The Mouse Immune Interferon Receptor Gene Is Located on Chromosome 10*

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When mouse L cells are incubated with $^{32}$P-labeled recombinant murine immune interferon ($^{32}$P-Mu-IFN-$\gamma$) and subsequently cross-linked with disuccinimidyl suberate, a major complex with an apparent molecular mass of 95,000-125,000 daltons can be visualized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The complex was not formed when the binding was performed in the presence of excess unlabeled Mu-IFN-$\gamma$ or when Chinese hamster ovary cells were used. This complex therefore represents the Mu-IFN-$\gamma$ receptor (or its interferon-binding subunit). The chromosomal location of the Mu-IFN-$\gamma$ receptor (or the binding subunit of the receptor) gene, termed Ifgr, was identified by performing the binding and cross-linking reactions on a series of mouse-hamster somatic cell hybrids with different subsets of mouse chromosomes. The presence of mouse chromosome 10 was shown to be necessary and sufficient for the formation of the cross-linked complex. Thus, the gene coding for the binding subunit of the Mu-IFN-$\gamma$ receptor was localized to mouse chromosome 10. The presence of this chromosome in the hybrid cells was not sufficient, however, to confer antiviral resistance to the hybrids when they were treated with Mu-IFN-$\gamma$ and challenged with encephalomyocarditis virus.

The interferons (IFNs) are a family of proteins which display antiviral, antiproliferative, and immunoregulatory activities. To date, three classes of interferons have been isolated and characterized (reviewed in Refs. 1-5). These are the leukocyte (IFN-$\alpha$), fibroblast (IFN-$\beta$), and immune (IFN-$\gamma$) interferons. In order to elicit a response, the interferons must first bind to specific cell surface receptors (6-9). The preparation of radiolabeled interferons has stimulated many studies of the interferon receptors (reviewed in Refs. 9 and 10). So far, it seems that IFN-$\alpha$ and IFN-$\beta$ share a common receptor which is distinct from the IFN-$\gamma$ receptor.

Most of the IFN receptor studies have utilized $^{125}$I-labeled interferons as ligands. Recently, recombinant human (11, 12) and murine (13) IFN-$\gamma$ have been radiolabeled to high specific radioactivities with $[^\gamma^{32}P]$ATP and the catalytic subunit of bovine heart muscle cAMP-dependent protein kinase. The $^{32}$P-labeled recombinant human (Hu) IFN-$\gamma$ was initially used to measure equilibrium and kinetic parameters for the interaction between the $[^3P]$Hu-IFN-$\gamma$ and its receptor (12). Subsequently, human-mouse and human-hamster somatic cell hybrids were cross-linked with $[^3P]$Hu-IFN-$\gamma$ in order to localize the gene for the Hu-IFN-$\gamma$ receptor to human chromosome segment 6q (14).

In the present study, mouse-hamster somatic cell hybrids were cross-linked with $[^3P]$Mu-IFN-$\gamma$ to determine the chromosomal location of the Mu-IFN-$\gamma$ receptor gene.

EXPERIMENTAL PROCEDURES

Interferon, Radiolabeling, and Interferon Assay—Recombinant murine IFN-$\gamma$ with a specific activity of 1.02 x 10$^8$ units/mg was isolated from Escherichia coli as described previously (15) and kindly supplied by Dr. H. M. Shepard (Genentech). The protein concentration of the interferon was determined by amino acid analysis.

Mu-IFN-$\gamma$ was phosphorylated according to procedures developed for the phosphorylation of Hu-IFN-$\gamma$. Briefly, 0.9 µg of Mu-IFN-$\gamma$ was incubated at 37°C for 15 min with 0.5 mCi $[^\gamma^{32}P]$ATP ($>$5000 Ci/mmol; Amersham Corp.) and 5 units of the catalytic subunit of cAMP-dependent protein kinase from beef heart (Sigma) in a 30-µl reaction volume containing 20 mM Tris-HCl, pH 7.4, 100 mM NaCl, 12 mM MgCl$_2$, and 1 mM dithiothreitol. The reaction was terminated by cooling on ice and adding 0.4 ml of 5% bovine serum albumin in 10 mM sodium pyrophosphate (NaPPi), pH 6.7. After extensive dialysis at 4°C against 10 mM NaPPi, pH 6.7, aliquots were stored in liquid nitrogen.

The antiviral activity of the Mu-IFN-$\gamma$ was routinely measured on mouse L cells by a cytopathic effect inhibition assay (16) with encephalomyocarditis virus. The antiviral effect of Mu-IFN-$\gamma$ on the mouse-hamster hybrid cells was measured in the same manner. Somatic Cell Hybrids—Somatic cell hybrids were made by fusing cells of the Chinese hamster line E36 and peritoneal or spleen cells of BALB/c, A/J, or NFS/Au congenic mice. The production and characterization of these hybrids has been described previously (17-19).

Cells—Chinese hamster ovary K1 cells were grown in 10% CO$_2$ in monolayer culture in F12 medium (Gibco) containing 15% heat-inactivated fetal calf serum and 50 µg/ml gentamicin sulfate. Mouse L cells and hybrid cells were grown in monolayer culture in Dulbecco's modified Eagle's medium (Gibco) containing 4.5 g/liter glucose, 10% heat-inactivated fetal calf serum, 12.5 mM sodium HEPES, pH 7.3, and 50 µg/ml gentamicin sulfate in tightly-capped flasks. All cells were incubated at 37°C in 75-cm$^2$ flasks to 80-90% confluency.

Binding and Cross-linking of $[^3P]$Mu-IFN-$\gamma$ to Cells—Cells were rinsed twice with 5 ml of Dulbecco's phosphate-buffered saline lacking Mg$^{2+}$ and Ca$^{2+}$ and then with 2.5 ml of trypsin-EDTA solution (1 x phosphate-buffered saline: Gibco). Trypsinization was performed at 37°C until the cells were released from the tissue culture flask. The cells were resuspended in the appropriate medium and counted. After centrifugation at 500 x g at 4°C for 10 min, the cells were resuspended at 5 x 10$^5$ cells/ml.

The binding and cross-linking reactions were done as described previously (14) with slight modification. About 10$^5$ cpm of $[^3P]$Mu-IFN-$\gamma$ ($>$516-2,110 Ci/mmol), with or without 1 µg of unlabeled Mu-

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§ The abbreviations used are: IFNs, interferons; Hu-IFN-$\gamma$, human immune interferon; Mu-IFN-$\gamma$, murine immune interferon; HEPES, 4-(2-hydroxyethyl)-1-piperazineneethanesulfonic acid.
IFN-γ as a competitor, were added to 0.3 ml of cells in a 1.5-ml polypropylene tube. Binding was allowed to proceed at 22 °C for 60 min with gentle resuspension every 15 min. The reactions were chilled on ice, and the cells were pelleted for 20 s at 15,000 x g in a Brinkman microfuge. The cells were washed once with and resuspended in 500 μl of cold phosphate-buffered saline. A 50-mM solution of disuccinimidyl suberate (Pierce Chemical Co.), freshly prepared in dimethylsulfoxide, was added to a final concentration of 0.5 mM. After 20 min on ice, the cross-linking reaction was quenched by the addition of 10 μl of 1 M Tris-HCl, pH 7.5. After 5 min on ice, the cells were pelleted as described above and extracted with 100 μl of 0.5% Triton X-100 in phosphate-buffered saline containing 5 mM EDTA at 4 °C for 20 min. Insoluble material was sedimented at 15,000 x g for 10 min. The supernatants were then analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 1.5-mm thick slab gels containing 8% acrylamide (20). Gels were dried under vacuum and subjected to autoradiography at -70 °C with Kodak XRP-1 film and Cronex Lightning Plus intensifying screens (DuPont).

RESULTS

The [32P]Mu-IFN-γ, bound to mouse L cells and then cross-linked to its receptor with disuccinimidyl suberate, migrates predominantly as a broad radioactive band of 110,000 ± 15,000 upon analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 1) as previously reported (13). This band can be eliminated by the addition of excess unlabeled IFN-γ during the binding reaction (Fig. 1). Several less intense bands are also seen which are not eliminated completely by the addition of unlabeled IFN-γ and may represent nonspecific interactions between [32P]Mu-IFN-γ and other cell surface polypeptides. The large amount of radioactivity that migrates with the tracking dye represents [32P]Mu-IFN-γ which was bound to the receptor but was not cross-linked.

When mouse-hamster somatic cell hybrids were examined in this manner, the 110,000-dalton band characteristic of the mouse L cells was noted with several of the hybrids (e.g. hybrid 2A5E5 in Fig. 1). In all hybrid cells where this band was present, it was completely eliminated when the binding reaction contained excess unlabeled IFN-γ.

The presence of the major cross-linked complex was correlated with the mouse chromosome complements of the mouse-hamster hybrids (Table 1). The presence of mouse chromosome 10 always correlated with the presence of the 110,000-dalton complex, and there was no discordant segregation for this chromosome. This association is particularly striking since the set of hybrids included 2 pairs of sister subclones in which each pair was karyotypically identical except for the presence of chromosome 10 in only one member of each pair. For all of the other mouse chromosomes, there are hybrids in which the presence of the 110,000-dalton band does not correlate with the presence of the chromosome.

The ability of Mu-IFN-γ to confer antiviral resistance to various hybrid cells was examined. Neither Chinese hamster ovary K1 cells nor any of the mouse-hamster hybrids, including those that contained mouse chromosome 10 and displayed the 110,000-dalton cross-linked complex, were protected against encephalomyocarditis virus infection by the addition of up to 2.6 x 10⁴ units/ml of Mu-IFN-γ (data not shown). On the other hand, mouse L cells were protected by 3.2 units/ml of the Mu-IFN-γ.

DISCUSSION

When [32P]Mu-IFN-γ is cross-linked to mouse L cells, a predominant complex of 95,000-125,000 daltons is formed.

| Mouse +/− | TOP | 200 | 92.5 | 69 | 46 | TD |
|-----------|------|-----|------|-----|-----|----|
| L | CHO-K1 | BM34 | BM220 | 2A5E5 |
| − | − | − | − | − |

![Electrophoretic analysis of [32P]Mu-IFN-γ covalently cross-linked to L cells and a representative set of mouse-hamster hybrid cells](image)

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**Table 1**

| Mouse chromosome markers | Mu-IFN-γ receptor/chromosome retention* | Percent discordance |
|--------------------------|----------------------------------------|---------------------|
| 1 | 2 | 4 | 0 | 7 | 54 |
| 2 | 2 | 3 | 1 | 8 | 64 |
| 3 | 0 | 3 | 1 | 6 | 70 |
| 4 | 1 | 5 | 2 | 7 | 60 |
| 5 | 0 | 10 | 3 | 3 | 38 |
| 6 | 2 | 5 | 1 | 8 | 56 |
| 7 | 3 | 2 | 0 | 11 | 69 |
| 8 | 2 | 6 | 1 | 5 | 43 |
| 9 | 2 | 4 | 1 | 7 | 57 |
| 10 | 3 | 13 | 0 | 0 | 0 |

* Nine hybrids were karyotyped and seven were typed only for the presence or absence of specific chromosome markers. + and − designate the presence and absence, respectively, of either the Mu-IFN-γ receptor (110,000-dalton complex) or the specified chromosome. The percent discordance is the percent of cell lines tested in which the presence (or absence) of the Mu-IFN-γ receptor does not correlate with the presence (or absence) of the specified chromosome.
Formation of this complex is completely inhibited by excess unlabeled Mu-IFN-γ (Fig. 1). If this represents a 1:1 complex between Mu-IFN-γ and its receptor, then the binding subunit of the murine IFN-γ receptor has a molecular mass of 80,000–110,000 daltons as was recently reported elsewhere (13, 21).

Of the 16 mouse-hamster somatic cell hybrid lines analyzed for the Mu-IFN-γ receptor by cross-linking with [32P]Mu-IFN-γ, three lines were clearly positive. Examination of the chromosome content of the 16 lines showed that all three positive lines contained mouse chromosome 10 and that this chromosome was missing in all other cell lines tested. Since no other chromosome showed any correlation with the formation of 110,000-dalton complex, the data strongly support the assignment of the Mu-IFN-γ receptor gene (or the gene of the receptor-binding subunit) to mouse chromosome 10.

Although several hybrid lines expressed the binding subunit of the Mu-IFN-γ receptor, the cells were not protected from encephalomyocarditis virus killing by prior treatment with Mu-IFN-γ. This failure to establish an antiviral state was also observed in human-mouse and human-hamster hybrid cells which expressed the Hu-IFN-γ receptor-binding subunit (14). Several explanations for this phenomenon may be offered. First, the number of IFN-γ receptors expressed on the surface of the hybrid cells may not be sufficient to elicit an antiviral effect when the cells are treated with IFN-γ. In the case of the Hu-IFN-γ receptor, hybrid cells seemed to express the binding subunit at one-fifth to one-half the level expressed by human A431 cells (14). However, in the present case, two of the positive hybrid lines seemed to express the murine receptor-binding subunit at the same level as mouse L cells (data not shown). Another possibility is that the binding subunit of the IFN-γ receptor is not fully functional in somatic cell hybrids either because of some modification in the protein itself (e.g. post-translational modifications affecting glycosylation or proteolytic cleavage) or because the binding subunit fails to couple efficiently with other host cell-encoded factors involved in the antiviral response. Perhaps this coupling has a high degree of species specificity, and the mouse binding subunit cannot function in a hamster cell.

It should be noted that the gene coding for Mu-IFN-γ has also been localized to chromosome 10 (22). This contrasts with results for Hu-IFN-γ where the Hu-IFN-γ gene resides on chromosome 12 (23), and the gene coding for the binding subunit of the receptor is on the long arm of chromosome 6 (14).

A comparison of the human and mouse gene maps indicates that human chromosome 6 has some homology to mouse chromosome 10. The human and murine homologs for the avian myeloblastosis virus (myb) proto-oncogene have also been localized to the same chromosomes as the genes encoding the binding subunit of the IFN-γ receptor (24, 25). All of the mouse-hamster hybrids used in the present study were tested for the presence of the murine myb homolog and perfect correlation with mouse chromosome 10 and Ifgr was observed (data not shown). Since a comparison of all the other known genes on these two chromosomes indicates extensive gene rearrangement, it is likely that the myb proto-oncogene and the IFN-γ receptor gene are genetically closely linked. This interesting observation may provide a means for the isolation of the IFN-γ receptor gene and the eventual structural characterization of the receptor itself.

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