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Ligand-Induced Association of the Type I Interferon Receptor Components

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Two transmembrane polypeptides, IFNAR and IFN-α/βR, were previously identified as essential components of the type I interferon (IFN) receptor, but their interrelationship and role in ligand binding were not clear. To study these issues, we stably expressed and characterized the two polypeptides in host murine cells. In human cells, native IFN-α/βR is a 102-kDa protein but upon reduction only a 51-kDa protein is detected. In host murine cells human IFN-α/βR was expressed as a 51-kDa protein. Host cells expressing IFN-α/βR bound IFN-α_2 with a high affinity (K_d of 3.6 nM), whereas cells expressing IFNAR exhibited no ligand binding. Upon coexpression of IFNAR and the 51-kDa IFN-α/βR, the affinity for IFN-α_2 was increased 10-fold, approaching that of the native receptor. We show by cross-linking that both the cloned (51-kDa) and native (102-kDa) IFN-α/βR bind IFN-α_2 to form an intermediate product, while IFNAR associates with this product to form a ternary complex. Hence, IFNAR and IFN-α/βR are components of a common type I IFN receptor, cooperating in ligand binding. Ligand-induced association of IFNAR and IFN-α/βR probably triggers transmembrane signaling.

Type I human interferons (HuIFNs) (the multiple HuIFN-α species, one HuIFN-β, and one HuIFN-ω) are a family of cytokines defined by their ability to establish an antiviral state in cells. IFNs also induce many other physiological responses, including antiproliferative activity; stimulation of cytotoxic activity in lymphocytes, natural killer cells, and phagocytic cells; modulation of cellular differentiation; and stimulation of class I major histocompatibility complex antigens and other surface markers (23).

Like that of all other cytokines and growth factors, the action of type I IFNs is mediated by their interaction with a specific cell surface receptor. A type I IFN receptor was identified in human cells by specific binding of radiolabeled IFN (4). Cross-linking of radioiodinated IFN-α to whole cells or to isolated cell membranes revealed 110- to 150-kDa protein bands, indicating that the type I IFN receptor has a molecular mass of 100 to 130 kDa (12). Complexes of >200 kDa were seen as well, indicating that the ligand-receptor complex either self aggregates or binds additional proteins (9). The type I IFN receptor is present in almost every cell type, albeit at a rather low abundance (100 to 5,000 copies per cell).

An HuIFN-α receptor (IFNAR) was cloned by transferring human DNA into mouse cells and selecting for cells resistant to virus upon treatment with HuIFN-α_B. However, these cells were not protected by any other type I HuIFN and except for IFN-α_B, no high-affinity ligand binding to these cells could be demonstrated. Since human cells bind and respond equally well to most of the >20 species of type I HuIFN, it was proposed that additional components must be present in the functional type I IFN receptor (29). Studies with monoclonal antibodies, raised against the receptor on the cell surface, provided additional evidence for a multicomponent receptor containing at least two different components of 115 and 100 kDa as well as higher-molecular-mass complexes (7). The 115-kDa component was recently identified as IFNAR (6). Studies with knockout mice have confirmed that IFNAR is essential (but not sufficient) for response to all type I IFNs (17).

Recently, we characterized and cloned an additional component of type I IFN receptor (IFN-α/βR [22]). This transmembrane protein binds equally well to various type I HuIFNs, including IFN-β, and is essential for IFN activity, since antibodies raised against a soluble form of IFN-α/βR completely blocked the activity of all type I HuIFNs, including IFN-α_B. IFN-α/βR is expressed on the cell surface mainly as a 102-kDa protein, but upon reduction by dithiothreitol only a 51-kDa subunit was detected. Host murine cells transiently expressing HuIFN-α/βR but lacking HuIFNAR bound IFN-α_2 but did not respond in the antiviral assay.

In the present study we stably expressed IFNAR and the 51-kDa IFN-α/βR, either separately or together, in murine cells. Binding studies with ^{125}I-HuIFN-α_2 revealed that IFN-α/βR is the major ligand-binding component, while IFNAR does not bind IFN-α_2 when expressed separately. Upon coexpression of these two proteins, a composite receptor exhibiting a 10-fold-increased affinity for IFN-α_2 was obtained. Cross-linking and immunoprecipitation with specific antibodies enabled us to identify a ternary complex of IFN-α_2 with IFN-α/βR and IFNAR. Hence, these two proteins are subunits of the same receptor, IFN-α_2 induces their association, and this event probably elicits transmembrane signaling.

MATERIALS AND METHODS

Cells and reagents. Human HeLa (CCL 2.1) cells, Daudi (CCL 213) cells, murine NIH 3T3 (CRL 1658) cells, and vesicular stomatitis virus Indiana strain (ATCC VR-158) were obtained from the American Type Culture Collection. Cells were grown in Dulbecco's modified minimal essential medium supplemented with 10% fetal bovine serum in a 5% CO_2 atmosphere. Murine IFN-α/β (MuIFN-α/β) was obtained from Lee Biomolecular Research Laboratories. The following recombinant HuIFNs were used: IFN-α_2 (*Escherichia coli*) (Reprogen, Nes-Ziona, Israel), IFN-α_B (*E. coli*) (Ciba Geigy, Basel, Switzerland), and IFN-β (CHO) (InterLab Ltd., Nes-Ziona, Israel). A monoclonal antibody (IFNaR3) directed against HuIFNAR (6) and a rabbit polyclonal antibody directed against IFN-α/βR (22) were previously described. ^{125}I-IFN-α_2 was prepared by a modification of the chloramine T method (21).

Cross-linking, immunoprecipitation, coimmunoprecipitation, immunoaffinity purification, and immunoblotting. Cross-linking, immunoprecipitation, coimmu-

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TABLE 1. Identification of murine NIH 3T3 clones expressing HuIFN-α/βR and HuIFNAR

Cell line (receptor[s])	Binding activity (cpm)[a]		Sp act (U/mg)	
	IFN-α/βR antibody	^{125}I-IFN-α_2	IFN-α_2	IFN-α_B
369.11 (IFN-α/βR)	9,960 ± 1,900	14,840 ± 1,320	$<10^2$	$<10^2$
385.7 (IFN-α/βR)	8,510 ± 1,530	12,550 ± 2,360	$<10^2$	$<10^2$
470.6 (IFNAR)	236 ± 62	90 ± 39	$<10^2$	10^7
467 (IFNAR)	162 ± 92	112 ± 55	$<10^2$	10^7
508.12 (IFN-α/βR, IFNAR)[b]	7,730 ± 1,300	70,240 ± 4,100	$<10^2$	10^6
1306 (IFNAR, IFN-α/βR)[c]	3,370 ± 610	32,100 ± 1,850	$<10^2$	10^6
NIH 3T3	197 ± 33	78 ± 49	$<10^2$	$<10^2$
HeLa (human)	850 ± 156	4,230 ± 186	10^8	10^8

[a] Average of triplicates ± standard deviation.
[b] Derived from clone 369.11.
[c] Derived from clone 470.6.

noprecipitation, immunoaffinity purification, and immunoblotting procedures were performed as previously described (22). Briefly, ^{125}I-IFN-α_2 was cross-linked to washed cells (5×10^7 to 1.7×10^8 cells) with disuccinimidyl suberate. Cells were then solubilized with a lysis buffer containing CHAPS {3-[(3-cholamidopropyl)-dimethyl-ammonio]-1-propanesulfonate} and protease inhibitors. Cell lysates were immunoprecipitated either with rabbit IFN-α/βR antiserum (1:100) and protein A-Sepharose or with monoclonal antibody IFNaR3 (1:200) and protein G-Sepharose. Bound proteins were eluted by boiling in sample buffer containing dithiothreitol and were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography. In other experiments, cells were first solubilized with detergent and then subjected to cross-linking with ^{125}I-IFN-α_2, followed by immunoprecipitation, SDS-PAGE as noted above, and autoradiography. Detergent-solubilized cell extracts (of 5×10^7 cells) were subjected to immunoprecipitation as described above, followed by SDS-PAGE under reducing or nonreducing conditions and immunoblotting with rabbit anti-IFN-α/βR antibody (1:400) and ^{125}I-protein A. Immunoaffinity purification was done with Affigel-10 (Bio-Rad, Richmond, Calif.)-bound monoclonal anti-IFN-α/βR antibody 5.73 as described previously (22).

IFN assay. The antiviral activities of various HuIFNs and MuIFNs were measured in miscellaneous cells by a cytopathic effect inhibition assay with vesicular stomatis virus (24). IFN titers were calibrated against human and murine National Institutes of Health standards.

Expression plasmids. The coding region of the HuIFN-α/β receptor cDNA was amplified from plasmid pCEV9-m6 DNA (22) by PCR with specific oligonucleotides. A DNA construct (IFNABRΔC) having two Lys residues instead of the entire cytoplasmic domain was similarly prepared. The cDNA corresponding to IFNAR (29) was generated by reverse transcription PCR (10). All of the above-mentioned products were cloned into the *Xba*I site of the pEF-BOS expression vector (15), kindly provided by S. Nagata, to yield pEF-BOS-IFNABR, pEF-BOS-IFNABRΔC, and pEF-BOS-IFNAR. The oligonucleotide 5′GATCT TCGGGAAATGGAAACTTCGGGAAATGGAAACAT, corresponding to a duplicated IFN-stimulated response element of the murine (2′-5′)oligoadenylate synthetase gene (5), was annealed to an antisense strand and then inserted in front of the thymidine kinase promoter of the pTK-luciferase reporter vector (3, 20) to yield pxmuISRE-TKluc. All constructs were confirmed by DNA sequencing.

Stable expression of receptor components in host cells. NIH 3T3 cells (1.5×10^6 cells in 10-cm-diameter plates) were cotransfected with pSV2neo (2 μg), together with either pEF-BOS-IFNAR or pEF-BOS-IFNABR (10 μg of DNA each), by the calcium phosphate precipitation method (11). Independent G418-resistant colonies expressing IFN-α/βR were identified by binding of anti IFN-α/βR antibody and by binding of radiolabeled IFN-α_2 (as described below). Independent G418-resistant colonies expressing IFNAR were identified by an antiviral assay with HuIFN-α_B and by a luciferase induction assay with HuIFN-α_B (as described below). In both sets of cells, positive colonies were subcloned. For stable coexpression, G418-resistant clones expressing either IFNAR or IFNABR were transfected with pSV2hygro (2 μg), together with either pEF-BOS-IFNABR, pEF-BOS-IFNAR, or pEF-BOS-IFNABRΔC as detailed above. Hygromycin- and G418-resistant clones coexpressing both IFNABR and IFNAR or both IFNABRΔC and IFNAR were similarly selected and subcloned. HeLa cells (1.5×10^6 cells in 10-cm plates) were cotransfected by the calcium phosphate precipitation method (11) with pSV2neo (2 μg), together with either pEF-BOS-IFNABR or pEF-BOS-IFNABRΔC (10 μg of DNA each). Independent G418-resistant colonies were identified by increased binding of both anti IFN-α/βR antibody and radiolabeled IFN-α_2 and then were subcloned.

Determination of IFN response by IFN-stimulated response element-dependent luciferase induction. Plasmid pxmuISRE-TKluc was transiently transfected into various cells (0.8×10^6 cells per 35-mm-well-diameter plate) by the DEAE-dextran method (26). The cells were stimulated at 24 h posttransfection with various IFNs (500 U/ml) for 18 h, and the level of luciferase activity in the cell extracts was then determined (19).

Binding of anti-IFN-α/βR antibody to cells. Early confluent cultures of adherent cells in 2-ml aliquots of culture medium containing 2% fetal bovine serum and 0.1% sodium azide (wash medium) in 35-mm-well plates were incubated for 2 h at room temperature with a rabbit anti-IFN-α/βR antibody (1:500). The cells were washed, ^{125}I-protein A (2 ml at 250,000 cpm in the wash medium) was added, and the cells were further incubated for 45 min. The cells were washed again, collected into trypsin, and counted.

Binding of ^{125}I-IFN-α_2 to cells and Scatchard analysis. Early confluent cells in 1 ml of wash medium (as described above) in 35-mm-well plates were incubated for 2 h at room temperature with ^{125}I-IFN-α_2 (2×10^5 to 3×10^5 cpm; 10^8 U/mg; 5×10^7 cpm/μg) and the appropriate concentrations of nonlabeled IFN-α_2. The cells were then washed, collected into trypsin, and counted. Nonadherent cells (10^6 cells) in 96-conical-well plates were similarly incubated with labeled and nonlabeled IFN-α_2 in wash medium (200 μl) before being washed and counted. Binding data were analyzed by the LIGAND program (18).

RESULTS

The 51-kDa IFN-α/βR has an intrinsic ligand-binding activity. We stably expressed the cloned cDNA of the 51-kDa IFN-α/βR in murine NIH 3T3 cells. Twenty independent clones, expressing IFN-α/βR, were identified by binding of both ^{125}I-IFN-α_2 and anti-IFN-α/βR antibody (representative clones are shown in Table 1). SDS-PAGE under nonreducing conditions of the affinity-purified receptor revealed that these clones expressed the receptor as a 51-kDa protein, corresponding in size to the 1.5-kb cDNA of IFN-α/βR. In human cells, IFN-α/βR is expressed mainly as a 102-kDa protein but upon reduction with dithiothreitol only a 51-kDa IFN-α/βR is detected by immunoblotting (Fig. 1A).

We then stably expressed HuIFNAR by transfection of NIH 3T3 cells with plasmid pEF-BOS-IFNAR. Twenty-five IFNAR-positive clones were identified by an antiviral assay and by luciferase induction upon treatment with HuIFN-α_B to which murine cells expressing HuIFNAR are responsive (representative clones are shown in Tables 1 and 2). As expected, such IFNAR-positive clones were not responsive to HuIFN-α_2, while the integrity of the intracellular components of the IFN system in these clones was demonstrated by their response to MuIFN-α/β (Table 2).

Cells expressing either one of the two receptor components were tested for binding of ^{125}I-HuIFN-α_2, and the binding data were evaluated by a Scatchard analysis. Cells expressing IFNAR only (clone 470.6) did not exhibit any specific binding of ^{125}I-IFN-α_2 (Fig. 2A), and hence, no K_d value could be derived for IFNAR. In contrast, high-affinity, specific, and saturable binding was obtained with cells expressing the 51-kDa IFN-α/βR (clone 369.11). Scatchard analysis indicated a single type of binding sites, having a K_d of 3.6 nM, while the affinity of the native receptor in human Daudi cells (K_d of 0.16 nM) was about 22 times higher (Fig. 2C). Hence, IFN-α/βR and not

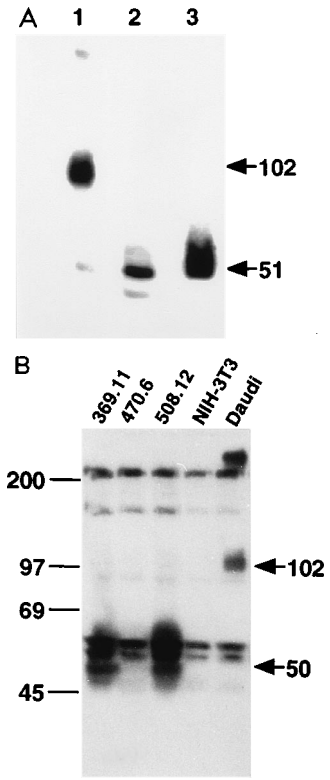

FIG. 1. Expression of HuIFN-α/βR in various cells. (A) Immunoblotting of immunoaffinity-purified receptors derived from Daudi cells (lanes 1 and 2) and from clone 369.11 expressing HuIFN-α/βR (lane 3). One sample (lane 2) was reduced with dithiothreitol prior to SDS-PAGE, while no others were reduced. The 51- and 102-kDa forms of IFN-α/βR are indicated by arrows. (B) SDS-PAGE (7.5% acrylamide under nonreducing conditions) of detergent cell extracts after immunoblotting with rabbit anti-IFN-α/βR antibody. NIH 3T3 clone 369.11 cells express HuIFN-α/βR, clone 470.6 cells express IFNAR, and clone 508.12 cells express both proteins. Control NIH 3T3 cell and human Daudi cell results are shown as well. Molecular mass standards (in kilodaltons) are marked on the left.

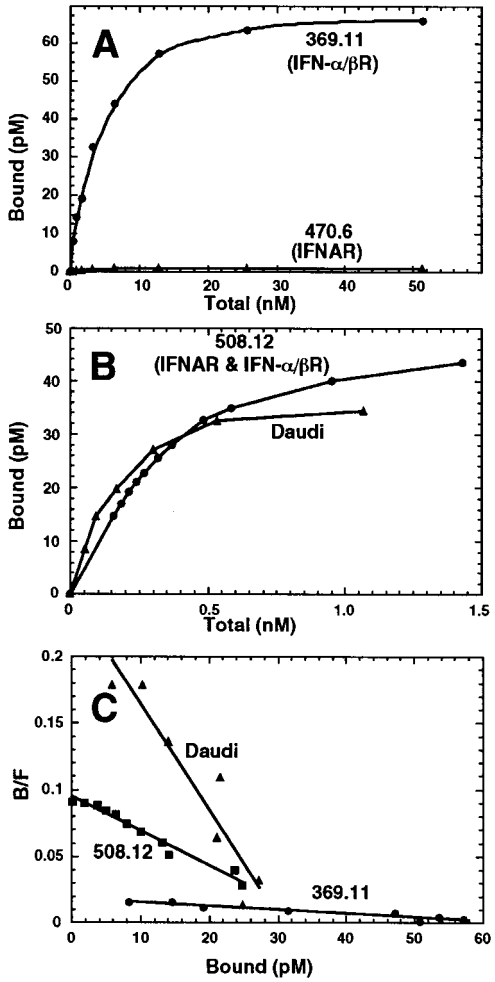

FIG. 2. Binding of ^{125}I-IFN-α_2 to various type I IFN receptor configurations. (A) Saturation binding of ^{125}I-IFN-α_2 to NIH 3T3 cells expressing IFN-α/βR (clone 369.11 [●]) and lack of binding to cells expressing IFNAR (clone 470.6 [▲]). (B) Saturation binding of ^{125}I-IFN-α_2 to cells expressing both IFN-α/βR and IFNAR (clone 508.12 [●]) and to human Daudi cells (▲). (C) Scatchard analysis of ^{125}I-IFN-α_2 binding. Binding data were analyzed by the LIGAND program. The following cells showed high-affinity saturable binding: human Daudi cells (▲), IFN-α/βR-positive cells (clone 369.11 [●]), and clone 508.12 cells expressing both IFNAR and IFN-α/βR (■). B/F, ratio of bound antigen to free antigen.

IFNAR exhibits intrinsic ligand-binding activity. The binding parameters are summarized in Table 3.

IFN-α/βR and IFNAR cooperate in ligand binding. We coexpressed IFNAR and IFN-α/βR in NIH 3T3 cells. Either clone 369.11, expressing the 51-kDa HuIFN-α/βR, or clone 470.6, expressing HuIFNAR, was transfected with expression vectors harboring the complementary receptor cDNA. Twenty double transfectants derived from clone 470.6 were identified by binding of both ^{125}I-IFN-α_2 and anti-IFN-α/βR antibody, while an additional set of 20 clones derived from clone 369.11 were identified by their antiviral response and luciferase induction with IFN-α_B (representative clones are shown in Tables 1 and 2). However, such cells were not responsive to other type I HuIFNs, probably because IFN-α/βR was expressed as a 51-kDa protein rather than the native 102-kDa protein as shown by immunoblotting (Fig. 1B).

Host cells coexpressing 51-kDa IFN-α/βR and IFNAR

TABLE 2. Induction of luciferase activity in cells expressing various type I IFN receptor components

Cell line (receptor[s])	Luciferase activity[a] (arbitrary U) in cells treated with:			
	No IFN	MuIFN-α/β	HuIFN-α_2	HuIFN-α_B
HeLa	0.055 ± 0.015		3.48 ± 0.78	2.35 ± 0.63
470.6 (IFNAR)	0.43 ± 0.11	6.8 ± 1.77	1.0 ± 0.31	7.8 ± 1.1
369.11 (IFN-α/βR)	0.440 ± 0.14	13.04 ± 2.9	0.83 ± 0.24	0.83 ± 0.13
508.12 (IFN-α/βR, IFNAR)[b]	0.54 ± 0.2	14.7 ± 3.2	1.7 ± 0.57	4.9 ± 0.9

[a] Average ± standard deviation.
[b] Derived from clone 369.11.

TABLE 3. Binding characteristics[a] of the type I IFN receptor in various configurations with ^{125}I-IFN-α_2 as the ligand

Cell line (receptor[s])	No. of binding sites/cell	K_d (M) at 20°C	ΔF (kcal/mol) at 37°C
Daudi	4,900 ± 11%	1.6×10^{-10} ± 20%	14
470.6 (IFNAR)	0		
369.11 (51-kDa IFN-α/βR)	80,000 ± 11%	3.6×10^{-9} ± 17%	12
508.12 (51-kDa IFN-α/βR, IFNAR)	59,000 ± 10%	4.07×10^{-10} ± 20%	13.4

[a] The values listed for binding sites and K_d are averages ± percent standard deviations. 1 kcal = 4.184 kJ.

(clone 508.12) were then tested for their affinity for the ligand. Such cells exhibited a saturable ligand binding of IFN-α_2 with about 10-fold-greater affinity than that of 369.11 cells expressing IFN-α/βR alone (Fig. 2B and C). This result indicated that IFNAR and IFN-α/βR cooperate in ligand binding and hence are functionally associated. The resulting affinity was close to that of the native receptor in Daudi cells (Table 3). The number of high-affinity receptors in clone 508.12 was similar to that in its parental clone 369.11, indicating that the expression levels of IFNAR and IFN-α/βR in the composite, high-affinity receptor are similar (Table 3).

A ternary complex of IFN-α_2 with IFN-α/βR and IFNAR. In order to study possible interactions between IFNAR and IFN-α/βR, control and IFN-treated Daudi and 508.12 cells were solubilized with detergent, immunoprecipitated either with anti-IFN-α/βR antibody or with anti-IFNAR antibody, and then immunoblotted with both antibodies. Each antibody immunoprecipitated its receptor, but coimmunoprecipitation was not observed (data not shown). This result did not rule out the possibility that IFNAR and IFN-α/βR become associated in the presence of their ligand, as it is possible that such association products dissociate back upon solubilization of the cells with the detergent-containing buffer.

In order to overcome the possible problem of dissociation upon detergent solubilization, we cross-linked ^{125}I-IFN-α_2 to various cell types and identified the resulting complexes by immunoprecipitation with specific antireceptor antibodies. This procedure provides a double specificity, that of the ligand-receptor interaction coupled with immunorecognition. ^{125}I-IFN-α_2 was cross-linked to intact cells, the cells were then solubilized with detergent, and cross-linked products were identified by immunoprecipitation with the specific antireceptor antibodies. In addition, the procedure was reversed: cells were first solubilized with detergent and then cross-linked to ^{125}I-IFN-α_2 and immunoprecipitated with the two antibodies. Under such conditions, only receptor components which have intrinsic ligand-binding activity form cross-linked products.

In the first set of experiments, we cross-linked ^{125}I-IFN-α_2 to intact murine cells expressing individual receptor components (Fig. 3A). Cells expressing IFNAR only (clone 470.6) did not yield any cross-linked complex, indicating once more that IFNAR alone does not bind IFN-α_2 (lane 1). In contrast, anti-IFN-α/βR antibody immunoprecipitated an ~70-kDa band from cells expressing the 51-kDa IFN-α/βR (clone 369.11 [Fig. 3A, lane 4]). The ~70-kDa product is therefore an equimolar complex of the 51-kDa IFN-α/βR and IFN-α2 (apparent mass, 17 kDa).

In the second set of experiments, murine cells coexpressing IFNAR and 51-kDa IFN-α/βR (clone 508.12) were first cross-linked with ^{125}I-IFN-α_2 and then solubilized and immunoprecipitated with either anti-IFN-α/βR antibody or anti-IFNAR

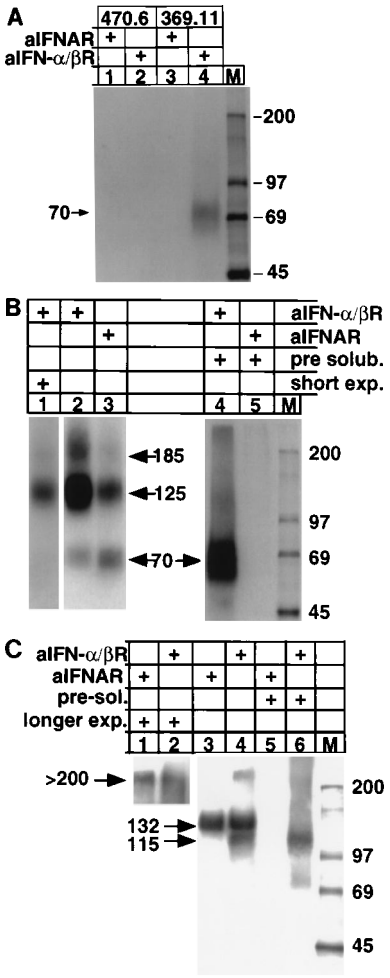

FIG. 3. Identification of various type I IFN receptor components by cross-linking to ^{125}I-IFN-α_2. From 5×10^7 to 1×10^8 cells were cross-linked to ^{125}I-IFN-α_2, detergent solubilized, and immunoprecipitated either by monoclonal anti-IFNAR antibody (aIFNAR) or by rabbit anti-IFN-α/βR antibody (aIFN-α/βR). Alternatively, cells were presolubilized (pre solub.) with a detergent and then cross-linked to ^{125}I-IFN-α_2 and immunoprecipitated as described above. The immunoprecipitates were analyzed by SDS-PAGE and autoradiography. The sizes (in kilodaltons) and positions of the resulting cross-linked complexes are indicated by arrows. (A) Cross-linked complexes of NIH 3T3 cells expressing either HuIFNAR (clone 470.6) (lanes 1 and 2) or HuIFN-α/βR (clone 369.11) (lanes 3 and 4). (B) Cross-linked complexes of NIH 3T3 cells expressing both IFNAR and IFN-α/βR (clone 508.12). (C) Cross-linked complexes of Daudi cells. exp., exposure. Molecular mass standards (in kilodaltons) (lanes M) are indicated on the right.

antibody (Fig. 3B). Both antibodies immunoprecipitated a 185-kDa product. This product corresponds in terms of size and immunoreactivity to a ternary complex of 51-kDa IFN-α/βR, 115-kDa IFNAR, and 17-kDa IFN-α_2 (Fig. 3B, lanes 2 and 3). Since covalent cross-linking produces low yields, it was expected that most of the 185-kDa complexes would not be fully cross-linked and that following immunoprecipitation by either antibody, they would be dissociated by SDS to yield partially cross-linked products. Indeed, two lower-molecular-mass products (~70 and 125 kDa) were obtained together with the 185-kDa complex (lanes 2 and 3). In order to sort these partially cross-linked products, we used a reversed procedure in which cells were first solubilized and then cross-linked and immunoprecipitated. Under these conditions, only the ~70-kDa complex was obtained by immunoprecipitation with anti-

TABLE 4. Identification of the complexes obtained by cross-linking of ^{125}I-IFN-α_2 with various type I IFN receptor configurations

Size of complex (kDa)	Formation in cell line[a]:				Component(s) participating in formation of complex
	Daudi	369.11 (IFN-α/βR)	470.6 (IFNAR)	508.12 (IFN-α/βR, IFNAR)	
~70	−	+	−	+++	51-kDa IFN-α/βR
115	++	−	−	−	102-kDa IFN-α/βR
125	−	−	−	+++	IFNAR
132	+++	−	−	−	IFNAR
185	−	−	−	++	51-kDa IFN-α/βR and IFNAR
>200	++	−	−	−	102-kDa IFN-α/βR and IFNAR

[a] −, no complex formed; +, low level of complex formed; ++, medium level of complex formed; +++, high level of complex formed.

IFN-α/βR antibody (lane 4), while no product was immunoprecipitated by anti-IFNAR antibody (lane 5). The ~70-kDa band is therefore the already-identified complex of IFN-α_2 and 51-kDa IFN-α/βR, while the 125-kDa band is a cross-linked complex of IFN-α_2 and IFNAR. The band identification is further supported by the observation that it was not formed in cells expressing IFN-α/βR alone (clone 369.11 [Fig. 3A, lanes 3 and 4]).

At this point, it became possible to identify the cross-linked products of IFN-α_2 with its native receptor on human cells. We cross-linked ^{125}I-IFN-α_2 to intact Daudi cells, solubilized the cells, and immunoprecipitated the resulting products with the two antibodies. In analogy with 508.12 cells, three products of >200, 132, and 115 kDa were obtained. These products were immunoprecipitated by the two antibodies, albeit at different efficiencies (Fig. 3C, lanes 1 to 4). The >200-kDa band represents in terms of size and immunoreactivity the fully cross-linked ternary complex of IFN-α_2 with the 102-kDa IFN-α/βR and the 115-kDa IFNAR. It is analogous to the 185-kDa ternary complex of 508.12 cells, except that it contains the native 102-kDa IFN-α/βR instead of the 51-kDa cloned receptor component. When the reversed procedure of solubilization followed by cross-linking was employed, only the 115-kDa complex was obtained by immunoprecipitation with anti-IFN-α/βR antibody (lane 6), while no product was immunoprecipitated by anti-IFNAR antibody (lane 5). Therefore, the 115-kDa band is the complex of IFN-α_2 and 102-kDa IFN-α/βR, while the 132-kDa band is a cross-linked complex of IFN-α_2 and IFNAR, analogous to the 125-kDa complex of 508.12 cells. The data on the various complexes and their proposed compositions are summarized in Table 4.

The apparent size of the IFN-α_2–IFNAR complex seems to vary with the cell type, and the reason for this variation is not known. The complex of human Daudi cells measures 132 kDa (corresponding to the 115-kDa IFNAR and 17-kDa IFN-α_2), while that of murine 508.12 cells (Fig. 3B) and the human fibrosarcoma cell line 2ftgh (data not shown) migrates as a 125-kDa protein.

IFNAR and IFN-α/βR associate at their ectodomains and are not preassociated. In order to localize regions of IFN-α/βR which participate in the association with IFNAR, we constructed an expression vector harboring an IFN-α/βR cDNA coding for a truncated (~44-kDa) receptor lacking the entire cytoplasmic domain. Clone 470.6 (expressing HuIFNAR) was transfected with this vector (pEF-BOS-IFNABRΔC), and stable double transfectants were isolated. Upon cross-linking of ^{125}I-IFN-α_2 to one of these clones (clone 2634ΔC) and immunoprecipitation with either anti-IFNAR antibody or anti-IFN-α/βR antibody, a 125-kDa band was obtained (Fig. 4, lanes 3 and 4). This band was previously identified as a cross-linked complex of ^{125}I-IFN-α_2 and IFNAR. Hence, the extracellular domain of IFN-α/βR is sufficient for interaction with IFNAR. The bands of 53 and 64 kDa are cross-linked complexes of ^{125}I-IFN-α_2 and IFN-α/βRΔC, since they were immunoprecipitated by the anti-IFN-α/βR antibody. The expected molecular mass of the cross-linked complex of ^{125}I-IFN-α_2 and IFN-α/βRΔC is ~60 kDa, and the mechanism by which a smaller complex (53 kDa) was formed is not known.

The experiments done so far did not rule out the possibility that IFNAR and IFN-α/βR were preassociated even before addition of the ligand. In order to study this issue, we cross-linked intact Daudi cells (1.75×10^8 cells) with disuccinyl suberate in the absence of IFN-α_2 and immunoprecipitated and immunoblotted the detergent-solubilized cells with anti-IFN-α/βR antibody. Upon immunoblotting with the same antibody, a band corresponding to 102-kDa IFN-α/βR and a large ~50-kDa band corresponding both to the immunoglobulin heavy chain and to the 51-kDa IFN-α/βR were obtained. No cross-linked IFN-α/βR complexes with a molecular mass higher than 102 kDa were detected, in spite of the fact that a very large number of Daudi cells were used (Fig. 5). Hence, we consider it highly unlikely that IFN-α/βR and IFNAR are preassociated to form high-molecular-mass complexes.

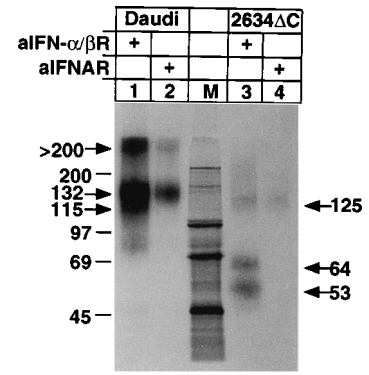

FIG. 4. The cytoplasmic domain of IFN-α/βR is not essential for interaction with IFNAR. NIH 3T3 cells expressing IFNAR and IFN-α/βR which lacks the entire cytoplasmic domain (clone 2634ΔC; 7×10^7 cells) were cross-linked to ^{125}I-IFN-α_2, detergent solubilized, and immunoprecipitated either by monoclonal anti-IFNAR antibody (aIFNAR) (lane 3) or by rabbit anti IFN-α/βR antibody (aIFN-α/βR) (lane 4). In both cases, the 125-kDa complex of IFNAR and ^{125}I-IFN-α_2 was detected. Immunoprecipitates of the IFN receptor of Daudi cells, cross-linked to ^{125}I-IFN-α_2 are shown as a control (lanes 1 and 2 for aIFN-α/βR and aIFNAR, respectively). The immunoprecipitates were analyzed by SDS-PAGE and autoradiography. The positions of the relevant bands (arrows) and molecular standards (lane M) (dashes) are indicated in kilodaltons.

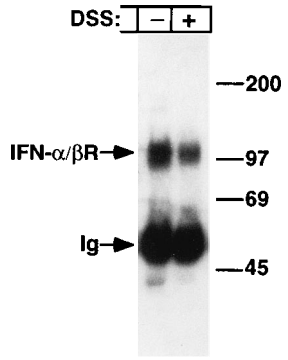

FIG. 5. The 102-kDa IFN-α/βR does not form high-molecular-mass complexes in the absence of a ligand. Human Daudi cells (1.75×10^8 cells) were either cross-linked with disuccinyl suberate (DSS) (+) or not cross-linked (−). The cells were detergent solubilized, immunoprecipitated with anti-IFN-α/βR antibody and protein A-Sepharose, resolved by SDS-PAGE (7.5% acrylamide) under reducing conditions (β-mercaptoethanol), and immunoblotted with anti-IFN-α/βR antibody and ^{125}I-protein A. A band corresponding to 102-kDa IFN-α/βR and an ~50-kDa band corresponding to both immunoglobulin heavy chain (Ig) and 50-kDa IFN-α/βR are indicated by arrows. In both lanes, no complex of more than 102 kDa is visible. Molecular mass markers are shown (in kilodaltons) on the right.

The 51-kDa IFN-α/βR is a component of a functional receptor. Since no functional receptor was reconstituted in murine cells expressing both the 51-kDa HuIFN-α/βR and HuIFNAR, it was necessary to check whether the 51-kDa HuIFN-α/βR was indeed a component of a functional type I IFN receptor. For this purpose, HeLa cells were transfected either with pEF-BOS-IFNABR or with pEF-BOS-IFNABRΔC. Stable transfectants were subcloned and selected by their ability to bind an anti-IFN-α/βR antibody. All cells were tested for their response to IFN by transient transfection with pxmuISRE-TKluc prior to treatment with IFN-α_2 or IFN-β and measurement of luciferase activity. Clone 191, exhibiting a twofold-increased expression of IFN-α/βR over that of HeLa cells, produced an activity three- to fourfold higher than that of IFN-treated native HeLa cells when it was treated with various type I HuIFNs, including IFN-α_B. In contrast, clone 225.51, expressing high levels of the truncated form of IFN-α/βR, produced the same level of IFN-induced luciferase activity as nontransfected HeLa cells (Fig. 6). These results indicate that 51-kDa IFN-α/βR is part of the functional type I IFN receptor and not a futile receptor (e.g., a clearance receptor or a receptor antagonist).

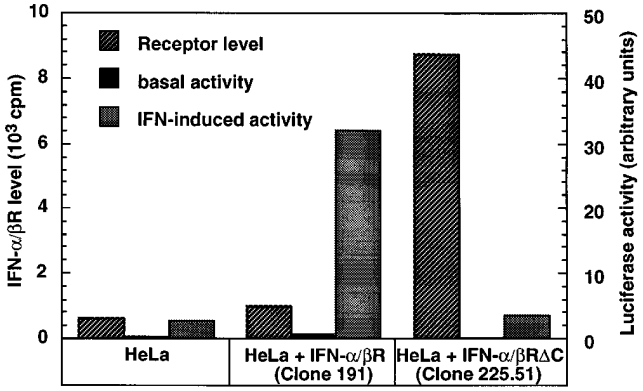

FIG. 6. The cloned IFN-α/βR is active in human cells. HeLa cells stably expressing either the cloned IFN-α/βR (clone 199) or a truncated form lacking the cytoplasmic domain (clone 225.51), as well as parental HeLa cells, were transiently transfected with the luciferase reporter vector pxmuISRE-TKluc, and the level of luciferase activity was determined before and after induction with HuIFN-α_2. The level of expressed IFN-α/βR was determined by binding of anti-IFN-α/βR antibody and ^{125}I-protein A to the cells.

DISCUSSION

This study elucidates the relationship between two components of the type I IFN receptor and the mode of their interaction with the ligand. We show here that the 102-kDa IFN-α/βR (22) and the 115-kDa IFNAR (29) are components of a common type I IFN receptor. Upon ligand binding, the two components associate with the ligand and form a ternary complex.

Our studies with cells expressing individual and combined receptor components show that IFN-α/βR is the primary ligand-binding subunit, while IFNAR has no intrinsic affinity for IFN-α_2. These results are in accordance with previous studies, showing that high-level expression of HuIFNAR does not provide binding sites for IFN-α_B (8, 14). The increased (10-fold) affinity exhibited by cells coexpressing IFNAR and IFN-α/βR indicates that these two polypeptides are functionally associated. Furthermore, cross-linked complexes of IFN-α_2 and IFNAR are obtained only in cells expressing IFN-α/βR. Hence, IFN-α_2 must bind to its receptor in two steps. First it binds to IFN-α/βR and forms an intermediate complex. The resulting intermediate is then attached to IFNAR to form a ternary complex. Cross-linked complexes of IFNAR and IFN-α_2 in Daudi and U266 cells were recently reported (1, 6). Since a variant IFN-α/βR lacking the cytoplasmic domain can still generate a ternary complex with IFNAR and IFN-α_2, we conclude that IFNAR and IFN-α/βR interact at their extracellular domains. Since we could not detect higher-molecular-mass complexes of IFN-α/βR upon cross-linking of human Daudi cells in the absence of ligand, preassociation between IFNAR and IFN-α/βR is highly unlikely.

We found that the levels of antiviral activity and the induction of luciferase activity by IFN-α_B in murine cells expressing both IFNAR and IFN-α/βR were lower than those detected in their parental cells, which express IFNAR only (Tables 1 and 2). In contrast with HuIFNAR, HuIFN-α/βR effectively binds HuIFN-α_B (22). Hence, we propose that the overexpressed 51-kDa HuIFN-α/βR probably acts as a dominant negative element, competing for HuIFN-α_B with the murine receptor. In contrast with the effect in murine cells, HuIFN-α/βR is agonistic in human cells, as we showed that a modest overexpression of IFN-α/βR increased considerably their response to several type I IFNs, including IFN-α_B. The relationship between the cloned 51-kDa IFN-α/βR and the native 102-kDa IFN-α/βR is still not clear. Apparently, the 102-kDa IFN-α/βR is not a homodimer of the cloned 51-kDa protein, since murine cells transfected with the 51-kDa IFN-α/βR cDNA expressed a 51-kDa protein rather than a 102-kDa protein.

The finding that the type I IFN receptor consists of IFNAR and the 102-kDa IFN-α/βR was not unexpected. A model of the receptor, based on two components, was suggested in previous studies (8, 28). This proposed structure of the type I IFN receptor bears structural and functional resemblance to other multicomponent cytokine receptors, e.g., the family of gp130 receptors (interleukin-6 [IL-6], CNTF, oncostatin M, LIF, and IL-11 receptors). The ligands of the type I IFN receptor, IL-6 receptor, and CNTF receptor bind to specific components (α-chains) and the resulting intermediates then bind to a signal transducing β-chain (either IFNAR [as discussed herein] or

gp130 [13]). Hence, IFN-α/βR may be considered as the α-chain and IFNAR as the β-chain of the type I IFN receptor.

Ligand-induced association of IFNAR and IFN-α/βR is probably the event that leads to signaling across the plasma membrane. The cytoplasmic tyrosine kinase JAK1 is constitutively associated with the 102-kDa IFN-α/βR, while Tyk2 is associated with IFNAR (2, 22). Ligand-induced association of IFNAR and IFN-α/βR may juxtapose their associated kinases, thereby enabling their cross-phosphorylation. The likelihood of such a mechanism of cross-phosphorylation is supported by the finding that phosphorylation of Tyk2 and JAK1 in IFN-α-treated cells required the mutual presence of both kinases (16). Similarly, cross-phosphorylation of JAK2 and JAK1 was suggested for the type II IFN receptor (25) and is the accepted signaling mechanism for many growth factor receptors and cytokine receptors (13, 27). Thus, the overall structure of the type I IFN receptor and its mode of interaction with the ligand suggest a rational mechanism of transmembrane signaling by this receptor.

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