Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 9901–9905, December 1989
Cell Biology

Cloning of murine interferon γ receptor cDNA: Expression in human cells mediates high-affinity binding but is not sufficient to confer sensitivity to murine interferon γ

(protein homology/major histocompatibility complex antigens)

SILVIO HEMMI*, PIETRO PEGHINI*, MARTINA METZLER*, GILLES MERLIN†, ZLATKO DEMBIC‡, AND MICHEL AGUET*§

*Institute of Immunology and Virology, University of Zürich, P.O. Box CH-8028, Zurich, Switzerland; †Unité Institut National de la Santé et de la Recherche Médicale 196, Institut Curie, 75231 Paris, France; and ‡Central Research Units, F. Hoffmann–La Roche, CH-4005, Basel, Switzerland

Communicated by Stanley G. Nathenson, September 5, 1989

ABSTRACT **A full-length cDNA encoding the murine interferon γ (IFN-γ) receptor was isolated from a λgt11 library using a human IFN-γ receptor cDNA probe. The deduced amino acid sequence of the murine IFN-γ receptor shows ≈53% homology to its human counterpart but no homology to other known proteins. Murine IFN-γ receptor cDNA was expressed in human HEp-2 cells, which do not bind murine IFN-γ and are insensitive to its action. Transfectants displayed the same binding properties as mouse cells. The biological responsiveness of such transfectants to various biological effects of both human and murine IFN-γ was investigated, including modulation of major histocompatibility complex class I and class II antigen expression, inhibition of cell growth, and antiviral activity. Like parental HEp-2 cells, these transfectants responded only to human, but not to murine, IFN-γ. Inversely, mouse L929 cells transfected with human IFN-γ receptor cDNA were insensitive to human IFN-γ. These results confirm and extend previous findings, suggesting that species-specific cofactors are needed for IFN-γ-mediated signal transduction.**

Interferon γ (IFN-γ) is produced by activated T cells and exerts a variety of biological effects, including antiviral activity, inhibition of cell growth, and activation of macrophages (1). It modulates the expression of major histocompatibility complex (MHC) antigens and probably plays a crucial role for regulating antigen presentation (2–4). IFN-γ exerts its effects through binding to specific cell-surface receptors (5). It enters the cells via typical endocytotic pathways (6–8), yet the second messengers involved in signal transduction are not known. To provide an access to these signaling events, we have recently cloned and expressed the gene for the human IFN-γ receptor (9). The human IFN-γ receptor is a 489-amino acid transmembrane receptor with about equally large extracellular (N terminal) and cytoplasmic domains. When expressed in mouse cells, this single chain exhibited binding properties indistinguishable from those of the natural human receptor, but preliminary results indicated that such transfected cells were insensitive to human IFN-γ. To extend these findings, we have cloned a cDNA encoding the murine IFN-γ receptor and tested human cells expressing the murine receptor for their sensitivity to both human and murine IFN-γ with regard to various biological effects of IFN-γ.¶ Clearly, our results suggest that additional species-specific signal transducing elements are needed.

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MATERIALS AND METHODS

Isolation of Murine IFN-γ Receptor cDNA Clones. RNA was extracted from SL/Am splenocytes or murine EL-4 cells by the guanidinium thiocyanate/CsCl procedure (10), and poly(A)$^+$ mRNA was purified according to standard techniques (11). Oligo(dT)-primed cDNA synthesis and cloning into λgt11 were done with commercial kits (Amersham). A human IFN-γ receptor cDNA probe was prepared by subcloning a blunt-ended *Cfo* I/*Bst*EII fragment containing the complete coding domain and parts of the 3′ untranslated region of the human IFN-γ receptor cDNA (9) into the *Sma* I site of pUC19 (Pharmacia). The probe was labeled with ^{32}P by the random oligonucleotide primer labeling method (12) and was hybridized to the libraries at low stringency in 20% formamide at 42°C. The filters were washed twice in 0.3 M NaCl/0.03 M sodium citrate/0.1% SDS at room temperature, twice in 0.3 M NaCl/0.03 M sodium citrate/1% SDS at 50°C, and once in 0.015 M NaCl/0.0015 M sodium citrate at room temperature. Insert DNA from plaque-purified specific clones was excised by *Eco*RI and subcloned into pUC19 and M13 vectors for further analysis.

Construction of Expression Plasmids. A blunt-ended *Cfo* I/*Ava* I fragment containing the complete coding region of the murine IFN-γ receptor cDNA was subcloned into the *Eco*RV site of pHMG. This plasmid, which contains the promoter, the first untranslated exon, and the first intron of the murine 3-hydroxy-3-methylglutaryl coenzyme A reductase gene, was kindly provided by R. Lathe (Unité de Biologie Moléculaire et de Génie Génétique, Institut de Chimie Biologique, Strasbourg, France) and will be described elsewhere (30). The resulting expression vector containing the murine IFN-γ receptor cDNA was designated pHMG-A7′. An analogous expression vector was constructed with a blunt-ended *Cfo* I/*Bst*EII fragment containing the complete coding domain and parts of the 3′ untranslated region of the human IFN-γ receptor cDNA (9), and the resulting vector was designated pHMG-16′.

Stable Transfection of Human HEp-2 and Murine L929 Cells with Murine or Human IFN-γ Receptor cDNA. Human HEp-2 and murine L929 cells obtained from American Type Culture Collection were grown in DMEM (GIBCO) supplemented with 10% fetal calf serum. Exponentially growing subconfluent cell monolayers of 1–2 × 10^6 cells were transfected with 10 μg of CsCl purified supercoiled plasmid DNA by the

Abbreviations: IFN-γ, interferon γ; MHC, major histocompatibility complex.

§To whom reprint requests should be addressed at the present address: Institute of Molecular Biology I, University of Zürich, P.O. Box CH-8097, Zurich, Switzerland.

¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. M28233).

calcium phosphate precipitation method (13). DNA from expression plasmids was coprecipitated with pSV2neo DNA (14). G418 (GIBCO) -resistant colonies were isolated and subcloned 3–4 weeks after transfection.

Ligand Binding Assay. Recombinant murine IFN-γ with a specific activity of 10^7 units per mg of protein was generously provided by G. Adolf (Ernst-Boehringer-Institute für Arzneimittelforschung, Vienna). Radiolabeling was as described for human IFN-γ (15). Recombinant human IFN-γ with a specific activity of 10^7 units per mg of protein was a gift from Ch. Weissmann (Institut für Molekularbiologie I, University of Zürich). IFN binding to HEp-2 or L929 cells was determined as described (16). Saturation curves were determined by incubation of the cells for 2 hr at 4°C at various concentrations of ^{125}I-labeled IFN-γ. Background binding was determined in the presence of 3×10^{-8} M unlabeled IFN-γ.

Western Blot Analysis of Solubilized Receptor Protein from HEp-2 Transfectants or L1210 Cells. Mouse leukemia L1210 cells were grown as suspension cultures in RPMI 1640 medium (GIBCO) supplemented with 10% fetal calf serum. For ligand blotting, a lysate of enriched membranes of 10^7 L1210 or HEp-2 cells [detached with 10 mM EDTA in phosphate-buffered saline (PBS)] was prepared as described (15), subjected to SDS/PAGE on a 10% gel under nonreducing conditions, and transferred to nitrocellulose. To reveal receptor protein, milk saturated nitrocellulose strips corresponding to the SDS/PAGE lanes were incubated with 3×10^{-10} M ^{125}I-labeled murine IFN-γ as reported (15). Back-

A

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1     GAATTCCGGGCCGCTTGCACTTGGCGACTAGTCTGCGGCGGACGTGACGCCAAGGCCACGGGCAGCGCGGGTCCCCTGTCAGAGGTGTCCCTCGCGCAGGAATGGGCCCGCAGGCGGCA
                                                                                                          MetGlyProGlnAlaAla
                                                                                                            s1
120   GCTGGCAGGATGATTCTGCTGGTGGTCCTGATGCTGTCTGCGAAGGTCGGGAGTGGAGCTTTGACGAGCACCGAGGATCCTGAGCCTCCCTCGGTGCCTGTACCGACGAATGTTCTAATT
      AlaGlyArgMetIleLeuLeuValValLeuMetLeuSerAlaLysValGlySerGlyAlaLeuThrSerThrGluAspProGluProProSerValProValProThrAsnValLeuIle
         s10              s20              .1                   10.                  20
240   AAGTCTTATAACTTGAACCCTGTCGTATGCTGGGAATACCAGAACATGTCACAGACTCCTATTTTTACTGTACAGGTAAAGGTGTATTCGGGTTCCTGGACTGATTCCTGCACCAACATT
      LysSerTyrAsnLeuAsnProValValCysTrpGluTyrGlnAsnMetSerGlnThrProIlePheThrValGlnValLysValTyrSerGlySerTrpThrAspSerCysThrAsnIle
                        30.                  40.                  50.                  60
360   TCTGATCATTGTTGTAATATCTATGAACAAATTATGTATCCTGATGTATCTGCCTGGGCCAGAGTTAAAGCTAAGGTTGGACAAAAAGAATCTGACTATGCACGGTCAAAAGAGTTCCTT
      SerAspHisCysCysAsnIleTyrGluGlnIleMetTyrProAspValSerAlaTrpAlaArgValLysAlaLysValGlyGlnLysGluSerAspTyrAlaArgSerLysGluPheLeu
                        70.                  80.                  90.                  100
480   ATGTGCCTAAAGGGAAAGGTCGGGCCCCCTGGCCTGGAGATCAGGAGGAAGAAGGAAGAACAGCTCTCCGTCCTCGTATTTCACCCTGAAGTCGTTGTGAATGGAGAGAGCCAGGGAACC
      MetCysLeuLysGlyLysValGlyProProGlyLeuGluIleArgArgLysLysGluGluGlnLeuSerValLeuValPheHisProGluValValValAsnGlyGluSerGlnGlyThr
                        110.                 120.                 130.                 140
600   ATGTTTGGTGACGGGAGCACCTGTTACACATTCGACTATACTGTGTATGTGGAGCATAACCGGAGTGGGGAGATCCTACATACGAAACATACGGTCGAAAAAGAAGAGTGTAATGAGACT
      MetPheGlyAspGlySerThrCysTyrThrPheAspTyrThrValTyrValGluHisAsnArgSerGlyGluIleLeuHisThrLysHisThrValGluLysGluGluCysAsnGluThr
                        150.                 160.                 170.                 180
720   CTGTGTGAGTTAAACATCTCAGTATCCACACTGGATTCCAGATATTGTATTTCAGTAGACGGAATCTCATCTTTCTGGCAAGTTAGAACAGAAAAATCGAAAGACGTCTGTATCCCTCCT
      LeuCysGluLeuAsnIleSerValSerThrLeuAspSerArgTyrCysIleSerValAspGlyIleSerSerPheTrpGlnValArgThrGluLysSerLysAspValCysIleProPro
                        190.                 200.                 210.                 220
840   TTCCATGATGACAGAAAGGATTCAATTTGGATTCTGGTGGTTGCTCCTCTTACCGTCTTTACAGTAGTTATCCTGGTATTTGCGTATTGGTATACTAAGAAGAATTCATTCAAGAGAAAA
      PheHisAspAspArgLysAspSerIleTrpIleLeuValValAlaProLeuThrValPheThrValValIleLeuValPheAlaTyrTrpTyrThrLysLysAsnSerPheLysArgLys
                        230.                 240.                 250.                 260
960   AGCATAATGTTACCTAAGTCCTTGCTCTCTGTGGTAAAAAGTGCCACGTTAGAGACAAAACCTGAATCGAAGTATTCACTTGTCACACCGCACCAGCCAGCTGTCCTAGAGAGTGAGACG
      SerIleMetLeuProLysSerLeuLeuSerValValLysSerAlaThrLeuGluThrLysProGluSerLysTyrSerLeuValThrProHisGlnProAlaValLeuGluSerGluThr
                        270.                 280.                 290.                 300
1080  GTGATCTGTGAAGAGCCCCTGTCCACAGTGACAGCTCCAGACAGCCCCGAAGCAGCAGAACAGGAAGAACTTTCAAAAGAAACAAAGGCTCTGGAGGCTGGAGGAAGCACGTCTGCCATG
      ValIleCysGluGluProLeuSerThrValThrAlaProAspSerProGluAlaAlaGluGlnGluGluLeuSerLysGluThrLysAlaLeuGluAlaGlyGlySerThrSerAlaMet
                        310.                 320.                 330.                 340
1200  ACCCCAGACAGCCCTCCAACTCCGACACAAAGACGCAGCTTTTCCCTGTTAAGTAGTAACCAGTCAGGCCCTTGTAGCCTCACCGCCTATCACTCCCGAAACGGCTCTGACAGTGGCCTC
      ThrProAspSerProProThrProThrGlnArgArgSerPheSerLeuLeuSerSerAsnGlnSerGlyProCysSerLeuThrAlaTyrHisSerArgAsnGlySerAspSerGlyLeu
                        350.                 360.                 370.                 380
1320  GTGGGATCGGGCAGCTCCATATCGGACTTGGAATCTCTCCCAAACAACAACTCAGAAACAAAGATGGCAGAGCACGACCCTCCACCCGTGAGAAAGGCCCCCATGGCCTCCGGTTATGAC
      ValGlySerGlySerSerIleSerAspLeuGluSerLeuProAsnAsnAsnSerGluThrLysMetAlaGluHisAspProProProValArgLysAlaProMetAlaSerGlyTyrAsp
                        390.                 400.                 410.                 420
1440  AAACCGCACATGTTGGTGGACGTGCTTGTGGATGTTGGGGGAAGGAGTCTCTCATGGGGTATAGACTCACAGGAGAGGCCCAGGAGCTGTCCTAAGGTCTCCCGAGGCCTGCTGGTGGT
      LysProHisMetLeuValAspValLeuValAspValGlyGlyLysGluSerLeuMetGlyTyrArgLeuThrGlyGluAlaGlnGluLeuSerStop
                        430.                 440.                 450.
1560  AAAGAAACTGACCTTTTAGGCAGTTTTTCTGCATTGATTTCATGAAAGAAGCTATACATTAGCTAATACTAACCACATAGAATATCAGACTTAGATACGTGAATAAGGATCCTGTGGGCA

1680  CTGCTGGGTCCACTCTGCAAATGCCAAGACTATCAAAGGAACGTATTGTCGCTTCTGGCTCCTTCCCAGGTGGGCTAGCATCTGTGAGTTTGCCTCGGCTAGCCTTGCTTCCTACAGCCG

1800  CCACTGCTCCTCCACCCTGATCATCTCACAGGACAGGGTGGACCGGGTTTTTTTTTTTTTTCACACACCTTTGTATATGTAAGTTCATGTATATAATATGTTTACATGTTTCACTTTGA

1920  ACTGAAAGCTACTCAAAGCCAGCCGTAAGTCTATGGTAGAATGTGATGGAACATGTTGGTGGAAGCTTGTACAATAGAACACATTGGTGGGAGCTTGTACATACTTTTTTATGGAGCATT

2040  ACTTACGATTTTTTAAGTAAAATGTTTTGAAACCAAAAAAAAAAAAAAGGAATTC
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B

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                        1
human          MALLFLLPLVMQGVSRAEMGTADLGPSSVPTPTNVTIESYNMNPIVYWEYQIMPQVPVFTVEVKNYGVKNSEWIDACINISHHYCNISDHVG   75
murine  MGPQAAAGRMI--VV-M-SAKVG-G-LTS-E-PE-P---V----L-K---L--V-C----N-S-T-I---Q--V-SGS...-T-S-T---D-C---YEQIM

human   DPSNSLWVRVKARVGQKESAYAKSEEFAVCRDGKIGPPKLDI.RKEEKQIMIDIFHPSVFVNGDEQEVDYDPETTCYIRVYNVYVRMNGS.EIQYKILTQK 174
murine  Y-DV-A-A----K------D--R-K--LM-LK--V---G-E-R--K-E-LSVLV---E-V---ES-GTMFGDGS---TFD-T---EH-R-G--LHTKH-VE

human   EDDCDEIQCQLAIPVSSLNSQYCVSAEGVLHVWGVTTEKSKEVCITIFNSSIKGSLWIPVVAALLLFLVLSLVFICFYIKKINPLKEKSIILPKSLISVVR 275
murine  KEE-N-TL-E-N-S--T-D-R--I-VD-ISSF-Q-R-----D---PP-HDDR-D-I--L---P-TV-T-VI---AYW-T--.-SF-R---M-----L---K

human   SATLETKPESKYVSLITSYQPFSLEKEVV.CEEPLSPATVPGMHTEDNPGKVEHTEELSSITEVVTTEENIPDVVPGSHLTPIERESSSPLSSNQSEPGSI 375
murine  ------------.--V-PH--AV--S-T-I------TV-A-DSP.-.....AAEQ----KE-KALEAGGSTSAMT-D-PP--TQ-R-F-L------G-C-L

human   ALNSYHSRNCSESDHSRNGFDTDSSCLESHSSLSDSEFPPNNKGEIKTEGQELITVIKAPTSFGYDKPHVLVDLLVDDSGKESLIGYRPTEDSKEFS    472
murine  TA..-----GS..........--GLVG-G--I--L-SL---NS-T-MAEHDPPP-R---MAS------M---V---VG-----M---L-GEAQ-L-
```

FIG. 1. (*A*) Murine IFN-γ cDNA nucleotide and predicted amino acid sequences. Amino acid numbering starts at the predicted cleavage site for the murine signal sequence (S1–S26). (*B*) Comparison of human (9) and murine IFN-γ receptor amino acid sequences (single-letter code). Homologous amino acid residues are marked as dashes. Deletions or insertions are marked as dots. Numbering starts at the predicted cleavage site for the human signal sequence and refers to the human sequence. Hydrophobic putative leader and transmembrane regions are double underlined. Sites of potential asparagine-linked glycosylation are underlined.

ground binding was determined in the presence of 3×10^{-8} M unlabeled murine IFN-γ.

Cytofluorometry of MHC Antigen Expression. Parental or transfected HEp-2 cells were incubated in 25-cm^2 culture flasks for 36 hr at 37°C with 50 units of human or murine IFN-γ per ml. Subsequently, the cell monolayers were detached by treatment with 10 mM EDTA in PBS, washed with culture medium, and incubated for 90 min at 4°C with mouse monoclonal antibodies specific for common human MHC class I or class II antigen determinants (monoclonal antibodies W6/32 and L243 from Serotec and Becton Dickinson, respectively). Subsequently, the cells were washed by centrifugation and incubated for another 60 min at 4°C with a phycoerythrin-conjugated goat anti-mouse IgG second antibody (Serotec). After a further washing step, cell-associated fluorescence was quantified in a fluorescence-activated cell sorter (FACS analyzer, Becton Dickinson).

Antiviral Assay. Human or murine IFN-γ was assayed on human HEp-2 (American Type Culture Collection) or murine L929 cells challenged with vesicular stomatitis virus. One unit of IFN per ml is defined as the concentration that results in 50% protection from the cytopathic effect.

RESULTS

Cloning of a Murine IFN-γ Receptor cDNA. A human IFN-γ receptor cDNA probe containing the complete coding region and parts of the 3′ untranslated region (9) was used for Northern blot analysis of mRNAs from different murine tissues and cell lines. Significant cross-hybridization was notably found with poly(A)$^+$ mRNA from murine EL4 cells, which express a high number of IFN-γ receptors (17), or from splenocytes of various mouse strains. Two λgt11 cDNA libraries were constructed with oligo(dT)-primed poly(A)$^+$ mRNA from either EL4 cells or splenocytes from SL/Am mice that were chosen for convenience. Screening of $\approx 10^6$ colonies of the two unamplified libraries with the human probe revealed 12 cross-hybridizing clones containing *Eco*RI inserts with superimposable or overlapping restriction enzyme maps. From these, 9 proved to contain full-length cDNA inserts of 2.2 kilobases (kb). Insert fragments were subcloned into M13 vectors to determine the nucleotide sequence by the chain-termination method (18). Both cDNA strands from two independent full-length clones were sequenced with superimposable results (Fig. 1*A*). The 2093-base cDNA nucleotide sequence of the longest insert showed an open reading frame of 1431 bases starting at nucleotide 48 and with a first initiation codon (ATG) embedded in a consensus sequence typical of translation initiation in vertebrates (19). This cDNA could encode a protein of 477 amino acids containing a putative N-terminal signal peptide with a predicted cleavage site after amino acid residue 26 (20). A potential transmembrane anchoring domain is found in the middle of the molecule (amino acids 228–252). Computer-assisted searches in the EMBL/GenBank sequence data bank revealed no significant similarity to known proteins except to the human IFN-γ receptor. The comparison with the human amino acid sequence revealed ≈53% homology within aligned sequences (versus 40% between murine and human IFN-γ, ref. 21). While the murine signal peptide is significantly longer than its human counterpart and possesses classical properties of a signal peptide (20), the potential transmembrane domains are concordant (Fig. 1*B*). The eight cysteine residues of the human extracellular (N terminal) domain are conserved in the murine counterpart, which contains two additional cysteines. Like the human receptor, the murine receptor protein is remarkably rich in serines (12%) and several serine- and threonine-rich regions are indicative for O-linked glycosylation. The extracellular portions of both receptors contain five potential N-linked glycosylation sites, but only two are conserved. The putative cytoplasmic portion of the murine receptor contains one site in addition to two sites also found in the human receptor. Glycosylation could well account for the discrepancy between the apparent M_r of ≈90,000 for the natural receptor (22) protein and the M_r of ≈50,000 predicted from the deduced amino acid sequence.

Northern blot hybridization using the murine IFN-γ receptor cDNA probe revealed a single 2.2-kb transcript versus 2.3 kb for human IFN-γ receptor mRNA (data not shown). A superimposable transcript signal was invariably observed in

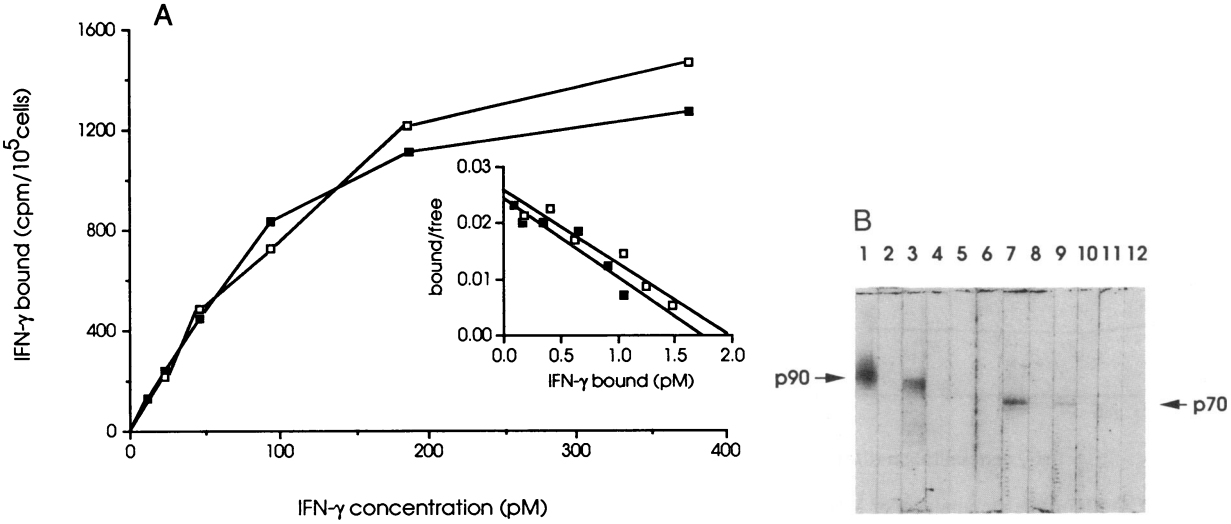

FIG. 2. (*A*) Saturation curve with ^{125}I-labeled human (■) or mouse (□) IFN-γ on human HEp-2 cells transfected with murine IFN-γ receptor cDNA. The cells were incubated for 90 min at 4°C at various concentrations of labeled IFN-γ as described elsewhere (16). Nonspecific binding was determined by simultaneous addition to labeled IFN-γ of 30 nM unlabeled human or murine IFN-γ. The specific binding is depicted as the difference between total and nonspecific binding. (*Inset*) Scatchard plot of the same data. (*B*) Ligand blot analysis of solubilized murine receptor protein from untreated (lanes 1-6) versus tunicamycin treated (lanes 7–12) L1210 cells (lanes 1, 2, 7, and 8), transfected HEp-2 cells (lanes 3, 4, 9, and 10), and parental HEp-2 cells (lanes 5, 6, 11, and 12). Membrane lysates were subjected to SDS/PAGE and transferred to nitrocellulose. Nitrocellulose strips were incubated with ^{125}I-labeled murine IFN-γ in the absence (odd numbered lanes) or presence (even numbered lanes) of excess unlabeled murine IFN-γ. The autoradiograph shows samples from one typical experiment.

a broad panel of normal murine tissues and cell lines (P.P., unpublished results).

Expression of IFN-γ Receptor cDNA. To study both binding and biological properties of the cloned murine IFN-γ receptor, human HEp-2 cells were chosen for transfection experiments since they are highly responsive to various biological effects of human IFN-γ, while, like all human cells, they do not bind murine IFN-γ and are insensitive to its action. To express mouse IFN-γ receptor cDNA, the promoter of the human 3-hydroxy-3-methylglutaryl coenzyme A reductase gene was chosen. Like the IFN-γ receptor gene, this housekeeping gene is ubiquitously expressed in a constitutive manner (23). Stable transfectants were obtained that expressed the murine receptor to a similar extent as the human receptor (≈2 × 10^3 receptors per cell; Fig. 2*A*) and bound murine IFN-γ with the same affinity as human IFN-γ (K_d, ≈5 × 10^{-11} to 10^{-10} M; Fig. 2*A*). Receptor protein from such transfectants displayed a significantly lower apparent M_r as compared to the natural receptor of murine L1210 cells (Fig. 2*B*). This difference was attributed to different N-linked glycosylation, since, upon preincubation of the cells for 18 hr at 37°C with tunicamycin (2 μg/ml) (Boehringer Mannheim), the major receptor protein band became indistinguishable in both transfected HEp-2 and L1210 cells (Fig. 2*B*) and migrated with an apparent M_r of ≈70,000.

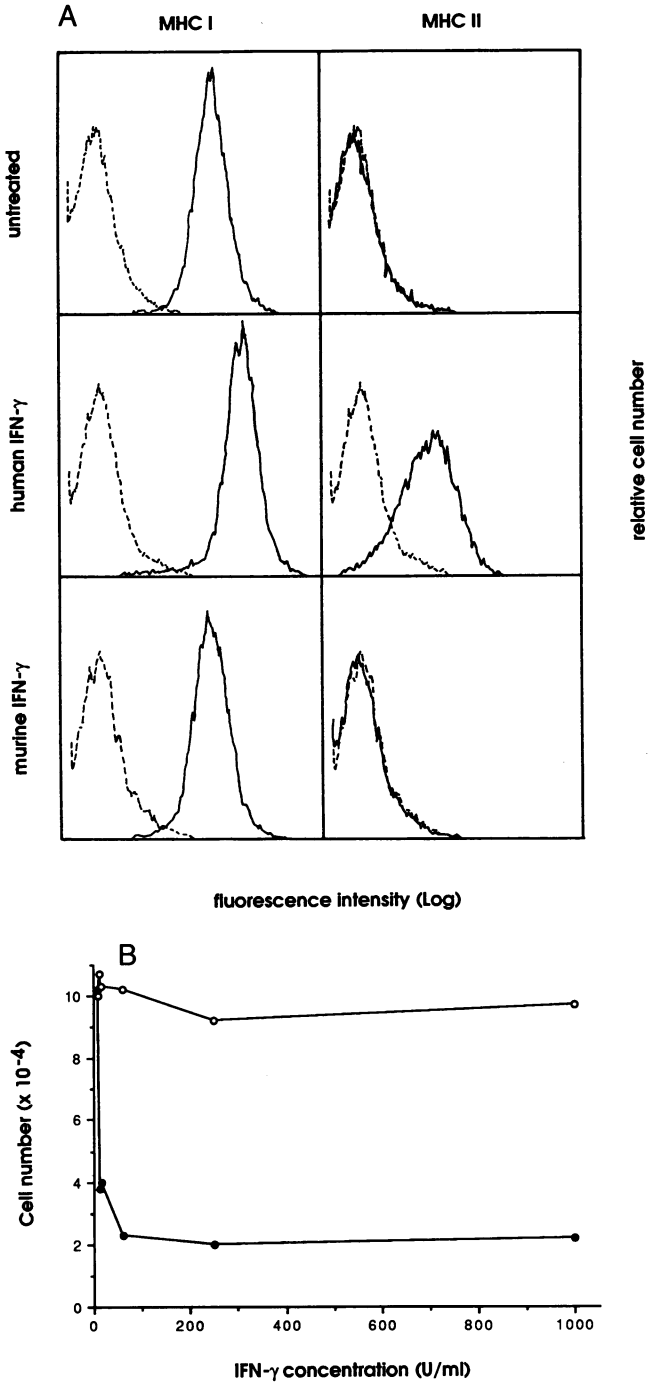

FIG. 3. (*A*) Cytofluorometric analysis of MHC class I and class II antigen expression on human HEp-2 transfectants expressing the murine IFN-γ receptor and treated with human or murine IFN-γ. Dotted lines represent background binding of the fluorescent antibody. (*B*) Effect of human versus murine IFN-γ on the growth of human HEp-2 transfectants expressing the murine IFN-γ receptor. The cells were seeded in duplicate 2-cm^2 wells at an initial density of 10^4 cells per well, cultured for 72 hr at 37°C at various concentrations of human (●) or murine (○) IFN-γ, and counted. Standard deviations were <10%.

Functional Properties of the Transfected IFN-γ Receptor. HEp-2 cells expressing the murine IFN-γ receptor remained insensitive to the biological effects of murine IFN-γ. As shown in Fig. 3*A*, MHC antigen expression was only modulated by human but not by murine IFN-γ. Likewise, the growth rate of transfected HEp-2 cells remained unaltered in the presence of up to 1000 units of murine IFN-γ per ml (Fig. 3*B*). Finally, transfected HEp-2 cells were protected from infection with vesicular stomatitis virus upon preincubation with 1 unit of human IFN-γ per ml, while 1000 units of murine IFN-γ per ml had no antiviral effect.

Inversely, mouse L929 cells that were transfected with human IFN-γ receptor cDNA and expressed human IFN-γ receptors to a similar extent and with apparently identical binding properties as human HEp-2 cells (data not shown), remained insensitive to the antiviral effect of human IFN-γ, even at 1000 units/ml, although they were sensitive to 1–2 units of murine IFN-γ per ml. Mouse L929 cells are insensitive to murine IFN-γ with regard to enhancement of MHC antigen expression and cell growth inhibition. As an alternative response marker, the induction of 2′–5′-oligo(A) synthetase was investigated (24, 25). In contrast to HEp-2 cells, which displayed a high constitutive level that was not modulated by human IFN-γ, an up to 10-fold enhancement of 2′–5′-oligo(A) synthetase activity was observed in mouse L929 cells treated with murine IFN-γ (Table 1). But again, L929 transfectants expressing the human IFN-γ receptor were insensitive to human IFN-γ. The marked differences observed in the constitutive 2′–5′-oligo(A) synthetase activity of the three independent clones tested remain unexplained.

Table 1. Induction of 2′–5′-oligo(A) synthetase in L929 transfectants expressing the human IFN-γ receptor

	L929		
	Clone 12	Clone 13	Clone 15
Untreated	2,925 ± 26	4,706 ± 62	14,724 ± 882
Murine IFN-γ (100 units/ml)	29,602 ± 379	18,332 ± 2,770	74,958 ± 5,321
Human IFN-γ (20 units/ml)	3,058 ± 379	4,917 ± 740	11,020 ± 274
Human IFN-γ (200 units/ml)	2,549 ± 401	4,819 ± 16	13,869 ± 180

Subconfluent 25-cm^2 cultures of three independent clones of murine L929 cells transfected with the human IFN-γ receptor cDNA and selected for stable high-affinity binding of ^{125}I-labeled human IFN-γ were incubated overnight at 37°C with murine or human IFN-γ or were left untreated. Cell extracts were prepared and assayed for 2′–5′-oligo(A) synthetase as described (24). The results are indicated as cpm corresponding to ^{32}P incorporated into oligo(A) oligomers.

DISCUSSION

IFN-γ is a lymphokine with potent immunoregulatory effects (1–3). Elucidating the structure of its receptor should open new ways not only to investigate the mechanisms of signal transduction but, more generally, to define the physiological role of IFN-γ. The availability of mouse models with the possibility to modulate the action of IFN-γ at the receptor level could represent an important step toward new therapeutic concepts in a variety of immunological disorders.

Here we report the cloning of the murine IFN-γ receptor from murine λgt11 cDNA libraries by hybridization with a human IFN-γ receptor cDNA probe and the expression of the murine receptor in human cells. Like the human IFN-γ receptor (9), the murine IFN-γ receptor is a transmembrane receptor that bears no resemblance to known proteins except a 53% amino acid homology to its human counterpart (Fig. 1). Since the cysteine backbone is widely conserved and the hydropathy index plots are virtually superimposable (data not shown), the global structure of these two receptors must be very similar. As in its human analogue, the putative transmembrane anchor of the murine receptor subdivides the polypeptide chain into an N-terminal presumably extracellular portion and an almost equally large cytoplasmic domain. The homology between the human and the murine IFN-γ receptor is distributed rather evenly and since both ligand binding and signal transduction (see below) are species specific, it is difficult to speculate on relevant functional domains.

Like the human IFN-γ receptor expressed in mouse cells (9), the murine receptor expressed in human HEp-2 cells displayed binding properties indistinguishable from those of the resident receptor (Fig. 2). However, the murine IFN-γ receptor expressed in human HEp-2 cells was not able to transduce an IFN-γ-specific signal. In these transfectants, enhanced MHC class I and class II antigen expression (Fig. 3*A*), growth inhibition (Fig. 3*B*), and antiviral protection were only observed upon treatment with human but not murine IFN-γ. We have reported previously (9) that mouse L1210 cells transfected with the human IFN-γ receptor gene were insensitive to human IFN-γ with regard to enhancement of MHC class I antigen expression and 2′–5′-oligo(A) synthetase activity. Consistent with these preliminary findings, murine L929 cells transfected with human IFN-γ receptor cDNA remained insensitive to the antiviral effect of human IFN-γ and only murine, but not human, IFN-γ was able to stimulate 2′–5′-oligo(A) synthetase activity (Table 1).

Obviously, biological responsiveness to heterologous IFN-γ of human and murine cells transfected with the IFN-γ receptor of the other species depends on additional species-specific signal transducing elements. From experiments with mouse–human somatic cell hybrids, it is known that stimulation of MHC class I antigen expression by human IFN-γ requires human chromosome 21 besides human chromosome 6 (26), which carries the gene for the IFN-γ receptor (9, 26). Here we show that the missing element(s) is essential for biological effects as diverse as antiviral protection, enhancement of MHC class I/II antigen expression, and growth inhibition. It has been shown that a murine MHC class I antigen promoter transfected into human cells is responsive to human IFN-γ (27), and, likewise, the 5′ flanking region of the human IFN-γ-induced gene 9–27 becomes responsive to murine IFN-γ when transfected into mouse cells (28). Clearly, the IFN-γ-stimulated regulatory events at the level of gene transcription are much less species specific than the interactions at the receptor level. For all these reasons, it is tempting to assume that the missing component(s) is probably interacting with the receptor chain identified so far. Unlike the case of the interleukin 2 receptor, in which a complex of at least two receptor chains is needed to mediate high-affinity binding (29), the IFN-γ receptor expressed in heterologous cells displays binding properties indistinguishable from those of the resident receptor (Fig. 2*A*). It is improbable, therefore, that a second receptor chain involved in ligand binding is missing and most data from crosslinking experiments do not support the hypothesis of such a second chain (for review, see ref. 5).

Transfectants expressing a nonfunctional heterologous IFN-γ receptor besides the functional constitutive receptor provide a tool to search for components involved in IFN-γ signaling.

Note Added in Proof. A report by Gray *et al.* describing very similar findings has been published (31).

We are grateful to Dr. G. Adolf (Ernst-Boehringer-Institut für Arzneimittelforschung, Vienna) and to Dr. Ch. Weissmann (Institut für Molekularbiologie I, University of Zürich) for generously providing recombinant murine and human IFN-γ, respectively. The excellent technical assistance of E. Huf and G. Kunz is gratefully acknowledged. This work was supported in part by the Kanton of Zürich and the Swiss National Science Foundation.

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