HUMAN IMMUNE INTERFERON GENE IS LOCATED ON CHROMOSOME 12*

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Three classes of human interferon (IFN)[1] have been characterized on the basis of antigenic, biological, and biochemical properties (1). IFN-α and IFN-β[2] are synthesized by leukocytes and fibroblasts, respectively, that have been treated with virus or synthetic polynucleotides. Unlike IFN-α and IFN-β, immune interferon (IFN-γ) is acid labile and is produced by mitogen-stimulated lymphocyte cultures (1). The IFN-α gene family consists of twelve or more members (2–8), whereas IFN-β (9–15) and IFN-γ (16, 17) are encoded by single genes. The structures of IFN-α and IFN-β proteins are similar and, in fact, are recognized by the same receptor. The genes for IFN-α and IFN-β do not contain introns and share 30–95% sequence homology (5, 18). Both the IFN-α genes and a IFN-β gene have been localized to the short arm of chromosome 9 (19–22). In contrast, IFN-γ is not homologous to the other IFN and is encoded by a single gene that contains three introns (16, 17).

Because immune interferon differs from IFN-α and IFN-β by many criteria, we expected that the chromosomal gene location would also differ from the other two classes of interferon. To chromosomally map the IFN-γ gene, Southern blot analysis was used to analyze DNA isolated from human-rodent somatic cell hybrids. The gene for IFN-γ was located to human chromosome 12 by these analyses, and further studies with a hybrid containing a portion of human chromosome 12 places the gene for IFN-γ[2] in the p1205→qter region.

Materials and Methods

Somatic Cell Hybrids. Human-rodent somatic cell hybrids were constructed by fusing rodent cell lines (LMTK$^-$ and RAG) to various human fibroblasts using polyethylene glycol or Sendai virus (23, 24). The hybrid clones were isolated using the hypoxanthine, aminopterin, thymidine medium, which selects for the retention of human hypoxanthine phosphoribosyltransferase in the case of RAG hybrids or human thymidine kinase in hybrids made with LMTK$^-$. WIL (WI38 × LMTK$^-$), REW (WI38 × RAG), and ICL (GM1006 × LMTK$^-$) were derived from

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[1] Abbreviations used in this paper: CHO, Chinese hamster ovary; IFN, interferon; kb, kilobase pair; LDHB; lactate dehydrogenase-B; PEPB, peptidase-B; SDS, sodium dodecyl sulfate.

[2] Conforming to human gene mapping nomenclature, these genes have been designated *IFA*, *IFB*, and *IFG* corresponding to the interferons IFN-α, IFN-β, and IFN-γ, respectively.

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karyotypically normal human parental cells. Other hybrids segregate human chromosomes containing translocations as indicated in the Table legends.

The hybrid line 12A resulted from the fusion of human lymphocytes with a glyA$^-$ mutant of Chinese hamster ovary (CHO) cells (25, 26). Since the glyA gene codes for serine hydroxymethyl transferase (SHMT), which is necessary for growth of cells on glycine-free medium (25), these hybrids retain human chromosome 12 encoding the human SHMT gene (26). In fact, the 12A line contains only human chromosome 12, whereas A9, a derivative of 12A, has all but the p terminus of chromosome 12 (12p1205→12qter) (27).

Chromosome Composition of Cell Hybrids. Hybrid cell lines were characterized for their human chromosome content by assaying marker enzymes previously assigned to each of the 22 human autosomes and the X chromosome (28, 29) and by direct karyotyping (30). Human isozymes were separated from mouse on starch gel systems and detected by histochemical staining or autoradiography as described in reference 28. The enzyme assays were ACO1, aconitase (soluble); ACO2, aconitase (mitochondrial); ACY1, aminoacylase-1; ADA, adenosine deaminase; AK1, adenylate kinase-1; AK2, adenylate kinase-2; APRT, adenine phosphoribosyl transferase; ESD, esterase-D; G6PD, glucose-6-phosphate dehydrogenase; GALK, galactokinase; GOT1, glutamate oxaloacetate transaminase (soluble); GPI, glucose phosphate isomerase; GSR, glutathione reductase; GUS, β-glucuronidase; HEXB, hexosaminidase-B; IDH1, isocitrate dehydrogenase (soluble); LDHA, lactate dehydrogenase-A; LDHB, lactate dehydrogenase-B; MDH1, malate dehydrogenase (soluble); ME1, malic enzyme (soluble); MPI, mannose phosphate isomerase; NP, nucleoside phosphorylase; PEPA, peptidase-A; PEPB, peptidase-B; PEPC, peptidase-C; PEPS, peptidase-S; PGK, phosphoglycerate kinase; PKM2, pyruvate kinase (muscle form); SOD1, superoxide dismutase (soluble); and SOD2, superoxide dismutase (mitochondrial). DNA markers were also assayed for human chromosomes 3, 5, 17, 18, and 22.

Molecular Probe for the Human Immune IFN genes. The IFN-γ gene was detected by hybridization with an IFN-γ cDNA clone p69 (16). The fragment used was a 887 bp Sau3A fragment encompassing the coding sequences of the IFN-γ gene. The fragment was labeled with ^{32}P to a specific activity of ~10^8 cpm/μg by random calf thymus DNA fragment-primed replication (31).

DNA Isolation and Southern Blot Hybridization. DNA was isolated from cultured cells essentially as described by Wigler et al. (32). Approximately 10^8 cells were removed from plastic flasks by trypsinization and washed several times with serum-free medium. DNA was digested with proteinase K in 1% sodium dodecyl sulfate as described (32); however, extractions were done repeatedly with buffer saturated (10 mM Tris, pH 8.0, 1 mM EDTA) phenol containing 0.1% 8-hydroxyquinoline and then with chloroform isoamyl alcohol (24:1). After bringing the salt concentration to 0.3 M with sodium acetate buffer, pH 5.5, the DNA was precipitated with 2.0 vol of cold isopropanol. The DNA was washed with 70% isopropanol, partially dried, and resuspended in 10 mM Tris, 1 mM EDTA, pH 8.0. The DNA was then RNAse treated (32) and extracted in a similar manner.

10 μg of DNA was cleaved with endodeoxynuclease (EcoRI) using buffers suggested by the manufacturer (4 U of enzyme/μg DNA for 3 h at 37°C). The samples were then heated to 65°C for 10 min. DNA fragments were separated by electrophoresis through a 0.8% agarose gel and transferred to nitrocellulose by the method of Southern (33).

Hybridizations (10^7 cpm probe/blot) were at 42°C for 24 h in 50% formamide, 5× 0.15 M NaCl, 0.015 M NaCitrate pH 7.0 (SSC), 5× Denhardt's, 50 mM sodium phosphate buffer, pH 6.5, 200 μg/nl denatured salmon sperm DNA, and 10% dextran sulfate (34). The filters were washed in 0.2× SSC, 0.1% SDS at 50°C as described (34).

Results

Hybridization of IFN-γ cDNA Probe to Human and Hybrid DNA. DNA was isolated from 37 human rodent somatic cell hybrids and their parental lines for analysis of the IFN-γ gene. The IFN-γ gene was detected by hybridization with an IFN-γ cDNA probe (887 bp Sau3A fragment [16]). Southern hybridization of the ^{32}P-labeled IFN-γ probe to EcoRI digested human genomic DNA results in two DNA fragments of 8.8 and 2.0 kilobase pair (kb) (Fig. 1). Under the hybridization conditions used, the probe

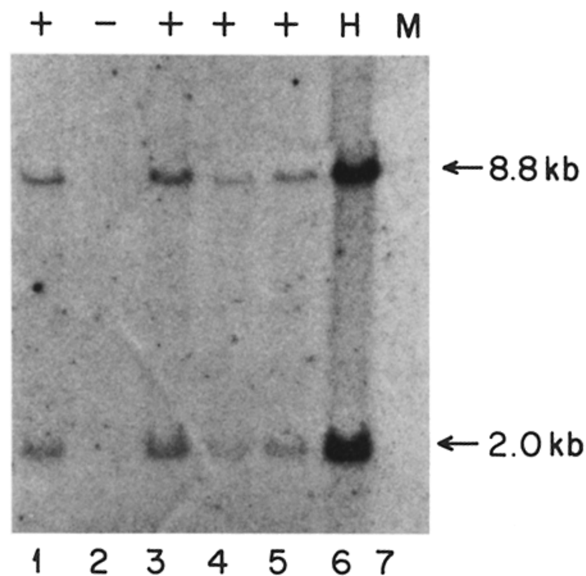

FIG. 1. Hybridization of human immune interferon sequences to EcoRI digested human, mouse, and hybrid DNA. The IFN-γ hybridization patterns shown are for human-mouse hybrids (lanes 1–5), human diploid fibroblast (MLD; lane 6); and $LMTK^-$ mouse cells (lane 7). Cell hybrid DNA in lanes 1, 3, 4, and 5 are positive for IFN-γ gene.

did not hybridize to mouse or Chinese hamster DNA. Consequently, EcoRI digested DNA from cell hybrids segregating human chromosomes yielded either the 8.8- and 2.0-kb human IFN-γ specific fragments (16, 17) or did not hybridize to the IFN-γ probe.

Chromosomal Mapping of Human IFN-γ Gene. Hybrid cell lines were characterized for their human chromosome content by assaying marker enzymes previously assigned to each human chromosome (28, 29) and by direct karyotyping. Table I compares marker enzymes and DNA fragments specific for each human autosome and the X chromosome and their segregation with the IFN-γ gene in somatic cell hybrids. Only the markers for chromosome 12, lactate dehydrogenase-B (LDHB) and peptidase-B (PEPB), segregated concordantly with the gene for IFN-γ. That is, the human IFN-γ gene was present in a hybrid clone only when PEPB and LDHB were present; conversely, the IFN-γ gene was absent when neither human PEPB nor LDHB could be detected. Southern blot analyses of 20 karyotyped hybrid cells confirmed the assignment of the IFN-γ gene to human chromosome 12 (Table II). In fact, DNA from the hybrid line 12A, which contains only human chromosome 12, was positive for IFN-γ hybridization.

To regionally localize the IFN-γ gene, DNA from a hybrid containing a broken chromosome 12 was analyzed by Southern hybridization. The hybrid A9 is a derivative of 12A that was isolated after X ray-induced chromosome breakage in the 12A line (26), and contains the p1205→qter region of human chromosome 12 (27). This region of chromosome 12 contains the gene for peptidase-B and SHMT, a marker that complements the $glyA^-$ deficiency of the CHO parent (25). As a result, the p1205→qter region of chromosome 12 was stably retained in the A9 line grown in glycine-free medium. The IFN-γ probe hybridized with human specific fragments

TABLE I
Segregation of IFN-γ with Human Genetic Markers

Chromosome marker	IFN-γ gene/marker				Percent discordancy
	+/+	+/−	−/+	−/−	
1 AK2, PEPC	4	3	2	8	29
2 MDH1, IDH1	1	5	2	9	41
3 ACY1, HS-3	3	4	6	4	59
4 PEPS	4	3	3	7	35
5 HEXB, 12-65	3	4	5	5	53
6 ME1, SOD2	1	6	2	8	47
7 GUS	4	3	4	6	41
8 GSR	4	2	4	6	37
9 AK1, ACO1	0	7	0	10	41
10. GOT1	4	3	3	7	35
11 LDHA	4	3	4	6	41
12 LDHB, PEPB	7	0	0	10	0
13 ESD	3	4	1	9	29
14 NP	5	2	6	4	47
15 MPI, PKM2	6	1	2	7	18
16 APRT	2	5	1	9	35
17 GALK, 12-2	7	0	9	1	53
18 PEPA, 12-62	6	1	7	3	47
19 GPI	3	3	2	8	35
20 ADA	4	3	4	6	41
21 SOD1	6	1	9	1	59
22 ACO2, MS3-18	0	7	4	6	65
X G6PD, PGK	6	1	7	3	47

Human genetic markers have previously been assigned to each of the 22 human autosomes and the X chromosome (28, 29). Human isozymes were separated from mouse on starch gel systems and detected by histochemical staining or autoradiography as described (28). HS-3, 12-65, 12-2, 12-62, and MS3-18 are DNA markers which we have assigned to chromosomes by Southern blot analysis (Naylor, S. L., A. Y. Sakaguchi, D. Barker, R. White, and T. B. Shows, manuscript in preparation). The columns indicate the number of hybrids in which genetic markers were present together with human immune interferon sequences (+/+), absent together (−/−), or showed discordant segregation (+/− and −/+). The only enzyme markers that segregate concordantly with IFN-γ gene are PEPB and LDHB.

of 8.8 and 2.0 kb in EcoRI digested A9 DNA (Table II). Therefore, the IFN-γ gene is located in the p1205→qter region of human chromosome 12.

The hybrid cell lines used in this study were derived from more than a dozen unrelated individuals. As only a single hybridization pattern was seen when EcoRI was used to cleave the DNA, there apparently is not a frequent DNA polymorphism in IFN-γ detectable by EcoRI within a 10.8-kb region of the gene (8.8 + 2.0 kb). Previously, the enzymes PvuII, HincII, XbaI, PstI, HinfI, and RasiI were used to screen human DNA, and no DNA polymorphisms were found (17). These data indicate that the IFN-γ gene and surrounding sequences are relatively conserved.

Discussion

Many genes coding for proteins with related functions have sequence homology and seem to be derived from a common progenitor; for example, the globin genes.

TABLE II

Segregation of IFN-γ with Human Chromosomes in Somatic Cell Hybrids

Cell hybrid	IFN-γ gene	Human chromosomes																							Trans-location chromosomes
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	
WIL-14	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	
ICL-15	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−	−	+	+	−	−	
REW8DCSAz3	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	
MH21 15-2-2-0	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	
IT22XWeRi	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	
XER-7	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	−	−	+	−	−	−	−	+	11/X*
XER-11	+	+	−	+	+	−	+	+	+	−	+	−	+	−	−	+	+	+	+	−	+	−	+	−	11/X; X/11*
JSR-17S	+	+	−	+	−	+	−	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	7 q$^-$‡
TSL-2	+	−	+	−	−	+	+	−	−	−	+	−	+	−	−	−	−	−	+	−	+	+	−	+	17/3§
ATR-13	+	+	+	+	+	−	+	+	+	−	+	−	+	−	+	+	−	+	+	−	−	−	−	−	5/X‖
EXR-5CSAz	+	+	−	+	+	+	+	+	+	−	+	−	+	+	+	+	−	−	+	+	+	+	+	+	X/11¶
DUM-13	+	+	+	+	−	+	+	−	−	−	+	+	+	−	+	−	+	+	+	+	+	+	+	−	X/15; 15/X**
NSL-7	+	−	−	−	−	−	+	−	−	+	−	−	+	+	+	−	−	−	−	+	−	+	−	−	
NSL-9	+	−	−	−	−	+	−	−	+	−	+	−	+	+	+	+	+	+	−	−	+	+	+	−	
NSL-15	+	−	+	−	+	+	−	+	+	−	−	−	+	+	+	+	−	+	+	+	−	+	+	+	
NSL-16	+	−	−	+	+	+	−	+	−	−	−	−	+	−	−	+	−	+	+	−	+	+	−	−	17/9; 9/17‡‡
12A	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	
A9	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	12p$^-$§§

Chromosomes of hybrid cells were karyotyped and banded by Giemsa-trypsin staining. Enzyme markers assigned to each chromosome except the Y have been tested on each hybrid, confirming the chromosome analysis. The translocation chromosomes are as follows:

* 11/X (Xqter→Xq13::11p11→11qter) and X/11 (Xpter→Xq13::11p11→11pter).

‡ 7q$^-$ (7pter→7q22).

§ 17/3 (17qter→17p13::3p21→3pter).

‖ 5/X (Xqter→Xq22::5q35→5pter).

¶ X/11 (Xpter→Xq22::11q13→11qter).

** X/15 (Xqter→Xq11::15q11→5qter) and 15/X (Xpter→Xq11::15q11→15pter).

‡‡ 17/9 (17qter→17p11::9p11→9pter) and 9/17 (17pter→17p11::9p11→9qter).

§§ 12p$^-$ (12p1205→12qter).

The non-α globin genes show a high degree of sequence homology (73–93%) (35, 36) and are all located in a 62-kb region of human chromosome 11 (37). This cluster of genes apparently arose by duplication of an ancestral gene (36, 38). The α globins, on the other hand, show less sequence homology with β globin (35, 36) and their structural genes are located on human chromosome 16 (39). The IFN-α family and IFN-β are acid stable proteins that have similar mechanisms of induction and are ~30% homologous (1, 18, 40). Evidence for the clustering of these genes on chromosome 9 is suggested by somatic cell hybrid studies (19) and demonstrated directly by the isolation of closely linked IFN-α genes from a human genomic DNA library (2, 6, 8, 41, 42). Although IFN-γ also is a powerful antiviral agent, it differs from IFN-α and IFN-β in many properties. The gene structure and sequence of IFN-γ is unrelated to the other IFN (17). IFN-γ is acid labile and its mode of induction is distinct from that of IFN-α and IFN-β (1, 40). Therefore, it is not especially surprising that the gene coding for IFN-γ is located on a different chromosome, as demonstrated in the present study.

It has been reported (21, 43) that human chromosomes 2 and 5 are involved in interferon production in human-mouse hybrids. Neither of these chromosomes can be the location of the structural gene for IFN-γ since both show a high rate of discordance with the IFN-γ gene (41%, Tables I and II). Likewise, the previous study mapping several leukocyte and a single fibroblast IFN genes to chromosome 9 (19) further demonstrates that neither chromosomes 2 nor 5 contain these structural genes, although there may be other distantly related leukocyte or fibroblast interferon genes.

Any genes on chromosomes 2 and 5 encoding for human interferon in cell hybrids would have to be distinctly different from the described cloned IFN-α, -β, and -γ genes.

Summary

A cDNA clone for human immune interferon (IFN-γ) gene sequences, plasmid p69, was used to chromosomally map the IFN-γ gene by detecting human IFN-γ gene sequences in DNA isolated from human-rodent somatic cell hybrids. We were able to map the IFN-γ gene by correlating the human chromosomes present in these hybrids with the human specific 8.8 and 2.0 kilobase pair fragments produced by EcoRI digestion of genomic DNA. Southern blot analysis of 37 hybrid cell lines indicated that the gene for IFN-γ was on human chromosome 12. A hybrid containing a portion of chromosome 12 localized the IFN-γ gene to the p1205→qter region.

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