Intrinsic ligand binding properties of the human and bovine α-interferon receptors

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Abstract

The Type I interferon receptor (IFN-αR) interacts with all IFN-αs, IFN-β and IFN-ω, and seems to be a multisubunit receptor. To investigate the role of a cloned receptor subunit (IFN-αR1), we have examined the intrinsic ligand binding properties of the bovine and human IFN-αR1 polypeptides expressed in *Xenopus laevis* oocytes. Albeit with different efficiencies, *Xenopus* oocytes expressing either the human or bovine IFN-αR1 polypeptide exhibit significant binding and formation of crosslinked complexes with human IFN-αA and IFN-αB. Thus, the IFN-αR1 polypeptide most likely plays a direct role in ligand binding.

Key words: Interferon α receptor; Interferon α; *Xenopus laevis* oocyte

1. Introduction

Human Type I interferons (IFN), including multiple nonallelic IFN-αs, 1 IFN-β and 1 IFN-ω, are structurally similar but they differ in their ability to elicit biological responses in various human cells [1,2–6]. Bovine cells respond almost equally to all human IFN-αs, but not to HuIFN-β [7–9].

Type I IFNs bind to a common receptor which is composed of several subunits ([10–13]; denoted Type I receptor, IFN-α/β receptor, or IFN-α receptor). Two distinct cDNAs encoding putative human IFN-α receptors or receptor subunits have been cloned thus far. A cDNA for a human IFN-αR, here designated IFN-αR1**, conferred on transfected mouse cells responsiveness to human IFN-αB, but not to other Type I IFNs [14]. However, this HuIFN-αR1 polypeptide is not a specific receptor for HuIFN-αB, since antibodies raised against the HuIFN-αR1 protein neutralize the activities of diverse human type I IFNs on human cells [15,16].

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Abbreviations: IFN-α, interferon alpha; IFN-αR, Type I IFN-α receptor; IFN-αR1, cloned subunit of the IFN-αR, designated as bovine (Bo-) or human (Hu-).

**The cloned IFN-αR is denoted as IFN-αR1 because of current evidence that other IFN-αR subunits exist (see text). For IFN-αR subunits, we suggest Arabic numerals, rather than Greek letters since: (i) the interferon receptors already include a Greek letter to denote the α or γ interferon receptor, and a second Greek letter denoting the various subunits of the receptor might be confusing; and (ii) the genetic nomenclature rules do not permit Greek letters, so it will simplify the correspondence between protein nomenclature and gene nomenclature if Greek letters are minimized in the protein nomenclature.

Recently, a second cDNA for a human IFN-αR, here referred to as IFN-αR2, has been cloned and initially characterized [17]. This cDNA encodes both membrane-bound and soluble forms of an IFN receptor which has the intrinsic ability to bind several IFN-αs and IFN-β. As with antibodies to IFN-αR1, antibodies to IFN-αR2 block the binding of a variety of Type I IFNs to human cells [17]. A multi-subunit structure for the IFN-αR is also indicated by the results of previous affinity crosslinking experiments, which show multiple bands representing complexes of cell surface proteins with HuIFN-αs, and by the characterization of several monoclonal antibodies that appear to recognize cell surface polypeptides involved in Type I IFN-α binding [18–21]. The total number of receptor chains is not yet known, but a human chromosome 21-derived YAC, encoding HuIFN-αR1 and other polypeptides, is capable of conferring on hamster CHO cells responsiveness to a variety of human Type I IFNs [22].

Current results are ambiguous as to whether the human IFN-αR1 protein is directly involved in ligand binding. In addition to the original functional characterization of the protein expressed in murine cells ([14]; see above), monkey COS-1 cells expressing large amounts of HuIFN-αR1 do not show enhanced binding of human IFN-αA or -αB ([23]; unpublished data). These and other results have led some investigators to suggest that the IFN-αR1 is primarily involved in signal transduction rather than ligand binding [17,24,25].

In contrast to the experiments with the HuIFN-αR1 cDNA, monkey COS cells transfected with cDNA encoding the bovine homologue (BoIFN-αR1) [23,26] display large numbers of high affinity binding sites for HuIFN-αA and -αB [23]. Thus, the BoIFN-αR1 polypeptide appears sufficient for high-affinity binding of

human IFN-αA and -αB [23]. Similarly, the BoIFN-αR1 cDNA confers on human cells an enhanced binding and response to HuIFN-αD, an IFN-α subtype that is notably weak in its effects on human cells, but which has strong interactions with bovine cells [26].

To investigate the intrinsic ligand binding properties of the human and bovine IFN-αR1 proteins in a cellular background devoid of other receptor subunits, we have expressed these proteins in *Xenopus laevis* oocytes. *Xenopus laevis* is evolutionarily distant from mammals, and there are no reports of IFN-like antiviral activity in amphibians or reptiles [27]. It is therefore reasonable to consider *Xenopus* as a 'null cell' with regard to the introduction of mammalian IFN receptor components. The ability of *Xenopus* oocytes microinjected with cRNA encoding human or bovine IFN-αR1 to bind human IFN-αA and -αB has been examined, and the resulting IFN-α/IFN-αR1 complexes have been characterized by covalent crosslinking. These observations suggest that the IFN-αR1 subunit of the Type I IFN receptor plays a direct role in ligand binding.

2. Materials and methods

Genetically engineered phosphorylatable IFN-αA and -αB were phosphorylated with the catalytic subunit of bovine heart cAMP-dependent protein kinase and [γ- 32P]ATP, as described [28,29]. Human Burkitt lymphoma-derived Daudi cells [30] and bovine kidney-derived MDBK cells [31] were used for binding and crosslinking controls.

A full-length bovine IFN-αR1 cDNA was previously cloned and characterized [23]. HuIFN-αR1 cDNA in the vector pYH12 was the gift of Drs. Y. Hibino and S. Pestka. The coding regions of the receptor cDNAs were cloned into the transcription vector pSP64T [32] by blunt-end ligation [33], and the orientation of the inserts was confirmed. These clones were designated as BoIFNαR/pSP64T and HuIFNαR/pSP64T. Capped in vitro transcripts corresponding to the BoIFN-αR1 and HuIFN-αR1 cRNAs were made from BoIFNαR/ pSP64T and HuIFNαR/pSP64T linearized with SalI and XbaI, respectively. The integrity and size of the transcripts were analyzed on 1% denaturing agarose gels [33].

Stage V–VI *Xenopus laevis* oocytes were prepared as described [34,35], with the addition of 1% horse serum to the Barth's solution. After 12–24 h, cRNA (50 nl at 1 μg/μl) from HuIFN-αR1 or BoIFN-αR1 cDNA was injected into the cytoplasm of healthy oocytes. Control oocytes were injected with 50 nl of water. Injected oocytes were maintained in the above medium at room temperature with daily changes of medium until tested for IFN binding activity.

Injected oocytes were tested for IFN-α binding after incubation for about 68 h. Microinjected oocytes were placed in 100 μl of binding solution containing 1% non-fat dry milk (NFDM) and 2 × 10⁵ cpm (ca. 2 × 10⁻¹⁰ M) of [32P]IFN-αA or -αB in a 5 ml round bottom polypropylene tube (Falcon). Where appropriate, 3 μg of unlabeled HuIFN-αA was used as a competitor. Oocytes were incubated at room temperature for 1 h with rotation at 120 rpm, then washed 3 times with 0.5 ml of ice-cold binding solution and 3 times with 1 ml of ice-cold PBS. Oocytes were transferred to scintillation vials with scintillation fluid, vortexed for 1 min, and radioactivity was quantitated.

For measuring IFN binding to individual oocytes, the oocytes were transferred into a filter unit with a nylon membrane filter, and residual liquid was removed by gentle vacuum. A section of the membrane containing the oocyte was cut out and added to a scintillation vial containing 2 ml of scintillation fluid. The background level of radioactivity was determined for each vial prior to adding the washed oocytes.

Covalent crosslinking of [32P]HuIFN-αs to 50 oocytes/ batch was performed as described previously for mammalian cells [36,37]. Follow-

ing binding of [32P]HuIFN-αs and reaction with 0.5 mM disuccinimidyl suberate (DSS; Pierce Chemical Co.), oocytes were washed with 50 ml ice-cold PBS, allowed to settle, and liquid was removed by aspiration. Receptor–ligand complexes were extracted with 0.2 ml of 1% Triton X-100 with protease inhibitors. Insoluble material was then removed by centrifugation at 13,000 × g for 20 min at 4°C. Triton X-100 extracts were analyzed by SDS-UREA-PAGE containing 7.5% acrylamide and 6.7 M urea [38,39]. Gels were dried and subjected to autoradiography.
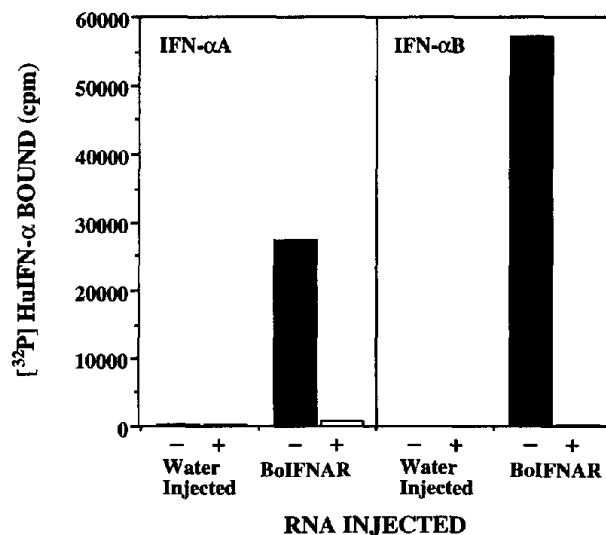
A

B
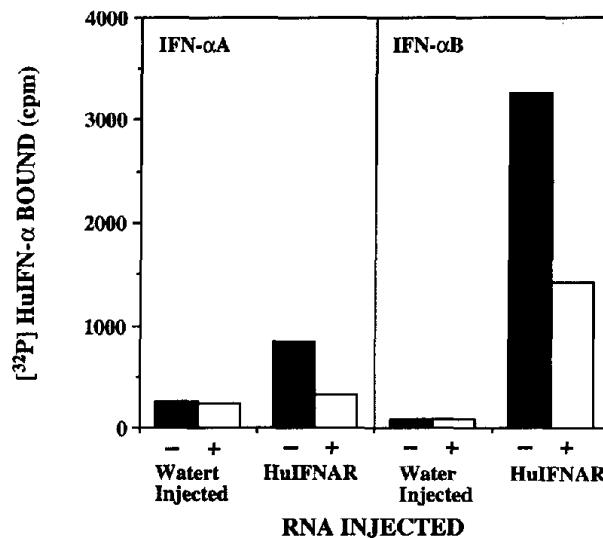
RNA INJECTED

Fig. 1. Binding of [32P]IFN-αA and -αB to *Xenopus* oocytes microinjected with in vitro-made transcripts of HuIFN-αR1 (A) and BoIFN-αR1 (B). 5 oocytes in 100 μl binding buffer were incubated with 2 × 10⁵ cpm of [32P]IFN-αA or -αB, in the presence or absence of 3 μg non-radioactive IFN-αA, with gentle rotating for 1 h at room temp. The oocytes were washed and bound IFN was measured as described in section 2. Filled bars, binding in the absence of competitor ('Total binding'); open bars, binding in the presence of non-radioactive IFN-αA ('Non-specific binding').
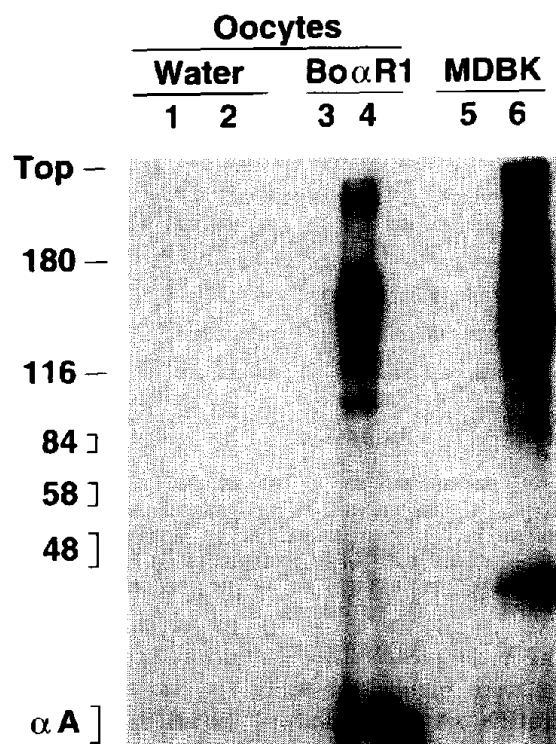
Fig. 2. Covalent crosslinking of [^{32}P]IFN-αA to receptors on oocytes and bovine MDBK cells. Control water-injected oocytes (lanes 1,2), oocytes injected with the BoIFN-αR1 transcript (lanes 3,4), and MDBK cells (lanes 5,6) were incubated with [^{32}P]IFN-αA in the absence (lanes 2,4,6) and presence (lanes 1,3,5) of 3 µg IFN-αA. The bound [^{32}P]IFN-αA was crosslinked to the receptors and analyzed by SDS-PAGE and autoradiography as described in section 2. Positions of ^{14}C-labeled molecular weight markers expressed in kDa are shown on the left. The 40 kDa band in lane 6 is a covalent dimer of IFN-αA.

3. Results and discussion

Xenopus laevis oocytes were chosen as an expression system since they are highly unlikely to have IFN receptors capable of interacting with mammalian IFN recep-

Table 1
Ligand binding properties of IFN-αR1 in different cell contexts

Expressed cDNA	Host cells	Human IFNs				Ref.
		IFN-αA	IFN-αB	IFN-αD	IFN-β	
HuIFN-αR1	Murine	–	+	nd	–	[14]
	CHO	nd	–	nd	+	[41]
	COS (monkey)	–	–	nd	nd	[23]*
	Xenopus	+	+	nd	nd	
BoIFN-αR1	Murine	++	++	+	nd	[40]*
	COS	++	++	nd	nd	[23]
	Human	nd	nd	+	nd	[26]
	Xenopus	++	++	nd	nd	

nd, not done or not reported.
*J.K.L. and J.A.L., unpublished data.

tor polypeptides, and, indeed, may not have an IFN system at all [27]. Consistent with this assumption, water-injected oocytes bound human IFN-αA and -αB poorly (Figs. 1 and 3), and produced no evidence of any IFN–receptor complexes in covalent crosslinking experiments (Figs. 2 and 4).

3.1. Ligand binding properties of the bovine IFN-αR1 polypeptide

Oocytes were microinjected with RNA transcripts made in vitro from the cDNA for the bovine IFN-αR1. Two-to-three days after injection, binding of both [^{32}P]HuIFN-αA and -αB, corresponding, respectively, to 1.3×10^9 and 3.0×10^9 IFN molecules per oocyte, was measured for oocytes injected with the BoIFN-αR1 RNA transcript (Fig. 1B). Most binding was blocked by the addition of excess IFN-αA, demonstrating that the binding is specific. Control water-injected oocytes displayed low levels of non-specific binding, and no specific binding of either ligand.

In affinity crosslinking experiments (Fig. 2), oocytes expressing the BoIFN-αR1 polypeptide displayed a broad band centered at 155 kDa, corresponding to the [^{32}P]HuIFN-αA/receptor complex, and several weaker bands, including one of >220 kDa, and several between 130 and 95 kDa. The nature of these minor bands is unknown, but may reflect heterogeneous processing of the BoIFN-αR1 polypeptide in *Xenopus*. Addition of excess non-radioactive IFN-αA abolished these bands (lanes 3 and 5). This pattern can be compared to the broad 140 kDa complex, sometimes discernable as a doublet, formed on control bovine MDBK cells (Fig. 2, lane 6). The difference in electrophoretic mobility of the receptor–ligand complex from injected *Xenopus* oocytes with that on MDBK cells may represent differences in glycosylation or processing. Alternatively, the difference

Table 2
Binding of [^{32}P]HuIFN-αA to bovine MDBK cells and murine cells transfected with the BoIFN-αR1 cDNA

Cell type	K_d (M^{-1})	Receptors/cell
MDBK	6.8×10^{-11}	1500
BoIFNAR/3T3	8.6×10^{-11}	2470
BoIFNAR/L	10.7×10^{-11}	1325

For expression of the BoIFN-αR1 in rodent cells, the BoIFN-αR1 cDNA was subcloned into expression vector pD5/IgH-En/Hygro B [44]. Plasmids derived from pD5/IgH-En/Hygro B were transfected into mouse NIH/3T3 or L cells by the polybrene-DMSO procedure [45], and transformants were selected with 250 µg/ml hygromycin B. After 10 days of selection, well-isolated healthy colonies were transferred to 24-well plates. These subclones were screened for binding of HuIFN-αs. The selected clones derived from NIH3T3 cells and L cells were designated BoIFNAR1/3T3 and BoIFNAR1/L, respectively. The binding of [^{32}P]HuIFN-αA to bovine MDBK and BoIFNAR1/3T3 cells was measured by incubating cells with various amounts of ^{32}P-labeled ligand (1.4×10^{-11} M–1×10^{-9} M) for 1 h at room temperature, and samples were processed as described [23,36,37]. Untransfected NIH-3T3 cells do not show high affinity specific binding of [^{32}P]HuIFN-αA.
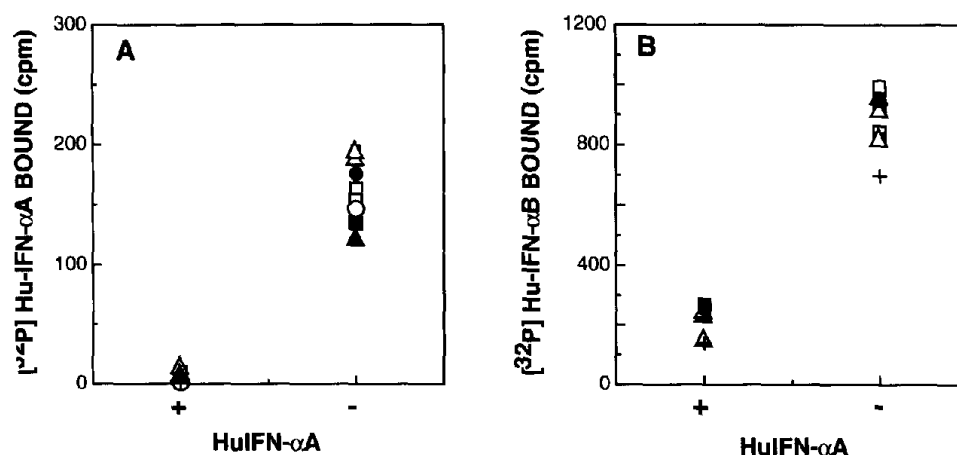
Fig. 3. Binding of [^{32}P]IFN-αA and -αB to individual *Xenopus* oocytes microinjected with in vitro-transcribed HuIFN-αR1 RNA. Individual oocytes were injected with 50 ng of HuIFN-αR1 transcript and assayed 3 days later (see section 2). The value for each oocyte is plotted as a separate symbol. (A) Binding of [^{32}P]HuIFN-αA. (B) Binding of [^{32}P]HuIFN-αB. The values above the symbol '–' represent total binding, in the absence of excess non-radioactive IFN-αA; values above the symbol '+' represent non-specific binding, measured in the presence of excess HuIFN-αA.

may result from cross-linking to other components of the IFN-α receptor in MDBK cells. Nevertheless, identification of the 155 kDa receptor/ligand complex in oocytes injected with the BoIFN-αR1 transcript constitutes direct evidence that the BoIFN-αR1 subunit is sufficient for IFN-α binding.

The results of the current experiments with *Xenopus* oocytes are consistent with our previous observation that COS cells transiently transfected with the BoIFN-αR1 in pcDNA1 have 0.25–1 × 10^6 high-affinity binding sites for HuIFN-αA and -αB, compared to <10^4 receptors for HuIFN-αA and -αB on untransfected COS cells ([23]; Table 1). The high-affinity binding of HuIFN-αs to the BoIFN-αR1 polypeptide may account for the fact that mouse or human cells stably transfected with the BoIFN-αR1 protein acquire the ligand binding affinities and specificities for human IFN-αs characteristic of bovine cells (e.g. Table 2, and unpublished data; [26,40]).

3.2. Ligand binding properties of the human IFN-αR1 polypeptide

Previous studies of the human IFN-αR1 polypeptide expressed in various heterologous host cells have not produced firm conclusions on its role in IFN binding, and the ligand specificity appears to be strongly modulated by factors in the host cell ([14,23,41]; Table 1).

In the present study, *Xenopus* oocytes microinjected with the HuIFN-αR1 transcript exhibited significant specific binding of both [^{32}P]IFN-αA and -αB (Fig. 1A), corresponding to 2.6 × 10^7 and 9.6 × 10^7 IFN molecules bound per oocyte. Binding to individual oocytes was also measured (Fig. 3). At the subsaturating concentration of IFN-αs used, the average specific binding per oocyte, estimated from measurements on 10 individual oocytes, was ca. 5.5 × 10^7 molecules of [^{32}P]HuIFN-αA and 1.7 × 10^8 molecules of [^{32}P]HuIFN-αB. The differences in

binding in the presence and absence of excess unlabeled IFN-α were highly significant (*t*-values of 18.36 for [^{32}P]HuIFN-αA and 20.58 for [^{32}P]HuIFN-αB).

The specific binding of [^{32}P]HuIFN-αB to the expressed HuIFN-αR1 was confirmed by crosslinking experiments. A single 140 kDa band representing the recep-
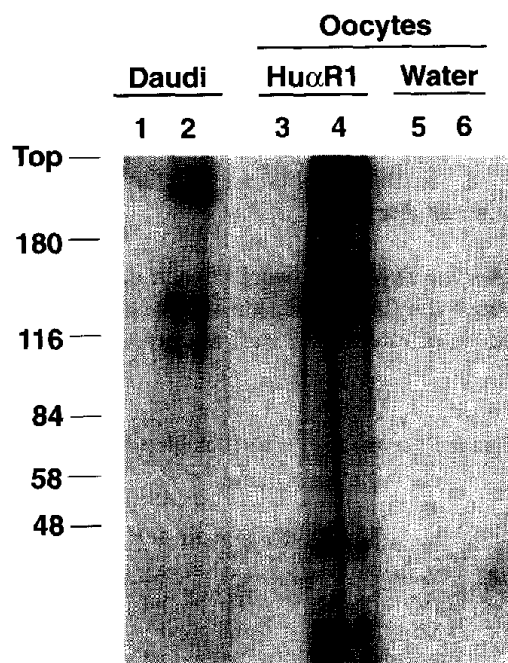

Fig. 4. Covalent cross-linking of [^{32}P]IFN-αB to the receptors on human Daudi cells and oocytes injected with the HuIFN-αR1 cRNA. Daudi cells or oocytes were incubated with [^{32}P]IFN-αB and processed as described in Fig. 2. Odd lanes represent reaction containing excess non-radioactive IFN-αA and even lanes represent without. (Because much less Daudi sample was loaded, the Daudi controls in lanes 1 and 2 were density- and contrast-enhanced by computer to better visualize the bands. The lanes from oocyte samples were not manipulated.)

tor–ligand complex was observed (Fig. 4, lanes 3 and 4). This complex displayed a slightly slower electrophoretic mobility than the upper band observed with Daudi cells (ca. 130–135 kDa) (Fig. 4, lane 2); it is possible that the IFN-αR1 subunit expressed on *Xenopus* oocytes is more heavily glycosylated or represents a different subunit from the major band seen with Daudi cells. It is significant that a single crosslinked band is seen with the oocytes, whereas the analogous experiment on human cells produces several bands (Fig. 4, lane 2; [19,42]). Some of these bands have been identified as complexes of IFN-α with other human cell surface proteins, presumably other subunits of the receptor [18,19,21]. These observations are also consistent with the possibility that the HuIFN-αR1 polypeptide expressed in oocytes is but one subunit of the putative multi-subunit Type I IFN receptor.

The low levels of IFN binding to oocytes expressing the HuIFN-αR1 preclude Scatchard analysis and determination of dissociation constants. Since high expression of the bovine homologue was observed in this system, it is likely that the low levels of IFN binding reflect a low affinity for HuIFN-αs, rather than a failure to express protein efficiently. This likelihood is speculative, since we currently lack an independent method for quantitating cell surface HuIFN-αR1 expression (e.g. appropriate antibodies). The high sensitivity of detection of IFN binding in the oocyte system may reflect the extremely low background for IFN binding on oocytes; in our previous experiments with COS cells ([23]; unpublished data), the high background arising from endogenous COS high affinity IFN-α receptors may have obscured low affinity binding by the HuIFN-αR1 polypeptide, despite strong expression of the protein.

We have demonstrated directly that the human and bovine IFN-αR1 polypeptides have the intrinsic ability to bind IFN-αA and IFN-αB; previously, such a role could only be inferred indirectly from immunochemical evidence [15,16]. The recently identified HuIFN-αR2 also seems to have a broad ability to bind Type I IFNs, probably with much higher affinity than the HuIFN-αR1, although the affinity has not yet been measured [17]. That these are both subunits of a common receptor is indicated by the ability of antibodies to either protein to neutralize the binding and activity of a variety of Type I IFNs to human cells [15–17]. The co-expression of these two polypeptides is of great interest, and it is not yet known whether these are the only subunits of the Type I receptor (e.g. [22]). There is also evidence suggesting a role for the HuIFN-αR1 in signal transduction [24,25]. Thus, the IFN-αR1 polypeptide may play roles in both ligand binding and signal transduction, and the simple schema [43] whereby cytokine ligand binding is assigned to an 'α subunit' and signal transduction is assigned to a 'β subunit' may not reflect the complexity of the Type I IFN receptor.

Finally, the contrasting abilities of the human and bovine IFN-αR1 proteins to bind human IFN-αs provides a powerful system for mapping IFN binding determinants of these proteins.

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