THE INTERFERON RECEPTORS

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I. INTRODUCTION: THE INTERFERON SYSTEM

In 1957, Isaacs and Lindenmann discovered a protein which was produced by virus-infected cells and was responsible for the phenomenon of viral interference.[1] This substance was named interferon and later it was characterized as a group of proteins ranging in molecular weight from 15,000 to 50,000. Interferons are produced and excreted by most vertebrate cells in response to a variety of viral and nonviral inducers. By and large interferons are "species specific" as was already observed in 1959.[2] Extensive studies over two decades led to a definition of interferons as "proteins which exert virus nonspecific, antiviral activity, at least in homologous cell, through cellular metabolic processes involving synthesis of both RNA and proteins."[3]

A. The Structure of Interferons

Three antigenically distinct groups of interferons were characterized in terms of proteins and gene structure as well as in terms of biological functions. The antigenic differences were used as a basis for the current nomenclature which names the three groups: interferon-α (IFN-α), IFN-β, and IFN-γ.[3] A previous nomenclature defining virus-induced interferons as "Type I" (corresponding to -α and -β) and mitogen-induced interferons as "Type II" (IFN-γ), seems to be more appropriate in terms of structure and biological functions, and as will be demonstrated later, in terms of receptor binding properties.

Human IFN-α is by itself a group of structurally related (50% sequence homology in 14 subtypes) polypeptides of 165 to 166 amino acid residues which are derived from an intronless multigene family.[4-6] Most members in this group lack glycosylation signal sequences and therefore are not *N*-glycosylated, however, *O*-glycosylation of some subtypes was observed.[7] All IFN-α subtypes contain two disulfide bonds at positions 1 to 99 and 29 to 139.[8] IFN-α mixtures are produced and excreted by leukocytes in response to virus challenge. Most of these subtypes are completely resistant to acid treatment (pH 2, 4°C, 24 hr)[9] and to denaturation by Na dodecylsulfate ($NaDodSO_4$).[10] All IFN-α subtypes are antigenically cross reactive, but some monoclonal antibodies can distinguish between certain subtypes.[11] Recently, a group of α-like (αL)[12] or class-IIα[13] genes, of which only one is active and the rest are pseudogenes were described. No unique properties or functions were found so far for this somewhat longer polypeptide (172 residues). An acid labile form of IFN-α was detected in sera of lupus erythematosus patients[14] and in vitro in uninduced cultures of low density peripheral blood lymphocytes.[15] Nothing is so far known about the structure of this IFN-α.

Human IFN-β consists of a major subtype (IFN-β_1) derived from a single intronless gene

and having a significant (29%) sequence homology with IFN-α. It is produced and excreted by fibroblasts in response to virus (or dsRNA in vitro). IFN-β_1 is an acid-stable glycoprotein having 166 amino acid residues, one disulfide bond at position 32 to 141, and one glycosylation site at Asn-80.[16] All IFN-α subtypes, as well as IFN-β_1, are hydrophobic proteins as was demonstrated, e.g., by their interaction with reversed-phase silicas.[17,18] Minor species of IFN-β were described.[19,20] Of these, IFN-β_2 was isolated from supernatants of dsRNA-induced fibroblast cultures.[21]

Human IFN-γ is a product of a single gene bearing three introns.[22,23] The mature translation product has 143 amino acid residues but it is partially shortened at the C-terminus.[24] It lacks disulfide bonds but it has two glycosylaiton signal sequences in positions 25 and 97. There is a very limited sequence homology between IFN-γ and either IFN-α or -β.[25] Gel electrophoresis of pure IFN-γ in the presence of $NaDodSO_4$ reveals several immunologically cross-reactive protein bands having apparent molecular weights of 25,000, 21,000, and 17,500. This heterogeneity results from variability in the degree of glycosylation and from the C-terminal processing. IFN-γ is a rather hydrophilic, acid labile, and basic (pI = 8.5)[26] protein which appears as a dimer (Mr = 45,000 to 50,000) on gel filtration.

B. Biological Activities of Interferons and their Mechanisms

By definition, all interferons exert an antiviral activity at least on some homologous cells. However, the various IFN subtypes display many other biological activities which in some cases, depend on the type of cell and on the IFN subtype. In most cases the various actvities are exerted only by homologous IFNs, namely, species specificity is maintained. The antiviral activity of IFN is in itself a whole spectrum of activities since the various IFNs may act on one or more of the phases of the virus replication cycle. Thus, IFNs can inhibit the uptake and processing of virions, their early transcription, DNA or RNA replication, viral protein synthesis, assembly of virions, and finally their release from the cells.

There are several known mechanisms by which IFNs can exert these effects and both rapid biochemical changes as well as slow changes which involve induction of specific genes to produce certain proteins were described. Three interferon-induced enzymes were identified as mediators of its antiviral activity. The best characterized one is (2′ - 5′) oligo adenylate synthetase, a dsRNA-dependent enzyme which polymerizes ATP into $ppp(A2'p)^{\wedge}_{n}$, (n = 2 to 15).[27] These oligomers activate a latent ribonuclease (RNase F) which in turn, degrades ribosomal RNA and polysomes, thus inhibiting viral protein synthesis. A second IFN-induced enzyme is a dsRNA-dependent protein kinase which phosphorylates the small subunit of initiation factor eIF-2. Phosphorylated eIF-2 forms a stable complex with GDP, which prevents GTP binding, thereby reducing the rate of initiation of protein synthesis.[28]. A third IFN-induced enzyme is 2′-phosphodiesterase which requires high concentrations of IFN. This enzyme degrades the 2′ - 5′ adenylate oligomers, thus acting as an inhibitor of antiviral response. However, it also cleaves the 3′ terminal trinucleotide CCA of tRNA, a process which reduces the amount of tRNA that is available for viral protein synthesis.[29]

The antiviral activity of IFN is mediated both via inhibition of viral replication as described above and by augmentation of killing of virus-infected cells. IFN, and particularly IFN-γ, induces Class I MHC and thus increases the lysis of virus-infected cells by cytotoxic T lymphocytes. In addition, IFN acts directly on monocytes, cytotoxic T lymphocytes, and NK cells and increases their ability to lyse virus-infected cells.[30] IFN causes lymphopenia which is due to redistribution of lymphocytes. Mobilized lymphocytes are seen around blood vessels and since foci of viral infections are located in tissues, this mobilization could be part of the indirect antiviral effects of IFN.[31] Pure IFN preparations are pyrogenic and therefore it is likely that fever which is associated with many viral diseases is caused by IFN. Since high body temperature is inhibitory to virus replication, IFN-induced fever may represent another antiviral mechanism which probably involves the induction of prostaglandin and/or interleukin 1.[32,33]

IFNs are inhibitors of cell growth and replication and as such they are considered to be negative growth factors. Control of cell proliferation occurs primarily during the G_1 phase of the cell cycle. A variety of serum-derived (positive) growth factors such as insulin and EGF include the expression of several proteins during G_1 phase that are necessary for the onset of DNA replication. IFN prolongs all phases of the cell cycle but particularly G_1 and G_2 and therefore IFN and EGF have antagonistic effects.[34,35] As a negative growth factor IFN diminishes the large increase in the rate of protein synthesis which normally occurs during the transition from Go/G_1 to S phase. This effect is probably mediated via the induction of (2′ to 5′) oligo A synthetase.[36] IFN can exert its effects both by inducing specific genes as that of synthetase and by blocking other genes. Thus, it was recently found that IFN inhibits in human Daudi cells the induction of c-*myc* oncogene in a specific manner and this was correlated with the profound growth inhibitory effect of IFN on these cells.[37] Another example of this kind is the ability of IFN to inhibit the increase in ornithine decarboxylase activity which normally occurs in late G_1.[38] This is a rate-limiting enzyme in the biochemical pathway of polyamine synthesis which occurs in the S phase and is associated with DNA replication.

Several studies have demonstrated the involvement of IFN in the process of cell differentiation. Thus, when erythroleukemic cells are treated with IFN prior to induction of differentiation, the extent of differentiation as determined by the number of hemoglobin-containing cells, and the amount of hemoglobin produced per cell is increased.[39] Similar effects of IFN are seen in promyelocitic cells[40] and myeloid leukemic cells.[41] In several cases, a significant increase (up to 30-fold) in the activity of (2′ - 5′) oligo A synthetase was associated with the process of differentiation.[42]

Prolonged treatment of transformed cell cultures with IFN progressively changes their phenotype into normal morphology. These changes are transient and the transformed phenotypes reappears upon IFN withdrawal.[43,44] Such changes are associated with the inhibitory effects on oncogene expression and with the ability of IFN to restore part of the actin microfilament structures and the microtubules as present in nontransformed cells.[45,46]

IFN, and particularly IFN-γ, have profound immunoregulatory effects. IFN-γ activates monocytes towards tumor cell killing. It is in fact identified as a macrophage activating factor (MAF),[47,48] and the activation is achieved by increasing the release of cytotoxin.[48a] IFN activates NK cells,[49] probably in a similar manner, however, its overall effect on lysis by NK cells is minimal since it protects the target cells from lysis by NK cells.[50] The increased expression of Class I and II[51,52] MHC on the surface of cells treated with IFN (particularly IFN-γ)[53] is probably the most impressive immunoregulatory function of IFN. Class I MHC which appears on all cell surfaces restricts the activity of cytotoxic T lymphocytes, while Class II MHC is normally present only on certain immunocytes and restricts the process of antigen presentation to T helper cell. This is a key process in immune response and its modulation by IFN-γ places this lymphokine at a central point in immune response. Over the years many additional immunoregulatory functions of IFN were identified but not related to a specific biochemical mechanism. Among these are inhibition of antibody formation (by IFN-α),[54] inhibition of suppressor T cell activity,[55] modulation of delayed type hypersensitivity,[56] increased release of histamine, increased phagocytosis by monocytes,[57] induction of B-cell maturation,[58] and antibody formation.[59] Other studies revealed the induction of 12 proteins by IFN-α and -β; these and 12 additional proteins by IFN-γ.[60] Therefore, one may conclude that only a small fraction of the biochemical pathways related to IFN activities have been revealed so far.

II. BINDING OF INTERFERONS TO THEIR RECEPTORS

A. Early Evidence for the Existence of Interferon Receptors

Characterization of binding sites for a given ligand is possible only if a pure preparation

of this ligand is available in sufficient quantities. Pure IFN-β was obtained by Knight in 1976 while pure IFN-α has only been available since 1978,[61,62] nevertheless, indirect evidence was used long before that date to suggest the presence of such a receptor. The very fact that IFN had a high specific activity ($\sim 10^9$ units per milligram were estimated) and was present in minute quantities implied that it had a high affinity binding site which is in fact a simplified definition for a receptor. Indeed, earlier studies have suggested the presence of such a receptor on the cell surface.

In 1967, Friedman demonstrated that incuation of chick cells for 10 to 20 min at 1°C with IFN, followed by washing excess IFN and further incubation at 37°C without IFN, were sufficient for eliciting the antiviral activity.[63] Moreover, treatment of cells with trypsin after binding of IFN at 0°C prevented the establishment of the antiviral state. Friedman concluded from these studies that binding of IFN to a superficial cell site was the first step in its action. Stewart et al.[64] discovered that cell-bound IFN activity can be recovered by extraction and that this phenomenon of binding is species specific and proportional to the extent of cell response to IFN. By this way they have localized the phenomenon of species specificity at the level of the binding site. In other words, heterologous IFN was inactive because it did not bind to the specific cell surface receptor. By using a similar extraction procedure, Berman and Vilcek[65] could measure some kinetic parameters of the binding. Maximal binding was found after 30 min both at 4 and 37°C. Moreover, spontaneous release of bound IFN was demonstrated at 37°C with a $t^1/_2$ of about 30 min. Finally, they could show that IFN was bound in a stable manner to the membranal fraction of the cells, however, the specificity of this binding was not demonstrated.

The role of internalization was studied with the aid of immobilized IFN. Sepharose-bound IFN was used to demonstrate that external contact with IFN was sufficient for the establishment of the antiviral state.[66] Mechanical obstruction of the agarose bead movement caused a restriction in the protected area on the cell monolayer, thus showing that leakage of IFN from the agarose beads was not responible for the observed IFN activity. Therefore it was concluded that internalization of IFN was not essential for its activity. Another study to prove the importance of external interaction with IFN was performed with IFN-producing cells. Antibodies to IFN-β were found to inhibit the establishment of the antiviral state in fibroblasts that were stimulated to produce IFN-β by poly(rI):(rC).[67] This observation indicated that IFN must be excreted before it could act on the cell and an external binding site was therefore postulated. All these studies were based on the ability to detect and quantitate IFN by highly sensitive and specific bioassays based on its antiviral activity. These studies led to the conclusion that IFN acts by binding to a specific cell surface moiety, probably a receptor.

B. Binding Studies with Labeled Interferons

The interaction of a ligand with its cellular receptor can be studied by measuring the amount of the bound ligand as a function of its concentration. This is best done by the use of a radiolabeled active ligand and a typical saturation curve is obtained in many cases. Analysis of the binding data according to Scatchard[68] enables to calculate both the affinity constant and the number of binding sites per cell. A linear dependency (Scatchard plot) is usually obtained when the ratio of bound to free ligand is plotted against the concentration of bound ligand. The slope of the resulting straight line is equal to $^{-1}/Kd$, where Kd is the dissociation constant which is the reciprocal of the affinity constant while extrapolation of this line to the abscissa (concentration of bound ligand) gives an intercept from which the number of receptors per cell can be calculated. These methods were used to calculate data related to a large number of hormone-receptor systems.[69]

1. Interferon Receptors: their Abundance and Affinity

In 1980, Aguet[70] presented for the first time evidence for the existence of IFN receptors

with the aid of ^{125}I-labeled mouse IFN. By using an IFN-sensitive mouse L1210 cell and an IFN-resistant mutant of these cells, Aguet was able to demonstrate specific binding, namely, that the binding of labeled IFN was saturable and was inhibited by an excess of unlabeled IFN. In contrast, binding of ^{125}I-IFN to the IFN-resistant mouse cell line or to chick embryo fibrobalsts was nonspecific. Saturation of the binding site was achieved within 2 hr both at 4 and at 37°C. From the binding curve at 37°C Aguet estimated a dissociation constant (*Kd*) of 1 to 2 $\times$ 10^{-11} *M* for the IFN-receptor complex.

As many IFN subtypes recently became available in a pure state, more and more binding studies were performed and in most cases high-affinity binding sites were found. The data on affinity constants and the number of receptors per cells is summarized in Table 1. All *Kd* values are in the range of 10^{-9} to 10^{-12} *M*, indicating high affinity and specificity of the interaction between IFN and its receptor. The number of receptors per cell is 300 to 20,000, a rather low value in comparison to that of insulin and EGF receptors. One implication of this low value is the difficulty in isolating the receptor molecule in a pure form and in sufficient quantities as needed for protein sequence analysis.

In addition to *Kd* values and receptor abundance, it is possible to determine from the Scatchard plots whether there are one or more classes of binding sites. A linear Scatchard plot indicates a single class of noninteracting binding sites. Such a situation was reported for the receptor of murine IFN-α,β in mouse embryonal carcinoma cells and mouse L cells.[71,72] A single class of high-affinity binding sites for human IFN-α and β was found on various human B lymphoblastoid cells (Daudi,[73,74,76,78,81,86,87,96] Raji,[73] and Namalva[82]); in bovine MDBK cells;[75,79,80] in human fibroblasts (FL[79]); in human embryonic carcinoma cells (RSa[84]); and in human neuroblastoma cells (T98G[85]). The receptor for interferon-γ was characterized in several human cells and a single class of binding sites was found in human fibroblasts (GM-258,[88,89] FS11[90]) and in human amnion cells (WISH[90,92]). Similarly, the receptor for murine IFN-γ in mouse macrophages consisted of a single class of binding sites.[91]

Nonlinear Scatchard plots were obtained in many hormone-receptor systems, however, the interpretation of these plots varied. In most of the reports a curvilinear plot with an upward concavity was obtained. It could be interpreted either as the result of a binding site multiplicity or negative cooperativity among receptors belonging to the same class. However, the most likely explanation is that there is a second class of binding sites which do not induce any biological activity. This interpretation is supported by the fact that in most cases the second binding site is very abundant and its *Kd* value is very high (low affinity).

There are several reports which demonstrate nonlinear Scatchard plots in the IFN-receptor systems. Mogensen et al.[73] reported that the binding of ^{125}I-IFN-α to a variety of human cell lines at 4°C gave more or less linear Scatchard plots. However, at 37°C curvilinear plots were obtained for the lymphoblastoid lines Daudi and P3HRI. Similar curves were reported for human fibroblasts.[77] In these cases a second binding site having a 100-fold lower affinity was suggested, but its nature was not exactly determined. The difference between these results and those that have shown linear Scatchard plots could stem from the type of IFN used since in these two cases Namalva IFN-α which is a mixture of several subtypes was used. Indeed, we and others have shown that different IFN-α subtypes exhibited different affinities for a common receptor in human cells and IFN-α_1 (also named IFN-αD) had a 50 to 100 times lower affinity for the receptor when compared to IFN-α_2.[79,96-99] Thus, when the binding studies were repeated (at 37°C) with IFN-α_2 instead of Namalva IFN, linear Scatchard plots were obtained.[78,79] However, the possible existence of a second binding site was suggested from studies that revealed a dependence of *Kd* on the time of incubation with ^{125}I-IFN-α. Two binding sites were postulated; an early, low affinity site having a *Kd* of 10^{-10} *M*, and a late, high affinity ($Kd = 2.65 \times 10^{-12}$ *M*) site. It was suggested therefore that IFN-α binds first to the low affinity site and then it is transferred within 3 hr at 37°C

Table 1
BINDING OF RADIOLABELED INTERFERONS TO RECEPTORS IN VARIOUS CELLS

Ligand[a]	Specific activity (units/mg)	Label[b]	Recovery of bioactivity %	Cell line	Type of cell	Temp. (°C)	Binding specificity (%)	*Kd*	Receptors per cell	Ref.
MuIFN-α,β	5×10^8 to 10^9	A	25 to 50	L1210	Mouse leukemia	37	40 to 50	1 to 2×10^{-11}		70
MuIFN-α,β	5×10^8	A	25 to 50	PCD3,PCC4	Mouse embryonal carcinoma	37 & 4	50 to 80	10^{-10}	20,000	71
MuIFN-α,β	5×10^8 to 10^9	A	25 to 30	L1210		4	40 to 50	10^{-10}	1,000	72
HuIFN-α (Namalva)	62.5% pure	A	≥75	Daudi, etc.	3 Hu. B. lymphoblastoid	4	60 to 70	2 to 5×10^{-10}		73
				Daudi		37		8×10^{-12}[c]		
				P3HRI		37		9×10^{-12}[c]		
						37		3×10^{-11}[c]		
					Peripheral leukocytes	4		10^{-10} to 2×10^{-9}[d]		
HuIFN-αA (*E. coli*)[e]	2×10^8	A	50 to 75	Daudi		37	~90	1.4×10^{-10}	5,000	74
HuIFN-α (Namalva)	2×10^8	B	100	MDBK	Bovine kidney cells	4	80	6×10^{-11}	650	75
HuIFN-α_2 (*E.coli*)	1.2×10^8	A	50	Daudi		37	N.R.	3×10^{-10}	2,000	76
						4	N.R.	4.5×10^{-10}	2,000	
HuIFN-α (Namalva)	7.5×10^8	C	100	FL	Human fibroblasts	21	N.R.	9×10^{-12}	1,300	77
				FS-7, etc.	Human fibroblasts	21	N.R.	25×10^{-11} to 3.3×10^{-11}	1,200 to 4,000	
				MDBK		21	N.R.	1.5×10^{-11}	1,000	
HuIFN-α_2 (*E.coli*)	1.5×10^8	A	100	Daudi		4	95	10^{-10}		78
						37	95	2.6×10^{-12}[f]		
HuIFN-α_1 (*E.coli*)	4.4×10^6	C	100	FL, Daudi.		21	~20	$> 6 \times 10^{-10}$	1,000	79

HuIFN-α_2 (*E.coli*)	3.2×10^8	C	100	FL, Daudi.		21	~85	~ 3×10^{-11}	1,000	
HuIFN-α_1				MDBK		21	80	4.2×10^{-11}	800	
HuIFN-α_2 (*E.coli*)				MDBK		21	~85	1.6×10^{-11}	800	
HuIFN-α_2 (*E.coli*)	2×10^8	B	100	MDBK		4	80	4×10^{-11}		80
HuIFN-α_2 (*E.coli*)		B	30	Daudi[g]		30	85	1×10^{-11}		81
HuIFN-α (Namalva)	2×10^8	A		Namalva[g]	Lymphoblastoid	26		$2\text{-}5 \times 10^{-8}$		82
HuIFN-α_2 (*E.coli*)	2×10^8	A	100	Daudi-S	(IFN sensitive)	0		1×10^{-10}, 1×10^{-8}	35,000	83
HuIFN-α_2 (*E.coli*)				Daudi-R	(IFN resistant)	0		4×10^{-9}	24,000	
HuIFN-αA (*E.coli*)	2×10^8	A		RSa	Human embryonic	25	90	8.7×10^{-10}	1,110	84
HuIFN-α_2 (*E.coli*)	2×10^8	A	50 to 75	T98G	Human neuroblastoma	4	78	6.2×10^{-10}	11,000	85
HuIFN-β[ser^{17}] (*E.coli*)	1 to 2×10^8	A	100	Daudi		0	50 to 75	2.4×10^{-8}	4,000	86
HuIFN-β[ser^{17}] (*E.coli*)	2×10^8	B	>95	Daudi		4	50 to 60	2.7×10^{-8}	10,000	87
HuIFN-γ (PBL)	4×10^6	B	100	GM-251	Human fibroblast chr.21 trisomy	37	65	1.5×10^{-10}	2,400	88
HuIFN-γ (PBL)	6×10^6	B		GM-258		4	75 to 80	2 to 6×10^{-9}	8,000 to 20,000	89
HuIFN-γ (PBL)	4×10^7	B	72	WISH	Human amnion	37	74	2.6×10^{-9}	19,500	90
				FS-11	Foreskin fibroblasts	37	72	1×10^{-9}	5,500	
MuIFN-γ (*E.coli*)	7.2×10^6	B	100		Mouse bone marrow macrophages	4	80	1.1×10^{-9}	12,000	91
HuIFN-γ (*E.coli*)	1×10^7	B	No loss	WISH		4	70	7.3×10^{-9}	50,000	92
						37	70	6.2×10^{-9}	100,000	
HuIFN-γ (*E.coli*)	1.2×10^7	B	10	Daudi		4	90	3.8×10^{-10}	13,000	93
				HeLa	Human cervical carcinoma	4	90	6.3×10^{-10}	5,000	

Table 1 (continued)
BINDING OF RADIOLABELED INTERFERONS TO RECEPTORS IN VARIOUS CELLS

Ligand[a]	Specific activity (units/mg)	Label[b]	Recovery of bioactivity %	Cell line	Type of cell	Temp. (°C)	Binding specificity (%)	*Kd*	Receptors per cell	Ref.
HuIFN-γ (*E.coli*)	2×10^7	B	N.R.		Human monocytes	4	70 to 95	1 to 2×10^{-10}	4,000	94
HuIFN-γ (CHO)	5×10^7	B	82		Human monocytes	4	80	6.8×10^{-9}	2,400	95

[a] Mu — Murine; Hu — Human. The source of IFN is given in parenthesis.
[b] (A) Labeling of tyrosine with I_2; (B) labeling of lysines (and α amines) with Bolton & Hunter reagent; (C) metabolic labeling.
[c] The high value from extrapolation of a curvilinear Scatchard plot.
[d] A range obtained from nine normal and leukemic donors.
[e] HuIFN-α_2 and HuIFN-αA are essentially identical.
[f] High affinity binding site which is apparent after 3 hr of incubation.
[g] Membranal fraction or solubilized receptor from these cells.

to the high affinity site.[78] A similar study by Hannigan[83] confirmed the existence of two binding sites in Daudi cells, stating that in earlier studies the low affinity site could not be identified due to a lack of IFN-α with high specific radioactivity. It should be stressed that a "low affinity" site of $Kd = 10^{-10}$ M is in fact a very high affinity site. Measurements of binding at 37°C are further complicated by the process of ligand-receptor internalization which leads to nonequilibrium conditions. This phenomenon affects both *Kd* values and the number of receptors and therefore measurements at 37°C should be avoided.

Littman et al.[93] observed curvilinear Scatchard plots with an upward concavity for the interaction of ^{125}I-IFN-γ with its receptor on Daudi and on HeLa cells. However, by using a certain mathematical model they found that the low affinity site was in fact a reflection of the nonspecific binding. Recent studies in our laboratory have shown that ^{125}I-IFN-γ, having a high recovery (82%) of bioactivity and high binding specificity gives a linear Scatchard plot when it is bound to WISH cells. However, the same ^{125}I-IFN-γ preparation gave a curvilinear plot with an upward concavity when bound to peripheral-blood monocytes.[95] The specificity of the interaction with the low affinity second binding site for IFN-γ in monocytes remains to be established.

2. Type I and Type II Interferon Receptors

The multiplicity of IFN subtypes indicated the possibility that IFN receptors display a similar heterogeneity. If each IFN subtype had a specific receptor which induces a specific function, then it would provide a rationale for the existence of multiple IFN subtypes. However, as we already know, the various IFN-α subtypes as well as IFN-β have very similar biological activities when tested in homologous cells and only IFN-γ exhibited some unique activities. Moreover, a structural homology was found between all IFN-α subtypes and IFN-β while only IFN-γ had a minimal homology to the others (see Section I.) Therefore, it was not surprising that Branca and Baglioni[74] reported that labeled HuIFN-α can be displaced from its receptor in Daudi cells by unlabeled IFN-α and -β, but not with IFN-γ. A similar observation was made in the mouse system.[100] The report from Anderson et al. claiming that ^{125}I-IFN-γ can be displaced with IFN-β but not with IFN-α was quite surprising since it implied that the receptor for IFN-γ can bind IFN-β (but not IFN-α). Our studies with two different human cell lines and with homogeneous IFN preparations have shown that neither IFN-α nor IFN-β inhibit the binding of ^{125}I-IFN-γ.[90] This observation was further confirmed in a number of studies with human cells using either ^{125}I-IFN-γ[92,93] or ^{125}I-IFN-β.[86,92] A similar observation was made in the murine system where neither IFN-α nor IFN-β inhibited the binding of ^{125}I-IFN-γ to mouse macrophages.[91] In contrast, a study with human Daudi cells showed that IFN-γ can compete with ^{125}I-IFN-β.[87] At this point one can conclude that in most human cells tested as well as in mouse cells, IFN-γ has a distinct receptor. In a previous nomenclature of IFN subtypes, virus-induced IFNs were referred to as Type I interferons, wheras mitogen-induced IFN was named Type II interferon. The current nomenclature has lost the distinction between these two groups. In view of most studies we proposed to assign this relevant nomenclature for the two distinct IFN receptors, namely, Type I interferon receptor for the one which binds IFN-α and IFN-β and Type II interferon receptor for the one specific for IFN-γ.[90]

3. Kinetics of Interferon Binding

Earlier studies on interferon binding have already differentiated between the binding step which was found to be very fast (10 to 20 min) even at 0°C and the development of antiviral state which required several hours.[63] Moreover, by using the extraction of IFN-β activity from human cells postbinding it was shown that maximal binding was already attained after 30 min both at 4 and 37°C.[64] Using mouse ^{125}I-IFN-α,β, Aguet[70] observed maximal binding to mouse L cells after 120 min both at 4 and 37°C. Similar results were obtained with ^{125}I-

IFN-α and Daudi, FL (human amniotic), and bovine MDBK cells.[74,75,77,81,87,101,102] Being a second-order reaction, the kinetics of IFN binding to the receptor linearly depends on IFN concentration. Therefore, saturation can be achieved faster at higher IFN concentrations.[103] A more rigorous analysis has shown that the model of second-order reaction is in fact too simplistic. Two stages were suggested: initial binding to a surface receptor which was a fast process, followed by a transfer of IFN to a second moiety on the cell membrane which was the rate-limiting step.[78] In addition, the kinetics of binding depended on the type of IFN. Thus, when IFN-α_1 was compared to IFN-α_2 it was found that the rate of association of IFN-α_2 to its receptor was about 60-fold higher than the equivalent values of IFN-α_1. This difference correlated with the difference in their specific activity.[99] Fast saturation (15 min) of IFN-α receptors was found in RSa cells[84] and in the human T cell line Molt-4.[104] The kinetics of IFN-β binding to Daudi cells was determined and was found to be similar to that of IFN-α.[87] Similar results were obtained with ^{125}I-IFN-γ and human fibroblasts.[88]

4. Critical Evaluation of the Binding Data

The actual values of *Kd* and the number of receptors per cell should be taken with caution, particularly when the recovery of biological activity after iodination is low or not reported. At least two modes of inactivation are theoretically possible; either all IFN molecules were partially inactivated or part of the molecules are completely inactivated. It is possible to calculate *Kd* only if one assumes the second possibility is correct and further assumes that fully inactivated IFN does not bind to the receptor. However, the last assumption is not always correct as was demonstrated in the case of IFN-γ and WISH cells, where acid-inactivated (95%) IFN-γ completely retained its affinity for the receptor.[95] If all the molecules are partially inactivated or, as should be expected, an intermediate situation exists, then the exact calculation is impossible. It should be mentioned that both labeling of tyrosines with free I_2 and labeling of lysines with a hydroxysuccinimide ester actually gives heterogeneous products with labeling at different sites and at different degrees of substitution. Therefore, a mixed population exhibiting a range of biological activity and affinity for the receptor is obtained. In this respect "metabolic labeling" with ^{3}H or ^{35}S amino acids is superior, however, the specific radioactivity is significantly lower than that obtained with [^{125}I].

The kinetics of IFN binding to its receptor was not determined accurately in most studies. In fact, the intention of the investigators was only to determine the time required for achieving maximal binding. Another limitation was the possible involvement of the internalizaiton process at 37°C. This could be avoided by working at lower temperatures, however, the kinetic values are temperature dependent and therefore measurements at low temperatures should be somehow extrapolated to the actual values at 37°C.

In spite of all these limitations, by correlating the compiled data on IFN receptors with analogous data on other hormones and their surface receptors, it is possible to conclude that IFN interacts with its receptor in much the same way as other hormones do.

C. Are Gangliosides Inolved in Interferon Action?

In 1974, Besancon and Ankel[105] reported that the antiviral activity of mouse IFN bound to Sepharose can be blocked by preincubation of this IFN with certain gangliosides. It was further shown that IFN-Sepharose probably interacts with the carbohydrate portion of the gangliosides in a lectin-like fashion.[106] In a similar study, it was reported that both human IFN-α and particularly -β are neutralized by preincubation with gangliosides. However, the "complex" IFN-α:gangliosides could be dissociated by dilution and the activity restored.[107] It seems therefore, that highly concentrated IFN as is the case in Sepharose-bound IFN was needed for demonstration of the binding to gangliosides and this is in fact a reflection of the rather low affinity of IFN to gangliosides. Additional experiments demonstrated the binding of IFN to Sepharose-bound gangliosides, however, this is even less convincing since

unlike the case of membranal gangliosides the hydrophobic ceramide portion of the ganglioside was free and therefore it could bind IFN in the same way as octyl Sepharose does. The inhibition of mouse IFN activity by thyrotropin on one hand and the inhibition of the binding of both thyrotropin and cholera toxin by IFN were interpreted as evidence for a common receptor, probably a ganglioside.[108] However, this study was made before pure IFN was available and the indirect evidence that was presented could be interpreted in other ways. Indeed, when pure mouse IFN-α,β became available it was shown that cholera toxin does not inhibit binding of IFN-α,β and vice versa.[100] A similar study with human IFNs have shown that preincubation of IFN-β and not -α with gangliosides abolished its activity.[109] Since both IFN-α and β share a common receptor, the possible interaction of gangliosides with IFN-β may bear little relevance to the IFN-receptor complex. The observed selective effect on IFN-β may stem from the fact that IFN-β is less stable than IFN-α and it is possible that gangliosides have selectively increased the rate of IFN-β denaturation during the step of preincubation.

At this point in time, it seems as if gangliosides are not involved in IFN action, however, preliminary studies in our laboratory indicate that this is not the final verdict. Treatment of human WISH cells with a monoclonal antibody made against thyrotropin receptor reduced the antiviral activity of human IFN-α_1 20-fold while the activity of IFN-α_2 was reduced only 3-fold. Preincubation of the target cells with gangliosides increased the activity of IFN-α_1 fourfold and that of IFN-α_2 only twofold. Since IFN-α_1 has a lower specific activity than IFN-α_2 it seems to be "aided" by binding to gangliosides which could serve as an initial interaction point on the cell surface. The significance of these preliminary observations remains to be studied.

D. Correlation of Binding to Biological Activities

For obvious reasons binding of IFN to cells is a prerequisite for exerting its activity on these cells. That data presented so far indicates that IFN acts via specific cell surface receptors. Moreover, there are several examples showing that IFN-resistant cells do not bind ^{125}I-IFN in a specific manner.[70,72,75,76,110] However, there are certain cells that bind IFN but do not display biological activity. Since binding to the receptor is only the initial step, a block at a later step can produce such a mutant cell. Moreover, since IFN has many biological activities, it is possible that some are blocked while others persist. This situation may stem either from mutations or from the natural course of cell differentiation. The mouse embryonal carcinoma cells PCC4 bind IFN-α,β but IFN does not exhibit antiviral or growth inhibitory activities in these cells.[71] Similarly, Daudi cells bind IFN-γ but they neither produce (2′ - 5′) oligo A synthetase nor do they enter into an antiviral state.[93,93a] In spite of all the reports, the possibility of interacting not via a receptor but, e.g., by nonspecific endocytosis cannot be excluded.

A correlation between the magnitude of the biological response and the degree of receptor saturation was studied in responsive cells. The three factors which determine the number of IFN-receptor complexes, namely, IFN concentration, receptor abundance, and the affinity constant were probably optimized during evolution but there is no reason to assume that maximal response correlated with maximal saturation. The actual number of receptors per cell is such that it gives a sufficient number of IFN-receptor complexes at physiological IFN concentrations. This number does not need to be very high since the antiviral activity of IFN is of a catalytic nature. Thus, the initial signal which may come from binding of a few IFN molecules to their receptor is in fact amplified in a cascade-like process which includes transcription, translation, and finally the enzymatic or hormone-like activity of the translation products. In this way the signal given by a few IFN molecules is transferred into a major change in cellular physiology. (In the [2′ - 5′] oligo A synthetase pathway an additional amplification stage is provided by the product [2′ - 5′] oligo adenylate which activates

another enzyme: RNase F.) Calculation of the receptor saturation level confirmed this view of IFN action. No saturation of mouse Type I receptors was observed at the concentration range of IFN that gives maximal biological activity.[70-72] In fact, binding of IFN to only 1% of the available surface receptors was sufficient to elicit maximal antiviral activity.

The antiviral state by its nature is a rapidly developed and transient situation and so are probably most of the other IFN-induced activities. Maximal response at a low receptor occupancy is a common feature among these activities. There are, however, other activities such as growth inhibition which require much higher IFN concentrations and hence a higher receptor occupancy. In some cases, doses that exceed maximal receptor occupancy manifold were needed.[73] In another case, Mogensen and Bandu[78] found a correlation between the occupancy of the high affinity Type I receptor on Daudi cells and the extent of growth inhibition. As was noted by these authors, as well as by Hannigan,[97] it is very risky to compare binding and response since these differ significantly in their kinetics. Thus, binding of IFN to its high affinity receptor reaches a steady-state within 3 hr, while most biological responses such as growth inhibition takes days to measure. Finally, it was demonstrated that unlike the case of antiviral activity, a continuous stimulation by free IFN is required for growth inhibition and therefore the biological response depends on the rate of association of IFN to its receptor rather than on the degree of receptor saturation.[99]

Type II IFN receptor is also present in excess numbers and low receptor occupancy was reported at IFN-γ concentrations that give maximal antiviral or HLA-inducing activities.[89,90,92,93]

III. POSTBINDING EVENTS

Studies with several polypeptide hormones such as insulin, EFG, transferrin, etc. produced a general description of a specific internalization process which is named receptor mediated endocytosis.[111,112] According to this description, the receptors are initially mobile and diffusely distributed in the membrane. After binding of the ligand, the receptor-hormone complexes cluster in specific membrane areas that are defined by the presence of a hexagonal lattice made mainly of clathrin on their cytoplasmic site. The clustered receptor-hormone complexes are now immobile and appear as indentations or "coated pits" on the cell surface. At a later stage the pits deepen, roundup, and finally are pinched off to form intracellular coated vesicles. This energy-dependent process leads to internalization of the hormone-receptor complex. In many cases several coated vesicles fuse to give a larger structure called endosome. The pH inside the endosome is low and this causes a dissociaiton of the receptor-ligand complex. In some cases, the free receptor accumulates in a newly formed tubular portion of the endosome and it finally separates from the endosome and returns to the cell surface. The endosome containing either the hormone alone or both the hormone and the receptor fuse with a lysosome and its content is degraded.

In the case of iron-transferrin the purpose of the process is to gather very effectively this essential element from the environment. However, in the case of polypeptide hormones this process bears little relevance to the mode of action of a specific hormone. Nevertheless, many questions can be asked and answered. Which of these steps (formation of coated pits, internalization, degradation, recovery of receptors) actually occurs in the case of IFN? More important, which of these steps is needed for exhibiting one or more of the biological activities? With luck the answers to these questions could shed some light on the mechanisms of IFN action.

A. Internalization and its Role in Interferon Action

Surface-bound polypeptide hormones and IFN can be removed by incubating the cells at a low pH or with trypsin, and no appreciable cell lysis is observed. This procedure permits

distinguishing between surface-bound and internalized IFN, while degradation of IFN can be tested by precipitation of intact IFN with trichloroacetic acid (TCA). Finally, labeling of pure IFN with heavy atoms (e.g., by binding to ferritin or colloidal gold) enables localization of IFN molecules within the cells by electron microscopy. Even before these procedures and pure IFN were available, it was shown that at least part of the cell-bound IFN could be recovered as determined by its activity.[64] Moreover, it was shown that bound activity is spontaneously released from cells upon incubation at 37°C in the absence of external IFN.[65] Several studies dealt with the question: "is internalization needed for activity?" Aguet and Blanchard[72] studied the binding of murine IFN-α,β to mouse cells and concluded from the identity of the binding levels at 4 and 37°C that no internalization took place at 37°C. Analysis of solubilized cells by SDS polyacrylamide (12.5%) gel electrophoresis (SDS-PAGE) did not reveal degradation products. These results should be taken with caution since the lack of internalization was concluded on the basis of indirect evdience and no release at low pH was made. Moreover, the conclusion that no degradation took place could not be actually reached by SDS-PAGE since this method is not suitable for detecting fragments smaller than 10,000 da.

A study with IFN-αA and Daudi cells revealed rapid internalization at 37°C. After 60 min of incubation, about 50% of cell-associated IFN could not be released by 0.2 N acetic acid.[113] This is indicative of internalization but by itself it cannot be used to distinguish between internalized and nonspecifically bound IFN. However, methylamine reduced significantly the amount of nondissociable IFN and this observation indicated that internalization rather than nonspecific binding was taking place. A temperature-dependent degradation and release of the degradation products was observed by measuring TCA soluble radioactivity. This process could also be inhibited by methylamine and chloroquine which are known as inhibitors of lysosomal proteases. Finally, no effect of chloroquine was observed on the level of (2′ - 5′) oligo A synthetase induction, thus indicating that IFN degradation was not essential for its activity.

Internalization and degradation of IFN-α were demonstrated in Daudi cells,[85,114,115] in bovine MDBK cells,[96,115] and in human fibroblasts.[102] The concomitant inhibition of both internalization and induction of (2′ - 5′) oligo A synthetase by diethyldithiocarbonate was interpreted as a proof that internalization was important for the antiviral activity.[102] However, such a reactive inhibitor is probably nonspecific and may inhibit many independent cellular processes due to its high reactivity with most proteins. Internalization of HuIFN-γ and the release of degradation products was found in human trisomic fibroblasts (GM-258).[83] The situation in WISH cells seems to be somewhat different, internalization was very slow and although degradation of IFN-γ was found, it was not related only to lysozomes since both chloroquine and methylamine failed to inhibit it completely. Even more, degradation was found in the presence of Na-azide, indicating that it took place outside of the cell.[115a]

The fate of receptor bound IFN can be studied by labeling IFN with electron-opaque tracers and visualizing the complex IFN-receptor by electron microscopy. Kushnaryov et al.[116,117] used partially purified (2 to 3% pure based on specific activity) human and mouse IFN, polyclonal anti-IFN sera, and ferritin-labeled second antibodies to visualize bound IFN on the cell surface and in coated pits. The validity of these observations would have been aided by use of pure IFNs. A more rigorous study with pure IFN-α_2 that was labeled with colloidal gold without loss of activity revealed that at 37°C IFN is localized in coated pits within 1 min and then it is internalized inside receptosomes within 8 min.[115] It should be noted that the establishment of an antiviral state in various cells requires a much longer time. In another study, murine IFN-γ was coupled to fluorescent microspheres and specific binding to the surface of mouse macrophages was demonstrated.[91] However, internalization and degradation could not be tested in this case.

In conclusion, most studies have shown so far that IFN is internalized and degraded in

much the same way as other polypeptide hormones. However, the various studies have confirmed the original observation with immobilized IFN,[66] namely, that internalization is not an essential part of IFNs mechanism of action. It should be mentioned, however, that only the antiviral activity was studied in detail and the other IFN-induced activities may still depend on receptor-mediated endocytosis.

B. Down Regulation of Inferferon Receptors

The phenomenon of down regulation is related to the biosynthesis and degradation of receptors. In the absence of ligands, the amount of available receptors on cell surface is determined by the rate of its synthesis and by the rate of its degradation which together determine its turnover. The receptor half-life can be determined experimentally by incubating cells with inhibitors of protein synthesis and in the case of IFN-α it was found to be about 3 to 5 hr.[77,118] When a ligand is added, the balance is disturbed since the process of receptor-mediated endocytosis, which is initiated by the ligand, causes a rapid drop in the level of surface receptors. In many cases, the biosynthetic machinery does not catch up and hence the level of the receptors on the surface stays low until the free ligand is removed. This phenomenon of down regulation was observed in the case of IFN-α and Daudi cells. A 50 to 60% drop in available receptors was obtained after incubation of the cells with 100 units per milliliter of IFN-α_2 for about 2 hr.[118] A somewhat lower value was obtained with IFN-γ in HeLa[93] and WISH cells. Incubation of these cells with 100 units per milliliter of IFN-γ for at least $1^1/_2$ hr caused a 30% drop in the specific binding but on longer incubations the binding capacity returned to a normal value.[115a]

Is down regulation a control mechanism in IFN action? In most cases IFN titers are transient but that is sufficient for triggering certain biochemical pathways. Its presence for longer times is required only if the viral infection persists. Moreover, the extent of down regulation of IFN receptors is rather limited and since low receptor occupancy is sufficient for maximal biological activity, it seems that down regulation has a limited physiological significance. The situation may be somewhat different when one considers the clinical usage of external IFN. In this case it is likely that administration at intervals, rather than continuous infusion may be somewhat more effective.

C. Induction of Biological Response: The Missing Link

If internalization of IFN is not essential for its activity, we still lack knowledge on how the signal is transferred from cell surface to the responsive genes in the nucleus. Internalization could account for part of the way but the process of degradation which occurs within the receptosomes makes this pathway very unlikely. There are even some studies which demonstrate that the introduction of IFN to cells by microinjection does not elicit antiviral activity.[110,119,120] It seems that a breakthrough in this topic will probably result from the biochemical characterization of the receptor molecule. In a recent study the presence of high affinity Type I murine receptors was observed on the nuclear membrane.[121] This very appealing observation implies that IFN is internalized and reaches the nuclear membrane in an intact form, but this assumption is in contrast to most of the published data on the fate of interalized IFN. It would be interesting to repeat this study with other types of IFN and particularly in the human system.

IV. PURIFICATION AND BIOCHEMICAL CHARACTERIZATION OF THE RECEPTORS

Assuming that internalization and degradation of IFN-receptor complexes probably occur after triggering of the steps that lead to the establishment of the antiviral state, then further studies should be concentrated on early activities of this complex. Isolation and character-

izaiton of the interferon receptors is essential for such a study. Evidence that IFN receptors are proteins comes from the observation that trypsin-treated cells are not responsive to IFN and that they lose the capacity to bind IFN.[65] Two possible approaches can be taken for further characterizing the receptor. Either the receptor is isolated and its structure determined, or the gene coding for the receptor is identified, isolated, and used for expression of receptor molecules in a host cell or bacterium. In fact, both approaches are complementary and will eventually lead to a complete sequence determination and to production of sufficient quantities of receptors. As was the case with other proteins, partial characterization is possible even before achieving purification to homogeneity.

A. Determination of the Molecular Weight

Determination of the molecular weight of Type I receptor in a variety of human cells was achieved by chemical cross linking of receptor-bound ^{125}I-IFN-α followed by analysis on SDS-polyacrylamide gel electrophoresis. Joshi et al.[76] reported that this procedure revealed a radiolabeled band of molecular weight 150,000. This band was present in IFN-sensitive cells and absent in IFN-resistant cells. Moreover, the addition of unlabeled IFN-α before cross linking prevented the formation of this radiolabeled complex.[76] Scatchard analysis of the binding data (see Section II) revealed in most cases a single binding site for IFN-α on the receptor. Therefore, the net molecular weight of this receptor is 130,000. Similar values were reported in other studies that used the same analytical system.[81,114,87,92] Treatment of this complex with reducing agents did not change its electrophoretic mobility and this indicated that Type I receptor was made of a single polypeptide chain. Treatment with neuraminidase increased the mobility somewhat, indicating that the receptor was in fact a glycoprotein.[76] A similar study with Daudi cells, but with ^{125}I-IFN-β, revealed two complexes of Mr 145,000 and 120,000. The authors of this study suggest, but bring no evidence, that these two bands are related to putative IFN-α,β and IFN-β,γ receptors.[76] Proof of this hypothesis could be obtained by selective competition with IFN-α and IFN-γ.

An alternative method to determine the molecular weight of an IFN-receptor complex is to solubilize it and analyze the complex by gel filtration chromatography. This procedure was used by Eid and Mogensen[122] who succeeded in solubilizing the ^{125}I-IFN-α_2-receptor complex from Daudi cells with digitonin.[99] Gel filtration chromatography of an extract obtained after 30 min of incubation revealed a complex of molecular weight 230,000. Extraction of the cells after 90 min gave an additional peak of apparent molecular weight 10^6. The authors suggest that this aggregate correlates with the high-affinity binding site which they have previously identified in these cells.[78] Faltynek et al.[81] applied an assay that measures binding of IFN-α_2 to solubilized receptors. This assay was based on selective precipitation of ^{125}I-IFN-receptor complexes in the presence of free ^{125}I-IFN with the aid of polyethylene glycol. Using it and a combination of several physical methods such as sedimentation in sucrose gradients and gel filtration, a molecular weight of 170,000 was determined for the complex of Type I receptor and Triton® X-100. By subtracting the weight of bound Triton® X-100, a molecular weight of 95,000 was estimated for this receptor. The reason for the discrepancy between this value and the one obtained from SDS-PAGE (120,000 to 130,000) remains to be established, however, both methods of molecular weight determinations are not very accurate. The band width of the IFN-receptor complex spans 30% of the distance between molecular weight markers 92,500 and 200,000 in SDS-PAGE, while the use of sedimentation in sucrose does not produce much better resolution.

The molecular weight of human Type II receptor was determined by SDS-PAGE of ^{125}I-IFN-γ cross-linked to its receptor on WISH and GM-258 cells. A molecular weight of 85,000 $\pm$ 5,000 was calculated for the receptor in WISH cells based on a band of Mr 105,000 $\pm$ 5,000, corresponding to the IFN-γ-receptor complex.[92] A molecular weight of 205,000 $\pm$ 7,000 was found in the case of GM-258 fibroblasts.[123] Assuming that both studies were

based on electrophoresis under reducing conditions, these results indicate that different human cells may have different Type II receptor. Alternatively, it is possible that the smaller complex was obtained by cleavage of a larger receptor molecule.

B. Purification of the Receptors

The low abundance of IFN receptors on the cell surface makes their isolation and purification a task that is even more difficult than the task of purifying natural IFN itself. If we take the values calculated from Scatchard analyses for receptor abundance (1,000 to 20,000 per cell) and assume that 1 kg of tissue contains approximately 10^{12} cells, then a purification of 5×10^5 to 5×10^6-fold would give 0.2 to 4 mg receptor at a very optimistic recovery of 100%. These staggering values are manifested by the fact that very little was achieved so far. Traub et al.[82] used the human lymphoblastoid Namalva cells as a source of IFN-receptor. In a three-step procedure which included membrane solubilization, chromatography on wheat-germ lectin Sepharose, and an interferon-α Sepharose, a 182-fold purificaiton and 28% recovery of binding activity were achieved. In another attempt, human foreskin fibroblasts were solubilized and the Type II receptor was purified by affinity chromatography on IFN-γ Sepharose. IFN-γ binding activity was demonstrated in the column eluate and cross linking in solution with ^{125}I-IFN-γ followed by SDS-PAGE revealed a specific band of Mr ~115,000. A purification factor of 2700 was obtained in one step and further attempts to purify this receptor are in progress.[123a]

C. Receptor Heterogeneity

The data presented so far indicates the presence of two receptor types, one for IFN-α,β and the other for IFN-γ. It is possible that each type of receptor is in fact a family of subtypes but the limited amount of available data on the structure of human IFN receptors makes this discussion a very speculative one. Discrepancies in data on receptor structure that are obtained from different laboratories can sometimes be dismissed as resulting from technical reasons. However, there are some data comparing receptors from different sources or indicating receptor heterogeneity in the same cell. Analysis of IFN-α-receptor complexes by gel filtration reveals several peaks of different molecular weights, however, these could be aggretates since such chromatography must be done under nondenaturing conditions.[81,99,122] Most studies based on SDS-PAGE of IFN-receptor complexes yielded single bands which indicate a single molecular weight class of polypeptides. This, however, does not exclude the possibility of several polypeptides having the same electrophoretic mobility but different sequences. In one case, two bands of molecualr weights 103,000 and 128,000 were obtained by cross linking of ^{125}I-IFN-β to its receptor on Daudi cells.[87] Since other laboratories obtained only the higher molecular weight band with ^{125}I-IFN-α, this was interpreted as proof for a specific IFN-β receptor. However, the obvious specific competition test with unlabeled IFN-β was not performed. Treatment of mouse cells with pronase prior to IFN addition, specifically blocked the antiviral activity of IFN-β but not that of IFN-α. It was therefore suggested that in these cells the receptors for IFN-α and β are different.[124] However, one may equally argue that a certain proteolytic damage to a single class of receptors may selectively abbrogate the binding of IFN-β and not that of IFN-α. In any case, this phenomenon was found only in one type of mouse cells in their logarithmic growth phase. In spite of this, the difference observed between resting and growing cells does point toward receptor heterogeneity.

Functional differences can also be used as indicators of receptor heterogeneity. We have compared Type II receptor in human WISH cells to that in peripheral-blood monocytes. ^{125}I-IFN-γ was specifically bound to both cells (Figure 1), however, different binding characteristics were obtained. Scathchard analysis gave, in the case of monocytes, an upward concave dependency curve, indicating either multiple binding sites or a negative cooperativity

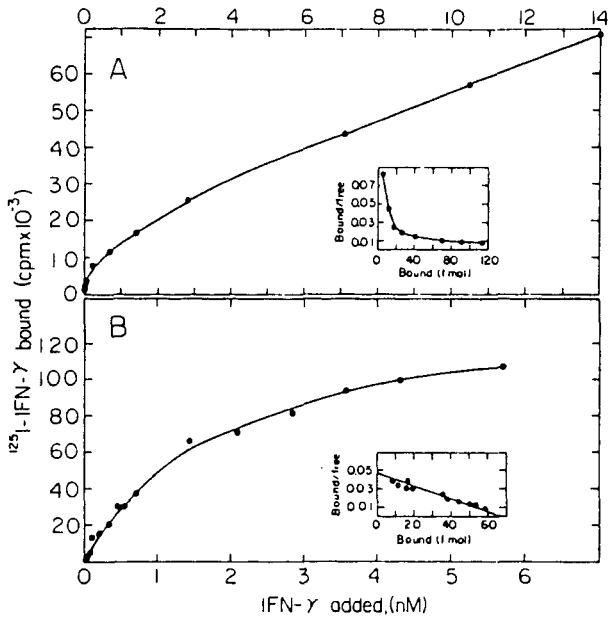

FIGURE 1. Binding of ^{125}I-IFN-γ to human peripheral blood monocytes (A) and to WISH cells (B). Cells were incubated with increasing concentrations (15 to 15,000 units per milliliter) of ^{125}I-IFN-γ at 4°C for $2^1/_2$ hr and cell-bound radioactivity was then measured. Insets: Scatchard plots of the binding data.

among the binding sites. In contrast, a linear Scatchard plot was obtained for the binding in WISH cells. However, it is possible that nonspecific binding to monocytes produced the concave Scatchard plot. Competition studies gave more striking differences. Acid treated-IFN-γ (95% inactivated) effectively competed with ^{125}I-IFN-γ for binding to the receptor on WISH cells but not on monocytes (Figure 2). The significance of these differences was evaluated by analyzing the various biological activities of IFN-γ in these two cell types. IFN-γ was found to induce an antiviral state in WISH cells but not in monocytes. Acid treated-IFN-γ was found to be almost as active as IFN-γ itself in inducing HLA-DR in WISH cells, but was almost completely inactive as an HLA-DR inducer in monocytes. It was proposed that these variations in biological activity stem from the presence of different Type II receptors in monocytes and in WISH cells. Moreover, we suggested that the immunoregulatory functions of IFN-γ in monocytes are related to the presence of a distinct IFN-γ receptor in these cells.[95]

D. Antibodies to the Receptors

Both purification of the receptor and preparations of antibodies against it are hampered by the low abundance of the receptors on the cell surface. The use of human-mouse somatic cell hybrids as immunogens enabled Revel et al.[125] to prepare an antiserum that blocked the human Type I receptor. A hybrid cell bearing human chromosome 21 was used as an immunogen and the antiserum obtained could block the activity of human IFN-β even if added after 3 hr of contact between IFN and the cells at 37°C. Incubation of similar antibodies with Daudi cells blocked the binding of radiolabeled IFN-α but not IFN-γ to cell surface receptors.[126] In this way the existence of different receptors for IFNs -α,β on one hand and IFN-γ on the other was proven again by an independent method. Monoclonal antibodies to this receptor have been prepared as well.[125a] Such antibodies will be useful in characterizing receptor heterogeneity and in studying the mode of action of IFN.

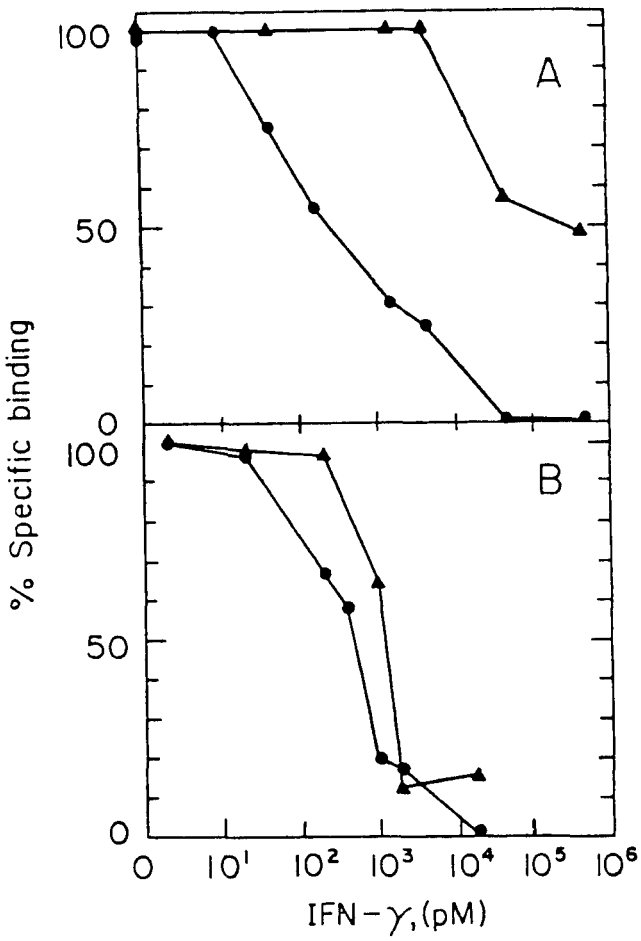

FIGURE 2. Binding site competition between ^{125}I-IFN-γ and unlabeled IFNs in human peripheral blood monocytes (A) and in WISH cells. (B) [^{125}I]-IFN-γ (300 units per milliliter, 300 pM in A, 100 units per milliliter in B) were added together with various concentrations (1 to 6 × 10^5 pM) of either unlabeled IFN-γ (●—●) or unlabeled acid-treated IFN-γ (▲—▲). Following incubation at 4°C for $2^1/_2$ hr, bound radioactivity was determined. Specific binding was 80% for monocytes and 70% for WISH cells.

V. GENETICS OF THE INTERFERON RECEPTORS

Using human mouse somatic cell hybrids it was possible to show that the responsiveness to human IFN-α and -β can be transferred to mouse cells by human chromosome 21.[127—129] The protein responsible for the transfer was proven to be on the cell surface since antibodies to whole cells blocked IFN activity.[125] Recently, it was proven that this is actually the IFN receptor since similar antibodies blocked IFN binding.[126] The receptor gene is on the distal segment of the long arm of chromosome 21, close to region q22 which is involved in Down's syndrome.[130] Further evidence to the chromosomal localization comes from the finding that cells having chromosome 21 trisomy were more sensitive than diploid cells both in the antiviral[125,130,131] and the antimitogenic effects.[132—134] The increased sensitivity of chromosome 21 trisomic cells can be used as a tool to distinguish between Type I and Type II IFN receptors. Indeed, it was found to be limited to IFN-α and -β.[135] Moreover, mouse

human hybrid cells containing chromosome 21 were responsive to IFNs α and β but not to IFN-γ. Finally, ^{125}I-IFN-α and not ^{125}I-IFN-γ could be bound specifically to these cells.[136] There is, however, one study which finds increased sensitivity of trisomic cells to IFN-γ as well.[137]

In conclusion, the majority of the studies clearly indicate that Type II IFN receptors are not coded by chromosome 21. The novel techniques of molecular biology enable identification of the genes coding for various IFN receptors, and once this is achieved a better understanding of the mechanism of IFN action is expected.

VI. LOCALIZATION OF RECEPTOR-BINDING EPITOPES ON INTERFERONS

The phenomenon of biological recognition is based on high affinity interaction between two biomolecules. The ability of proteins to fold into exact three-dimensional structures and to form "pockets" of a defined structure enables them to bind small ligands with high affinity. Such interactions exist, e.g., between enzymes and substrates. Another example is that of avidin and biotin (molecular weight 244) which bind together at a record *Kd* of 10^{-15} *M*. Therefore, it is not surprising that when two macromolecules interact, only small portions of the molecules are actually sufficient for a strong interaction. In the case of antigen-antibody binding, the antigenic epitope is usually made of no more than six to seven amino acid residues[138] and this space-limited interaction displays dissociation constants in the range of 10^{-7} to 10^{-10} *M*. A similar situation probably exists in the hormone-receptor system, namely, it should be possible to localize small and defined binding sites both on IFNs and on their receptors.

Several approaches can be taken in order to identify amino acids that are directly involved in the binding. It is possible to generate antibodies against synthetic peptides whose sequences represent parts or the IFN molecule and to study their effects in IFN binding and activity. By using modern methods of molecular biology, it is possible to replace or delete codons of one or more amino acids and in this way to study their role in binding. It is also possible to perform various chemical modifications which are specific for certain amino acids. Finally, one can generate fragments of IFN either by chemical or by enzymatic means and to study their receptor-binding ability. The last approach is quite limited since most binding epitopes are conformational, namely, the amino acids involved in the binding are not necessarily adjacent to each other and therefore small fragments rarely contain all the amino acids of a given binding epitope. Consequently, only large fragments which contained most of the IFN molecule were studied so far.

Levy et al.[139] observed that the mixture of natural human IFN-α subtypes obtained by virus induction of leukocytes contains several subtypes which are shorter at the C-terminus by ten amino acids in comparison to the sequence expected from the gene.[139] These subtypes were fully active, thus indicating that the C-terminal ten amino acids are not involved in either binding or activity. Indeed, a monoclonal antibody prepared against a synthetic peptide having a sequence of the 16 C-terminal amino acid residues of IFN-α neither inhibits the receptor binding nor the activity of IFN-αA.[80] Streuli et al.[140] studied the activity of IFN-α_1, α_2 and their hybrids in various cells. They found that molecules containing the N-terminal half of IFN-α_1 are active in bovine cells but are poorly active in human cells, while molecules containing the N-terminal half of IFN-α_2 were highly active in both human and bovine cells. In cotnrast, molecules having the C-terminal half of IFN-α_1 were more active on mouse cells than those having the C-terminal -α_2. Therefore, they proposed that IFN-α has two binding epitopes, one in the N-terminal half and the other in the C-terminal half.[140] In another study limited proteolysis of IFN-α_2 with thermolysin produced two major complementary fragments 1 to 110 and 111 to 153. After cleavage of the disulfide bonds with 2-mercaptoethanol and separation, the antiviral and growth inhibitory activities persisted in the larger,

Mr 12,000 fragment consisting of the N-terminal 110 amino acids.[141] The activity of the fragment was about 3% of the original and this drop can be interpreted with the aid of the theory on the existence of two binding sites, namely, that one or more of the amino acids that are part of the binding epitope were localized in the second fragment. Further support for this theory comes from another study in which two fragments obtained by CNBr cleavage of reduced and *S*-carboxymethylated IFN-αC were found to compete with IFN-αC for binding to the receptor. Although these fragments exhibited no antiviral activity, they could inhibit the activity of IFN, thus acting as antagonists. These fragments were identified by sequence analysis as IFN-αC (1 to 59) and IFN-αC (112 to 148) and their apparent affinity was 10 to 15 times lower than that of IFN-αC. These observations indicate that the binding epitope is actually made of amino acids residues that are present in two distinct regions.[140a]

Studies at the binding site of IFN-γ were performed as well. It was reported that antibodies made against the N-terminal synthetic fragment of IFN-γ inhibited its activity.[142] In another study, several monoclonal antibodies directed against epitopes at the N-terminus and C-terminus of murine IFN-γ were prepared. All these antibodies inhibited the antiviral activity of IFN-γ to a similar extent but only those directed against the C-terminal epitope inhibited macrophage activation, Ia induction and binding of ^{125}I-IFN-γ to mouse macrophages.[143] Therefore, the authors concluded that the binding epitope is present at the C-terminal portion of IFN-γ. A study with the human IFN-γ system gave opposite results. Limited proteolysis of the C-terminus with carboxypeptidases had only a marginal effect on the affinity for the receptor, while cleavage of the first two N-terminal amino acids, with dilute acid at 100°C (Asp-Pro cleavage), completely abolished the binding of the receptor on human WISH cells.[143a] Thus, it appears that the N-terminus and not the C-terminus of IFN-γ is involved in binding to the receptor. The difference between the binding sites in WISH cells and in monocytes is one of the indications for the presence of different Type II receptors in these cells (see Section IV.C). It is not due to differences between the human and the murine systems since denatured IFN-γ could not compete for the binding of ^{125}I-IFN-γ to its receptor in human monocytes.

VII. CORRELATIONS WITH OTHER CELL SURFACE COMPONENTS

The interferon receptors are minor constituents of the cell membrane, however, they may profoundly affect other membrane components. In princple, IFN can induce or inhibit the production of other surface components and it can modulate the binding of other ligands to their receptors on the cell surface. The best known example is that of HLA surface antigens. Class I HLA antigens (HLA-A,B,C in human cells) are present in all human cells, while Class II antigens (HLA-DR) are present in certain immunocytes and in nonhemopoietic cells involved in autoimmune diseases. Interferons, and particularly IFN-γ are the only known inducers of these surface antigens.[51—53] They elevate HLA levels and in some cases HLA-DR is absent on cell surface unless this cell was exposed to IFN. In many cases a low concentration of 1 unit per milliliter or even less is sufficient for a significant response. In this respect, the induction of HLA-DR can be used as an IFN assay that is more sensitive than the antiviral assay. So far, HLA is the only known example of IFN-induced surface component. The induction is at the genomic level since a parallel (and sometimes even larger) increase in HLA mRNA is observed.[53]

A decrease in surface components is seen as well. Incubation of lymphoblastoid cells such as Daudi and Raji with IFN-α_2 for 3 days caused a 50% reduction in their ability to bind insulin. This effect was quite specific since binding of transferrin was not inhibited and serum starvation did not mimic the effect of IFN-α_2. Analysis of insulin binding revealed that the number of insulin receptors was decreased by 50% while their affinity for insulin did not change.[144] Since insulin receptors undergo a turnover of synthesis and degradation,

IFN could affect either of these processes. It is likely, however, that the synthesis was inhibited.

A similar study with bovine MDBK cells revealed that IFN-α_2 reduced the number of EGF receptors. Treatment of cells with Hu IFN-α_2 for 20 hr reduced the number of receptors from 11,800 to 7100. No change was observed in the rates of degradation or internalization hence an effect on protein synthesis is involved.[145]

VIII. EPILOGUE

There are still many open questions regarding the IFN receptors and some of them will probably be answered while this review is being printed.

The major unanswered question is what is the link between binding of IFN to its receptor and inducing (or blocking) the genes involved in IFN action? If it likely that at least part of the answer will be found once the receptor molecule is isolated and characterized. Because of the observed immunoregulatory activities of IFN-γ it is possible that interesting findings will be obtained by studying the regulation and functions of the Type II IFN receptor in immunocytes. These problems and others related to IFN receptors will probably be intensively studied in the near future.

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