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Functional characterization of a hybrid human–mouse interferon γ receptor: Evidence for species-specific interaction of the extracellular receptor domain with a putative signal transducer

(human chromosome 21/signal transduction)

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ABSTRACT **The human interferon γ (IFN-γ) receptor expressed in mouse cells displays binding properties indistinguishable from those of the resident receptor on human cells. Still, mouse cells expressing the human IFN-γ receptor remain insensitive to human IFN-γ. It is widely accepted that at least one species-specific cofactor encoded within human chromosome 21 is required for signal transduction. To define structural domains of the human IFN-γ receptor responsible for this species-specific interaction, a hybrid between the human and the murine receptor was constructed and expressed in mouse L929 cells or in mouse L cell-derived SCC16-5 cells, which contain human chromosome 21. This hybrid receptor, which consisted of the extracellular domain of the human IFN-γ receptor and the transmembrane and cytoplasmic domains of the murine IFN-γ receptor, was found to bind human IFN-γ with high affinity. However, only SCC16-5 cells expressing the human/mouse hybrid receptor were responsive to human IFN-γ as revealed by enhanced expression of major histocompatibility complex class I antigens, induction of the transcription factor IRF-1, and induction of a partial antiviral state. These findings strongly suggest that IFN-γ-mediated signal transduction requires a species-specific interaction of the extracellular portion of the known ligand-binding IFN-γ receptor chain with an additional, presumably membrane-anchored receptor subunit.**

Interferons (IFNs) are antiviral cytokines that exert additional functions such as immunomodulatory and cell growth-inhibitory effects. While IFN-α and -β (type I) are produced ubiquitously in the course of viral infections and are of primordial importance as an early antiviral defense system, IFN-γ (type II) is a T-cell-derived immunoregulatory cytokine (1, 2). IFN-γ exerts pleiotropic effects such as macrophage activation (3), induction of major histocompatibility complex (MHC) antigens (4), and regulation of B-cell development (5), and it cooperates with various other cytokines (6). IFNs may also play a physiological role in early embryonic development (7, 8). IFN receptors cannot be assigned to any known cytokine or growth factor receptor families (9), even though certain structural characteristics may be indicative of an evolutionary relationship to other cytokine receptors (10). The mature human IFN-γ receptor (huIFN-γR) is a novel cell surface receptor that consists of 472 amino acids and is subdivided into almost equally large extracellular and cytoplasmic portions (11). It is rich in serines and threonines that are in part constitutively phosphorylated, and ligand binding induces additional serine and threonine phosphorylation (12, 13). The biological role of these phosphorylation events has not been elucidated, but it is clear that the cytoplasmic receptor domain is needed for signal transduction (14). The huIFN-γR expressed in mouse cells displays binding properties indistinguishable from those of the resident receptor on human cells. However, such transfected mouse cells remained insensitive to huIFN-γ (11, 15), in agreement with early observations on somatic cell hybrids, which suggested that in addition to human chromosome 6, which carries the gene for huIFN-γR, human chromosome 21 is required for responsiveness to huIFN-γ (16). Accordingly, mouse–human or hamster–human somatic cell hybrids containing human chromosome 21 became sensitive to huIFN-γ upon transfection with huIFN-γR cDNA (17, 18).

To define domains of huIFN-γR involved in species-specific interaction with the presumed chromosome 21-encoded cofactor(s), a hybrid receptor consisting of the extracellular domain of huIFN-γR and the transmembrane and cytoplasmic domains of murine IFN-γR (muIFN-γR) (15, 19–22) was expressed in mouse L929 cells or mouse–human SCC16-5 hybrid cells, which are L cells that contain chromosome 21 as the only human chromosome (23). Since only SCC16-5 cells expressing huIFN-γR became sensitive to huIFN-γ, these experiments strongly suggest that species-specific interaction between huIFN-γR and putative cofactor(s) encoded within human chromosome 21 involves the extracellular portion of the receptor.

MATERIALS AND METHODS

Construction of hu/muIFN-γR Hybrids. A hu/muIFN-γR hybrid was constructed by using a synthetic double-stranded 34-base-pair oligodeoxynucleotide to link a 5′-*Kpn* I–*Nsp*HI fragment of the huIFN-γR cDNA (11) to a 3′-*Aat* II–*Bam*HI fragment of the muIFN-γR cDNA (15). The resulting cDNA was sequenced to verify that it encoded a hybrid receptor consisting of 215 N-terminal (extracellular) amino acid residues of the mature huIFN-γR and 233 C-terminal amino acid residues of muIFN-γR. Thus, the C-terminal murine receptor fragment comprised 9 amino acid residues of the extracellular portion and the complete transmembrane and cytoplasmic domains. A *Kpn* I–*Bam*HI fragment containing the entire coding region for the hu/muIFN-γR hybrid was blunted and subcloned into the *Eco*RV site of the expression plasmid pHMG (15, 24), to generate the expression plasmid pHMGhu/muIFN-γR.

Stable Transfection of Mouse L929 and SCC16-5 Cells with hu/muIFN-γR cDNA. Mouse L929 cells (ATCC) and SCC16-5 mouse–human hybrid cells containing only human chromosome 21 (kindly provided by R. D. Schreiber, Wash-

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Abbreviations: IFN, interferon; IFN-γR, IFN-γ receptor; huIFN, human IFN; muIFN, murine IFN; mAb, monoclonal antibody; MHC, major histocompatibility complex; VSV, vesicular stomatitis virus.

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ington University School of Medicine, St. Louis) were grown in Dulbecco's modified Eagle's medium (GIBCO) supplemented with 10% fetal bovine serum. Exponentially growing subconfluent cell monolayers of 1–2 × 10^6 cells were cotransfected with 10 μg of pHMGhu/muIFN-γR and 1 μg of pSV2neo (25) or pX343 DNA by the calcium phosphate precipitation method (26). pX343, which was constructed and kindly provided by K. Blöchlinger (Institut Suisse de Recherches Expérimentales sur le Cancer, Lausanne, Switzerland) contains the hygromycin-resistance gene (27) subcloned into pSV2 (25). Colonies resistant to G418 (GIBCO) at 0.8 mg/ml or hygromycin (Boehringer Mannheim) at 0.2 mg/ml were isolated and subcloned 2–3 weeks after transfection. Recombinant hu- and muIFN-γ with specific activities of 10^7 units/mg of protein were obtained from C. Weissmann (Institute of Molecular Biology I, University of Zurich) and G. Adolf (Ernst Boehringer Institute for Pharmacological Research, Vienna), respectively. Radiolabeling was as described (28). Binding to stably transfected cells was assayed as reported elsewhere (11).

Cytofluorometry of MHC Antigen Expression. Parental or transfected mouse L929 or SCC16-5 cells were incubated in 10-cm^2 culture wells for 48 hr at 37°C in the presence or absence of hu- or muIFN-γ (100 units/ml). Subsequently the cells were detached by treatment with 20 mM EDTA in phosphate-buffered saline (pH 7.2), washed with culture medium, and incubated for 60 min at 4°C with a mouse monoclonal antibody (mAb) specific for the mouse MHC class I antigen H-$2K^k$ (ref. 29; ATCC TIB 95) diluted in balanced salts solution (BSS: 140 mM NaCl/1.0 mM $CaCl_2$/5.4 mM KCl/0.8 mM $MgSO_4$/0.3 mM Na_2HPO_4/0.4 mM KH_2PO_4, pH 7.0) containing 5% fetal bovine serum. The cells were washed by centrifugation with BSS/2% fetal bovine serum and incubated for 60 min at 4°C with a fluorescein-conjugated rabbit anti-mouse IgG $F(ab')_2$ antibody (Serotec). After two further washing steps, cell-associated fluorescence was quantified in an EPICS Profile fluorescence-activated cell sorter (Coulter).

Antiviral Assay. hu- and muIFN-γ were assayed on human HEp-2 (ATCC) and murine L929 cells, respectively, challenged with vesicular stomatitis virus (VSV). One unit of IFN per ml is defined as the concentration that results in 50% reduction of the cytopathic effect. Parental or transfected mouse L929 or SCC16-5 cells were incubated in 10-cm^2 culture wells for 72 hr at 37°C with hu- or muIFN-γ (100 units/ml) or were left untreated. Subsequently the cells were exposed for 12–14 hr at 37°C to VSV (Indiana strain) at an approximate multiplicity of infection of 1, detached as described above, washed with culture medium, and incubated for 60 min at 4°C with a rat anti-VSV G-protein mAb (kindly provided by H. P. Roost, Institute of Pathology, University of Zürich). The cells were washed twice by centrifugation and incubated for another 60 min at 4°C with a fluorescein-conjugated rabbit anti-rat IgG $F(ab')_2$ antibody (Serotec). Cell-associated fluorescence was quantified by flow cytometry as described above.

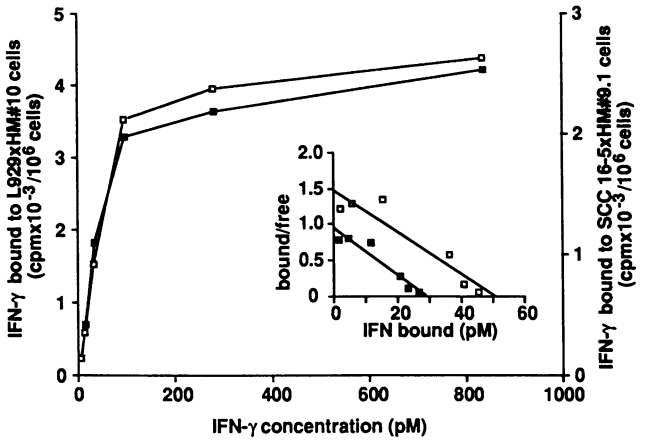

FIG. 1. Binding of ^{125}I-labeled huIFN-γ to mouse L929 cells (□) or SCC16-5 mouse–human cell hybrids (■) transfected with the hu/muIFN-γR hybrid. The cells were incubated for 90 min at 4°C with various concentrations of labeled huIFN-γ, as described (11). Nonspecific binding, determined by simultaneous addition of labeled huIFN-γ and a 100-fold excess of unlabeled huIFN-γ, was <10% of specific binding at 800 pM ^{125}I-labeled IFN-γ. Specific binding is depicted as the difference between total and nonspecific binding. (*Inset*) Scatchard plot of the same data.

RESULTS

Binding of Radiolabeled huIFN-γ to Mouse L929 Cells or SCC16-5 Mouse–Human Cell Hybrids Transfected with a hu/muIFN-γR Hybrid. To identify receptor domains that interact with the chromosome 21-encoded cofactor, mouse L929 and SCC16-5 cells were stably transfected with pHMGhu/muIFN-γR, a cDNA construct designed to express a hybrid receptor consisting of the extracellular domain of huIFN-γR (215 amino acid residues) and the transmembrane and cytoplasmic domains of muIFN-γR (233 amino acid residues). Transfectants were assayed for binding of radiolabeled huIFN-γ (11, 15). Comparison of L929 and SCC16-5 transfectants revealed no significant difference in the dissociation constant for binding of ^{125}I-labeled huIFN-γ (Fig. 1). Saturation curves were almost superimposable and Scatchard plots revealed parallel slopes with extrapolated K_d values of about 3 × 10^{-11} M for both cell lines. Expression of the hu/muIFN-γR hybrid was comparable to the expression

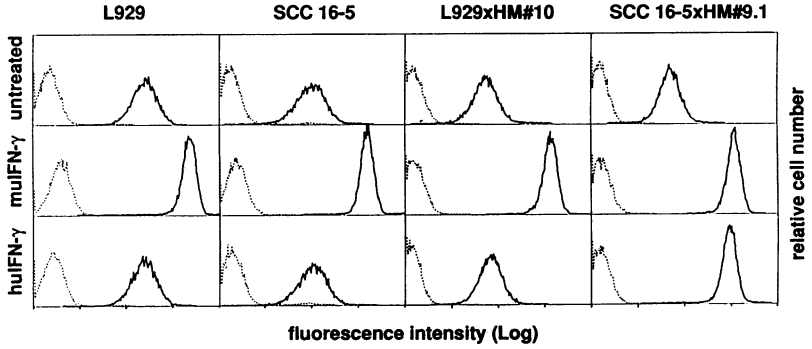

FIG. 2. Cytofluorometric analysis of MHC class I antigen expression on mouse L929 cells or SCC16-5 mouse–human cell hybrids transfected with the hu/muIFN-γR hybrid (L929×HM#10, SCC16-5×HM#9.1) as compared to parental L929 and SCC16-5 cells. Cells were treated with mu- or huIFN-γ (100 units/ml) as described in *Materials and Methods*. Background binding of the fluorescent antibody in the absence of the anti-H-$2K^k$ mAb is represented by dotted lines.

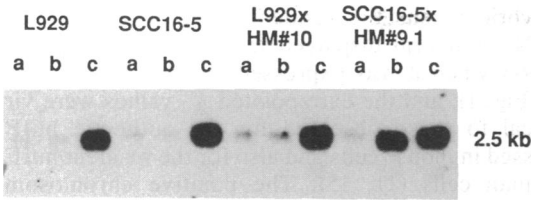

FIG. 3. Northern blot analysis of transcription factor IRF-1 mRNA in untreated (lanes a), huIFN-γ-treated (lanes b), or muIFN-γ-treated (lanes c) parental L929 and SCC16-5 cells and L929 and SCC16-5 cells expressing hu/muIFN-γR. Total RNA was extracted from cells cultured for 24 hr in the presence or absence of mu- or huIFN-γ (100 units/ml). Hybridization was carried out according to standard techniques (32) with 10 μg of total RNA per lane. The murine IRF-1 cDNA hybridization probe (30) was labeled by random oligonucleotide priming. Hybridization with a murine actin cDNA probe revealed no significant difference in the amount of RNA loaded in the different lanes (data not shown). kb, Kilobases.

levels previously observed with the integral huIFN-γR in L929 cells using the same promoter (15) and varied between 10^3 and 5×10^3 binding sites per cell (data not shown).

IFN-γ-Mediated Enhancement of MHC Class I Antigen Expression in Mouse L929 or SCC16-5 Cells Transfected with a hu/muIFN-γR Hybrid. Two independent G418-resistant and two independent hygromycin-resistant clones each of L929 and SCC16-5 cells expressing the hu/muIFN-γR hybrid (hereafter, L929×HM and SCC16-5×HM cells) were used for characterizing their biological responsiveness to huIFN-γ as compared with muIFN-γ. Treatment of both parental and transfected L929 and SCC16-5 cells with muIFN-γ (100 units/ml) for 48 hr at 37°C resulted in an ≈10-fold increase in MHC class I antigen expression (Fig. 2). However, only SCC16-5×HM cells responded to huIFN-γ with a similar increase in MHC class I antigen expression, whereas mouse L929×HM cells, like parental L929 or SCC16-5 cells, remained insensitive to the action of huIFN-γ. Thus, a hybrid receptor consisting of the human extracellular portion and murine transmembrane and cytoplasmic domains, when expressed in mouse cells, was not able to overcome the human/mouse species barrier and still needed the presumed human chromosome 21-encoded cofactor(s) for signal transduction. This cofactor, therefore, most likely interacts with the extracellular portion of the known receptor chain.

IFN-γ-Mediated Induction of IRF-1 and Antiviral Activity in Mouse L929 or SCC16-5 Cells Transfected with the hu/muIFN-γR Hybrid. To extend our observations, two additional markers for responsiveness to IFN-γ were investigated. Transcription factor IRF-1 (30) mRNA was found to be strongly and stably induced in both L929 and SCC16-5 cells upon treatment with muIFN-γ for up to 24 hr (ref. 31; unpublished observation). Fig. 3 shows a Northern blot analysis of IRF-1 mRNA from parental or L929×HM and SCC16-5×HM cells in the presence or absence of IFN-γ. Again, only SCC16-5×HM cells proved responsive to huIFN-γ; however, the level of IRF-1 mRNA induced at a saturating concentration of huIFN-γ was significantly lower than that induced by incubation with muIFN-γ. No increase in IRF-1 mRNA was observed in huIFN-γ-treated L929×HM cells. These findings were reproduced with four independent clones of transfected L929 or SCC16-5 cells. When IFN-γ was assayed for its ability to protect cells from the cytopathic effect of VSV, all cell clones tested proved fully responsive to muIFN-γ. Parental L929 and SCC16-5 cells, like L929×HM cells, were insensitive to the action of huIFN-γ, but a slight and inconsistent protection from the cytopathic effect of VSV was observed in SCC16-5×HM cells (data not shown). To further investigate this marginal effect, appearance of VSV G protein at the surface of VSV-infected cells was monitored by indirect immunofluorescence. Both parental and transfected L929 and SCC16-5 cells could be completely protected from VSV replication by murine IFN-γ, since no VSV G-protein became detectable upon challenge with VSV (Fig. 4). Treatment with huIFN-γ resulted in a significant reduction of VSV G-protein staining in SCC16-5×HM cells, whereas L929×HM cells, like parental L929 and SCC16-5 cells, remained insensitive to huIFN-γ. Even at a saturating dose, 100 units/ml (300 pM), huIFN-γ was not as effective as muIFN-γ in protecting transfected SCC16-5 cells. This incomplete antiviral response was reproducibly found with three independent clones of SCC16-5×HM cells. A fourth clone used in the experiments described above was lost. A comparison between SCC16-5×HM cells and SCC16-5 cells transfected with the integral huIFN-γR (SCC.hgR cells, kindly provided by R. D. Schreiber; see ref. 14) revealed the same partial response to the antiviral action of huIFN-γ (Fig. 5).

DISCUSSION

The murine and human receptors for IFN-γ display about 53% amino acid identity with a rather even distribution of conserved residues (15, 18–21). Still, mouse and human cells do not bind, and are insensitive to, IFN-γ of the other species. This species barrier extends to further elements in the IFN-γ signaling pathway, since transfection of huIFN-γR into mouse cells or, conversely, of muIFN-γR into human cells does not confer responsiveness to the cognate IFN-γ (11, 15, 19). At least one species-specific cofactor encoded within human chromosome 21 is needed to render mouse cells transfected with huIFN-γR susceptible to the action of huIFN-γ (17, 18). Here we present evidence suggesting that this cofactor interacts in a species-specific manner with the

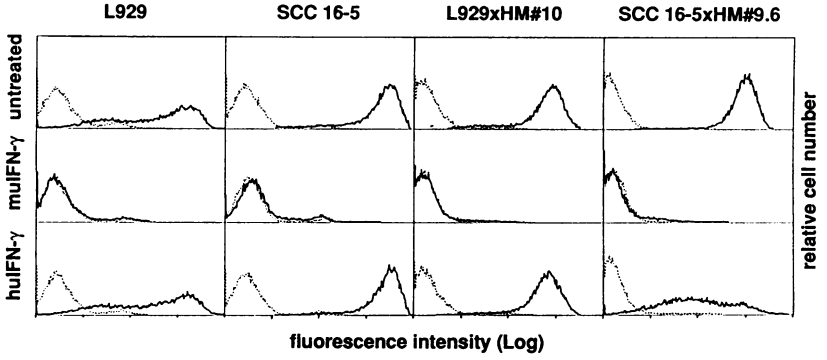

FIG. 4. IFN-γ-mediated antiviral effect in parental and hu/muIFN-γR-expressing L929 and SCC16-5 cells. Inhibition of viral replication was determined by cytofluorometric analysis of VSV G-protein expressed in VSV-infected cells after incubation for 72 hr in the presence or absence of mu- or huIFN-γ. Background binding of the fluorescent antibody in the absence of the anti-VSV mAb is represented by dotted lines.

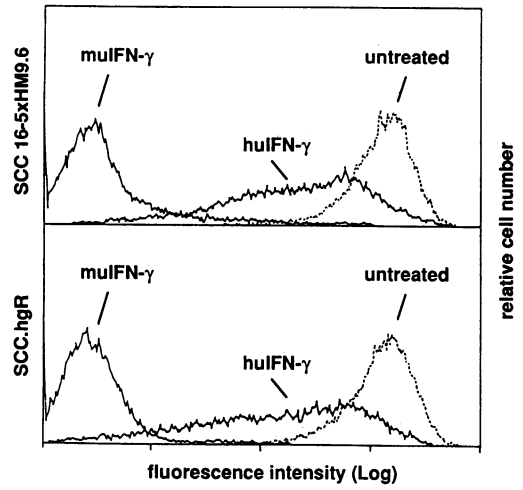

FIG. 5. IFN-γ-mediated antiviral effect in SCC16-5 cells expressing either the hu/muIFN-γR hybrid (SCC16-5×HM#9.6) or the entire huIFN-γR (SCC.hgR). Inhibition of VSV replication was determined by cytofluorometric analysis of VSV G-protein as described in Fig. 4. (Background binding of the fluorescent antibody in the absence of the anti-VSV mAb was determined for untreated cells only and was superimposed on the staining of muIFN-γ-treated cells.)

extracellular portion of the known huIFN-γR receptor chain. Thus, a hybrid receptor consisting of the extracellular domain of huIFN-γR and the transmembrane and cytoplasmic domains of muIFN-γR expressed in mouse L929 cells was still not able to overcome the human/mouse species barrier (Figs. 2–4). Likewise, a mu/huIFN-γR hybrid constructed as the inverse counterpart of the hu/muIFN-γR hybrid remained nonfunctional when expressed in human HEp-2 cells. Indeed, like HEp-2 cells expressing the entire muIFN-γR (15), HEp-2 cells expressing mu/huIFN-γR remained unresponsive to muIFN-γ, as assayed by monitoring MHC class I and class II antigen expression (data not shown). The hu/muIFN-γR hybrid became functional, however, when expressed in SCC16-5, a mouse–human somatic cell hybrid line that contains human chromosome 21. In terms of up-regulation of MHC class I and IRF-1 expression, SCC16-5 cells expressing the hybrid receptor (SCC16-5×HM cells) proved clearly responsive to hu- and muIFN-γ, although, even at saturating doses, the response to huIFN-γ was consistently slightly lower (Figs. 2 and 3). The antiviral action of huIFN-γ on SCC16-5×HM cells, as measured by the cytopathic-effect assay, was markedly less efficient than that of muIFN-γ (data not shown). Immunofluorescence staining, however, revealed a significant reduction of VSV G-protein expression at the surface of huIFN-γ-treated SCC16-5×HM cells, whereas incubation with muIFN-γ resulted in a complete inhibition of VSV G-protein appearance (Fig. 4). This partial antiviral response to huIFN-γ was also observed in SCC16-5 cells expressing the entire huIFN-γR (SCC.hgR cells, Fig. 5), indicating that the murine cytoplasmic domain was not able to overcome the residual species barrier that obviously exists in SCC16-5 cells expressing either the hybrid receptor or the entire huIFN-γR. It remains to be elucidated whether this partial antiviral effect is due to accumulation of incomplete responses as observed for MHC class I antigen and IRF-1 mRNA expression (Figs. 2 and 3) or whether signaling mediated by the chimeric receptor fails to activate a distinct pathway required for a fully protective antiviral state.

The incomplete antiviral response could not be compensated by increasing the concentration of huIFN-γ, suggesting that it was not due to differences in ligand binding affinity of the hybrid versus the resident receptor. Indeed, the hu/muIFN-γR hybrid displayed the same binding affinity for huIFN-γ when it was expressed in L929 cells or SCC16-5 cells (Fig. 1), and the extrapolated K_d values were virtually identical to those observed for the authentic huIFN-γR expressed in mouse cells and also for the resident huIFN-γR in human cells (11, 15). The putative chromosome 21-encoded cofactor, therefore, probably does not contribute significantly to ligand binding. This is consistent with the finding that the soluble huIFN-γR chain binds huIFN-γ with almost the same affinity as the natural receptor expressed on the cell surface (33).

Cytokine-mediated transmembrane signaling probably occurs quite generally through formation of oligomeric complexes triggered by ligand binding to one or several receptor subunits (34). It is believed that such complexes acquire cytoplasmic configurations that allow for interaction with downstream substrates. Several variations of this principle have been observed: The epidermal growth factor receptor and other members of the tyrosine kinase receptor family form homodimers upon ligand binding and thereby activate their catalytic domains (34). Several hemopoietic growth factor receptors, such as the interleukin 2 receptor (35), consist of multiple ligand-binding chains (36). Interestingly, some of these oligomeric receptors, the interleukin 3, interleukin 5, and granulocyte/macrophage-colony-stimulating factor receptors, seem to have one chain in common (36). The IFN-γR is reminiscent of the interleukin 6 receptor, which consists of a ligand-binding subunit that associates with a signal-transducing chain upon ligand binding (37). In contrast to the interleukin 6 receptor, however, the ligand-binding chain of the IFN-γR contains a cytoplasmic domain that is essential for signal transduction, since deletion of C-terminal domains (14) or microinjection of mAbs specific for a cytoplasmic domain (M. Metzler, D. Stüber, and M.A., unpublished observation) abrogates signal transduction. Since both the integral huIFN-γR and a hu/muIFN-γR hybrid are functional in mouse cells containing human chromosome 21, the mechanism that involves signaling from the cytoplasmic domain may not be species-specific. Clearly, the huIFN-γR consists of a ligand-binding subunit that needs to interact with a species-specific second extracellular chain for signal transduction. The same conclusion was recently reached by Gibbs *et al.* (38), based on similar findings of huIFN-γ-mediated MHC class I antigen expression in SCC16-5 cells transfected with hybrid receptor constructs. Identification of this putative second receptor chain is a prerequisite for answering questions on how IFN-γ, which seems to exist as a dimer (39), can bring about the assembly of a signal-transducing complex.

Note Added in Proof. Using a similar approach, Hibino *et al.* (40) recently also found that an accessory factor required for signal transduction interacts with the extracellular IFN-γR domain.

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