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Role of interferon α/β receptor chain 1 in the structure and transmembrane signaling of the interferon α/β receptor complex

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ABSTRACT **A previously cloned cDNA encodes one subunit of the human interferon α/β receptor (IFNαR), denoted IFNαR1. To study the expression and signaling of IFNαR1, we used monoclonal antibodies (mAbs) generated against the baculovirus-expressed ectodomain of IFNαR1. Immunoprecipitation and immunoblotting of lysates from a variety of human cell lines showed that IFNαR1 has an apparent molecular mass of 135 kDa. Binding analysis with ^{125}I-labeled mAb demonstrated high levels of cell surface expression of IFNαR1 in human cells and in mouse cells transfected with IFNαR1 cDNA, whereas no cross-reactivity was observed in control mouse L929 cells expressing only the endogenous mouse receptor. The subunit was rapidly down-regulated by IFNα (80% decrease within 2 hr) and degraded upon internalization. The IFNαR1 chain appeared to be constitutively associated with the 115-kDa subunit of the IFNα/β receptor, since the mAbs coprecipitated this protein. IFNα/β treatment induced tyrosine phosphorylation of IFNαR1 within 1 min, with kinetics paralleling that of the IFN-activated protein-tyrosine kinases Jak1 and Tyk2. Ligand-induced tyrosine phosphorylation of IFNαR1 was blocked by the kinase inhibitors genistein or staurosporine. Although IFNαR1 cDNA-transfected mouse cells expressed high levels of this subunit when compared with empty vector-transfected cells, the number of binding sites for human IFNα (50–75 sites per cell) was not increased. Human IFNα induced the expression of a mouse IFNα/β-responsive gene (the 204 gene) in mouse L929 cells transfected with the IFNαR1 cDNA, but not in mock-transfected cells. These results suggest that the IFNαR1 subunit acts as a species-specific signal transduction component of the IFNα/β receptor complex.**

The type I interferons (IFNs), α and β, are cytokines which exert major biologic effects (antiviral, antiproliferative, immunomodulatory). While IFNs α and β appear to compete with one another for binding to a common cell surface receptor, immune IFN (IFNγ) binds to a distinct receptor (1). Binding sites for IFNα/β are ubiquitously present on cells, including tumor cell lines resistant to the antiproliferative and antiviral actions of IFN (2, 3). The crosslinking of ^{125}I-IFNα to several human cell lines indicates that the IFNα/β receptor is a multiprotein complex, consisting of 95-, 115-, and 135-kDa subunits (3–5).

Type I IFNs induce the transcription of specific early genes, the IFN-stimulated genes (ISGs), through the activation of the Jak1 and Tyk2 protein-tyrosine kinases (PTKs) (6, 7). PTK activation mediates the IFN-induced rapid tyrosine phosphorylation of the multiprotein latent cytosolic transcription factor ISGF3, which consequently translocates to the nucleus, where it interacts with conserved promoter elements in ISGs (8, 9). This model of PTK activation resulting in the tyrosine phosphorylation of specific transcription factors and gene activation has been described for several cytokines (6, 10, 11) and serves as a paradigm for cytokine signaling. In contrast, the early events triggered by IFNα receptor occupancy and the functions of the various subunits are poorly characterized.

A cDNA coding for one subunit (IFNαR1) of the human IFNα/β receptor (IFNαR) has been cloned and, when transfected into mouse hepatoma cells, confers an antiviral response to one human IFNα/β subtype (IFNα8) (12). The mouse and bovine homologues of IFNαR1 have been cloned and also confer sensitivity to the antiviral action of IFNα when transfected into nonresponsive cells (12–14). These results clearly demonstrate that the IFNαR1 chain plays a central role in signal transduction through the IFNαR. Sequence analysis of this subunit identified it as a member of the cytokine receptor superfamily (15). The predicted translation product of this cDNA is a 63-kDa protein with 10 potential N-linked glycosylation sites in the extracellular domain (12). Transfection of this subunit into IFNα-nonresponsive mouse or human cells produces high antiviral sensitivity to multiple IFNα subspecies (IFNα2, -α8, and -αCon1), without increasing cellular binding of any of these subspecies (16). These results led to the working hypothesis that human IFNαR1 is a signal-transducing subunit of the human IFNαR, analogous to the recently cloned β subunit of the IFNγ receptor (17, 18).

In the present study we have used five different monoclonal antibodies (mAbs) generated against the extracellular domain of IFNαR1 expressed in baculovirus. We report that human cell lines express an excess of IFNαR1 chains over IFNα/β binding sites. IFNαR1 was down-regulated upon IFNα treatment within 2 hr and was degraded. Both IFNα and -β induced tyrosine phosphorylation of IFNαR1 in a variety of human cells within minutes, an effect blocked by PTK inhibitors. The time course was equivalent to that of Jak1 and Tyk2 tyrosine phosphorylation. Immunoblot analysis and immunoprecipitation of surface-iodinated material showed that IFNαR1 was 135 kDa. A receptor subunit of 115 kDa appeared to be constitutively associated with IFNαR1, since the mAbs against the IFNαR1 chain coprecipitated this subunit. Further, mouse cells transfected with IFNαR1 cDNA had high levels of cell surface expression of this chain compared with control mouse cells, although they expressed the same number of binding sites for human IFNα (75 sites per cell) as the empty vector-transfected or parental cells. Mouse IFNα/β induced expression of the IFN-responsive mouse 204 gene (19) in mouse L929 cells transfected with IFNαR1 cDNA or with empty vector. However, human IFNα induced 204 gene expression only in cells expressing

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Abbreviations: BCS, bovine calf serum; IFN, interferon; IFNαR, IFNα/β receptor; ISG, IFN-stimulated gene; IU, international unit(s); mAb, monoclonal antibody; PTK, protein-tyrosine kinase; pTyr, phosphotyrosine.

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human IFNαR1. Thus, the IFNαR1 chain of the type I IFN receptor transduces signals generated by human IFNα/β binding to cells.

MATERIALS AND METHODS

Cells. Human Daudi cells and a resistant Daudi subclone were maintained at 2.5–10 × 10^5 cells per ml in RPMI 1640 containing 10% defined bovine calf serum (BCS; HyClone). Mouse L929 cells, mouse–human hybrid WA-17 cells (mouse L cells containing three copies of human chromosome 21), and the human fibroblast HFS cell line were maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% BCS. Human chromosome 21 codes for binding as well as transducing subunits of human IFNαR. Mouse L929 transfectants that express IFNαR1 (16) or empty-vector transfectants were maintained in DMEM with 10% BCS and G418 at 250 μg/ml.

IFNs. Human IFNαCon1 was generously provided by L. Blatt (Amgen). Human IFNβser17 (Betaseron) was from Berlex Biosciences. IFN activities are expressed in international units (IU)/ml as assayed by protection against the cytopathic effect of vesicular stomatitis virus on human fibroblasts, using the National Institutes of Health human IFNα standard for reference.

Production of mAbs Against the Ectodomain of IFNαR1. IFNαR1 ectodomain containing a FLAG peptide (GIBCO/BRL) at the C-terminus was expressed in baculovirus, purified on immunoaffinity columns, and used to immunize mice (unpublished work). Splenocytes derived from mice having a high serum titer for IFNαR1 were used for fusion with Pu38 myeloma cells. The resultant hybridomas were screened by ELISA, followed by flow cytometry using Daudi cells.

IFNα and Anti-IFNαR1 mAb Binding to Cells. IFNαCon1 was iodinated with Iodo-Gen (Pierce) (2) to specific activities of 75–100 Ci/g (1 Ci = 37 GBq). Iodinated IFN preparations were routinely >95% biologically active in antiviral and antiproliferative assays. mAb 4B1, directed to the ectodomain IFNαR1, was iodinated with Iodo-Gen to 75 Ci/g. Saturation and Scatchard analyses were performed as described (2). The equilibrium dissociation constants, K_d of ^{125}I-IFNαCon1 and ^{125}I-4B1 were 50 and 125 pM, respectively.

Surface Iodination of Cells. Surface proteins on 5 × 10^7 Daudi cells were iodinated with 1 mCi of Na^{125}I in Iodo-Gen (20 μg/ml)-coated tubes for 30 min. Cells were washed with phosphate-buffered saline (PBS) at 4°C and lysed in 1% Nonidet P-40 buffer [300 mM NaCl/50 mM Tris, pH 7.4/1% (vol/vol) Nonidet P-40/1 mM phenylmethanesulfonyl fluoride with soybean trypsin inhibitor (5 μg/ml), leupeptin (5 μg/ml), and benzamidine (1.75 μg/ml)] for 20 min on ice. Samples were clarified by centrifugation at 12,000 × *g* for 15 min at 4°C prior to use in immunoprecipitations.

Crosslinking of ^{125}I-IFN to Cell Surface Receptors. Daudi cells were incubated at 5 × 10^6 per ml in binding medium (RPMI 1640 with 5% BCS and 20 mM Hepes, pH 7.4) with 200 pM ^{125}I-IFNαCon1 for 2 hr at 15°C. Cells were washed with PBS at 4°C and incubated with 25 mM disuccinimidyl suberate (Pierce) for 30 min at 15°C. Samples were lysed in buffer with 1% Nonidet P-40 and used for immunoprecipitation.

Immunoprecipitations and Immunoblot Analysis. Cell lysates were immunoprecipitated overnight with either anti-IFNαR1 mAbs or mouse IgG1. In addition, immunoprecipitations were also performed with polyclonal rabbit serum directed against Jak1, Jak2, or Tyk2 (Upstate Biotechnology, Lake Placid, NY) or with nonimmune serum. The immunocomplexes were collected on protein A-Sepharose beads (Pharmacia). Proteins were eluted in sample buffer for SDS/PAGE in 7.5% or 5–15% gradient polyacrylamide gels (3). Radioactivity was quantitated on a Molecular Dynamics model 425 PhosphorImager.

For phosphorylation studies cells were treated with IFNα or -β at 37°C for the indicated times, washed with ice-cold PBS, and lysed for 20 min in lysis buffer [50 mM Tris, pH 7.4/150 mM NaCl/1 mM EDTA/0.5% Nonidet P-40/15% (vol/vol) glycerol] containing 1 mM NaF, 1 mM orthovanadate, 1 mM phenylmethanesulfonyl fluoride, soybean trypsin inhibitor (5 μg/ml), leupeptin (5 μg/ml), and benzamidine (1.75 μg/ml) (20). Samples were centrifuged at 12,000 × *g* for 15 min at 4°C and supernatants were immunoprecipitated with anti-IFNαR1 mAb, antibodies against Jak PTKs, or control mouse sera overnight at 4°C. Immune complexes were collected on protein A beads (precoated with rabbit anti-mouse IgG) and eluted in SDS/PAGE sample buffer. Samples were run in SDS/7.5% polyacrylamide gels, transferred to Immobilon (Millipore), and probed with anti-phosphotyrosine (pTyr) mAb (Ab-2, dilution 1:500; Oncogene Science) followed by peroxidase-coupled anti-mouse IgG and ECL detection (Amersham). The blots were stripped and reblotted with antibodies against the immunoprecipitated protein (to validate that equal amounts of protein were in each lane).

Gene Expression. Total RNA was isolated from cells by (*i*) the guanidinium thiocyanate/CsCl method and purified by phenol/chloroform extraction or (*ii*) a single-step method (21, 22). Total RNA (40 μg) was denatured, electrophoresed, and transferred onto nitrocellulose, and blots were hybridized to nick-translated ^{32}P-labeled DNA probes (204 gene and γ-actin) for 16–18 hr (23). Blots were exposed to phosphorimaging screens for 2–4 days.

RESULTS

Expression of IFNαR1 Protein. Saturation binding studies with ^{125}I-4B1 mAb against IFNαR1 and ^{125}I-IFNα showed 10,200 4B1-specific (IFNαR1) and 3800 IFNα binding sites per human Daudi lymphoblastoid cell (Fig. 1). A difference between mAb and ligand binding sites was demonstrated repeatedly, suggesting that the number of IFNαR1 chains expressed in a given cell line exceeds the number of ligand binding sites even in the most conservative estimation (binding of only one IFNαR1 chain per IgG molecule). In direct competition studies 4B1 mAb did not compete with IFNα for cell surface binding. IFNα treatment (20,000 IU/ml, 18 hr) of Daudi cells down-regulated (by ≈95%) cell surface expression of both IFNα binding sites and IFNαR1 sites. Similar numbers of IFNαR1 chains and IFNα binding sites were obtained for the mouse–human hybrid WA-17 cell line (Table 1), which contains three copies of human chromosome 21. In

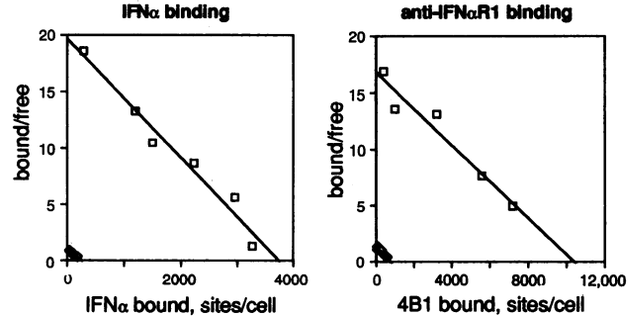

FIG. 1. Binding of IFNα and 4B1 anti-IFNαR1 mAb to Daudi cells. Control (□) and IFNα-pretreated (20,000 IU/ml, 18 hr) (◇) cells (1.5 × 10^6 per ml) were incubated with ^{125}I-ligand (0.01–0.3 nM) for 100 min at 15°C. Cell-associated radioactivity was determined after centrifugation through a mixture of phthalate oils (2). Specific binding is the difference in ^{125}I-ligand binding in the absence and presence of 20 nM unlabeled ligand. Data are plotted according to the method of Scatchard (24).

Table 1. Number of 4B1 anti-IFNαR1 mAb and IFNα binding sites on various human and mouse cell lines

Cell line	No. of binding sites per cell	
	4B1	IFNα
Daudi (human)	10,200 ± 900	3800 ± 1100
HeLa S3 (human)	2,500 ± 700	1600 ± 400
HEC1B (human)	2,300 ± 500	1300 ± 300
WA17 (mouse–human hybrid)	4,200 ± 300	2500 ± 600
L929 (mouse)	≤60 ± 20	≤75 ± 25
V2 (vector-transfected mouse)	≤60 ± 30	≤75 ± 25
T1 (IFNαR1-transfected mouse)	15,200 ± 900	≤75 ± 25
T2 (IFNαR1-transfected mouse)	12,700 ± 600	≤75 ± 25

Adherent cells plated in 24-well plates (10^5 cells per well) or suspension cells (Daudi and HeLa S3, 2×10^6 cells per ml) were incubated with ^{125}I-ligand for 4 hr at 4°C or 100 min at 15°C, respectively. The number of binding sites (mean ± SEM) was determined by Scatchard analysis of specific ligand binding.

contrast, no 4B1 sites were detected on control mouse L929 cells expressing the endogenous mouse receptor.

To further characterize the cell surface expression of IFNαR1, we chemically crosslinked ^{125}I-IFN to cell surface receptors, prepared cell lysates, and immunoprecipitated with 4B1 mAb. Various human cells express subunits of 95, 115, and 135 kDa which can be crosslinked to IFNα (3), as well as a complex of ≈260 kDa representing an association of receptor subunits (4). In chemical crosslinking the apparent size of receptor subunits is increased by the covalent coupling of the 20-kDa IFNα to each receptor subunit. mAbs 4B1, 40H2, and 25C3 immunoprecipitated 135-, 155-, and 260-kDa proteins corresponding to 115- and 135-kDa subunits (Fig. 2*A*). The 95-kDa subunit observed in samples directly subjected to SDS/PAGE was not precipitated by mAb. Therefore, the IFNαR1 chain was present in the IFN receptor complex consisting of 115- and 135-kDa subunits. Results were confirmed by coprecipitation of these subunits with anti-IFNαR1 mAb from surface-iodinated material (unpublished work).

Immunoprecipitation of cell lysates with 4B1 anti-IFNαR1 mAb followed by immunoblot analysis using 40H2 mAb showed that 4B1 specifically precipitated a protein of 135 kDa from various human cell lines (Daudi, HeLa S3, and fibroblasts) (Fig. 2*B*). In contrast, 4B1 immunoblots of cell lysates immunoprecipitated with control mouse IgG1 sera detected no specific bands. Furthermore, 4B1 immunoblotting of affinity crosslinked samples revealed a band at ≈155 kDa corresponding to the 135-kDa subunit crosslinked to IFNα (data not shown). Therefore, IFNαR1 corresponds to the 135-kDa protein observed in immunoblots, as well as to the ≈155-kDa complex detected in affinity-crosslinked complexes. The 135-kDa IFNαR1 subunit was down-regulated by overnight treatment of cells with IFNα. IFNα treatment reduced the level of this protein by 80% within 2 hr (Fig. 2*C*). Since immunoblotting detects total IFNαR1 protein levels in cells and not only surface-expressed chains (detected by binding), down-regulation of the cell surface IFNαR1 chains was followed by degradation.

Tyrosine Phosphorylation of IFNαR1. One of the earliest signal transduction events induced by human IFNα/β in human cells is the activation of the Jak1 and Tyk2 PTKs. The activation of Jak PTKs by cytokines results in the tyrosine phosphorylation of receptor chains (11). IFNα and IFNβ induced tyrosine phosphorylation of the 135-kDa IFNαR1 chain (Fig. 3*A*). Ligand-induced tyrosine phosphorylation of IFNαR1 was detected within 1 min, peaked at 5 min, and was still detectable at 1 hr after IFN addition. Immunoprecipitation of cells after IFNα or -β treatment with control mouse IgG1 detected no tyrosine-phosphorylated bands. Pretreatment of cells (15 min) with the tyrosine kinase inhibitors

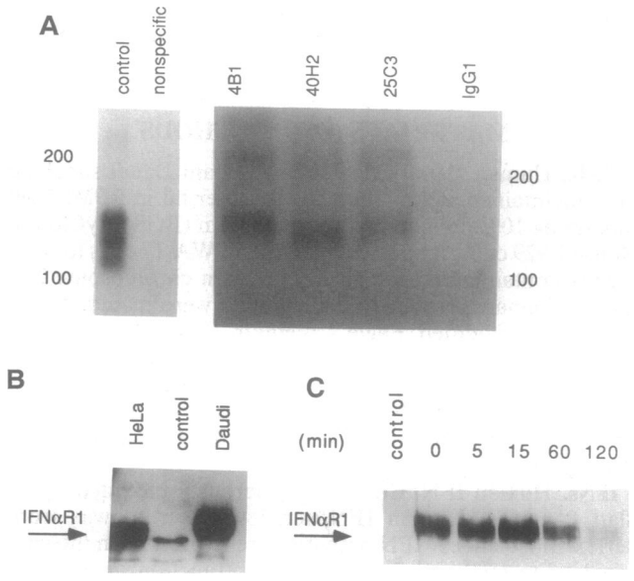

FIG. 2. Expression and down-regulation of the IFNαR1 chain. (*A*) ^{125}I-IFNα was affinity crosslinked to Daudi cells, cell lysates were prepared and used for immunoprecipitation with anti-IFNαR1 mAb (4B1, 40H2, and 25C3) or mouse IgG1, and proteins were separated by SDS/PAGE. All of these mAbs precipitated ≈20% of the input radioactivity. A nonprecipitated crosslinked sample (control) and a crosslinking performed in the presence of a 100-fold excess of unlabeled IFNα (nonspecific) are included for reference. Molecular size markers (100 and 200 kDa) are indicated. (*B*) Daudi and HeLa S3 cells (10^8 per lane) were immunoprecipitated with 4B1 anti-IFNαR1 mAb or control IgG1 and analyzed by SDS/7.5% PAGE followed by immunoblotting with 40H2 mAb. Similar results were obtained with other combinations of anti-IFNαR1 mAbs. (*C*) Daudi cells were treated with IFNα (20,000 IU/ml) for various times and subjected to immunoprecipitation and immunoblotting as in *B*.

genistein and staurosporine (25) abolished IFNα-induced tyrosine phosphorylation of IFNαR1 (Fig. 3*B*), demonstrating that the reactivity of the 135-kDa band with anti-pTyr mAb reflects ligand-dependent tyrosine phosphorylation. Stripping and reprobing immunoblots with anti-IFNαR1 mAb showed equivalent levels of IFNαR1 irrespective of inhibitor or IFN treatment.

We also examined the time course of tyrosine phosphorylation of the Jak1 and Tyk2 PTKs. At various times after IFN addition, cell lysates were immunoprecipitated with anti-Jak1 or -Tyk2, followed by anti-pTyr immunoblotting. Tyrosine phosphorylation of Tyk2 was detectable within 5 min and reached undetectable levels within 60 min (Fig. 3*C*). A similar time course was observed with Jak1. Stripping and reprobing of immunoblots with anti-PTK sera showed that the protein levels of these PTKs was unchanged irrespective of treatment. To test whether the IFNαR1 was associated with either of the IFN-activated PTKs, anti-IFNαR1 immunoprecipitates were immunoblotted with anti-Tyk2 or -Jak1. Neither Jak1 nor Tyk2 was detected in anti-IFNαR1 immunoprecipitates, even when cells were lysed with the less stringent detergent Brij 96 (26). However, antisera against Jak1 and Tyk2 precipitated the 95-kDa receptor subunit, as well as the IFNαR1 chain in affinity crosslinked material (data not shown). Thus, our results suggest that the IFN-activated PTKs are associated with the 95-kDa chain of the type I IFN receptor, in agreement with recent findings (27).

Expression of IFNαR1 and Induction of Biologic Effects. We recently reported on the expression of IFNαR1 cDNA in resistant human and mouse cells (16). Human K562 or mouse L929 cells transfected with this cDNA showed a restored antiviral response to human IFNα but showed no increase in binding for any human IFNα subtype, suggesting that this

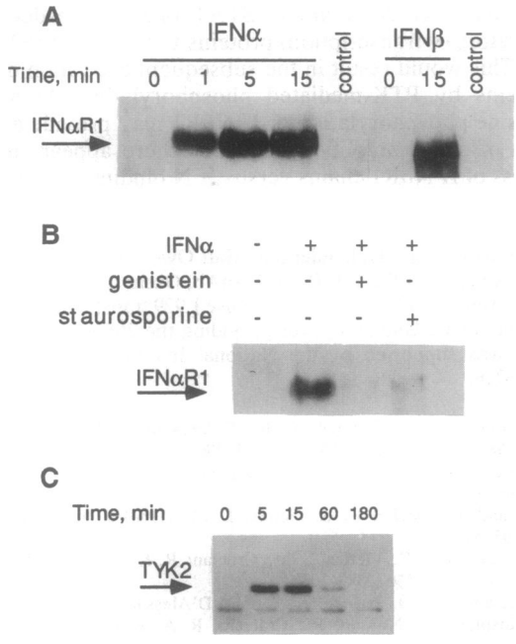

FIG. 3. IFNα/β-induced tyrosine phosphorylation of the IFNαR1 chain and Tyk2 PTK. (*A*) Daudi cells were incubated in the presence or absence of IFNα or -β (5000 IU/ml) for the indicated times. Cell lysates were immunoprecipitated with 4B1 anti-IFNαR1 or control IgG1 and analyzed by SDS/7.5% PAGE followed by immunoblotting with anti-pTyr mAb and detection by enhanced chemiluminescence. (*B*) Daudi cells were pretreated for 15 min with either genistein (100 μg/ml) or staurosporine (0.3 mM) and then incubated with IFNα (5000 IU/ml) for 15 min. Cell lysates were immunoprecipitated with 4B1 and immunoblotted with anti-pTyr mAb as described in *A*. (*C*) Daudi cells were incubated in the presence or absence of IFNα (5000 IU/ml) for the indicated times. Cell lysates were immunoprecipitated with anti-Tyk2 and analyzed by SDS/7.5% PAGE followed by immunoblotting with anti-pTyr mAb.

subunit functions as a signal transducer. Using anti-IFNαR1 mAb, we measured the cell surface expression of this receptor subunit, which was then compared with the number of IFNα binding sites. Parental L929 cells or empty vector-transfected cells had 50–75 high-affinity sites per cell for human IFNα and showed no detectable binding of iodinated 4B1 mAb (Table 1). WA-17 cells, which have three copies of human chromosome 21 (containing the whole IFN receptor complex locus) served as positive control. WA-17 cells had both IFNα/β and 4B1 (anti-IFNαR1) binding sites. Two independent IFNαR1 transfectants, T1 and T2 (formerly called SVX1 and SVX2, respectively), showing high sensitivity to the antiviral effect of IFNα against vesicular stomatitis virus (16), had >12,000 4B1 binding sites per cell, but the same low level (50–75 sites per cell) of high-affinity IFNα binding sites. Thus, expression of IFNαR1 alone did not confer high-affinity IFNα binding.

IFNαR1 expression confers antiviral responsiveness to several human IFNα/β subtypes (16). The induction of antiviral activity is believed to involve the selective expression of a group of early genes. We next investigated whether IFNαR1 expression in mouse cells conferred sensitivity to human IFN as determined by the induction of the mouse IFN-responsive 204 gene (19). Whereas human IFNα failed to induce 204 gene expression in L929 cells or empty vector-transfected cells (V2), human IFNα markedly induced expression in the two IFNαR1 transfectants (T1 and T2) (Fig. 4). Similar levels of 204 transcript were induced above the low constitutive level in the various L929-derived cell lines in response to mouse IFNα/β. The additional IFN-inducible

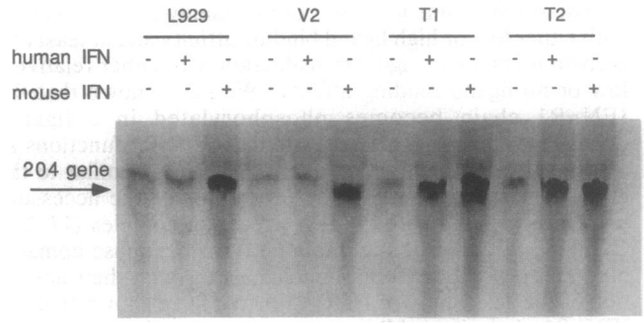

FIG. 4. Mouse 204 gene expression in mouse L cells transfected with IFNαR1 cDNA or empty vector. Parental L929 cells, empty vector-transfected cells (V2), and IFNαR1 cDNA-transfected cells (T1 and T2) were treated in the absence or presence of mouse IFNα/β or human IFNα (1000 IU/ml, 1 hr). Total RNA was isolated and examined by Northern blot analysis for the expression of the mouse IFN-responsive 204 gene.

mRNA reflects the cross-hybridization of the mouse 204 cDNA probe to the related IFN-responsive 202 mRNA (19). Thus, IFNαR1, when highly expressed in mouse cells, transduced the signal generated by low levels of human IFNα binding to mouse cell surface receptors, and thereby induced IFN-responsive genes and antiviral activity.

DISCUSSION

Using mAbs directed against the ectodomain of the IFNαR1 chain of the human IFNα/β receptor expressed in baculovirus, we have characterized the expression of the IFNαR1 chain in various cells. The mAbs detect expression of the IFNαR1 chain by flow cytometry, immunoblotting, and immunoprecipitation and are apparently directed against different extracellular epitopes of IFNαR1. In studies with ^{125}I-labeled anti-IFNαR1 mAb, we found ≈10,000 IFNαR1 chains expressed at the cell surface on Daudi cells. IFNα treatment (0.1 nM, 24 hr) of Daudi cells down-regulated surface expression of the IFNαR1 chains and IFNα binding sites by 95%, suggesting that more than one IFNαR1 chain interacts with each IFNα binding site.

IFNαR1 is expressed on the cell surface in a variety of human cell lines. Immunoblotting of cell lysates demonstrated that IFNαR1 migrates as a broad band at ≈135 kDa. The IFNαR1 cDNA codes for a translation product of ≈63 kDa (12) with 12 potential N-linked glycosylation sites, 10 of which are in the extracellular domain. Since the 135-kDa protein is recognized by mAb against the IFNαR1 chain of the human IFNαR in affinity-crosslinked material, surface-iodinated material, and immunoblots, our results indicate that IFNαR1 is subject to extensive glycosylation. Chemical crosslinking of ^{125}I-IFNαCon1 to a variety of human cell lines has shown that the IFNαR complex consists of 95-, 115-, and 135-kDa glycoproteins (3–5). Thus, our results indicate that the 135-kDa protein observed by affinity crosslinking represents IFNαR1. In addition, the 135-kDa chain coprecipitated with the 115-kDa protein in affinity-crosslinked and surface-iodinated material, suggesting that these proteins are associated prior to ligand binding. The 95-kDa component of the receptor was not detected in anti-IFNαR1 precipitates, although it is a major component of ^{125}I-IFNα crosslinked material (3) and was detectable in anti-Jak1 and -Tyk2 precipitates.

Structural analysis of the IFNαR1 chain classifies it as a member of the cytokine receptor superfamily (10, 28), which includes receptors for interleukins, IFNγ, ciliary neurotrophic factor, somatotropin, erythropoietin, nerve growth factor, tumor necrosis factor, leukemia inhibitory factor, and

oncostatin M. Some members of this family have an α chain with either low or high ligand binding affinity and at least one β chain involved in signal transduction with either relatively low or no ligand binding affinity. We have shown that the IFNαR1 chain becomes phosphorylated in a ligand-dependent manner. This suggests that IFNαR1 functions as a β signal-transducing component of IFNαR, similar to the gp130 chain of the interleukin 6 receptor or the accessory protein associated with the IFNγ receptor complex (17, 18). Cytokine receptors that contain no tyrosine kinase domains bind and generate cytoplasmic signals through their activation of the Jak family of PTKs (10). We have shown that the IFNαR1 chain is rapidly tyrosine-phosphorylated (within 1 min) in response to IFN addition. The tyrosine phosphorylation of the IFNα-activated PTKs Jak1 and Tyk2 occurs with similar kinetics. This leads us to suggest that one or both of these Jak PTKs mediate the tyrosine phosphorylation of the IFNαR1 chain. Although we can detect Jak1 and Tyk2 in precipitates with antisera to either PTK or with anti-pTyr, we have been unable to coprecipitate Jak1 or Tyk2 with anti-IFNαR1 mAb. In addition, anti-pTyr immunoblots of cell lysates immunoprecipitated with anti-IFNαR1 mAb show no bands corresponding to Jak1 or Tyk2. However, both anti-PTK sera and anti-IFNαR1 mAb recognize affinity-crosslinked IFNα complexes, demonstrating that these antibodies recognize components of the human IFNαR.

In a previous study (29) several mAbs were generated against a soluble form of IFNαR1 (aa 1–427); these mAbs neutralize the antiviral and antiproliferative activities of several type I IFN subtypes (IFNα, β, and ω). While these anti-IFNαR1 mAbs partially inhibit type I IFN binding, this does not show that the IFNαR1 chain is directly involved in high-affinity IFNα/β binding. In fact, this result may reflect the mAb interacting with a component of the IFNαR complex, thereby hindering accessibility of the IFNα/β binding site. Indeed, it has been difficult to demonstrate high-affinity type I IFN binding to purified IFNαR1 chain (unpublished data) or in mouse transfectants expressing 12,000–15,000 IFNαR1 chains per cell. Moreover, WA-17 mouse–human cells (containing three copies of human chromosome 21) expressed about one-third the number of IFNαR1 chains expressed by mouse transfectants but have $\geq$35 times the number of IFNα/β binding sites. Thus, expression of IFNαR1 does not correlate with high-affinity IFNα/β binding.

Expression of IFNαR1 cDNA conferred sensitivity to the antiviral (16) and gene-induction activities of human IFNα/β, without increasing IFNα/β binding to cells. Further, IFNαR1 functions in a species-specific manner, since the antiviral action of mouse IFN or its induction of the mouse 204 gene was not increased in IFNαR1 transfectants. The results lead us to propose a model of the function of the IFNαR1 chain in IFNα/β signaling. Binding of IFNα/β to the ligand-binding component of the type I IFN receptor activates members of the Jak PTK family (Tyk2 and Jak1) to rapidly (within 1 min) phosphorylate the IFNαR1 chain. IFNαR1 contains within its intracellular domain a highly conserved motif (DEDHKKYSSQTSQDSGNYSNNEDE) of two tyrosine residues separated by 10 aa which also has sites for protein kinase C-mediated phosphorylation (30). Multiple phosphorylation events are important, since the specificity of PTKs can be determined by previous phosphorylation events at serine/threonine residues (31) and IFNα rapidly activates selective protein kinase C subspecies (32, 33). A comparable motif is present in signaling subunits of the T-cell and B-cell receptors and is believed to couple to Src homology 2-containing proteins involved in signal transduction (34, 35). Thus, the PTK- and protein kinase C-mediated phosphorylation of IFNαR1 may initiate docking of Src homology 2-containing STAT (signal transducers and activators of transcription) proteins to the type I IFN receptor. This would result in the subsequent activation of STAT proteins by PTK-mediated phosphorylation. In addition, tyrosine phosphorylation of IFNαR1 may provide a mechanism of signal amplification, since there appears to be an excess of IFNαR1 chains versus IFN binding sites at the cell surface.

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