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Human IFNγ Receptor Cytoplasmic Domain: Expression and Interaction with HuIFNγ

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To investigate the structural basis for human interferon gamma (huIFNγ) binding to intracellular regions of the human IFNγ receptor (huIFNγR), we have subcloned and expressed the huIFNγR free of fusion proteins in the yeast strain *Pichia pastoris.* HuIFNγ bound to the cytoplasmic domain of the receptor via the IFNγ C-terminus. Binding was inhibited by both human and mouse C-terminus peptides. N-terminus peptides failed to inhibit cytoplasmic binding. Thus, while extracellular receptor domain binding is species specific, binding to the cytoplasmic domain of the receptor is species non-specific. In solid-phase binding assays, IFNγ had a K_d of 3.7 $\times$ 10^{-8} M for the newly expressed cytoplasmic domain. Peptide competitions showed that IFNγ bound to a receptor site corresponding to the membrane proximal residues 253-287, which is adjacent to the site of binding of the tyrosine kinase JAK2. The cytoplasmic binding affinity and binding site specificity suggest that the huIFNγR cytoplasmic domain can function independent of the extracellular domain to bind huIFNγ and induce the biological activity previously associated with internalized huIFNγ.
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Interferon gamma (IFNγ) is a secretory protein produced by activated T lymphocytes and natural killer (NK) cells. Upon binding to a species-specific cell surface receptor complex consisting of α and β subunits (1-3), IFNγ induces antiviral activity, up-regulation of class II major histocompatibility complex (MHC) molecule expression, B cell maturation, activation of cells to cytotoxic states, and release of mediators of inflammation (4, 5). The receptor α chain consists of a species-specific extracellular domain, transmembrane section, and cytoplasmic domain (1), and is the subunit that appears to be predominately responsible for ligand binding (1, 4).

Although the associated biological activities occur after IFNγ binds to the high affinity extracellular domain of the receptor (6), at least three independent lines of experimentation have identified an intracellular role for IFNγ in cell activation. These include the observations that 1) huIFNγ delivered by a liposome vector was able to activate murine macrophages to a tumoricidal state, 2) secretion-defective huIFNγ expressed in murine fibroblasts triggered antiviral activity, and 3) microinjected huIFNγ induced Ia expression in murine macrophages (7-9). The activity of huIFNγ in these murine cells defies the well known species specificity of exogenously applied huIFNγ which has no activity on murine cells. Thus, the IFNγ molecule most likely interacts with some intracellular element(s) to induce a biological response.

Previously, we identified IFNγ binding sites on the cytoplasmic domain of the soluble muIFNγR α chain subunit using synthetic peptides (10, 11). Briefly, C-terminal peptides muIFNγ(95-133) and huIFNγ(95-134) bound to the cytoplasmic domain of the muIFNγR α chain at residues 253-287. Binding to this membrane proximal region was demonstrated with peptides to the cytoplasmic domain of the receptor as well as with fixed/permeabilized mouse L cells where site-specific antibodies to residues 253-287 specifically blocked binding. The C-terminus of IFNγ contains a polycationic sequence that is required for intracellular binding (11, 12). Functionally, the C-terminal peptide induced an antiviral state and up-regulation of MHC class II molecules when taken up by pinocytosis by a macrophage cell line (11). Thus, intracellular binding of IFNγ C-terminus to the receptor cytoplasmic domain is associated with biological activity. Adjacent to this receptor cytoplasmic domain region is a binding site for the Janus tyrosine kinase JAK2 (13). JAK2 binding to this site is enhanced by IFNγ C-terminus (14).

Because the above binding experiments involved

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only peptides to the huIFNγR cytoplasmic domain, we have subcloned and expressed the huIFNγR cytoplasmic domain to evaluate its binding site specificity and relative affinity for huIFNγ and muIFNγ. Through direct and competitive binding experiments, we show that the newly expressed huIFNγR cytoplasmic domain protein can specifically bind both huIFNγ and muIFNγ via their C-terminal regions. Our data suggest that the expressed protein is ideal for structure studies on receptor complexes involving the interaction of the cytoplasmic domain with the tyrosine kinases, JAK1 and JAK2, and the latent cytosolic transcription factor, STAT1.

MATERIAL AND METHODS

Expression and protein purification. A 681-bp huIFNγR α chain cytoplasmic domain sequence was prepared by PCR in 50 μl reactions for 40 cycles of 94°C denaturation for 3 min, 50°C annealing for 2 min and 72°C extension for 4 min. Template was 100 ng of the huIFNγR α chain cDNA (1). Custom huIFNγR α chain forward primer (5′GGCTCGAGCATGGTATTCATCTGTTTTTATATTAAG 3′) was designed with an initiation ATG as part of a Kozak consensus sequence (15, 16), and a *Xho* I restriction site for subcloning into the same restriction site of the plasmid vector pPICZ-C (Invitrogen, Carlsbad, CA,). The reverse primer (5′GGCCGCGGTCATGAAAA TTCTTTGGAATCTTC 3′) was designed to include the huIFNγR α chain TGA stop codon and a *Sac* II restriction site for cloning into the same vector. Deoxynucleotide and primers were added in the concentrations as described previously (17). The PCR products were electrophoresed on and purified from a 1.2% low-melting agarose gel (FMC BioProducts, Rockland, ME) (17).

Ligation into the plasmid vector pPICZ-C with T_4 ligase (New England Biolabs, Beverley, MA), transformation into JM109 (Promega, Madison, WI), screening for plasmid associated Zeocin resistance (Invitrogen, Carlsbad, CA), plasmid amplification and purification using a midiprep wizard column (Promega, Madison, WI), and transformation of *Pichia pastoris* (Invitrogen,) with recombinant plasmid, after restriction digested with *Pme* I to generate Mut^+ (Methanol utilization plus) recombinant strains, were done according to the manufacturers suggestions. DNA sequencing of purified plasmid was done on a ABI 370 automated DNA Sequencer (Iowa State Sequencing Facility). Analytical and preparative scale expression of the cytoplasmic domain protein were done according to the manufactures suggestions (Invitrogen, Carlsbad, CA). Maximal cytoplasmic domain expression occurred in shaker culture at 48 h postinduction with methanol. Cells were harvested by centrifugation at 1500g for 10 min and resuspended in 10 ml of ice-cold ddH_20 per liter of induced shaker culture. This and all other manipulations, unless otherwise stated, were carried out at 4°C. Cell disruption was performed as previously described (18). The resuspended cell paste was squeezed into liquid nitrogen through a 60 ml syringe and ground to a fine powder in a Waring blender fitted with a stainless steel cup. After three successive 2 min bursts at high speed, the extract was checked for complete cell disruption under the microscope. The frozen yeast extract powder was diluted in two volumes of ice-cold storage buffer (20 mM Tris-Cl, pH 7.5, 0.1 mM EDTA, 1 mM PMSF, 2 μg/ml aprotinin, 0.5 μg/ml leupeptin, 0.1 mM benzamidine, and 0.1 mM sodium metabisulfite) and centrifuged 15 min at 5000g. The supernatant was then treated with bovine pancreatic ribonuclease A and bovine pancreatic deoxyribonuclease I at a final concentration of 1 μg/ml for 30 min at 25°C.

Extracts were brought to 30% saturation with ammonium sulfate, left on ice for 1 h, and centrifuged for 20 min at 48,000g. The supernatant was recovered and adjusted to 85% saturation with ammonium sulfate and left on ice for 1 h. After centrifugation at 48,000g, the supernatant was discarded, and the pellet was resuspended in 10 ml of storage buffer per liter of culture and dialyzed for 3 h against 2 liters of the same buffer. The extract (50 ml at 20 mg/ml) was applied to a DEAE-Cellulose column (1.5 × 30 cm) and eluted with a linear gradient of NaCl (0 to 0.5 mM). The cytoplasmic domain protein eluted between 0.3 and 0.4 M NaCl. Fractions containing the cytoplasmic domain protein were identified by immunoblot analysis as described below, pooled, and dialyzed for 1 h against Carboxymethyl-Cellulose (CM) column buffer (10 mM potassium phosphate, pH 6.8, 0.1 mM EDTA, 1 mM PMSF, 0.1 mM benzamidine, and 0.1 mM sodium metabisulfite), loaded onto a CM-Cellulose column and eluted with a linear gradient of NaCl (0 to 0.1 M). The cytoplasmic domain protein eluted between 1 and 10 mM NaCl.

Immunoblotting. Crude receptor protein preparations (4 μg) or purified samples (40 ng) were mixed with Laemmli's sample buffer and subjected to separation on a 12% SDS-PAGE mini-gel (Bio-Rad, Hercules, CA) at 15 Volts/cm for 1-1.5 h. These gels were either silver stained (BioRad, Hercules, CA) or transferred to nitrocellulose membranes at 6 volts/cm for 50 min at 4°C in a Bio-Rad Mini-Protean II apparatus. Membranes were immunoblotted with huIFNγ antipeptide antibody (466-485) (diluted 1:1000) (Santa Cruz, Santa Cruz, CA). After incubation with anti-rabbit secondary antibody (diluted 1:10,000), blots were developed by enhanced chemiluminescence (Amersham, Arlington Heights, IL).

Radioiodinations. Radioiodinations were performed as previously described (10). HuIFNγ (5 μg at 0.5 μg/μl) was radioiodinated with 5 μl (500 μCi) of $Na^{125}I$ (16.9 mCi/μg, Amersham) in the presence of 25 μl of 0.15 M potassium buffer, pH 7.4, and 10 μl Chloramine-T (5 mg/ml) for 2 minutes. After neutralization of the reaction with 10 μl each of sodium metabisulfite (10 mg/ml), potassium iodide (70 mg/ml), and BSA (20 mg/ml), the reaction mixture was chromatagraphed over a 10-ml Sephadex G-10 column equilibrated with a Tris/NaCl/BSA buffer (10 mM Tris-HCl, pH 7.4, 0.15 M NaCl, and 0.33 mg/ml BSA). Fractions of 500 to 600 μl were collected, and the fraction containing the greatest activity was used for receptor binding studies. The specific activity of ^{125}I-IFNγ was 90-120 μCi/μg of protein.

Binding assay. Binding assays were performed as previously described (10). EIA/RIA plates (Costar, Cambridge, MA) were seeded with huIFNγR cytoplasmic domain protein or soluble muIFNγR (10 ng) in 0.1 M carbonate/bicarbonate buffer, pH 9.6, and allowed to incubate for 18 h at 4°C. The remainder of the assay was performed at room temperature. Plates were washed three times with wash buffer (0.15 M NaCl, 0.05% Tween) then blocked for 1 h in 5% Carnation powdered milk dissolved in PBS. Following this, ^{125}I-huIFNγ was added to the wells at a final concentration of 5 nM for 1.5 h. For competition assays, competitors were incubated in the wells for 1 h prior to the addition of ^{125}I-huIFNγ. For saturation assays, various concentrations of unlabeled huIFNγ were added to the wells for 1 h prior to the addition of ^{125}I-huIFNγ (10 nM). When huIFNγR cytoplasmic domain protein or soluble muIFNγR were used as competitors, ^{125}I-huIFNγ was preincubated with the competing receptor in a microcentrifuge tube for 1 h then added to the plate wells for 1.5 h . The wells were then removed and the radioactivity quantified in a scintillation counter (Beckman instruments, Irvine, CA). Saturation and competition data were analyzed with the Equilibrium Binding Data Analysis (EBDA) program for "cold" saturation binding and ligand displacement analysis (19-22).

RESULTS

Expression and purification of the huIFNγR α chain cytoplasmic domain. To evaluate the binding properties of the huIFNγR α chain cytoplasmic domain independent of any structural or functional contributions

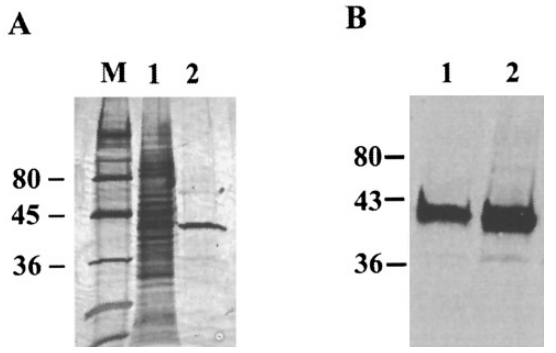

FIG. 1. Immunoblot analysis of the expressed huIFNγR cytoplasmic domain protein. Protein was separated by SDS-PAGE on 12% gels and silver stained (Panel A) or transferred to nitrocellulose and immunoblotted with IFNγ anti-peptide antibodies 466-485 (Panel B). Lane 1, crude huIFNγR cytoplasmic domain extract from *Pichia pastoris* (4 μg); lane 2, huIFNγR cytoplasmic domain protein (40 ng) purified as described in Materials and Methods. Molecular weight markers are indicated.

from the extracellular domain or fusion proteins, primers were designed to PCR amplify the cytoplasmic domain cDNA coding sequence corresponding to amino acids 262-489 of the huIFNγR α chain (1). The 5′ amplification primer contained a Kozak consensus translation initiation sequence (15, 16), and the 3′ primer was designed to include the original receptor TGA stop codon to avoid generating fusion proteins during expression. After subcloning into the plasmid pPICZ-C, DNA sequencing confirmed that the Kozak translation initiation ATG codon, and the cytoplasmic domain protein coding sequence were inframe with the receptor TGA stop codon.

The cytoplasmic domain was identified initially in crude extracts by immunoblotting using antibodies raised against a cytoplasmic domain peptide corresponding to amino acids 466-485 and migrated as a protein species of M_r 43KDa (data not shown). This 43 KDa band was purified by DEAE-cellulose and CM-cellulose chromatography to >90% purity as estimated by silver staining of the SDS-PAGE gel (Figure 1A). The purified protein was confirmed to be the huIFNγR cytoplasmic domain by immunoblot analysis (Figure 1B) and, as described in detail below, by its ability to bind specifically and with high affinity to huIFNγ in radioligand binding experiments using ^{125}I-labeled ligand. In immunoblots, a minor band at 38.5 KDa was also observed. This is most likely a result of proteolytic degradation similar to that observed with the purification of the full-length natural and cloned receptors from mammalian cells (1, 23), since protease inhibitors inhibited the formation of this species.

As has been previously observed with the intact natural and cloned full-length huIFNγR, the observed Mr of the expressed cytoplasmic domain was larger than that predicted from the deduced amino acid sequence (1, 23). As with these earlier studies, the altered electrophoretic mobility could not be explained by N-linked glycosylation since treatment of the purified huIFNγR cytoplasmic domain with N-glycanase did not alter the mobility of the expressed protein. The same N-glycanase did remove N-linked carbohydrates from a full-length cloned muIFNγR (data not shown). Thus, it is possible that either the cytoplasmic domain of the receptor contains O-linked glycosylation at one or more of the several serine and threonine-rich regions as previously proposed (1, 23), or regions like these make the domain unusually charged such that it could potentially affect its electrophoretic mobility.

Binding assays. Previously, we used synthetic peptides representing different regions of huIFNγ and muIFNγ to identify binding sites on the extracellular and cytoplasmic domains of the soluble muIFNγR (10, 11). Here, we wanted to determine whether the cytoplasmic domain of the huIFNγR, expressed without the structural and functional attributes of the extracellular domain, could function to bind IFNγ with the same relative affinity and site specificity determined for the cloned soluble muIFNγR containing the extracellular and cytoplasmic domains (10-12).

Initially, we established the ligand specificity of the newly expressed huIFNγR cytoplasmic domain in solid-phase competitive binding assays (Figure 2). Binding of ^{125}I-huIFNγ was effectively competed with 800 nM unlabeled huIFNγ and 100 μM C-terminus muIFNγ peptide, IFNγ(95-133). The N-terminal muIFNγ peptide, muIFNγ(1-39), previously shown to bind exclusively to the extracellular domain of the receptor (24), did not compete for binding in these experiments. The data show that binding of huIFNγ to intracellular regions of the huIFNγR and muIFNγR was mediated through cytoplasmic domain binding to the C-terminal region of huIFNγ in keeping with our previous peptide studies (10, 11).

To further test the specificity of huIFNγ binding, we determined dose responses of the inhibition of ^{125}I-huIFNγ binding to the huIFNγR cytoplasmic domain protein and the cytoplasmic domain of the soluble muIFNγR by huIFNγ and C-terminal peptides huIFNγ(95-134) and muIFNγ(95-133) (Figure 3). The effective concentrations of unlabeled huIFNγ needed to block half-maximal binding (EC_{50}) to the huIFNγR (Figure 3A, inset) and muIFNγR (Figure 3B, inset) cytoplasmic domains were similar, 1×10^{-8} M and 1.5×10^{-8} M, respectively. The EC_{50} of C-terminus huIFNγ(95-134) and muIFNγ(95-133) needed to block binding of ^{125}I-huIFNγ to the huIFNγR cytoplasmic domain protein were 4.2×10^{-6} M and 1.2×10^{-6} M, respectively (Figure 3A). Similar EC_{50} values were obtained for peptide inhibition of binding of ^{125}I-huIFNγ to the muIFNγR cytoplasmic domain (Figure 3B). Binding control IFNγ peptide, muIFNγ(1-39), was inef-

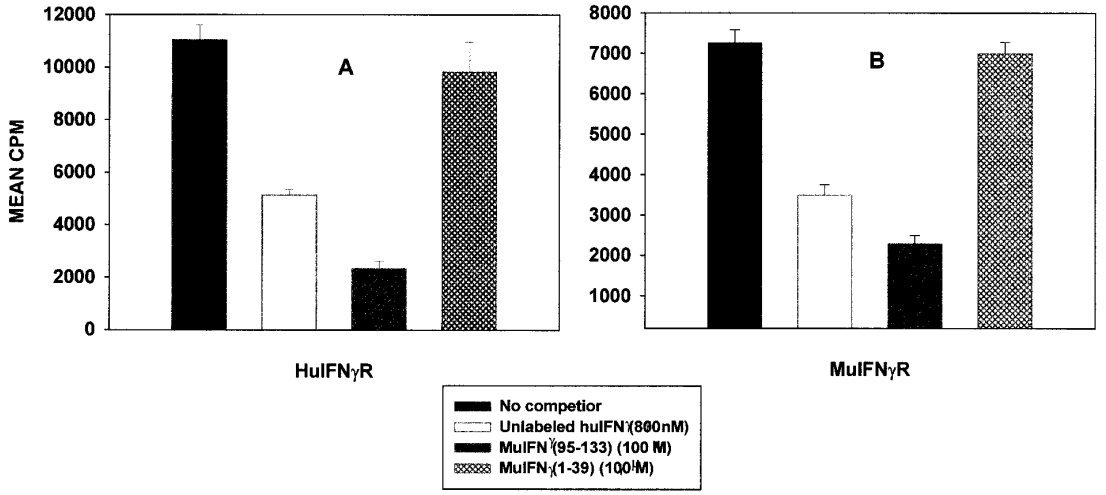

FIG. 2. Effect of huIFNγ and IFNγ peptides on ^{125}I-huIFNγ binding to huIFNγR and muIFNγR. Soluble huIFNγR cytoplasmic domain proteins (10 ng) (Panel A) or muIFNγR (10 ng) (Panel B) were absorbed to the wells of microtiter plates for 18 h at 4°C. Binding of ^{125}I-huIFNγ (5 nM) (specific activity of 112 μCi/μg) was competed in each well with unlabeled huIFNγ (800 nM), C-terminus muIFNγ(95-133) (100 μM), or N-terminus muIFNγ(1-39) (100 μM). Cpm data represent the mean of triplicate wells.

fective at blocking binding. Thus, huIFNγ was 200 to 500 times more effective than the peptides in blocking binding of ^{125}I-huIFNγ to the receptor cytoplasmic domain, which suggests that the C-terminus of the intact huIFNγ was better recognized by the receptor than C-terminus alone.

Analysis of receptor saturation was performed using a standard "cold" saturation experiment (20, 21) as shown in Figure 4A and 4B. Computer analysis of the binding data was used to obtain a Scatchard plot (Figure 3A and 3B insets) and K_d values (19, 21). The K_d of huIFNγ for the huIFNγR cytoplasmic domain protein (3.7×10^{-8} M) and the muIFNγR (7.2×10^{-8} M) were similar. The K_d of muIFNγ for the muIFNγR con-

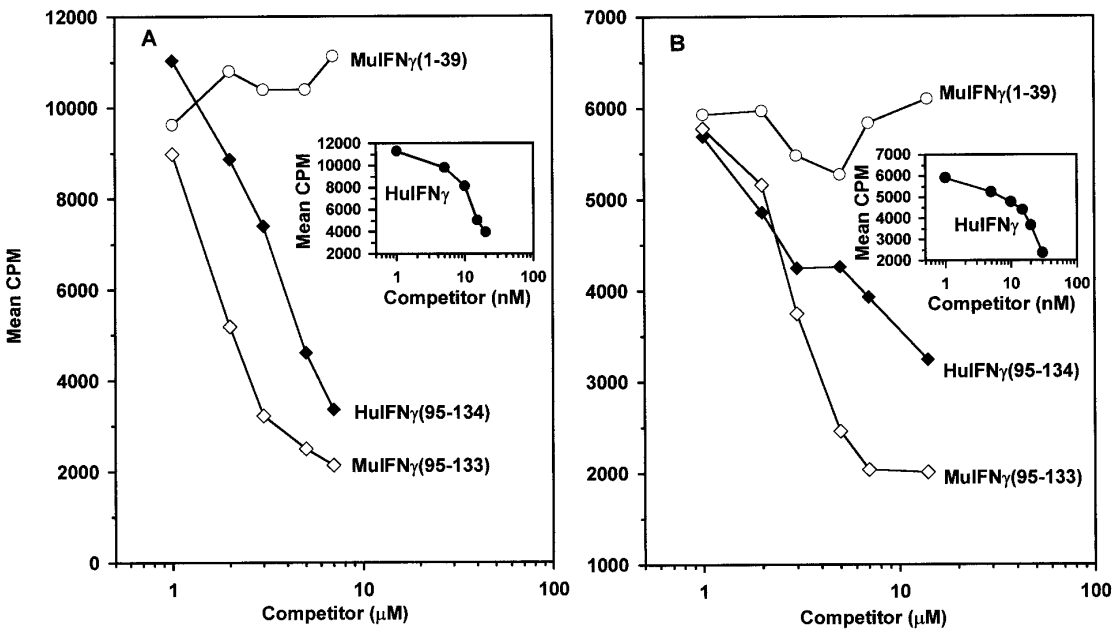

FIG. 3. Dose-response of huIFNγ and IFNγ peptides on ^{125}I-huIFNγ binding to huIFNγR and muIFNγR. Binding of ^{125}I-huIFNγ to solid-phase huIFNγR cytoplasmic domain protein (Panel A) or the soluble muIFNγR (Panel B) was performed as described in Fig. 2. The competitors were unlabeled huIFNγ (●; insert graphs), huIFNγ(95-134) (■), muIFNγ(95-133) (□), and muIFNγ(1-39) (○). Binding in the absence of competitor was 7,000 CPM and 11,031 CPM on the soluble muIFNγR and huIFNγR cytoplasmic domain, respectively. Data represent the mean of triplicate wells.

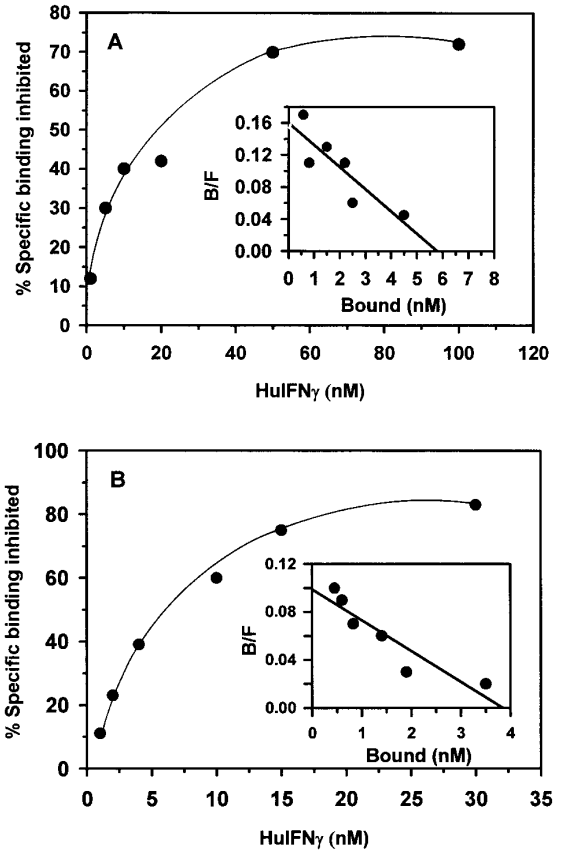

FIG. 4. Scatchard analysis of the binding of huIFNγ to the huIFNγR cytoplasmic domain protein and the soluble muIFNγR. Scatchard plots (insets) and K_d values were obtained using the EBDA program for "cold" saturation binding data (19-22). Saturation binding of unlabeled huIFNγ to the huIFNγR cytoplasmic domain (Panel A) and soluble muIFNγR (Panel B) were determined by incubating increasing concentrations of unlabeled huIFNγ with 10 nM ^{125}I-huIFNγ in microtiter plate wells absorbed with the receptor as in Figure 2. Control binding was determined in the absence of unlabeled huIFNγ. Nonspecific binding was determined in the presence of 800 nM unlabeled huIFNγ. Data represent the mean of triplicate wells. Scatchard plots of binding data (insets). B, bound; F, free.

taining both the extracellular and cytoplasmic domains (5.5×10^{-11} M) was used as an experimental control for K_d determinations in solid-phase binding assays (data not shown). The binding affinity of huIFNγ for the cytoplasmic domain of the huIFNγR α chain is similar to that of simian virus 40 (SV40) NLS binding to the nuclear transporter importin (29). Thus, IFNγ binding to the receptor cytoplasmic domain is of sufficient affinity for possible nuclear translocation.

The site of interaction of IFNγ on the huIFNγR cytoplasmic domain involves the receptor region adjacent to the cell membrane form the cytoplasmic side as indicated by the specific inhibition of ^{125}I-huIFNγ binding to the receptor by receptor peptides huIFNγR(252-291) and muIFNγR(253-287) (Figure 5A). HuIFNγR(102-130), which is involved in the extracellular binding of huIFNγ, had no effect on cytoplasmic binding. Unlabeled huIFNγR cytoplasmic domain protein (Figure 5A inset) and muIFNγR (Figure 5B inset) were similar in their ability to inhibit ^{125}I-huIFNγ binding to the huIFNγR cytoplasmic domain. Similar inhibition patterns were observed for ^{125}I-huIFNγ binding to muIFNγR (Figure 5B). Thus, receptor and receptor peptide competitions show that cytoplasmic binding of IFNγ occurs at corresponding

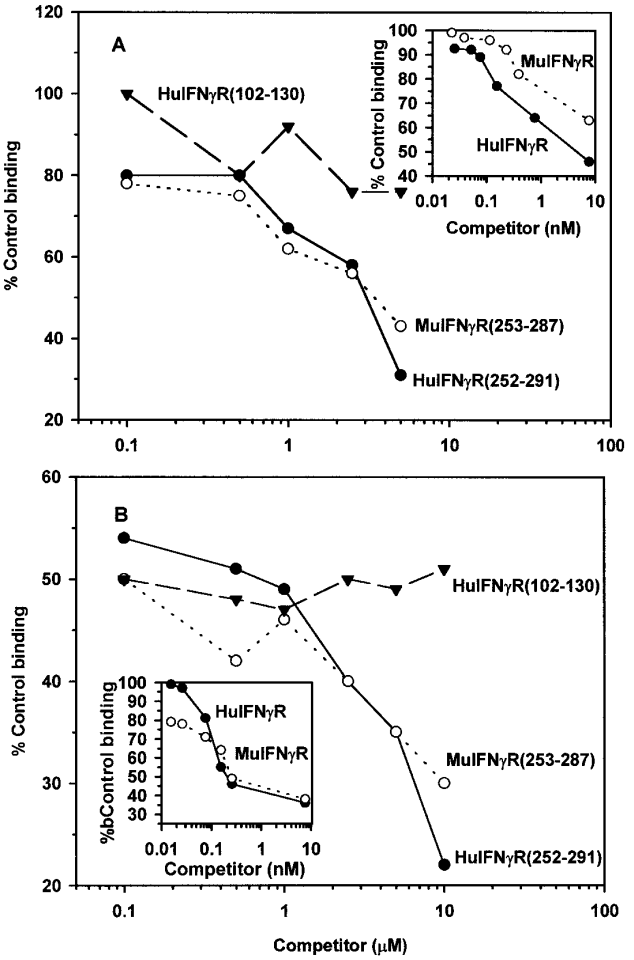

FIG. 5. Effect of huIFNγR cytoplasmic domain protein, muIFNγR, and IFNγR peptide dosage on ^{125}I-huIFNγ binding to receptors. HuIFNγR cytoplasmic domain proteins (10 ng) (Panel A) or the soluble muIFNγR (10 ng) (Panel B) were absorbed to the wells of microtiter plates for 18 h at 4°C. ^{125}I-huIFNγ (5 nM) was incubated with the receptor absorbed to the plate in the absence (control) or presence of increasing concentrations of competitors. The competitors were (Panel A) huIFNγR cytoplasmic domain protein (●; inset graph), muIFNγR (○; inset graph), huIFNγR(252-291) (●), huIFNγR(102-130) (▼), and muIFNγR (253-287) (○). (Panel B) muIFNγR (○; inset graph), huIFNγR (●; inset graph), huIFNγR(252-291) (●), huIFNγR(102-130)(▼), and muIFNγR (253-287) (○). Specific binding (total binding - nonspecific binding of BSA at various concentrations) is expressed as percent of control specific binding (100%=3700 ±250 cpm for huIFNγR, Panel A; and 4200 ± 300 cpm for muIFNγ, Panel B). Data represent the mean of triplicate wells.

sites for huIFNγR and muIFNγR, and this binding is species non-specific.

DISCUSSION

We have subcloned and expressed the huIFNγR cytoplasmic domain without its high affinity extracellular domain to correlate the previously observed biological activity of internalized IFNγ and IFNγ peptides with their site specific binding and relative affinity for the cytoplasmic domain. We also describe its purification from the yeast host *Pichia pastoris.* Expression of the cytoplasmic domain of the huIFNγR α chain free of fusion proteins should help in subsequent structural determinations.

The cloned and expressed cytoplasmic domain migrated at a M_r different from that predicted by the cDNA sequence. Previous studies have indicated that both natural and cloned full-length huIFNγR migrate anomalously on SDS-PAGE gels (1, 23). While the exact reason for this is not known, we have shown in this paper and others have shown previously that this behavior is not due to N-linked glycosylation. Our cloned cytoplasmic domain appears to behave similarly to full-length huIFNγR, suggesting that the cytoplasmic domain either has O-linked glycosylation or that it has charged regions that contribute significantly to this structural property.

The species non-specific nature observed for huIFNγ binding to the huIFNγR and muIFNγR cytoplasmic domains may be explained by the 88% amino acid sequence homology the receptors share in their membrane proximal cytoplasmic region. The site-specific binding of huIFNγ, C-terminus huIFNγ(95-134), and C-terminus muIFNγ(95-133) to the membrane proximal region of each receptor cytoplasmic domain was confirmed by dose-dependent binding inhibition and subsequent saturation of the available binding sites with these ligands. The affinity constants for huIFNγ binding to huIFNγR and muIFNγR cytoplasmic domains obtained from these assays, 3.7×10^{-8} M and 7.2×10^{-8} M, respectively, resembled those for SV40 large tumor antigen NLS binding to the nuclear import protein importin (29). The similarity in K_d values suggest that the affinity with which huIFNγ binds the cytoplasmic domain is sufficient to effect cytosolic and nuclear transport.

Past and present work in our laboratory has helped delineate a functional role for cytoplasmic domain binding in the biological response to IFNγ. Two of the three components known to be required for IFNγ signal transduction, tyrosine kinases JAK1 and JAK2 (30-34), bind to specific sites on the cytoplasmic domain of the IFNγR (10, 11). JAK1 and JAK2 then effect tyrosine phosphorylation of the third component, transcription factor STAT1, which translocates to the nucleus to initiate transcription of IFNγ-inducible genes (35-42). Thus, it appears that IFNγ, JAK1, JAK2, STAT and other components required for inducing the biological response attributed to IFNγ must associate directly or indirectly with the receptor cytoplasmic domain. We propose that this complex could then be translocated to the nucleus via a nuclear localization motif we have identified in the amino acid sequence of IFNγ (Subramaniam et al., unpublished work). Considered with the biological response elicited to IFNγ and C-terminal IFNγ peptides delivered to the cytoplasm by pinocytosis, liposomes or microinjection and the site specific binding of JAK1 and JAK2 in the cytoplasmic domain, our results establish a physiological connection between specific receptor-like binding of IFNγ to the cytoplasmic domain and IFNγ induced biological activity.

ACKNOWLEDGMENTS

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