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The Extracellular Domain of the Human Interferon Gamma Receptor Interacts with a Species-Specific Signal Transducer

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At least two species-specific gene products are required for signal transduction by interferon gamma (IFN-γ). The first is the IFN-γ receptor, which binds ligand with high affinity in a species-specific manner. The second is an undetermined species-specific signal transducer(s). To determine whether the human IFN-γ receptor (hIFN-γR) interacts directly with this signal transducer(s) and, if so, with what functional domain(s), we constructed expression vectors for the hIFN-γR and three hybrid human-murine IFN-γ receptors. The hybrid receptors contained the extracellular, human IFN-γ (hIFN-γ) binding domain of the hIFN-γR, either the human or murine transmembrane domain, and either the human or murine intracellular domain. The vectors encoding these receptors were stably transfected into two mouse cell lines, one of which (SCC-16-5) contains a single copy of human chromosome 21. The resulting cell lines were treated with hIFN-γ, and murine major histocompatibility complex class I antigen expression was analyzed by immunofluorescence flow cytometry. All transfected cell lines lacking human chromosome 21 remained insensitive to hIFN-γ. However, all four of the IFN-γ receptors were able to signal when expressed in the cell line containing human chromosome 21. We conclude that the extracellular domain of the IFN-γ receptor is involved not only in the species specificity of IFN-γ binding but also in signalling through interaction with an as yet unidentified species-specific factor(s) encoded by a gene(s) on human chromosome 21.

Interferon gamma (IFN-γ) is a cytokine produced by activated T cells and natural killer cells that has antiviral and antiproliferative effects on a wide variety of cells (53). Its most potent effects are immunomodulatory (50) and include the activation of macrophages (46), the regulation of major histocompatibility complex (MHC) class I and class II antigen expression (31, 49), and the regulation of immunoglobulin production (35, 48).

IFN-γ binds with high affinity (K_d = 10^{-9} to 10^{-11} M at 4°C) to a 90-kDa glycoprotein receptor present in low numbers (10^2 to 10^4) on a wide variety of cell types (7, 16, 19, 33, 36, 42, 51). Human and murine IFN-γ share 40% amino acid sequence identity (22) and exhibit species specificity with respect to receptor binding (15, 52) and biological activity (14, 22). Human and murine IFN-γ receptor (hIFN-γR and mIFN-γR, respectively) cDNAs have been cloned and expressed (2, 12, 23, 25, 32, 39). The mature hIFN-γR protein consists of 472 amino acids and shares 52% amino acid sequence identity with the mIFN-γR. Both IFN-γR proteins have equally large extracellular (EC) (228 amino acids) and intracellular (IC) (222 amino acids for human, 200 amino acids for mouse) domains and a single transmembrane (TM) domain.

The gene for the hIFN-γR is located on chromosome 6 (41, 43). When this chromosome is present in human-murine somatic cell hybrids (29, 41, 43) or after transfection of the hIFN-γR gene into mouse cells (2), hIFN-γ binds with high affinity to the hIFN-γR but no biological response is elicited. The hIFN-γR as expressed in murine somatic cell hybrids and transfected mouse cells has binding properties similar to those of the hIFN-γR on human cells (1, 7, 36, 42). These findings, in conjunction with results of cross-linking experiments (7, 19, 36, 40), suggest that in contrast to the interleukin-6 (26, 54) and granulocyte-macrophage colony-stimulating factor receptors (21, 24), a second IFN-γR chain is not involved in mediating high-affinity ligand binding.

Human-murine somatic cell hybrids containing human chromosomes 6 and 21 (29), as well as human-hamster and human-murine somatic cell hybrids containing human chromosome 21 and transfected with hIFN-γR expression vectors (17, 28), respond to hIFN-γ as measured by the induction of MHC class I antigen. These findings demonstrate that the human chromosome 21-encoded factor(s) is necessary for signalling by the hIFN-γR, at least with respect to histocompatibility antigen expression, and suggest that the signal transducer(s) and the IFN-γR must be from the same species. How these two species-specific molecules function is unknown.

To determine the region(s) of the IFN-γR that must be from the same species as the signal transducer to generate a functional response to IFN-γ, we constructed expression vectors for human and hybrid human-murine IFN-γ receptors. We transfected each expression vector into two different murine cell lines, one of which (SCC-16-5) contains a single copy of human chromosome 21. Transfectants were stimulated with hIFN-γ and analyzed for increased expression of murine MHC class I antigen. Using this approach, we identified the region of the IFN-γR that cooperates with the species-specific signalling element(s).

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MATERIALS AND METHODS

Reagents. Recombinant mIFN-γ (10^7 U/mg) and recombinant hIFN-γ (2×10^7 U/mg) were produced in *Escherichia coli* and supplied as highly purified proteins (Genentech, Inc., manufacturing group). hIFN-γ was radioiodinated with Bolton-Hunter reagent (Amersham, Arlington Heights, Ill.) to a specific activity of 60 μCi/μg as described previously (8). Recombinant hIFN-α2a (3×10^6 U/ml) was purchased from Roche Pharmaceuticals, Nutley, N.J. Purified GIR 208, a murine monoclonal antibody specific for the hIFN-γR (47), and GR20, a rat monoclonal antibody specific for the mIFN-γR (4), were provided by R. Schreiber, Washington University, St. Louis, Mo. Purified murine monoclonal antibody H-2K^k, specific for murine MHC class I antigen, was purchased from Becton Dickinson, Mountain View, Calif. Phycoerytherin (PE)-conjugated goat anti-mouse immunoglobulin G (IgG) F(ab')$_2$ was purchased from Caltag, South San Francisco, Calif., and PE-conjugated goat anti-rat IgG was purchased from Biomeda, Foster City, Calif.

Plasmid construction. The expression plasmid pRK-M-γR encoding the mIFN-γR cDNA has been described elsewhere (23). A 1,469-bp fragment, containing the entire coding region of the hIFN-γR cDNA, was inserted into the mammalian cell expression vector pRK5, creating plasmid pRK-H-γR. pRK-HHM-γR encodes a receptor with hIFN-γR sequences for the EC and TM domains and the first four amino acids (Lys-Lys-Ile-Asn) of the IC domain and mIFN-γR sequences for the remainder of the IC domain. By using site-directed mutagenesis, an *Ase*I site was created in pRK-M-γR at the junction between the TM and IC domains, resulting in plasmid pRK-M-γR Ase I. An *Ase*I site is already present in pRK-H-γR at this position. An *Ase*I-*Hin*dIII fragment from pRK-M-γR Ase I was inserted into pRK-H-γR to create the hybrid receptor construct pRK-HHM-γR. pRK-HMM-γR encodes a receptor with hIFN-γR sequences for the EC domain and mIFN-γR sequences for the TM and IC domains. It was made with pRK-H-γR and pRK-M-γR as templates, using a two-step polymerase chain reaction (PCR) technique (6, 55) to generate a precise fusion at the junction between the EC and TM domains. pRK-HMH-γR encodes a receptor with hIFN-γR sequences for the EC and IC domains and mIFN-γR sequences for the TM domain. It was made by digesting pRK-H-γR with *Ase*I and *Hin*dIII and isolating a 1,069-bp fragment. This fragment plus two synthetic oligonucleotides were ligated to pRK-HMM-γR which had been digested with *Acc*I and *Hin*dIII. The predicted coding sequences for all four vectors were confirmed by supercoiled DNA sequencing, using a dideoxy-chain termination technique (11).

Cell lines and transfection. The murine fibroblast cell line L929 was grown in Dulbecco's modified Eagle medium with 10% fetal calf serum, 2 mM L-glutamine, 50 U of penicillin per ml, 50 μg of streptomycin per ml, and 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES). The murine cell line SCC-16-5 (SCC; provided by R. Schreiber, Washington University, St. Louis, Mo.) is a human-murine L-cell somatic cell hybrid which contains a single copy of human chromosome 21 (27). It was grown in Dulbecco's modified Eagle medium with 10% fetal calf serum, 2 mM L-glutamine, 50 U of penicillin per ml, and 50 μg of streptomycin per ml. The cells were separately plated in 10-cm dishes at 5×10^5 cells per dish and cotransfected with 10 to 20 μg of expression plasmid and 1 to 2 μg of pSVEneo DNA per dish by a calcium phosphate precipitation technique (10). Forty-eight hours after transfection, cells were split 1:4 and transfectants were selected in 600 μg of G418 (Geneticin; GIBCO) per ml.

Isolation of human or hybrid human-murine IFN-γR-expressing clones. G418-resistant clones were pooled and incubated for 1 h at 4°C with 4 μg of GIR 208 per 10^6 cells, washed, and then incubated for 1 h at 4°C with 5 μg of PE-conjugated goat anti-mouse IgG per 10^6 cells. Cells were washed and analyzed on a Coulter Elite flow cytometer equipped with a single 15-mW argon laser tuned to 488 nm. Forward and 90° angle light scatter, propidium iodide exclusion for viable cell gating, and integrated log PE fluorescence signals (with a band pass filter [575 ± 25 nm] in place) were collected and analyzed. Receptor-positive cells were collected by sorting the brightest 5% viable cells in the sample, washed with medium, and plated on a 35-mm plate. After expansion, this sorting procedure was repeated four times. At the final sort, cells were collected on 96-well plates; after growth, wells containing single clones were picked.

Cytofluorometric analysis of IFN-γR expression. Cells (10^6) from each L929 and SCC clone were removed from 10-cm plates with 20 mM EDTA, washed, and incubated with 4 μg of GIR 208 for 1 h at 4°C. Cells were washed, incubated with 2.5 μg of PE-conjugated goat anti-mouse IgG for 1 h at 4°C, washed, and analyzed by immunofluorescence flow cytometry as described above. mIFN-γR expression was assessed on 10^6 untransfected L929 and SCC cells, using 4 μg of GR20 and 2.5 μg of PE-conjugated goat anti-rat IgG in the same manner.

Radioligand binding analysis. Cells from one clone of each receptor construct were harvested with 20 mM EDTA, washed, and resuspended in phosphate-buffered saline containing 0.1% bovine serum albumin and 0.02% sodium azide (PBSA buffer). Binding displacement assays were performed in duplicate in PBSA by incubating 5×10^5 cells with 50 pM ^{125}I-IFN-γ with or without increasing concentrations of unlabelled hIFN-γ in a final volume of 250 μl. Nonspecific binding was determined by the addition of 1 μM unlabelled hIFN-γ. Reaction mixes were incubated at 4°C for 2 h. Reactions were stopped by centrifugation at 12,000 × *g* for 10 min at 4°C. Unbound ^{125}I-IFN-γ in the supernatant was removed by aspiration; the pellets were washed once with 300 μl of ice-cold PBSA and then counted in an Isodata gamma counter. The data were plotted with the Scatchard equation, using nonlinear least-squares regression.

Functional analysis. Cells (2×10^5) from each receptor-expressing L929 and SCC clone were plated on six-well plates in 2 ml of either medium alone or medium plus hIFN-γ (100 ng/ml), mIFN-γ (100 ng/ml), or hIFN-α2a (1,000 U/ml) for 48 to 72 h. Cells were removed with 20 mM EDTA, washed and incubated for 1 h at 4°C with 2.5 μg of anti-H-2K^k, washed, and then incubated 1 h at 4°C with 2.5 μg of PE-conjugated goat anti-mouse IgG. MHC class I antigen expression was assessed by immunofluorescence flow cytometry.

PCR analysis. Cells from individual L929 and SCC clones were resuspended at 10^4 cells per ml in PCR buffer (50 mM KCl, 10 mM Tris HCl [pH 8.4], 1.5 mM $MgCl_2$) containing 0.5% Tween and 100 μg of proteinase K per ml, heated at 55°C for 45 min, and then heated at 95° for 10 min (30). PCR analysis was performed in PCR buffer containing 40 μl of lysate, 200 μM deoxynucleoside triphosphates, and 1 U of *Taq* DNA polymerase (Perkin-Elmer Cetus) in a Perkin-Elmer Cetus DNA thermal cycler, using sets of 30-mer oligonucleotide primers for the hIFN-γR, mIFN-γR, hIFN-αR, amyloid precursor protein, and superoxide dismutase 1 genes. Reaction conditions were 94°C for 5 min, 95°C for 25

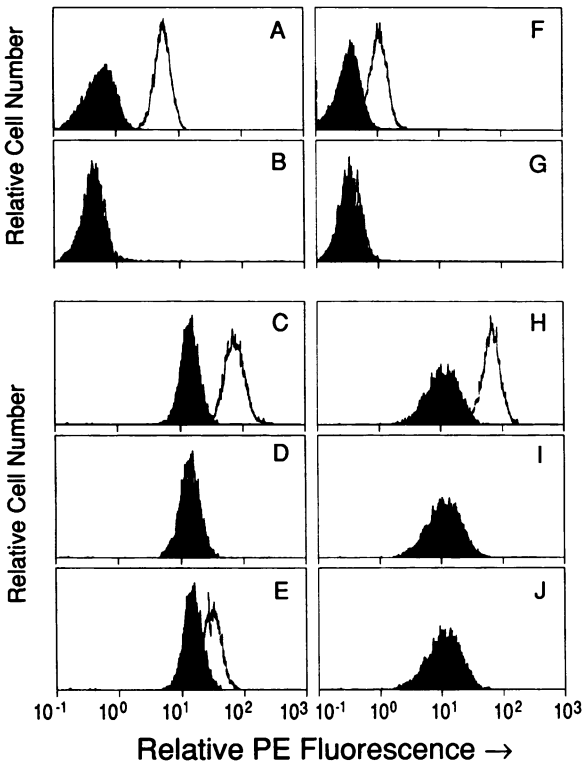

FIG. 1. Characterization of untransfected murine SCC (A to E) and L929 (F to J) cell lines. To analyze expression of mIFN-γR (A and F) and hIFN-γR (B and G), 10^6 cells were sequentially incubated with either anti-mIFN-γR or anti-hIFN-γR monoclonal antibody and PE-conjugated goat anti-rat or goat anti-mouse IgG and analyzed by flow cytometry as described in Materials and Methods. The black area represents background binding of the fluorescent antibody. To examine MHC class I antigen expression after treatment with 100 ng of mIFN-γ per ml (C and H), 100 ng of hIFN-γ per (D and I), or 1,000 U of hIFN-α2a per ml (E and J), after interferon treatment, 10^6 cells were sequentially incubated with anti-mouse H-$2K^k$ monoclonal antibody and PE-conjugated IgG and analyzed by flow cytometry as described in Materials and Methods. The black area represents constitutive level of class I antigen expression.

s, 60°C for 45 s, and 72°C for 2 min, for 30 cycles. Reaction aliquots were analyzed by 1.2% agarose gel electrophoresis.

RESULTS

SCC cells express about 3,000 mIFN-γR molecules per cell (13). The level of mIFN-γR expression on SCC cells, as demonstrated with a rat monoclonal antibody against the mIFN-γR, GR20, is shown in Fig. 1A. SCC cells do not express the antigen recognized by the anti-hIFN-γR monoclonal antibody GIR 208 (Fig. 1B). As determined by immunofluorescence flow cytometry, SCC cells constitutively express murine MHC class I antigen. After incubation with 100 ng of mIFN-γ per ml for 48 h, the level of MHC class I expression increases fivefold (Fig. 1C), whereas incubation with 100 ng of hIFN-γ per ml for 48 h results in no increase in class I expression (Fig. 1D). The gene for the hIFN-αR is located on chromosome 21 (9, 37, 44), so SCC cells contain this gene and respond weakly to hIFN-α2a. After incubation for 72 h with 1,000 U of hIFN-α2a per ml, a 1.5-fold increase in murine MHC class I antigen expression can be demonstrated (Fig. 1E). Human IFN-α2a is relatively species

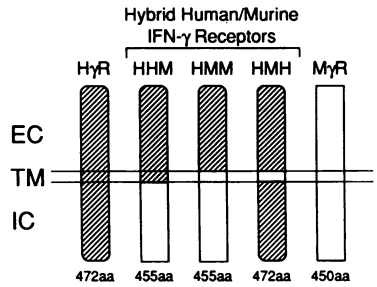

FIG. 2. Schematic view of the human, murine, and hybrid human-murine IFN-γ receptors. HγR, full-length hIFN-γR encoded by pRK-H-γR; HHM, hybrid receptor encoded by pRK-HHM-γR; HMM, hybrid receptor encoded by pRK-HMM-γR; HMH, hybrid receptor encoded by pRK-HMH-γR; mγR, full-length mIFN-γR encoded by pRK-M-γR. Stippled and open areas represent regions of human and murine origin, respectively.

specific, and at this dose, MHC class I antigen expression is preferentially stimulated through the hIFN-αR on the murine SCC cells. Higher doses are required to stimulate MHC class I expression through the murine IFN-αR (data not shown). Thus, SCC cells respond to mIFN-γ and hIFN-α2a but do not respond to hIFN-γ.

Murine fibroblast L cells have been reported to express 5 × 10^3 to 15 × 10^3 mIFN-γR molecules per cell (34). The level of mIFN-γR expression on L929 cells as demonstrated with the antibody GR20 is shown in Fig. 1F. L929 cells do not stain with antibody GIR 208 directed against the hIFN-γR (Fig. 1G). As seen with SCC cells, L929 cells constitutively express murine MHC class I antigen, and its expression increases about fivefold after incubation with mIFN-γ (Fig. 1H). However, there is no change in the level of MHC class I antigen expression following incubation with hIFN-γ or hIFN-α2a (Fig. 1I and J). Thus, L929 cells respond to mIFN-γ but do not respond to hIFN-γ or hIFN-α2a.

Characterization of mouse cell lines expressing human and hybrid human-murine IFN-γ receptors. We constructed and expressed in L929 and SCC cells one human and three hybrid human-murine IFN-γR expression vectors (Fig. 2). The first hybrid IFN-γR, designated HHM, contains the human EC and TM regions and a murine IC region. The second hybrid IFN-γR, HMM, contains the human EC domain and the murine TM and IC domains. The third hybrid IFN-γR, HMH, contains the human EC and IC domains and the murine TM domain.

For each of the IFN-γR constructs in L929 and SCC cells, at least 50 G418-resistant clones were isolated and examined (see Materials and Methods). Figure 3 illustrates the level of IFN-γR expression from a representative clone for each of the IFN-γR constructs, as determined by immunofluorescence flow cytometry using antibody GIR 208. The transfected murine cells expressed the hIFN-γR or a hybrid human-murine IFN-γR at different levels. In general, receptor expression was higher in L929 clones than in SCC clones. To confirm that each clone contained the correct expression vector, PCR analysis was performed on DNA from whole-cell lysates, using sets of species-specific primers to different regions of the receptors. All clones generated the expected fragments (data not shown).

To determine the affinity of hIFN-γ for the human and hybrid human-murine IFN-γ receptors, we performed competition binding assays on single clones of each receptor construct in both L929 and SCC cells. All clones exhibited

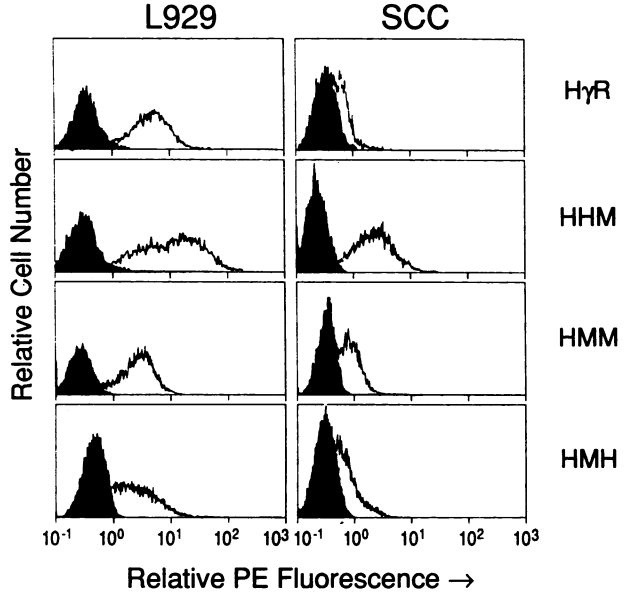

FIG. 3. Expression of human and hybrid human-murine IFN-γ receptors on transfected murine L929 and SCC cell lines expressing hγR, HHM, HMM, and HMH, as indicated. A total of 10^6 cells from one representative clone of each construct were sequentially incubated with anti-hIFN-γR monoclonal antibody GIR 208 and PE-conjugated goat anti-mouse IgG and analyzed by flow cytometry as described in Materials and Methods. The black area represents background binding of the fluorescent antibody.

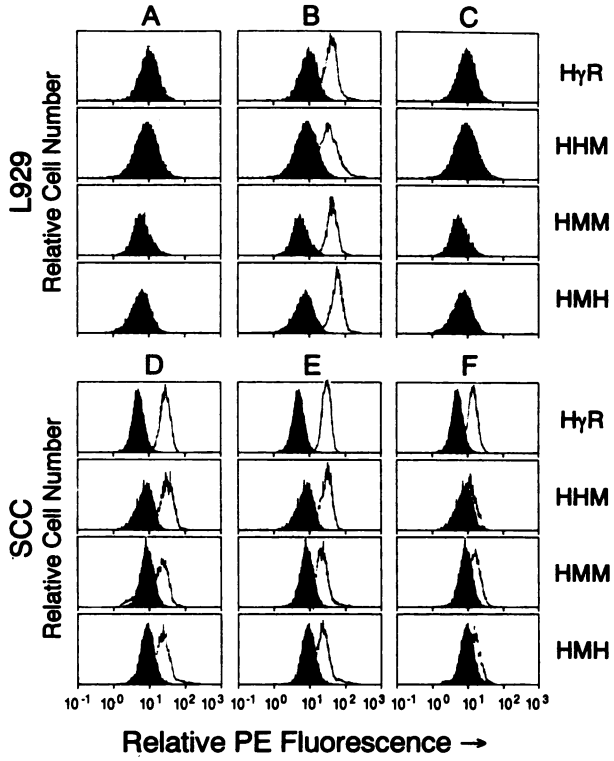

FIG. 4. MHC class I antigen expression after treatment with mIFN-γ, hIFN-γ, and hIFN-α2a on L929 and SCC cell lines expressing a human or hybrid human-murine IFN-γR. Stable cell lines expressing IFN-γR constructs were analyzed as described in the legend to Fig. 3. A total of 10^6 cells from one representative clone of each construct were sequentially incubated with anti-mouse H-$2K^k$ monoclonal antibody and PE-conjugated goat anti-mouse IgG and analyzed by flow cytometry after 48 h of treatment as follows: columns A and D, 100 ng of hIFN-γ per ml; columns B and E, 100 ng of mIFN-γ per ml; columns C and F, 1,000 U of hIFN-α2a per ml. The black area represents the constitutive level of class I antigen expression.

similar competition curves, and Scatchard transformation of the data indicated a single class of high-affinity binding sites (Table 1). The binding inhibition constants (K_i) ranged from 1.3×10^{-10} to 5.2×10^{-10} M, values similar to those reported for a variety of human cells (3, 7, 16, 36, 51). Whether a particular IFN-γR construct was expressed in L929 or SCC cells had no effect on its affinity for hIFN-γ.

SCC clones which express a human or hybrid human-murine IFN-γR respond to hIFN-γ. None of the L929 clones expressing the hIFN-γR or any one of the hybrid human-murine IFN-γ receptors showed increased levels of MHC class I antigen expression after stimulation with 100 ng of hIFN-γ per ml for 48 h (Fig. 4, column A). hIFN-γ doses of 1 to 1,000 ng/ml over 48 to 72 h were also without effect (data not shown). The expression of MHC class I antigen was modulated only by mIFN-γ (Fig. 4, column B). As expected, hIFN-α2a was without effect on L929 cells (Fig. 4, column C).

TABLE 1. Ligand binding constants[a] of IFN-γR constructs

Cell line	K_i (10^{-10} M) ± SD
H-L929	1.3 ± 0.3
H-SCC	1.7 ± 0.6
HHM-L929	1.9 ± 0.4
HHM-SCC	1.9 ± 0.6
HMM-L929	1.6 ± 0.2
HMM-SCC	3.3 ± 1.7
HMH-L929	5.2 ± 3.0
HMH-SCC	4.5 ± 1.0

[a] Derived from Scatchard plots and least-squares best-fit analysis of displacement binding experiments.

In contrast, most of the SCC clones which expressed the hIFN-γR or any one of the hybrid IFN-γ receptors responded to hIFN-γ with increased MHC class I antigen expression. All four IFN-γR constructs resulted in clones which responded equally well to hIFN-γ and mIFN-γ (Fig. 4, columns D and E). There was no correlation between the level of IFN-γR expression and the magnitude of response to hIFN-γ. For example, SCC clone HγR-SCC, which expressed low levels of IFN-γR, responded as well to hIFN-γ as did clone HHM-SCC, which had a higher level of receptor expression. SCC clones which responded to hIFN-γ and expressed the hIFN-γR or any one of the hybrid receptors also responded to hIFN-α2a. A 1.5- to 2-fold increase in murine MHC class I antigen expression can be seen after 72 h of incubation with hIFN-α2a (Fig. 4, column F).

SCC clones which fail to respond to hIFN-γ have lost chromosome 21. A number of SCC clones expressed the transfected human or hybrid human-murine IFN-γR at high levels but failed to respond to hIFN-γ or hIFN-α2a (data not shown). They responded normally (fivefold increase) to mIFN-γ (data not shown), demonstrating that there was no defect in their ability to increase MHC class I antigen

expression. We suspected that these clones might have resulted from transfection of cells which have lost all or part of human chromosome 21, since there is no selective pressure for the retention of this human chromosome. To test this hypothesis, PCR analysis was performed on whole-cell lysates from the untransfected SCC cell line and from all SCC clones to screen for the presence of three different human chromosome 21-encoded genes. The hIFN-αR, amyloid precursor protein, and superoxide dismutase 1 genes have been mapped to the long arm of human chromosome 21 (38). All of the genes were detected in DNA from the untransfected SCC cells and in all SCC clones which responded to hIFN-γ (data not shown). However, in the nonresponding SCC clones, none of the markers could be detected. Thus, these nonresponding SCC clones have probably lost human chromosome 21.

DISCUSSION

On the basis of an analysis of hIFN-γR-transfected human-hamster somatic cell hybrids, Kumar et al. (32) have suggested that the chromosome 21-encoded signal transducer(s) interacts with the intracellular domain of the homologous IFN-γR and that the species-specific event is intracellular. Others have hypothesized that the signalling element(s) might recognize extracellular species-specific domains (1). To identify the functional domain of the hIFN-γR required for the species-specific activation of the human chromosome 21-encoded signal transducer(s), we constructed expression vectors encoding the hIFN-γR and three hybrid human-murine IFN-γ receptors that contain the human EC domain. We transfected each expression vector into two mouse cell lines, one of which (SCC-16-5) contains human chromosome 21. We isolated individual clones which expressed each IFN-γR and analyzed each clone for the induction of murine MHC class I antigen expression after treatment with hIFN-γ.

HγR-L929 clones, as well as HγR-SCC clones which have lost chromosome 21, express the hIFN-γR and bind hIFN-γ. These clones respond normally to mIFN-γ, but there is no increase in the level of murine MHC class I antigen expression following treatment with hIFN-γ. Apparently the murine signal transducer(s) present in these clones can function with the mIFN-γR in response to mIFN-γ but not with the hIFN-γR in response to hIFN-γ. In contrast, HγR-SCC clones that contain human chromosome 21 bind hIFN-γ and possess a functionally active hIFN-γR. These data confirm previous findings (2, 17, 28, 29) that a functional hIFN-γR requires at least two components of human origin. One is a ligand-binding molecule, and another is a signal-transducing factor(s) encoded by a gene(s) on chromosome 21.

The L929 and SCC clones which express a hybrid human-murine IFN-γ receptors have different regions of the IFN-γR available to associate with the endogenous murine signal transducer(s). Neither the IFN-γR with a murine IC domain alone (HHM), a murine TM domain alone (HMH), or a murine TM and IC domain together (HMM) was active in L929 cells. All three hybrid human-murine IFN-γ receptors were functional in SCC cells that contained human chromosome 21. As the EC domain is the only region shared by the hIFN-γR and the hybrid human-murine IFN-γ receptors, we conclude that the IC domain of the hIFN-γR does not interact in a species-specific manner with the signal transducer(s). It is, in fact, the EC domain of the IFN-γR that is involved in mediating the species-specific response.

Studies of a 55-kDa fragment of the purified hIFN-γR (5), studies using monoclonal antibodies produced against the native hIFN-γR (20), and studies of a recombinant soluble hIFN-γR (18) have shown that the EC domain contains the IFN-γ binding site. Previous experiments have also shown that hIFN-γ binds with high affinity to an hIFN-γR whether or not the receptor is functional (2, 29, 41, 43). We demonstrate here that four different IFN-γR constructs containing the EC domain of the hIFN-γR, whether expressed in L929 or SCC cells, have similar affinities for IFN-γ. These findings suggest that the signal transducer(s) encoded by chromosome 21 does not substantially alter the affinity of IFN-γ for the IFN-γR. In addition, the human signal transducer(s) as expressed in SCC cells does not detectably bind hIFN-γ (45). These data, in conjunction with the findings reported here, suggest that the IFN-γR directly interacts with the signal transducer(s) and does not merely tether IFN-γ for association with the signalling element(s). We do not know whether IFN-γ binding to the IFN-γR precedes the interaction of the IFN-γR and the signal transducer(s) or whether the IFN-γR and the signal transducer(s) are in association in the absence of ligand. Cross-linking studies reported to date have not contained information that discriminates between these alternatives (7, 19, 36, 40). Finally, it is also possible that the signal transducer(s) makes contacts with IFN-γ as well as with the IFN-γR.

The IC domain of the IFN-γR has been shown to be essential for cellular activation after treatment with IFN-γ. Microinjection of monoclonal antibodies directed against portions of the IFN-γR IC domain blocked IFN-γ-induced MHC class II antigen expression and antiviral protection (1). In addition, the analysis of IC domain deletion mutants has identified specific regions in the IC domain that are required for ligand processing and biologic function (13). Whether the IC domain also interacts with the signal transducer(s) remains to be determined. However, our data demonstrate that the IC domain is not involved in the species-specific response.

The control of the species-specific response is a distinct function of the EC region of the IFN-γR. The EC domain is involved in the recognition of ligand and in the recognition of a signal transducer(s). On the basis of the data presented here and in analogy to gp130, the signal-transducing molecule associated with the interleukin-6 receptor (26), and KH97, the second subunit of the human granulocyte-macrophage colony-stimulating factor receptor (24), the IFN-γR signal transducer(s) may be a receptorlike molecule that possesses EC, TM, and IC domains. Further clarification of these issues should be facilitated by the identification and cloning of the IFN-γR signalling element(s).

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