Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 8497–8501, November 1989
Immunology

Cloning and expression of the cDNA for the murine interferon γ receptor

(lymphokine/cDNA sequence/interferon γ mechanism of action)

PATRICK W. GRAY*, STEVEN LEONG*, ELIZABETH H. FENNIE*, MICHAEL A. FARRAR†, JANINE T. PINGEL†, JOSE FERNANDEZ-LUNA†, AND ROBERT D. SCHREIBER†

*Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, CA 94080; and †Department of Pathology, Washington University School of Medicine, 660 South Euclid Avenue, Saint Louis, MO 63110

Communicated by Paul E. Lacy, July 21, 1989

ABSTRACT **A murine interferon γ (IFN-γ) receptor cDNA was isolated by screening a murine T-cell hybridoma library prepared in λgt10 with probes prepared from a human IFN-γ receptor cDNA. The 2.1-kilobase (kb) cDNA encoded a serine-rich polypeptide of 477 amino acids that was 52% identical to the human protein. Southern and Northern (RNA) blot analyses indicated the presence of a single receptor gene and a single predominant 2.3-kb receptor transcript. Human embryonic kidney fibroblasts, stably transfected with the murine IFN-γ receptor cDNA, expressed murine IFN-γ receptors as detected by flow cytometry with either ligand or a receptor-specific monoclonal antibody. Nontransfected cells bound neither ligand nor antibody. Radioligand-binding analysis demonstrated that the transfectants expressed 530,000 murine IFN-γ receptors per cell and bound murine IFN-γ with a K_a of 1×10^9 M^{-1}. However, despite high-level expression of murine IFN-γ receptors, the transfected human cells responded only to human and not to murine IFN-γ as detected by enhancement of major histocompatibility class I antigen expression and induction of antiviral activity. These results thus document the isolation and expression of a full-length murine IFN-γ receptor cDNA and suggest that additional species-specific components may be necessary to form a biologically active IFN-γ receptor.**

Interferon γ (IFN-γ) is a potent immunomodulatory cytokine produced by activated T cells and natural killer cells (1). IFN-γ is perhaps best recognized for its ability to enhance natural, cell-mediated, and humoral immunity by regulating the differentiation, activation and/or function of a wide variety of immune cell types, such as macrophages (2–4) and B cells (5, 6). However, it also induces a number of other biologic responses on cells, such as fibroblasts (7, 8) and endothelial cells (9, 10) that are not classically considered components of the immune system.

IFN-γ induces its pleotropic effects after interaction with a specific receptor at the cell surface. Radioligand binding and crosslinking techniques have confirmed the presence of IFN-γ receptors on a wide variety of human and murine cell types (11–13). Although these receptors are expressed only in limited numbers (200–10,000 receptors per cell), they bind ligand with high affinity ($K_a = 2 \times 10^9$–2×10^{10} M^{-1} at 4°C) in a species-specific manner. IFN-γ receptors have now been purified from different human (14–16) and murine (17) cells and have been characterized as 90- to 95-kDa single-chain integral membrane glycoproteins that display a modest degree of structural heterogeneity due to cell-specific glycosylation (18). The primary sequence of the human IFN-γ receptor has been elucidated by Aguet *et al.* (19), who cloned and sequenced a 2.1-kilobase (kb) human IFN-γ receptor cDNA from a Raji cell expression library prepared in λgt11. We recently confirmed this sequence by isolating a nearly identical cDNA from a human placental library prepared in λgt10 (P.W.G., S.L., W. Henzel, D. Pennica, D. Goeddel, J. Calderon, and R.D.S., unpublished data).

Although these studies have significantly elucidated the structure of the human IFN-γ receptor, they have failed to clarify whether this single protein is sufficient to convey biologic responsiveness to cells. This uncertainty is largely due to difficulties in obtaining high-level expression of the human IFN-γ receptor in mammalian cells. To date, expression has only been accomplished using the entire human IFN-γ receptor gene (19), and although the murine transfectants displayed the capacity to bind human IFN-γ, they failed to respond to it. Thus, it remains uncertain whether unresponsiveness was due merely to limited expression of the human IFN-γ receptor at the cell surface or to a deficiency of an additional human component needed to form a functionally active receptor.

To further investigate this issue, we examined the biologic responsiveness of human cells that expressed murine IFN-γ receptors. In the current communication, we report the cloning and sequencing of a murine IFN-γ receptor cDNA.‡ We also demonstrate that, unlike its human counterpart, the murine IFN-γ receptor cDNA can be stably expressed at high levels in human cells. However, despite expression of 530,000 murine IFN-γ receptors per cell, the human cells respond only to the homologous human ligand and not to the murine ligand. These results thus support the concept that additional species-specific components are necessary to form a functionally active IFN-γ receptor.

MATERIALS AND METHODS

Reagents. The murine and human IFN-γ preparations used in these studies were supplied as highly purified recombinant proteins derived from *Escherichia coli* and expressed specific antiviral activities of 1 and 3×10^7 units/mg, respectively. The proteins were radioiodinated with Bolton–Hunter reagent (ICN), as described (16). Polyvalent rabbit anti-recombinant murine IFN-γ (21) was affinity-purified by chromatography on murine IFN-γ–Sepharose columns. Purified GR20, a rat monoclonal antibody (mAb) specific for the murine IFN-γ receptor (17) was provided by Stephen Russell, Judith Pace, and Mitali Basu (University of Kansas Medical Center, Kansas City). Purified antibodies were conjugated to biotin using the ENZO biotinylating reagent (Enzo Biochemicals) as described (22).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: IFN-γ, interferon γ; iru, international reference units; PBS, phosphate-buffered saline; MHC, major histocompatibility complex; mAb, monoclonal antibody.

‡The sequence reported in this paper has been deposited in the GenBank data base (accession no. M26711).

Preparation of Oligonucleotide Probes Derived from the Human IFN-γ Receptor cDNA. A human IFN-γ receptor cDNA was isolated from a human placental library prepared in λgt10 by screening with synthetic oligonucleotides based on protein sequence. The cDNA displayed a nucleotide sequence that differed in 7 residues from the sequence reported by Aguet *et al.* (19). Two of these differences were a result of genetic polymorphism in the human IFN-γ receptor gene. Two ^{32}P-labeled probes were prepared from the human cDNA: a 5′-end restriction fragment of 660 base pairs (bp) (*Hin*dIII–*Sca* I) and a 3′-end restriction fragment of 1105 bp (*Sca* I–*Sca* I). The specific activities of the 3′- and 5′-end probes were 6×10^8 and 5×10^8 cpm/μg, respectively.

Isolation of Murine IFN-γ Receptor cDNA Clones. A cDNA library was prepared in λgt10 as described (23) from oligo(dT)-selected RNA derived from a murine T-cell hybridoma and screened at low stringency with the radiolabeled human IFN-γ receptor oligonucleotide probes. Filters were hybridized for 16 hr at 42°C with the probes (10^6 cpm/ml) in hybridization buffer (24) and then washed extensively with a solution of 0.06 M sodium chloride/0.006 M sodium citrate/0.1% SDS at 42°C. Positive clones were identified by autoradiography and plaque-purified (24); the cDNA insert size was determined by PAGE of *Eco*RI-digested phage DNA. The cDNAs were sequenced using the dideoxy chain-termination technique (25).

Southern and Northern (RNA) Blot Analysis. DNA was isolated from the PU5-1.8 murine macrophage cell line by published methods (26) and used for Southern blot analysis as described (27). Northern blot analysis was performed on RNA isolated from murine myeloma and EL-4 cells and selected on oligo(dT)-cellulose (28). For both analyses hybridization and washing were conducted under stringent conditions (29) by using a ^{32}P-labeled murine IFN-γ receptor cDNA.

Mammalian Cell Expression of Recombinant Murine IFN-γ Receptor. The coding region of the murine IFN-γ receptor cDNA was isolated as an *Spe* I–*Hin*dIII fragment of 1958 bp and cloned into the mammalian cell expression vector pRK5 (ref. 30; R. Klein and D. Goeddel, personal communication) between the *Xba* I and *Hin*dIII sites. The resulting prkMGR-24 plasmid and the pRSVneo plasmid containing the neomycin resistance gene (31) were cotransfected into a human kidney fibroblast cell line (293 cells) using the calcium phosphate/DNA precipitation method (32). Stable transfectants were first selected by culture in medium containing G418 at 400 μg/ml (GIBCO) and then subsequently identified by fluorescence activated cell sorting (FACS) based on their ability to bind murine IFN-γ.

Demonstration of Murine IFN-γ Receptors on Transfected Human Cells. Two flow cytometry protocols were used to identify transfected 293 cells expressing the murine IFN-γ receptor. In the first protocol, 1×10^6 cells were suspended in 0.5 ml of phosphate-buffered physiologic saline, pH 7.4 (PBS) supplemented with 10% heat-inactivated fetal calf serum (PBS-FCS) and incubated for 60 min at 4°C with 50 μl of heat-inactivated normal rabbit serum with or without 10 ng of purified recombinant murine IFN-γ. Cells were washed with PBS-FCS, resuspended in 250 μl of biotinylated, purified rabbit polyclonal anti-murine IFN-γ (16 μg/ml), and incubated for an additional 30 min at 4°C. Cells were washed with PBS lacking fetal calf serum and resuspended in 100 μl of streptavidin/phycoerythrin (Chromoprobe, Redwood City, CA) diluted 1/5 in PBS. Thirty minutes later, samples were washed, resuspended in 500 μl of PBS and analyzed on a Becton Dickinson FACS 440 equipped with a single 5-W argon laser. Receptor-positive cells were selected by sorting the brightest 5% viable cells in the sample. In the second protocol, cells were directly stained by sequential incubation with 10 μg of biotinylated receptor-specific GR20 mAb per 10^6 cells in the presence of 10% heated normal rat serum and streptavidin/phycoerythrin as described above.

Receptor expression on transfected cells was quantitated by radioligand-binding analysis performed at 4°C with ^{125}I-labeled recombinant murine IFN-γ and 1×10^5 transfected or control human 293 cells as described (33). Binding data was analyzed by the method of Scatchard (34).

Analysis of Transfected Human 293 Cells for Responsiveness to Murine IFN-γ. The ability of murine IFN-γ to enhance major histocompatibility complex (MHC) class I antigen expression on transfected 293 cells was examined by culturing $1.25–5 \times 10^5$ cells in 5 ml of either medium alone, recombinant human IFN-γ (1000 units/ml), or recombinant murine IFN-γ at concentrations of 1000 or 10,000 international reference units (iru)/ml for 1–4 days. MHC class I antigen expression was assessed by flow cytometry on cells that were stained with a murine mAb to human HLA-A, B, C antigens (Accurate Chemicals, Westbury, NY) and fluorescein isothiocyanate-labeled rabbit anti-murine immunoglobulin (Sigma). The capacity of cells to mount an IFN-γ-dependent antiviral response against encephalomyocarditis virus was assessed in a cytopathic effect assay that has been described (35).

RESULTS

Isolation and Characterization of a Murine IFN-γ Receptor cDNA. A murine IFN-γ receptor cDNA was isolated from a murine T-cell hybridoma cDNA library by using radiolabeled oligonucleotide probes derived from the 3′ and 5′ ends of the human IFN-γ receptor cDNA. Of 6×10^5 clones screened, 12 clones hybridized with both probes. Fig. 1 depicts the nucleotide and deduced amino acid sequences of the longest, 2085-base, cDNA clone isolated. The first potential ATG initiation site occurs at position 106 in the nucleotide sequence and is preceded by an upstream termination codon at base 28. The sequence displays an open reading frame of 1431 bases that code for a polypeptide of 477 amino acids. Based on an analogy to the amino terminus of the natural human IFN-γ receptor, we predict that the first 26 amino acids of the murine sequence represent the signal sequence. A hydropathy plot (data not shown) of the murine sequence predicts a single 23-amino acid transmembrane domain that bisects the protein into two nearly equivalent domains. The extracellular domain of 228 amino acids contains 10 cysteine residues and 5 potential N-linked glycosylation sites. The intracellular domain of 200 amino acids contains an additional 2 cysteine residues and is extremely rich in serine and threonine residues. The latter account for 24.6% of all amino acid residues in this portion of the polypeptide. Fig. 1 also compares the murine and human IFN-γ receptor sequences. IFN-γ receptors from the two species show a 62% homology at the cDNA level and a 52% homology at the protein level. The amino acid sequence homology is relatively constant over the entire length of the polypeptide.

Expression and Characterization of the Recombinant Murine IFN-γ Receptor. The identity of our putative murine IFN-γ receptor cDNA clone was established by monitoring whether human 293 cells transfected with a pRK5 plasmid containing the cDNA acquired the ability to bind murine IFN-γ. As detected by FACS analysis, 20% of the stably transfected human cell population expressed murine IFN-γ receptors. This population was sorted, expanded, and then analyzed again by flow cytometry (Fig. 2*A*). As expected, nontransfected 293 cells did not bind murine IFN-γ (lane 1). In contrast, transfected cells were strongly positive (100-channel shift) for murine IFN-γ receptor expression (lane 2). The magnitude of the staining was 2.5–5 times greater than that seen for murine EL-4 that expressed 50,000 receptors per cell (lane 3). Similar results were obtained when receptor

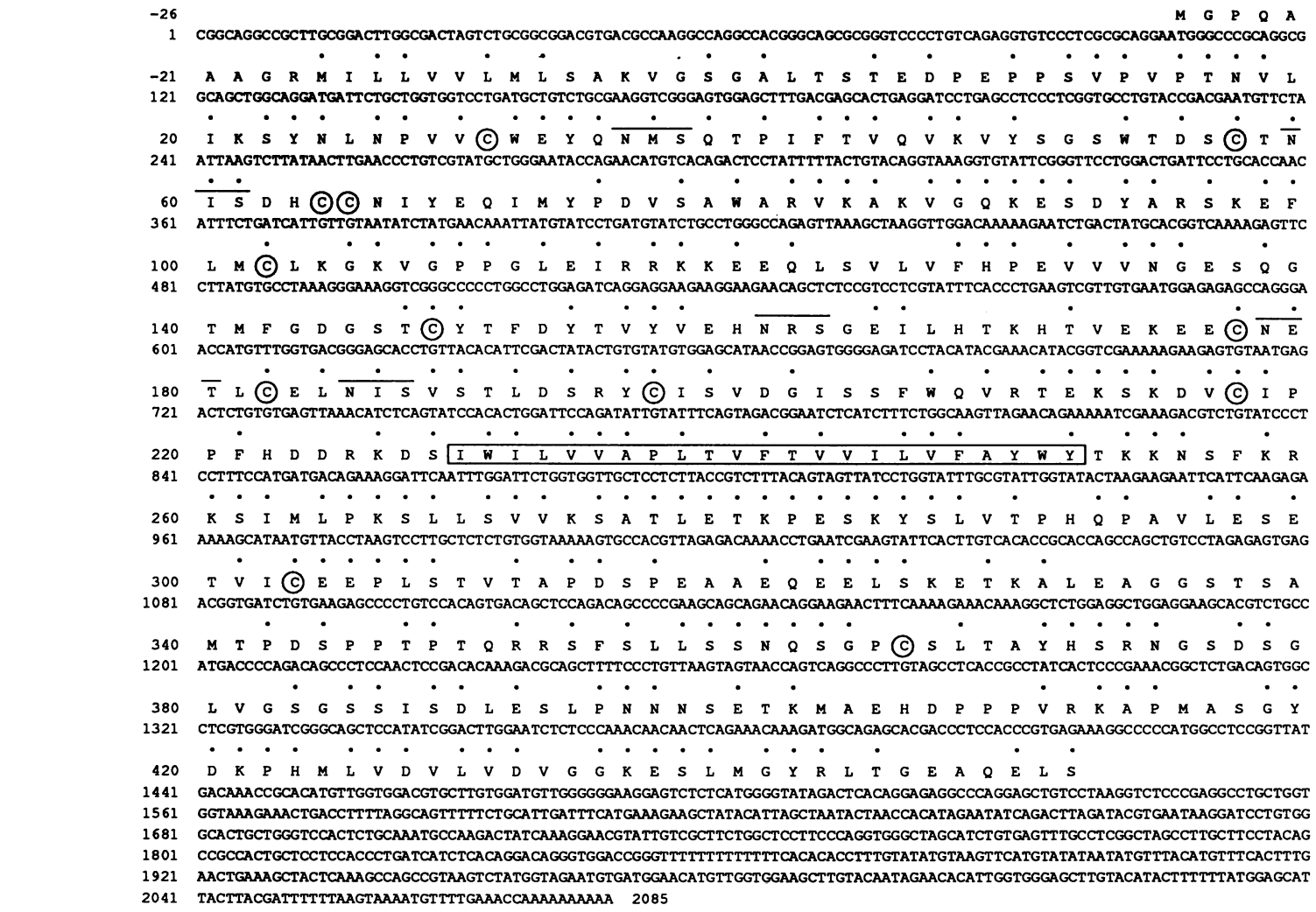

FIG. 1. Nucleotide sequence of the murine IFN-γ receptor cDNA and predicted amino acid sequence in single-letter code. Predicted signal sequence residues are numbered from −26 to −1. The residues homologous with the human sequence are identified by a dot. Cysteine residues are circled. The transmembrane domain is boxed, and potential N-linked glycosylation sites are overlined.

expression was monitored using the murine IFN-γ receptor-specific mAb. In this case, transfected cells displayed a 122-channel shift, whereas nontransfected cells were not stained (data not shown).

Recombinant murine IFN-γ receptors expressed on human 293 cells were directly quantitated using ^{125}I-labeled murine IFN-γ (Fig. 2*B*). Transfected 293 expressed 530,000 murine IFN-γ receptors per cell and bound the murine ligand with a K_a of 1×10^9 M^{-1} at 4°C. Specificity of the binding was established by two criteria: (*i*) binding of ^{125}I-labeled murine IFN-γ to transfected cells was inhibitable by a 1000-fold excess of unlabeled murine IFN-γ but not by unlabeled human IFN-γ and (*ii*) no binding of ^{125}I-labeled murine IFN-γ was detected on nontransfected 293 cells.

Northern and Southern Blot Analyses with Murine IFN-γ Receptor cDNA. Fig. 3*A* represents a Northern blot analysis performed on oligo(dT)-selected mRNA from murine myeloma (lane 1) and EL-4 (lane 2) cells using ^{32}P-labeled preparations of the murine IFN-γ receptor cDNA as probe. In both cases, only a single hybridizing species of 2.3 kb was seen, indicating that the isolated cDNA sequence represents the predominant (or only) transcript from the murine IFN-γ receptor gene. Fig. 3*B* depicts a Southern blot analysis conducted with a ^{32}P-labeled probe prepared from the 3′ end of the murine IFN-γ receptor cDNA. In four different restriction endonuclease digests of murine genomic DNA, only a single sequence was observed, suggesting that the murine IFN-γ receptor cDNA is encoded by a single gene.

Transfected Human 293 Cells Respond to Human IFN-γ but Not to Murine IFN-γ. We now studied whether the transfected human cells responded to the murine ligand (Fig. 4). As expected, human IFN-γ induced enhanced expression of MHC class I antigens on the *human* 293 cells. Increased expression was time dependent with a 52% increase detectable after 48 hr and a maximal enhancement of 72% seen after 72 hr. In contrast, no enhanced expression was observed when transfected cells were cultured for up to 96 hr in comparable or higher concentrations of the murine ligand. Thus, although the cells bound high amounts of the murine ligand, they failed to respond to it. Identical results were obtained when development of antiviral activity was monitored in the transfected cells. Although the cells responded to the homologous human ligand (3.3-fold reduction in virus yield), they did not respond to murine IFN-γ even when it was supplied in 1000-fold excess (data not shown).

DISCUSSION

This paper documents the cloning of a cDNA that encodes the murine IFN-γ receptor. The cDNA was isolated by screening a murine T-cell hybridoma cDNA library at low stringency with probes derived from a human IFN-γ receptor

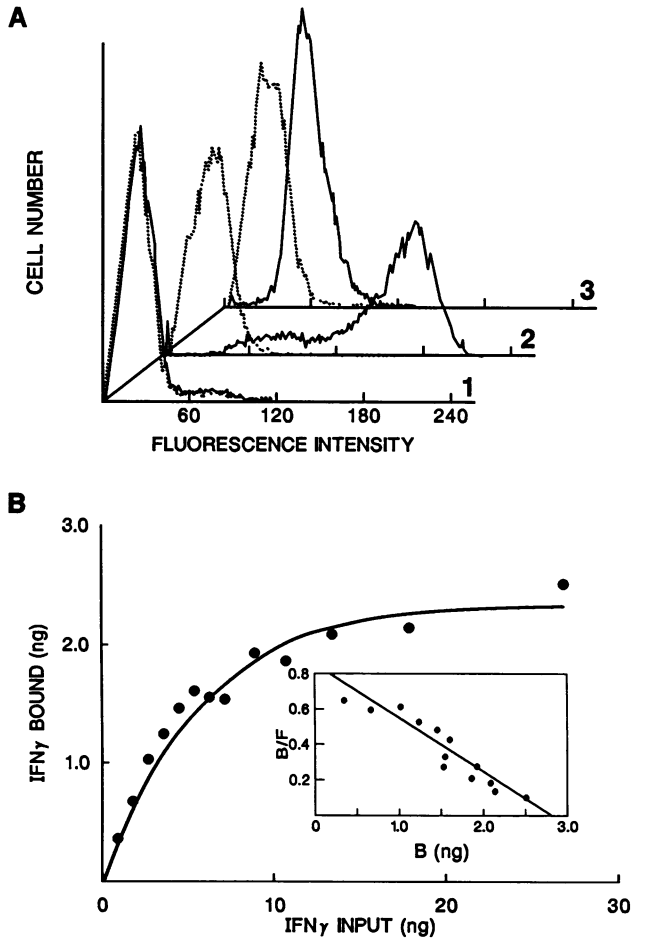

FIG. 2. Expression of murine IFN-γ receptors on transfected human 293 cells. (*A*) Demonstration by flow cytometry. Approximately 1×10^6 nontransfected human 293 cells (lane 1), 293 cells transfected with the murine IFN-γ receptor cDNA (lane 2), or murine EL-4 cells (lane 3) were sequentially incubated with (—) or without (····) 10 ng of purified recombinant murine IFN-γ, 4 μg of affinity-purified biotinylated rabbit polyvalent anti-murine IFN-γ, and streptavidin/phycoerythrin and analyzed by flow cytometry as described. (*B*) Specific binding of ^{125}I-labeled murine IFN-γ to transfected human 293 cells at 4°C. Reaction mixtures of 100 μl contained 1×10^5 transfectants and indicated amounts of radiolabeled murine IFN-γ. Cell-associated (B) and free radioactivity (F) were separated by centrifugation through phthalate oil. Specific binding was defined as the amount inhibitable by a 1000-fold excess of unlabeled IFN-γ. In this particular experiment, nonspecific binding was 0.6% of the total.

cDNA. The identity of the murine IFN-γ receptor cDNA was established by three criteria: (*i*) it displayed an overall 62% nucleotide sequence homology to its human counterpart; (*ii*) it coded for a protein that was similar in amino acid sequence (52% homology) and overall structure to the human IFN-γ receptor; and (*iii*) it directed the high-level expression of the murine IFN-γ receptor on transfected human cells. Southern and Northern blot analyses indicated that the murine IFN-γ receptor is encoded by a single gene that gives rise to one predominant transcript. This data thus supports the concept that only a single type of IFN-γ receptor is expressed on different cell surfaces (14–17). The murine IFN-γ receptor showed no significant sequence similarity to other lymphokine receptors or to any protein other than its human counterpart.

From the data presented herein and elsewhere (19, 22), the overall molecular organization of the murine and human IFN-γ receptors appears remarkably similar. The murine and

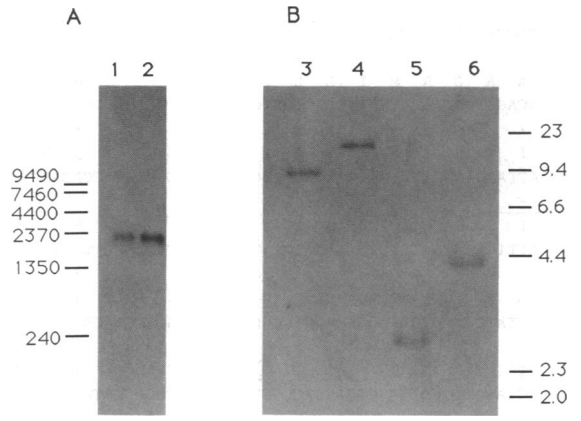

FIG. 3. (*A*) Northern blot of 10 μg of oligo(dT)-selected RNA from murine myeloma cells (lane 1) and murine EL-4 cells (lane 2) hybridized with murine IFN-γ receptor cDNA probe (1472-bp *Bam*HI fragment). Molecular size is indicated at left in bases. (*B*) Southern blot of murine genomic DNA digested with *Bam*HI (lane 3), *Eco*RI (lane 4), *Hin*dIII (lane 5), and *Pst* I (lane 6). The blot was hybridized with a probe from the 3′ end of the murine IFN-γ receptor cDNA (435-bp *Stu* I–*Hin*dIII fragment). Molecular size is indicated at right in kb.

human proteins are serine-rich polypeptides of 451 and 472 amino acids, respectively, have predicted molecular masses of 49.8 and 52.5 kDa, and are synthesized with additional 26- and 17-amino acid signal peptides. They are each composed of nearly equivalent-sized extracellular domains (228 and 229 amino acids for murine and human IFN-γ receptors, respectively) and intracellular domains (200 and 222 amino acids, respectively) that are separated by single 23- or 21-amino acid transmembrane domains. Both proteins contain complex oligosaccharides bound to at least some of five potential N-linked glycosylation sites (16, 18). Postsynthetic modifications thus give rise to mature receptor molecules of 90–95 kDa. Moreover, both receptors have intracellular domains that contain an unusually high content (20–25%) of serine and threonine residues. Nevertheless, despite their similar molecular structure, the two proteins are only 52% homologous

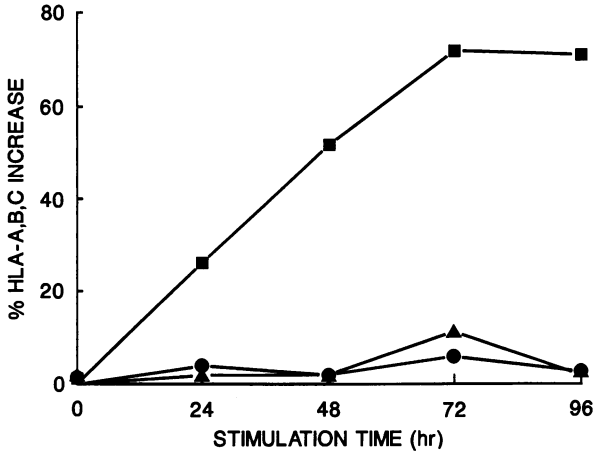

FIG. 4. Transfected human 293 cells display enhanced MHC class I antigen expression only when stimulated with human and not murine IFN-γ. Transfected cells were incubated for the indicated time periods with human IFN-γ at 1000 iru/ml (■), or murine IFN-γ at 1000 (▲) or 10,000 (●) iru/ml and analyzed for MHC class I antigen expression as described. Constitutive expression was determined as the difference between unstimulated cells stained with an irrelevant, isotype-matched mAb and cells stained with monoclonal anti-HLA A, B, C. The level of constitutive staining (38 channels) remained invariant throughout the experiment.

at the primary sequence level. Although it is not surprising that the extracellular domains of the two proteins show limited (50%) sequence homology (because they bind, in a species-specific manner, to ligands that are only 40% homologous), it is quite unusual to find such little sequence identity (55%) in intracellular domains of identical membrane proteins from different species. This observation suggests that the receptor may also bind to intracellular components in a species-specific manner.

For this reason, it is of particular interest that transfected human cells, expressing the recombinant murine IFN-γ receptor, only bound the murine ligand but did not respond to it. This observation is consistent with earlier reports from other laboratories. Using human–rodent somatic cell hybrids, Jung *et al.* (36) showed that responsiveness of murine or hamster cells to human IFN-γ required the concomitant expression of two human gene products: the IFN-γ receptor, the gene for which localizes to the q16–q22 region of human chromosome 6 (19, 36), and a second component, the gene for which localizes to chromosome 21. To date, no additional information is available about the second gene product. More recently, Aguet *et al.* (19) confirmed this observation by directly transfecting murine cells with the isolated human IFN-γ receptor gene and demonstrating that the transfectants bound, but did not respond, to the human ligand.

However, in both previous studies receptor expression on the hybrid or transfected cells was limited. Thus, it was not possible to distinguish whether unresponsiveness was due to a quantitative defect in receptor expression (that might be regulated by the additional gene) or to a deficiency of a second critical component. The data presented in the current paper now rules out the first possibility. Transfected human cells remained unresponsive to murine IFN-γ despite expression of 530,000 murine IFN-γ receptors per cell. In this case, expression of the murine receptor was at least 100 times greater than expression of the endogenous human protein. We also showed that the defect was not attributable to alterations in the ligand-binding affinity of the receptor. Radioligand-binding analyses revealed that the transfected human cells bound murine IFN-γ with a K_a that was virtually indistinguishable from that displayed by natural murine IFN-γ receptors constitutively expressed on murine cells (20). Thus, although no protein other than the 90-kDa IFN-γ receptor is needed to confer species-specific ligand-binding activity to cells, at least one additional component appears to be required to manifest IFN-γ-dependent cellular responses. The function of the additional component currently is undefined. This component could associate with the intracellular domain of the IFN-γ receptor in a species-specific manner and either mediate ligand-dependent signal-transduction mechanisms or direct the intracellular trafficking of the ligand–receptor complex. More work will be needed before these possibilities can be fully evaluated.

Note Added in Proof. We have recently become aware that similar data was obtained by a second group (S. Hemmi, P. Peghini, M. Metzler, G. Merlin, Z. Dembic, and M. Aguet) and will be published in a forthcoming issue of the *Proceedings*.

The authors are grateful to Drs. David Goeddel of Genentech and Matthew Thomas of Washington University School of Medicine for helpful discussions. We also acknowledge Dr. Ernst Rinderknecht for supplying purified murine IFN-γ, Thomas Camerato for performing the DNA sequencing, Dr. Glenn Rice (Genentech) and Gary Elliot (Washington University) for performing the FACS analyses, Beth Viviano for expert technical assistance, and Marian Simandl for secretarial assistance. This work was supported by United States Public Health Service Grants CA43059 and AI24854 and by a grant from Genentech, Inc.

1. Vilcek, J., Gray, P. W., Rinderknecht, E. & Sevastopoulos, C. G. (1985) *Lymphokines* **11,** 1–32.
2. Schreiber, R. D. & Celada, A. (1985) *Lymphokines* **11,** 87–118.
3. Adams, D. O. & Hamilton, T. A. (1987) *Immunol. Rev.* **97,** 5–27.
4. Steeg, P. S., Moore, R. N., Johnson, H. M. & Oppenheim, J. J. (1982) *J. Exp. Med.* **156,** 1780–1793.
5. Coffman, R. L. & Carty, J. (1986) *J. Immunol.* **136,** 949–954.
6. Snapper, C. & Paul, W. E. (1987) *Science* **236,** 944–947.
7. Youngner, J. S. & Salvin, S. B. (1973) *J. Immunol.* **111,** 1914–1922.
8. Maurer, D. H., Hanke, J. H., Mickelson, E., Rich, R. R. & Pollack, M. S. (1987) *J. Immunol.* **139,** 715–723.
9. Lapierre, L. A., Fiers, W. & Pober, J. S. (1988) *J. Exp. Med.* **167,** 794–804.
10. Jutila, M. A., Kroese, F. G., Jutila, K. L., Stall, A. M., Fiering, S., Herzenberg, L. A., Berg, E. L. & Butcher, E. C. (1988) *Eur. J. Immunol.* **18,** 1819–1826.
11. Langer, J. A. & Pestka, S. (1988) *Immunol. Today* **9,** 393–400.
12. Ucer, U., Bartsch, H., Scheurich, P., Berkovic, D., Ertel, C. & Pfizenmaier, K. (1986) *Cancer Res.* **46,** 5339–5343.
13. Rubinstein, M., Novick, D. & Fischer, D. G. (1987) *Immunol. Rev.* **97,** 29–50.
14. Aguet, M. & Merlin, G. (1987) *J. Exp. Med.* **165,** 988–999.
15. Novick, D., Orchansky, P., Revel, M. & Rubinstein, M. (1987) *J. Biol. Chem.* **262,** 8483–8487.
16. Calderon, J., Sheehan, K. C. F., Chance, C., Thomas, M. L. & Schreiber, R. D. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 4837–4841.
17. Basu, M., Pace, J. L., Pinson, D. M., Hayes, M. P., Trotta, P. P. & Russell, S. W. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 6282–6286.
18. Khurana, G. K. & Schreiber, R. D. (1989) *J. Biol. Chem.* **264,** 11981–11988.
19. Aguet, M., Dembic, A. & Merlin, G. (1988) *Cell* **55,** 273–280.
20. Celada, A., Gray, P. W., Rinderknecht, E. & Schreiber, R. D. (1984) *J. Exp. Med.* **160,** 55–74.
21. Schreiber, R. D., Hicks, L. J., Celada, A., Buchmeier, N. A. & Gray, P. W. (1985) *J. Immunol.* **134,** 1609–1618.
22. Sheehan, K. C. F., Calderon, J. & Schreiber, R. D. (1988) *J. Immunol.* **140,** 4231–4237.
23. Gubler, U. & Hoffman, B. J. (1983) *Gene* **25,** 263–269.
24. Maniatis, T., Hardison, R. C., Lacy, E., Lauer, J., O'Connell, C., Quon, D., Sim, G. K. & Efstratiadis, A. (1978) *Cell* **15,** 687–701.
25. Smith, A. J. H. (1980) *Methods Enzymol.* **65,** 560–580.
26. Blin, N. & Stafford, D. W. (1976) *Nucleic Acids Res.* **3,** 2303–2308.
27. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
28. Dobner, P. R., Kawasaki, E. S., Yu, L. Y. & Bancroft, F. C. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 2230–2234.
29. Lawn, R. M., Fritsch, E. F., Parker, R. C., Blake, G. & Maniatis, T. (1978) *Cell* **15,** 1157–1174.
30. Eaton, D. L., Wood, W. I., Eaton, D., Hass, P. E., Hollinghead, P., Wion, K., Mather, J., Lawn, R. M., Vehar, G. A. & Gorman, C. (1986) *Biochemistry* **25,** 8343–8347.
31. Gorman, C., Padmanbhan, R. & Howard, B. H. (1983) *Science* **217,** 551–553.
32. Graham, F. L. & van der Eb, A. J. (1973) *Virology* **52,** 456–467.
33. Celada, A., Allen, R., Esparza, I., Gray, P. W. & Schreiber, R. D. (1985) *J. Clin. Invest.* **76,** 2196–2205.
34. Scatchard, G. (1949) *Nature (London)* **227,** 680–685.
35. McManus, N. H. (1976) *Appl. Environ. Microbiol.* **31,** 35–38.
36. Jung, V., Rashidbaigi, A., Jones, C., Tischfield, J. A., Shows, T. B. & Pestka, S. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 4151–4155.