly in their cell type-specific antiproliferative actions. IFN-β exerts greater antiproliferative activity on many cell types, such as embryonal carcinomas, melanomas, and melanocytes (24,25). IFN-β has been reported to bind with higher affinity to the common IFN-α/β binding sites and to stimulate peripheral blood stem cells of patients with hairy cell leukemia to differentiate into erythroid burst- forming cells (26). IFN-β, but not IFN-α, inhibits the growth of vascular smooth muscle cells (27). Autocrine secretion of IFN-β seems to be the physiological mechanism by which proliferative signals induced by prostaglandin F (PDGF), IL-1, or TNF-α are muted in vascular smooth muscle cells (27). IFN-β can block human immunodeficiency virus (HIV) infection at a step prior to the reverse transcription of viral RNA (28). This is important, since HIV develops a tat-dependent mechanism to overcome the type I IFN—induced restriction of HIV replication (29). IFN-β increases steroid receptor expression in breast cancer cells (30), and it has promising antiproliferative effects on prostate cancer cell lines (31). Taken together, these data show that IFN-β may be active in the treatment of cancers which are resistant to the antiproliferative effects of natural IFN- α. Furthermore, IFN-β induces IFN-α production in mice after systemic administration or in transgenic mice carrying an IFN-α gene under control of a metallothionen-enhancer/promoter, whereas IFN-α does not induce IFN-β (32).

Recently, in several large clinical trials, IFN-β was found to lower the frequency of relapses and improve the symptoms of relapsing-remitting multiple sclerosis (MS) (33–37). In contrast, IFN-γ exacerbated MS symptoms, and in some studies IFN-α was detrimental (38) (see Chapters 11–14). MS is an inflammatory demyelinating disease of the central nervous system which is clinically characterized by relapses and remissions and leads to chronic disability. The pathology of the disease has an autoimmune element, with a proposed defect in the suppressor T-cell subset. Importantly, mice that had an inactivated gene for IRF-1 (an ISG) showed abnormalities in the development of CD8 (T suppressor cells), which are implicated in the pathology of MS (39). Interestingly, early studies indicate a selective inhibition by IFN-β of the generation in vitro of T suppressor cells (40). The molecular basis for any differential effects of IFN-α versus β in MS is unknown.

Furthermore, IFN-α-resistant cell mutants remain partially sensitive to IFN-β activation of the JAK/STAT pathway and gene induction (14,41). Although both IFN-α and IFN-β induce the rapid tyrosine phosphorylation of IFNIR subunits, a unique 105-kDa band is tyrosine phosphorylated only in response to IFN-β but not to several IFN-α subtypes (21–23).

III. TYPE I IFN RECEPTOR

A. Characterization

The four major antigenic types of IFNs (α, β, γ, and ω) are defined by the cellular source of their production. Type I IFNs (IFN α, β, and ω) compete with each other for cellular binding to IFNIR and thus share at least some components of a common multisubunit cell surface IFNIR, whereas the receptor for type II IFN (IFN-γ) is a distinct entity (42). Nearly all human cell lines and human tissues display the IFNIR, varying in number from 500 to 20,000 high-affinity (kd ≈ 50 pM) and 2000 to 100,000 low-affinity (Kd ≈ 1–10 nM) receptors/cell. Chemical cross linking of iodinated IFN-α to human tumor cells has demonstrated that the IFNIR apparently is composed of 100-, 110-, and 135-kDa glycoprotein subunits (21,43–45). The interaction of IFN with its cognate receptor is species specific, so that human cells respond preferentially to human type I IFNs over mouse IFNS. This suggests that a subunit (or subunits) of the huIFNIR is responsible for the species-specific interaction. Studies with monoclonal antibodies (MoAbs) directed against huIFNIR components suggest the existence of accessory proteins that may modulate the specificity of binding and signal transduction by the IFNIR (46,47). Furthermore, structure-function analysis of type I IFN subtypes identifies regions required for IFN binding, as well as those involved solely in signal transduction (48), providing further evidence for the complexity of the IFNIR structure.

Somatic cell genetics have established that both binding and transducing chains of IFNIR map to human chromosome 21 (Ch21). Antisera generated to Ch21-encoded proteins block the biological activity of type I IFNs but not of type II IFN (49,50). Furthermore, the biological effect induced by type I IFNs in cells and the number of receptor subunits directly correlates with the copy number of Ch21 (51,52). In addition, MoAbs generated to IFNIR components react with Ch21-encoded proteins (53,54). Furthermore, a gene on Ch21 in the region from q22.2 to q22.3 encodes a novel subunit of the IFNIR required for type I IFN signaling (55). Although the binding subunit of the type II IFN receptor maps to human chromosome 6, accessory factors involved in type II IFN signal transduction also map to Ch21 (56,57).

The nomenclature used for the components of the type I IFN receptor (IFNIR) is inconsistent and confusing, as shown in Table 1. The cDNAs coding for two IFNIR chains have recently been cloned and named the IFNAR and IFNABR subunits by the groups that isolated the cDNAs (58,59). In addition, MoAbs have been generated against cells expressing

Table 1 Terms Used to Describe IFNIR Subunits (Defined by Migration of Proteins from the Daudi Lymphoblastoid Cell Line) Cross-linked New Mr complex cDNA Subunit by name (kDa) (kDa) Designation MoAb IFNIR-1 135a 150 IFNAR α?IFNIR-2 100b 120 IFNABR βIFNIR-3 110 130 Not cloned α? aMigration depends markedly on cell line examined, generally between 115 and 135 kDa.bPresent in U937 cell line as a 50-kDa protein. Thus it may exist as homodimers or heterodimers of 50-kDa subunits.
IFNIR and identified by the ability either to precipitate IFN-α (or IFN- β) cross linked to cell surface IFNIR, or block ligand binding to IFNIR. The components of IFNIR recognized by these MoAbs were termed the α and β subunits, respectively. In this chapter we have designated IFNAR as IFNIR-1, and IFNABR as IFNIR-2 on the basis of the order in which they were identified. It thus follows that the subunits of the type II IFN receptor should be called IFNIIR-1, 2, and so forth in the order of their discovery. In the following sections, we attempt to relate these subunits to findings made on the α and β subunits. We consider this as the appropriate nomenclature, because at the present time, the biological role of each individual huIFNIR subunit in ligand binding and signal transduction has not been completely elucidated. Furthermore, our data suggest that a third component of the IFNIR also exists, but the roles of these subunits alone or in concert have not been elucidated.

Partial purification and characterization of a ligand binding subunit of the IFNIR from lymphoblastoid cell membranes has been achieved using a combination of wheat germ lectin and IFN-affinity techniques (60–62). The ligand binding subunit appears to be a highly asymmetrical membrane protein with a Stokes radius of ≈74 Å and a Mr of ≈110 kDa. These studies also provided evidence that the protein contains a sialic acid oligosaccharide moiety, a finding confirmed in affinity cross-linking studies (43). Using IFN-affinity chromatography, we have partially purified a ligand binding protein (IFNIR-3) of similar Mr, which apparently is distinct from the two cloned chains of the IFNIR (45).

B. Multisubunit Structure of IFNIR

1. Affinity Cross Linking

Several lines of evidence indicate that the huIFNIR binds the multiple type I IFNs (IFN-α subtypes, IFN-β, and IFN-ω) and consists of several subunits (43). Affinity cross linking of 125I-IFN-α2 to cells with the homobifunctional reagents such as disuccinimidyl suberate DSS result in formation of a broad IFN receptor complex of 120–150 kDa on a variety of human cells (60,63). The specificity of complex formation has been confirmed by its precipitation with anti-IFN sera and by competition with excess unlabeled IFN-α2. The glycoprotein nature of the IFNIR was demonstrated by the sensitivity of complex formation to the pretreatment of cells with trypsin and neuraminidase. In recent studies on the structure of the IFNIR in various human tumor cell lines, the broad 120–150-kDa IFN-α receptor complex has been resolved into 100-, 110-, and 130-kDa glycoproteins (120-, 130-, and 150-kDa affinity cross-linked complexes, respectively), as illustrated in Figure 1 (43). Treatment of affinity cross- linked material with glycosidases demonstrates the glycoprotein nature of all IFNIR subunits detected (43). In addition, numerous affinity cross- linking studies also identify high molecular weight IFN-α receptor complexes that reflect an association of receptor subunits, as illustrated by the ≈240-kDa complex in Figure 1. Affinity cross-linking studies with 125I-IFN-β reveal IFN receptor complexes with similar electrophoretic mobility to those formed with IFN-α (62,64).

Partial solubilization of IFN-α receptor complexes with the nonionic detergents, digitonin or CHAPS, resolves a complex on high-performance chromatography with a relative size of ≈600 kDa. In addition, solubilization of cells or cell membranes with CHAPS yields a similar sized complex that is capable of binding type I IFN, with an IFN binding site-containing component of ≈95 kDa (65). However, we have found that CHAPS solubilizes only a low-affinity binding component of the IFNIR. Use of the heterobifunctional Denny-Jaffe reagent to identify direct IFN receptor interaction reveals that a 110-kDa protein is selectively cross linked to IFN (66). The summary of results obtained by affinity cross-linking and gel chromatography shows that the IFNIR is a multisubunit complex.

2. Anti-IFNIR MoAbs Define Receptor Structure

Recent studies to define the exact structure of the IFNIR have been aided by the generation of MoAbs against various receptor components. Two different strategies have been used successfully: (1) mice were injected with human cells that express high levels of IFN-α binding sites, and

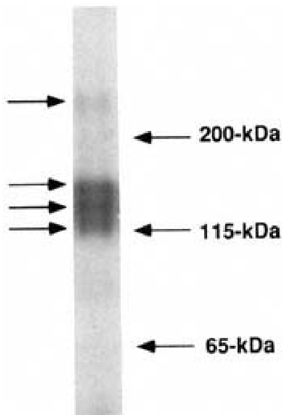

Figure 1 Affinity cross linking of type I IFN receptors on Daudi lymphoblastoid cells. After incubation of cells with iodinated IFNCon1, IFN was cross linked to the cell surface with disuccinimidyl suberate. Proteins were analyzed by SDS- PAGE and autoradiographed. Molecular weight markers are indicated on the right of the figure and the arrows point to cross-linked complexes. The Mr of the receptor chains is calculated by subtracting the Mr of IFNCon1 (20 kDa) from that of the cross-linked complex.

hybridomas generated from the responding B cells were screened for the ability to block IFN-α binding or to immunoprecipitate affinity cross- linked material (53,54); or (2) mice were injected with baculovirus-expressed ectodomain of IFNIR-1, and hybridomas were screened for the ability to detect IFNIR-1 cell surface expression by flow cytometry (21,67,68). Using the first strategy, MoAbs that detect the so-called α and β subunits of the IFNIR-1 were generated (53,54). Anti-α subunit MoAbs precipitate a 110-kDa protein from surface iodinated material, and a broad 130- to 150-kDa complex and ≈240-kDa complex from affinity cross-linked material. These MoAbs fail to precipitate the ≈100-kDa IFNIR subunit detected as the 120-kDa affinity cross-linked complex shown in Figure 1. The high molecular weight complex (≈240 kDa) presumably consists of the α subunit in IFNIR-association with another receptor subunit. Interestingly, the three individual anti-α subunit MoAbs were directed to one epitope, suggesting that this epitope is highly immunogenic. These results sug-