Contributions of cloned type I interferon receptor subunits to differential ligand binding

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Abstract The human type I interferons, including at least 12 IFN-αs, IFN-β and IFN-ω, bind to a receptor (IFNAR) composed of at least two transmembrane subunits, IFNAR-1 and IFNAR-2. The contributions of the receptor subunits to ligand binding were investigated by measuring the binding properties of IFNAR-1 or IFNAR-2 alone, or when co-expressed. The affinity of IFNAR-2 for IFN-α2 was increased by the co-expression of IFNAR-1, which itself binds ligand very weakly. Most type I IFNs inhibited the binding of IFN-α2 to IFNAR-2 alone with IC_{50} values of 2–20 nM. For cells co-expressing IFNAR-1 and IFNAR-2, the IC_{50} values decreased 3–20-fold for various ligands, relative to their values on IFNAR-2 alone. Thus, while IFNAR-2 plays the major role in affinity determination and differential recognition of type I IFNs, IFNAR-1 modulates both the ligand affinity and selectivity of the IFNAR-1/IFNAR-2 receptor complex.

Key words: Interferon receptor; Interferon; Cytokine

1. Introduction

Type I interferons (IFN-αs, IFN-β, IFN-ω)[2] bind a common receptor, the type I interferon receptor, denoted IFNAR [1–3]. The type I IFN receptor has perhaps the largest number of natural ligands of all cytokine receptors, and the differential binding and functional activation of cells by type I IFNs is of considerable interest.

IFNAR is composed of at least two subunits, IFNAR-1 and IFNAR-2, which are members of the class II cytokine receptor family [4]. Both subunits participate directly in ligand binding and intracellular signaling. Human IFNAR-1 (HuIFNAR-1) [5] has weak intrinsic affinity for at least two IFNs, IFN-α2 and -α8 [6,7]. Its expression confers on rodent cells limited responsiveness and binding of human type I IFNs [5], and enhances the ability of monkey-derived COS cells to bind human IFNs [8]. In contrast to HuIFNAR-1, the bovine homologue (BoIFNAR-1) fortuitously binds human IFN-αs with high affinity [6,9,10]. The ability of antibodies against HuIFNAR-1 to block the binding of various type I interferons to human cells [11,12] suggests that IFNAR-1 is involved in a complex that binds all type I interferons. The importance of IFNAR-1 was demonstrated most dramatically in *ifnar-1*$^{0/0}$ ('knockout') mice, which do not respond to type I IFNs [13–15]. Its role in signaling derives, at least in part, from its binding of the cytoplasmic tyrosine kinase Tyk2 [16,17], and possibly other factors (e.g. [18]).

HuIFNAR-2 [19] has three molecular forms generated by alternative splicing: a soluble secreted protein (IFNAR-2a), and two transmembrane forms, IFNAR-2b and -2c ('short form' and 'long form', respectively), which have identical extracellular and transmembrane domains, but differ in their cytoplasmic domains [19–22]. IFNAR-2 binds IFNs, including αB, αC and β, with the K_d for IFN-α2 being in the nM range [20–22]. In the presence of IFNAR-1, only the IFNAR-2c form appears to confer signaling and biological function [19–22], presumably from its ability to recruit the Jak1 tyrosine kinase, and the latent transcription factors Stat1 and Stat2 (reviewed in [23]).

The relative contributions of IFNAR-1 and IFNAR-2 to the IFN binding characteristics of the cellular IFNAR complex are not known. For murine cells co-expressing HuIFNAR-1 and HuIFNAR-2, the affinity of HuIFN-α2 is about 5–10-fold higher than for murine cells transfected only with HuIFNAR-2 [20,22], but the binding of other type I IFNs has not been systematically investigated. We have expressed HuIFNAR-1 and HuIFNAR-2, both individually and together, at high levels in COS cells, and have examined the interactions of these cloned receptors and the native receptor complex on human Daudi cells with a wide variety of human type I IFNs. We have thereby demonstrated variations in the relative contributions of the two IFNAR subunits for different type I IFNs: while IFNAR-2 has higher affinity and some selectivity for ligands, IFNAR-1 modulates both the affinity and the differential selectivity of the receptor complex.

2. Materials and methods

2.1. Reagents and antibodies[2]

The HuIFNAR-1 cDNA, HuIFNAR-2b cDNA and HuIFNAR-2c/pcDEF3 construct were provided by Dr. Sidney Pestka (this Department), as were the interferons: IFN-α2a (= IFN-αA; 1.56×10^8 IU/mg), IFN-α8b (= IFN-αB2; 4.95×10^8 IU/mg), and IFN-α1b (= IFN-

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Dedicated to the memory of Dr. Yakov Gluzman and his many contributions, including the development of COS cells.

[2]We adopt the nomenclature for type I receptors suggested by the Nomenclature Committee of the International Society for Interferon and Cytokine Research. IFNAR-1 corresponds to previous designations of: IFNAR, IFN-αR1, IFN receptor α subunit; IFNAR-2 corresponds to previous designations of IFN-α/β receptor, IFN-αR2, IFN receptor β subunit. The two transmembrane variants of IFNAR-2, denoted IFNAR-2b and IFNAR-2c, have previously been called either IFNAR 2-1 and IFNAR 2-2 [21], or IFN-α/β receptor short form (β_S) and long form (β_L), respectively [20]. For interferons used in our study, we follow the recommended international nomenclature (G. Allen and M.O. Diaz (1996) J. Interferon Cytokine Res. 16, 181–184): IFN-αA = IFN-α2a; IFN-αB2 = IFN-α8b; IFN-αD = IFN-α1b. For readers' ease, the nomenclature is simplified to IFN-α2, IFN-α8 and IFN-α1, respectively. For published citations, we maintain the nomenclature used by those authors.

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αD; 7.63×10^7 IU/mg). The synthetic consensus IFN protein, methionyl- IFN-αCon1 (1×10^9 IU/mg), was from Dr. Milton Taylor (Indiana University), originally from Dr. Lawrence Blatt (Amgen Corp.). IFN-ω (3×10^8 IU/mg) was from Dr. G.R. Adolf (Bender Wien, Vienna, Austria). CHO-derived IFN-β and the EA12 anti-IFNAR-1 monoclonal antibody were from Dr. Leona Ling (Biogen Corp., Cambridge, MA). The anti-HuIFNAR-1 4B1 monoclonal antibody was provided by Dr. Ed Croze (Berlex, Richmond, CA). The HuIFNAR-2 antibody IFNαRβ1 [24] was from Dr. Oscar R. Colamonici (University of Tennessee, Memphis, TN). The recombinant fusion protein ('immunoadhesin') of the extracellular domain of human IFNAR-2 with the human constant heavy immunoglobulin domain, produced in CHO cells and referred to as 'HuIFNAR-2/Ig', was supplied by Dr. Laura Runkel (Biogen, Inc.).

The HuIFN-αA analogue IFN-αA-P1 [25] was phosphorylated to high radiospecific activity with [γ-^{32}P]ATP and bovine heart cAMP-dependent protein kinase. For consistency with international nomenclature, [^{32}P]IFN-αA-P1 is referred to as [^{32}P]IFN-α2.

2.2. Plasmid construction and DNA transfections

IFNAR-1, IFNAR-2b, and IFNAR-2c were transiently expressed from the EF-1α promoter in the vectors pEF-BOS-CS [26] or pcDEF3 [26], derived from pEF-BOS [27] or pcDNA3 (InVitrogen, Inc.), respectively. COS-1 cells [28], derived from a simian kidney line, were transfected with plasmids by the DEAE-Dextran/DMSO shock protocol [10,29,30], and assayed after growth for 2–3 days.

2.3. Saturation binding and competition curves

Saturation binding were performed as described [10]. Briefly, COS cells were trypsinized and resuspended at $1–5\times10^6$ cells/ml. Aliquots of cells with and without excess cold IFN (1–3 μg/ml) were combined with [^{32}P]IFN-α2, serially diluted, and incubated for 1 h at room temperature. Cell-bound [^{32}P]IFN-α2 was separated from unbound by brief centrifugation, and bound and free radioactivity were measured. For competition binding assays, cells were combined with non-radioactive IFNs (generally 4-fold serial dilutions) in addition to [^{32}P]IFN-α2. Binding was generally measured in triplicate or quadruplicate. Occasional points more than 2 standard deviations from the mean were omitted from the analysis. Suppliers' determinations of IFN concentration (in mg/ml) were used for all calculations.

Data were analyzed by non-linear regression to one- or two-site binding or competition models, using the program Prism v.2.01 (GraphPad Software, Inc., San Diego, CA). Data sets were analyzed in two ways: (1) without any constraints, and (2) with the constraints that all curves in a single experiment have the same total binding in the absence of competitive ligand, and the same non-specific binding in the presence of saturating amounts of any competitor. Reported IC_{50} values and Figures are for constrained fits, unless the goodness of fit (R^2) was substantially better in the absence of constraints (e.g. Fig. 3B).

2.4. Solid-phase radioligand assay (RLA)

For binding of IFNs to the IFNAR-2/Ig immunoadhesin, the protocol was modified from an ELISA protocol of Mohammad R. Zafari (Biogen, Inc.). Immulon 2 plates (Dynatech Laboratories) were coated with 50 μl of 5 μg/ml of AffiniPure goat anti-human IgG Fcγ fragment (Jackson ImmunoResearch). After 1–2 h, the wells were blocked with BSA (5 mg/ml) and 1% normal goat serum (Jackson ImmunoResearch) in PBS. Wells were washed once with PBS with 0.05% Tween 20 and twice with PBS. The recombinant HuIFNAR-2/Ig fusion protein was added (50 μl of 1.25 μg/ml PBS), incubated for 1 h at room temperature, and washed. For competition binding assays, 50 μl PBS was added to each well, followed by approximately 1×10^5 cpm of [^{32}P]IFN-α2 and 50 μl of specific dilutions of different cold IFNs. After 1 h, plates were washed as described to remove unbound [^{32}P]IFN-α2. Bound [^{32}P]IFN-α2 was eluted with 1% SDS at 55°C for 10 min, and radioactivity was measured.

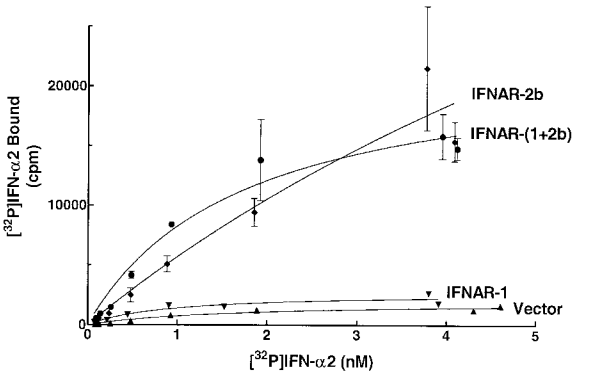

Fig. 1. Binding of [^{32}P]IFN-α2 to transfected COS cells. Binding assays were performed as described on COS cells transfected with: HuIFNAR-1/pEF-BOS-CS (▼); HuIFNAR-2b/pEF-BOS-CS (♦); HuIFNAR-1/pEF-BOS-CS and HuIFNAR-2b/pEF-BOS-CS (●); vector pEF-BOS-CS (lacking any insert) (▲).

2.5. Flow cytometric analysis of receptor surface expression

Transfected cells were resuspended in 5% DMEM with 0.1 mg/ml human IgG. Cells (25 μl) were incubated for 1 h at 4°C with 0.12–0.25 μg (25 μl) of primary antibody (anti-IFNAR-1 antibodies 4B1 or EA12, or anti-IFNAR-2 antibody IFNαRβ1 [24]), medium, or unrelated isotype-matched IgG. The cells were washed with PBS and resuspended in 0.125 μg (50 μl) of secondary antibody (R-Phycoerythrin-conjugated F(ab')2 Goat Anti-Mouse IgG; Jackson ImmunoResearch) and incubated for 1 h. The cells were washed and resuspended in 500 μl PBS for analysis with a Coulter Epics Profile II cell sorter.

3. Results

3.1. Transient expression of IFNAR subunits in COS cells and binding of [^{32}P]IFN-α2

The expression of IFNAR subunits on COS cells was analyzed by ligand binding, and by detection of surface receptors by flow cytometry. The transfection of COS cells with IFNAR-2b alone leads to very large increases in binding of [^{32}P]IFN-α2 when compared to COS cells transfected with vector alone, amounting to an average 16–25-fold increase in the calculated number of binding sites (Fig. 1; Table 1). These IFNAR-2 sites have an average K_d of about 7.9 nM, which is a somewhat lower affinity than the native COS receptor which is present at much lower abundance. The transfection of IFNAR-1 alone into COS cells leads to a slight

Table 1
Binding of [^{32}P]IFN-α2 to COS cells with human IFNAR components

Transfected receptor	K_d (nM)		Receptors/cell ($\times10^4$)		N
	Average	Range	Average	Range	
None	1.7	–	1.3	–	1
IFNAR-1	1.8	1.0–2.7	1.9	1.8–2.1	2
IFNAR-2b	7.9	2.7–11.7	56.0	23–89	5
IFNAR-(1+2b)	3.1	1.8–4.3	33.0	22–49	4
Daudi cells	0.27	–	1.1	–	1

N is the number of independent experiments.

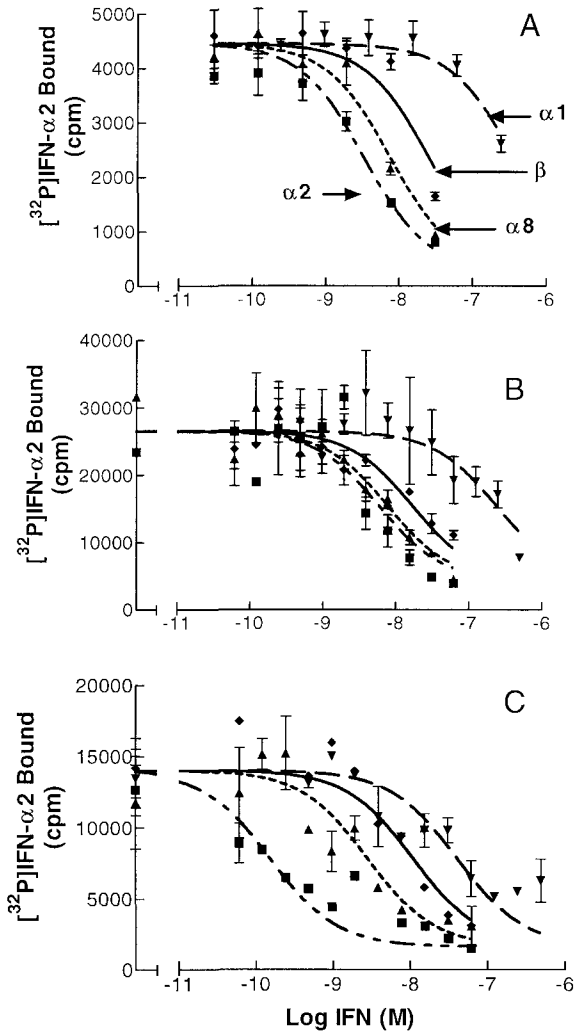

Fig. 2. Competition binding of type I interferons against [^{32}P]IFN-α2. The non-radioactive interferons were: IFN-α2 (■); IFN-α8 (▲); IFN-α1 (▼) and IFN-β (♦). Samples were: (A) soluble IFNAR-2/Ig fusion protein in a solid phase radioligand assay format; (B) COS cells transfected with IFNAR-2b; (C) COS cells cotransfected with IFNAR-1 and IFNAR-2b. All curves were constrained to a common value for binding in the absence of competitor IFN and a common value for the non-specific binding (see Section 2 for discussion).

increase over native COS cells in the number of sites, as reported previously [8]; the increase is so small, however, that it is not known whether this binding is to the transfected IFNAR-1 alone, or to a complex of ectopic IFNAR-1 with endogenous COS receptor subunits. Compared to COS cells or COS cells transfected with IFNAR-1 alone, cells cotransfected with IFNAR-1 and IFNAR-2b have greatly increased binding of [^{32}P]IFN-α2, but characterized by a reproducibly 2–3-fold higher affinity (3.1 nM) than that of COS cells transfected only with IFNAR-2b. Similar binding data were obtained with IFNAR-2c (data not shown), which differs from IFNAR-2b only in its cytoplasmic domain.

The cell surface expression of IFNAR-1 and IFNAR-2 was verified by flow cytometry (data not shown; see Section 2). This was particularly important for IFNAR-1, since its intrinsic binding of IFNs is poor. In these experiments, the surface expression of IFNAR-1 and IFNAR-2 are comparable: generally, 15–25% of transfected COS cells have fluorescence 10–100 times higher than negative controls. Negative controls included untransfected cells, mock-transfected cells, transfected cells reacted only with secondary antibody, and IFNAR-transfected cells reacted with an isotype-matched, unrelated primary antibody, but with the secondary antibody.

The large enhancement in ligand binding by cells expressing IFNAR-2, either alone or with IFNAR-1, compared to COS cells expressing only IFNAR-1, permits the confident use of this transient transfection system in the current studies.

3.2. Binding of type I IFNs to IFNAR-2

The ligand binding properties of IFNAR-2 expressed in COS cells were examined and compared to the properties of a soluble construct of IFNAR-2 (IFNAR-2/Ig) measured in a plate format, radioligand assay (RLA) (cf. Figs. 2A and 3A

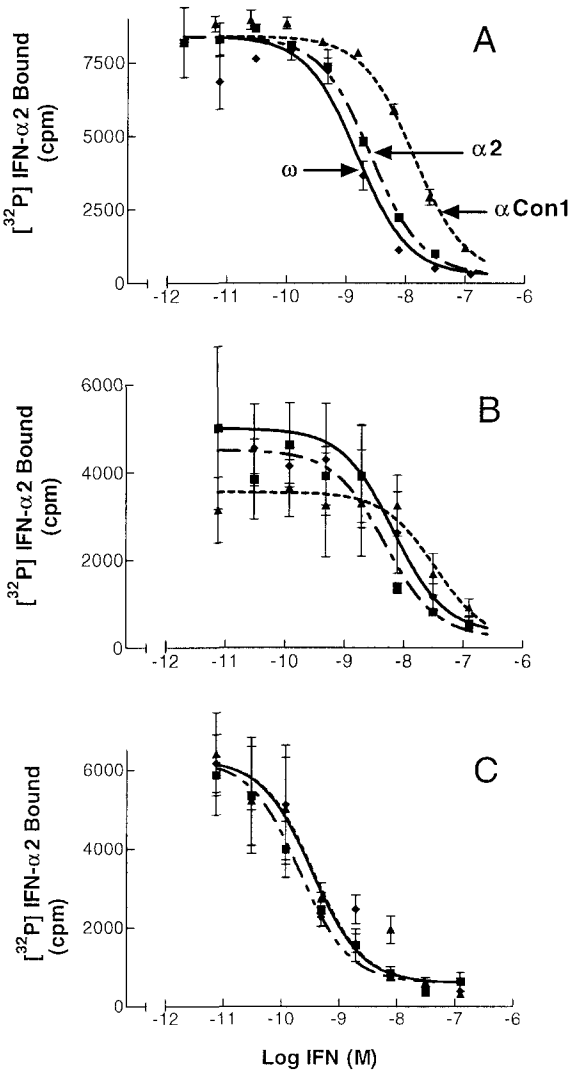

Fig. 3. Competition binding of type I interferons against [^{32}P]IFN-α2. The non-radioactive interferons were: IFN-a2 (■); IFN-aCon1 (▲); and IFN-ω (♦). Samples were: (A) soluble IFNAR-2/Ig fusion protein in a solid phase radioligand assay format; (B) COS cells transfected with IFNAR-2c; (C) COS cells cotransfected with IFNAR-1 and IFNAR-2c. Curves A and C were constrained to a common value for binding in the absence of competitor IFN and a common value for the non-specific binding; curve B was not constrained (see Section 2 for discussion).

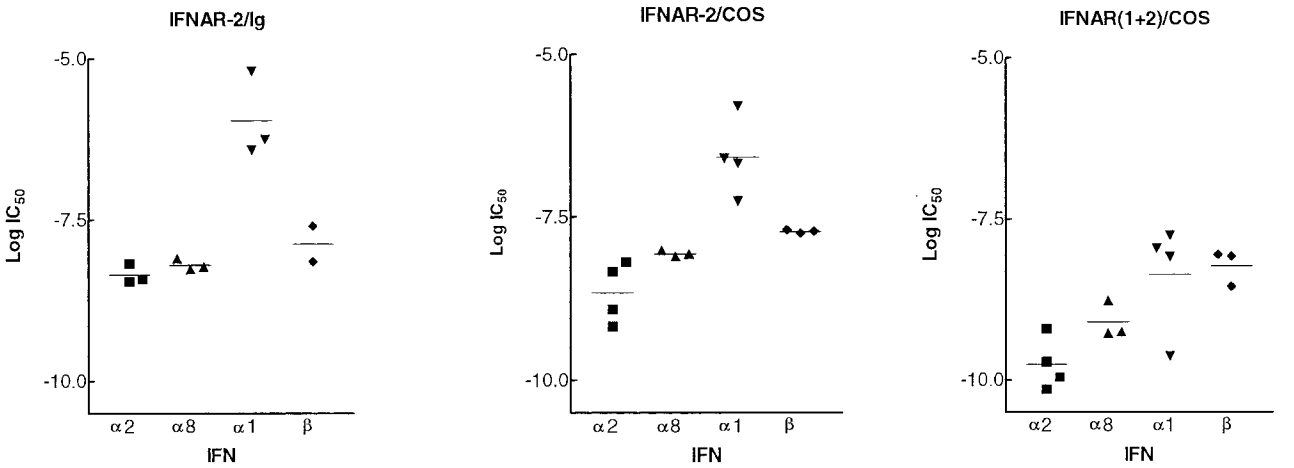

Fig. 4. Scattergram showing the IC_{50} values for independent experiments. Each IC_{50} value is derived from an independent competition curve like those in Figs. 2 and 3.

with Figs. 2B and 3B; Table 2). The selectivity of type I ligands was examined by their inhibition of [^{32}P]IFN-α2 binding to COS cells expressing IFNAR-2b or -2c (Table 2; Figs. 2B and 3B). The mean calculated IC_{50} values for independent experiments were reproducible (Fig. 4), and produced significant values for the IC_{50} for each ligand (Table 2). For IFN-α2 the mean IC_{50} is within experimental error (2-fold) in both sets of experiments shown in Table 2. This demonstrates that IFNAR-2b and -2c have experimentally indistinguishable binding affinity for IFN-α2. The two ligand sets can be merged to give a rank order of inhibition: IFN-ω ≈ IFN-α2 ≥ IFN-αCon1 ≈ IFN-α8 ≥ IFN-β ≫ IFN-α1. The placement of IFN-αCon1 is somewhat tentative because of its experimental variability.

Similar competition experiments of type I IFNs were performed with soluble IFNAR-2/Ig protein in a RLA (Table 2; Figs. 2A and 3A). Comparing the ligand specificities of IFNAR-2 expressed on COS cells or as a soluble protein, there is a good correspondence of the IC_{50} values and rank order for the diverse ligands tested, although there is a small (2-fold) discrepancy in the IC_{50} values for IFN-αCon1. The 4-fold discrepancy in the IC_{50} values for IFN-α1 in the two binding formats may reflect its low affinity and consequent fast dissociation rate; a fast dissociation rate increases the importance of some experimental variables, such as the slower washing times in the RLA format. Nevertheless, the measured binding of all ligands by IFNAR-2 expressed on COS cells appears to represent well the intrinsic binding properties of the IFNAR-2 extracellular domain as measured with the IFNAR-2/Ig soluble construct.

3.3. Binding of IFNs to the IFNAR-1/IFNAR-2 complex

The high-level co-expression of IFNAR-1 and IFNAR-2 on COS cells was used to investigate the ligand binding properties of the IFNAR-1/IFNAR-2 complex. In the competition experiments with diverse type I IFNs, two effects of co-expression of IFNAR-1 and IFNAR-2 are seen (e.g. Fig. 2C, Figs. 3C and 4; and Table 3, columns 1–3). First, the IC_{50} of all ligands decreases (Table 3, column 1), representing an increase in ligand-binding affinity over that measured for cells transfected with IFNAR-2 alone (column 2). Second, comparing the IC_{50} of each ligand with co-expressed IFNAR-1 and IFNAR-2, vs. IFNAR-2 alone, there are significant differences in the degree to which the IC_{50} values decreases (Table 3, column 3). The IC_{50} for the low affinity IFN-α1 increases most in the presence of IFNAR-1, followed by IFN-α2, IFN-α8, and IFN-αCon1. However, the effect of IFNAR-1 on the binding of IFN-ω and IFN-β is the smallest, only 3–7-fold. (The equivalence within experimental error of IFNAR-2b and IFNAR-2c was demonstrated in less extensive experi-

Table 2
Inhibition (IC_{50}, molar) by type I interferons of [^{32}P]IFN-α2 binding to soluble IFNAR-2/Ig, or COS cells transfected with IFNAR-2

	IFNAR-2/Ig		IFNAR-2/COS		Ratio (soluble/COS)
Ligand set 1:					
IFN-α2	4.4×10^{-9}	(3.2–6.3)	2.2×10^{-9}	(0.7–6.4)	2.0
IFN-α8	6.3×10^{-9}	(5.9–7.8)	8.6×10^{-9}	(7.7–9.5)	0.7
IFN-α1	1.1×10^{-6}	(0.2–5.5)	2.6×10^{-7}	(0.6–10)	4.2
IFN-β	1.3×10^{-8}	(0.5–3.3)	1.9×10^{-8}	(1.8–2.0)	0.7
Ligand set 2:					
IFN-α2	2.2×10^{-9}	(1.9–2.6)	3.5×10^{-9}	(1.4–8.7)	0.6
IFN-αCon1	1.2×10^{-8}	(1.0–1.5)	8.9×10^{-9}	(2.1–38)	1.3
IFN-ω	1.4×10^{-9}	(2.1–5.2)	2.6×10^{-9}	(0.9–7.6)	0.5

Values are the mean of several independent determinations of the IC_{50} (see Fig. 4 for individual IC_{50} values). Numbers in parentheses are the range of IC_{50} values at ± one standard deviation from the mean. Means were calculated from 3–4 independent measurements for the ligands in set 1, with the exception of IFN-β on IFNAR-2/Ig, which is the mean of two determinations. In ligand set 2, means for the transfected COS cells are from three determinations, whereas means for soluble IFNAR-2/Ig were calculated from two determinations. COS transfections for ligand set 1 utilized IFNAR-2b, whereas ligand set 2 transfections used the IFNAR-2c construct.

ments for all ligands; data not shown.) Thus, there is a clear increase in ligand affinity by co-expressing IFNAR-1 with IFNAR-2, but the effect varies significantly for different ligands.

4. Discussion

The ability of diverse IFNs to differentially activate cells through a common receptor poses the issue of how the receptor differentially recognizes these ligands and transmits signals of the appropriate type, strength or duration. Here we have examined the roles of IFNAR-1 and IFNAR-2 in differential ligand binding and recognition, with the goal of reconstituting the binding parameters and patterns seen on human cells.

The experiments exploited the high level expression of IFNAR-1 and IFNAR-2 provided by the COS transient expression system, as indicated by greatly elevated ligand binding (Fig. 1; Table 1), and confirmed by flow cytometric analysis of cell surface receptors (data not shown). Thus, the binding to the ectopic receptors was clearly distinguishable from the low-level binding to the endogenous COS receptor. Moreover, the strong agreement of the binding parameters for the soluble IFNAR-2/Ig fusion protein and IFNAR-2 expressed on COS cells (Table 2) confirms that the intrinsic ligand binding properties of the transfected receptors are being measured. However, the natural sensitivity of monkey-derived COS cells to human IFNs precludes correlative functional data of cellular activation resulting from transfected human IFNAR proteins.

IFNAR-2, whether expressed on COS cells or as the soluble IFNAR-2/Ig 'immunoadhesin', has moderately high but differential affinity for five of the type I IFNs examined here, with IC_{50} values in the range of 1.5×10^{-9} to 2×10^{-8} M (Table 2). Only IFN-α1 has a very low affinity (IC_{50} of 2.6×10^{-7} to 1.1×10^{-6}). These results extend previous qualitative results that soluble IFNAR-2 can bind IFN-α2, IFN-αB, IFN-αC and IFN-β [19], and demonstrate the fidelity of IFNAR-2 expressed on COS cells as a binding substrate.

The IFNAR-1/IFNAR-2 complex binds IFN-α2 with higher affinity than does IFNAR-2 alone (Table 1). This is qualitatively consistent with previous reports [20–22,31], although we observed a smaller increase in affinity than did workers using rodent cells. However, these reports also differed among them in their K_d values for the binding of radiolabeled IFN-α2 or -α8 to the IFNAR-1/IFNAR-2 complex expressed in different murine cells and at different expression levels; two reports suggested the existence of both high-affinity and low-affinity components [20,31]. It is difficult to clarify the quantitative differences among the reports, although experimental variation should not be underestimated.

While the IFNAR-1/IFNAR-2 complex binds all tested ligands with higher affinity (lower IC_{50}) than does IFNAR-2 alone (Table 3), the addition of IFNAR-1 to IFNAR-2 produces differential effects on the IC_{50} values of the various ligands. The strongest effects (12–22-fold) are on the binding of IFN-αs, with smaller effects (3–7-fold) measured for IFN-β and IFN-ω, the less 'typical' members of the type I IFN family.

Particularly interesting are the differences in the ligand specificity of COS cells cotransfected with IFNAR-1 and IFNAR-2 compared with human Daudi cells (Table 3, columns 2, 4, 5). Although the IC_{50} values for IFN-α2 and -α8 are comparable for transfected COS cells and Daudi cells, those for IFN-α1, -αCon1 and IFN-ω are about five times higher (lower affinity) on the transfected COS cells, and the value for IFN-β is about 18 times higher (lower affinity) than on Daudi cells. The discrepancy for IFN-β of IC_{50} values between Daudi cells and COS cells co-expressing IFNAR-1 and IFNAR-2 is noteworthy in view of reports of IFN-β-specific effects [32–38]. Other cellular factors that may modulate type I IFN receptor binding have been reviewed [39].

A direct comparison of our results with previous binding and competition studies on human cells is complicated by the variety of cell types and conditions used. However, in all studies, IFN-α1 has the weakest binding and lowest biological activity of all type I IFNs for human cells [40–45]. On the other hand, IFN-αCon1, a construct based on the consensus sequence of the human IFN-α family, is reported to have biological specific activity on some cells almost 10-fold higher than other IFN-αs (reviewed in [46]). While the basis for this high activity is said to be higher affinity interactions with the receptor [43,47], previous experimental results were incomplete [43], and our experiments did not show IFN-αCon1 to be distinguished by an unusually high affinity for IFNAR-2, for the IFNAR-1/IFNAR-2 complex on COS cells, or for Daudi cells (Tables 2 and 3).

Our current results demonstrate that IFNAR-2 binds the array of type I ligands with moderate affinity, and manifests

Table 3
Inhibition (IC_{50}, molar) by type I interferons of [^{32}P]IFN-α2 binding to soluble IFNAR-2/Ig, transfected COS cells and Daudi cells

	1. IFNAR-2/COS		2. IFNAR-(1+2)/COS		3. Ratio 1/2	4. Daudi		5. Ratio 2/4
Ligand set 1:								
IFN-α2	2.2×10^{-9}	(0.7–6.4)	1.8×10^{-10}	(0.7–4.4)	12	1.3×10^{-10}	(0.9–1.7)	1.4
IFN-α8	8.6×10^{-9}	(7.7–9.5)	7.9×10^{-10}	(4.1–15)	11	3.4×10^{-10}	(1.7–6.7)	2.3
IFN-α1	2.6×10^{-7}	(0.6–10)	1.2×10^{-8}	(0.8–1.7)	22	2.3×10^{-9}	(1.6–3.4)	5.2
IFN-β	1.9×10^{-8}	(1.8–2.0)	5.9×10^{-9}	(3.1–11)	3	3.2×10^{-10}	(0.9–11)	18.4
Ligand set 2:								
IFN-α2	3.5×10^{-9}	(1.4–8.7)	1.9×10^{-10}	(1.2–2.8)	18	1.4×10^{-10}	–	1.4
IFN-αCon1	8.9×10^{-9}	(2.1–38)	6.8×10^{-10}	(2.0–23)	13	1.4×10^{-10}	–	4.8
IFN-ω	2.6×10^{-9}	(0.9–7.6)	3.7×10^{-10}	(3.5–3.9)	7	0.6×10^{-10}	–	6.2

Values are the mean of several independent determinations of the IC_{50}. Numbers in parentheses are the range of IC_{50} values at ± one standard deviation from the mean. Means were calculated from 3–4 independent measurements for the ligands in set 1, with the exception of IFN-β on IFNAR-2/Ig, which is the mean of two determinations. In ligand set 2, means for the transfected COS cells are from three determinations, whereas means for soluble IFNAR-2/Ig were calculated from two determinations. For Daudi cells, IC_{50} values are an average of three determinations for ligands in set 1 (except for IFN-β, which represents two experiments), and represent a single measurement for ligands in set 2. COS transfections for ligand set 1 utilized IFNAR-2b, whereas ligand set 2 transfections used the IFNAR-2c construct.

differential affinity. IFNAR-1 was previously shown to bind IFNs weakly but directly, both when expressed on *Xenopus laevis* oocytes [6], and as a soluble ectodomain [7], confirming earlier inferences of such interactions [5,11,12,48,49]. The current experiments demonstrate that IFNAR-1 contributes both to enhancing the affinity of the IFNAR-1/IFNAR-2 complex and to modulating the differential recognition of type I IFNs.

The fact that both IFNAR-1 and IFNAR-2 bind directly to IFNs and presumably recognize different regions of the IFN is consistent with previous suggestions that type I IFNs have two or three receptor binding domains (e.g. [50–54]). It is now important to determine the IFNAR-1 and IFNAR-2 binding sites on type I IFNs, and vice versa. It is also important to ascertain whether contributions to the binding of some or all type I ligands, particularly IFN-β, are made by other cellular components, and whether there are variations in the ligand specificity between cell types.

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