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**Molecular cloning of human immune interferon cDNA and its expression in eukaryotic cells**

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**ABSTRACT**

Starting with mRNA derived from Staphylococcal enterotoxin A induced human splenocytes, dsDNA was synthesized and inserted into the unique *Bam*HI site of the eukaryotic expression vector pSV529 (1). A recombinant plasmid containing human immune interferon (IFN- $\gamma$ ) cDNA was identified by hybridization of plasmid inserted DNA bound onto nitrocellulose filters with mRNA derived from SEA-induced splenocytes, translation of the eluted RNA in *Xenopus laevis* oocytes and assaying for IFN activity. Plasmids containing the entire human IFN- $\gamma$  cDNA sequence were identified by colony hybridization and were sequenced. A unique coding region was identified which predicted a protein of 166 amino acids, the 20 N-terminal amino acids of which presumably represent a signal peptide. After transfection of monkey cells with plasmid DNA isolated from one of the recombinant clones (pHIIF-SV- $\gamma$ 1), IFN was excreted into the culture medium. This IFN was not distinguishable from human IFN- $\gamma$  by serological criteria or by cell target species specificity.

**INTRODUCTION**

Immune interferon (IFN- $\gamma$ ) can be induced in lymphocytes by mitogens (2), alloantigens (3) and other substances (4-7) as well as being induced in sensitized lymphocytes by specific antigens (8). Its production is dependent upon the presence either of macrophages or the macrophage product interleukin 1 (IL1) and this production is regulated by T-lymphocyte derived interleukin 2 (IL2) (9). Biologically, immune interferon is a very interesting lymphokine with important immunoregulatory properties (10). Reduced IFN- $\gamma$  production seems to occur in certain immunodeficiencies (11) and IFN- $\gamma$  has been found in the circulation of patients suffering from autoimmune diseases (12). It has been shown that IFN- $\gamma$  has a more potent anticellular activity (13, 14) and can activate natural killer cells to a higher extent (15) than other human IFNs. The antiviral effect (16) and the antitumour effect (17) of virus-induced IFNs (IFN- $\alpha$  and IFN- $\beta$ ) can be potentiated by IFN- $\gamma$ . The antiviral and antigrowth response of IFN- $\gamma$  on cells

selected for their resistance towards fibroblast IFN (18) and the differential antiviral activity of IFN- $\gamma$  (19) points towards a different mechanism of action. Further important functional differences between the type I IFNs (IFN- $\alpha$ s, IFN- $\beta$ ) and type II IFN (IFN- $\gamma$ ) include their different affinities for gangliosides (20) and their different membrane receptors (21).

IFN- $\gamma$  is produced by stimulated lymphocytes in minute quantities together with numerous other lymphokines. Only recently has extensive purification of IFN- $\gamma$  been achieved (22) and this has allowed a more precise determination of the molecular size (23). The mRNA for IFN- $\gamma$  isolated from human peripheral blood lymphocyte (PBL) cultures has been partially enriched and translated in *Xenopus laevis* oocytes (24-27). Recently, we reported the characterization of IFN- $\gamma$  mRNA derived from SEA-induced human splenocytes (28). We chose to use human spleen for the isolation of IFN- $\gamma$  mRNA because it is a source of appreciable and reproducible amounts of this mRNA, needed for the synthesis and identification of a cloned cDNA copy. In this report we describe the construction and identification of recombinant plasmids containing human IFN- $\gamma$  cDNA sequences, and the synthesis of human IFN- $\gamma$  by cultured monkey cells after transfection with the recombinant DNA in which the human IFN- $\gamma$  cDNA is under the control of SV40 late transcription. The results of similar work, i.e. on cloning, characterization and expression of IFN- $\gamma$  cDNA derived from human PBLs have recently been published by Gray et al. (29).

### MATERIALS AND METHODS

#### Construction of cDNA plasmids

Poly A<sup>+</sup>RNA was prepared from SEA-induced splenocyte cultures, purified by sucrose gradient centrifugation, and the IFN- $\gamma$  mRNA was identified by injecting aliquots into *Xenopus laevis* oocytes and after incubation for 72 hr assaying the incubation medium for IFN activity as described (28). The peak of IFN- $\gamma$  mRNA activity sedimented at around 13 S. cDNA was synthesized on 40  $\mu$ g poly A<sup>+</sup>RNA (from a single donor, spleen 7; ref 28) using oligo(dT)<sub>12-18</sub> (P-L Biochemicals) as a primer, followed by second strand synthesis, SI-treatment and sizing on a 4% polyacrylamide gel essentially as described (30). Molecules of dsDNA ranging in length between 800 and 1000 bp (g4), 1000 and 1250 bp (g3) and 1250 and 1400 bp (g2) were eluted from the gel, purified on hydroxyapatite and tailed with oligo dC. pSV529 DNA was used as a vector; it contains 1.4 genome equivalents of SV40 cloned between the EcoRI site and the PvuII site of pBR322 except that the VP1 gene has been deleted and replaced by a single BamHI site. This vector was cleaved with BamHI,

incubated with AMV reverse transcriptase in the presence of all four dNTPs to fill in the BamHI sticky ends, and tailed with oligo dG. After annealing, the mixture was used to transform E. coli strain DH1 (obtained from Dr. D. Hanahan through Dr. B. Hohn). Transformants grown on LB-agar plates containing 100  $\mu$ g/ml carbenicillin were picked up and grown overnight in microtiter plates. Dimethylsulfoxide was added to a final concentration of 10% and the plates were stored at  $-70^{\circ}\text{C}$ .

#### DNA preparation and screening of the cDNA library

Fifty individual clones were grown overnight on a single agar plate and the bacteria derived from these clones were suspended in 5 ml LB-medium. 0.5 l LB-medium was inoculated with this suspension and the bacteria were grown overnight (without amplification of the plasmids). Supercoiled plasmid DNA was prepared from these cultures by CsCl gradient centrifugation (31) and taken up in 10 mM Tris-HCl pH 7.5 - 1 mM EDTA at a concentration of 1 mg/ml. 150  $\mu$ g of this DNA was digested with BamHI followed by neutral density gradient centrifugation. The peak on the gradient which corresponded to excised "insert DNA" was collected, precipitated with ethanol, taken up in 10 mM Tris HCl pH 7.5 - 1 mM EDTA and used for binding on nitrocellulose filters (7 mm  $\phi$ , millipore HAWP 0.45 $\mu$ ). The conditions for the binding of insert DNA, hybridization of the filters with SEA-induced splenocyte poly A<sup>+</sup>RNA and elution of the RNA were essentially as described by Parnes et al. (32), except that the RNA was eluted in the presence of 4  $\mu$ g poly A<sup>-</sup>RNA (oligo(dT) cellulose runthrough RNA), and immediately precipitated without phenolization. After centrifugation, the eluted mRNA was taken up in 2  $\mu$ l sterile H<sub>2</sub>O, injected (in duplo) into each of 15-20 Xenopus laevis oocytes, and after three days at 23 C the medium was assayed for IFN activity. Colony hybridization was as described (33, 34).

#### Restriction enzyme analysis and DNA sequence determination

Plasmid DNA and sucrose gradient purified insert DNA were cleaved by restriction endonucleases (New England Biolabs, Boehringer-Mannheim) following the instructions of the supplier. Nucleotide sequences were determined by the chemical degradation method of Maxam and Gilbert (35) using 5'-end and 3'-end labelled DNA fragments.

#### DNA transfection of monkey cells and assay for IFN activity

Kidney cells of the African Green Monkey (AP8 cells) were transfected with recombinant SV40-plasmid DNA using a modification of the DEAE-dextran method (36) as described by Gheysen and Fiers (1). After 24 hr and 72 hr, aliquots of the incubation medium were withdrawn and stored at  $-70^{\circ}\text{C}$  until they were assayed for IFN activity. Assay and neutralization of IFN activity were performed as previously described (1, 28, 37).

RESULTS AND DISCUSSION

cDNA cloning

Recently, a eukaryotic expression vector (pSV529) designed to express a gene under late SV40 transcriptional control was constructed and successfully used for the expression of substantial quantities of IFN- $\beta$  (1). These experiments suggested that this vector could also be used as a direct screening vector for cloning eukaryotic genes corresponding to other proteins for which a sensitive assay is available, such as that for IFN- $\gamma$ . Such a screening method requires that the cDNA copy of the gene to be expressed is full length and is inserted in the correct orientation relative to the SV40 late promoter. If the length of the mRNA of the gene being studied is known, full length cDNA copies can be selected and this increases the chance of finding a biologically active clone. Since we had previously shown (28), that the mRNA coding for IFN- $\gamma$  has a length of between 900 and 1400 nucleotides, we chose to use this size range of cDNA to construct recombinant clones. mRNA was isolated from SEA-induced human splenocyte cultures, and purified by non-denaturing sucrose gradient centrifugation. dsDNA was fractionated by polyacrylamide gel electrophoresis and appropriate size classes were inserted into the unique BamHI site of pSV529 DNA and used for transformation of E. coli DH1. DNA was prepared from mixtures of 50 individual clones and used to transfect AP8 monkey cells. After incubation for 48 hr and 72 hr, aliquots of the medium were removed and assayed for IFN activity.

Screening of approximately 8000 clones by this method did not enable us to obtain any group of 50 clones which showed reproducible IFN activity (data not shown). We concluded that either the IFN- $\gamma$  was expressed at a level below the detection limit of our assay, or that there was no clone present in the cDNA library which contained a complete IFN- $\gamma$  cDNA copy inserted in the same orientation relative to transcription (see below).

Although the mRNA from which the recombinant plasmids were obtained was only purified 10-30 times after a single sucrose gradient step, relatively high titers of IFN- $\gamma$  were obtained after injection into Xenopus laevis oocytes (28). Furthermore, since we had found the human spleen to be a reliable source of appreciable amounts of IFN- $\gamma$  mRNA (28), we believed that a positive screening of the clones by cDNA-mRNA hybridization followed by translation in oocytes (32, 37) could be successful. Such a screening method could be rendered more sensitive by binding only "insert DNA" onto nitrocellulose filters, instead of binding total recombinant plasmid DNA. Since the BamHI site of the vector pSV529 was restored by the procedure used, "insert DNA" could be prepared by BamHI digestion of DNA from mixtures of 50 individual clones. This DNA was purified by sucrose gradient centrifugation (Fig. 1), and bound onto

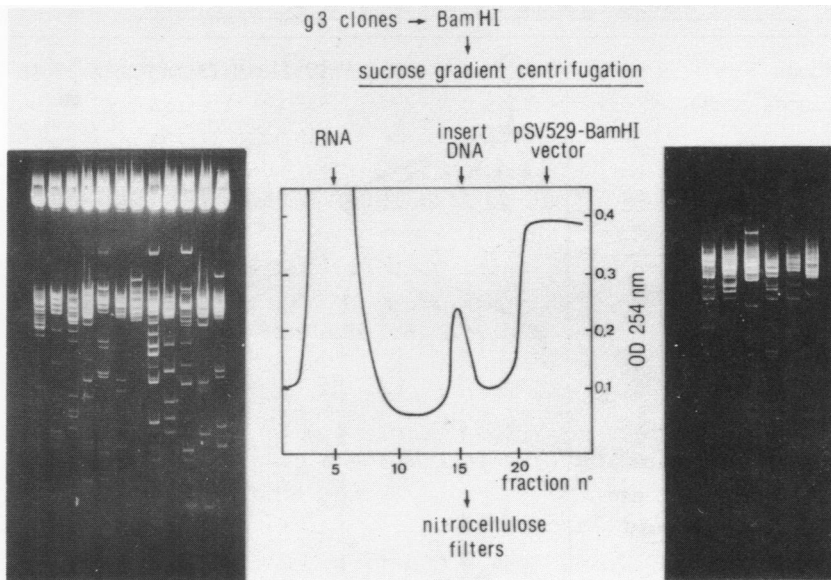
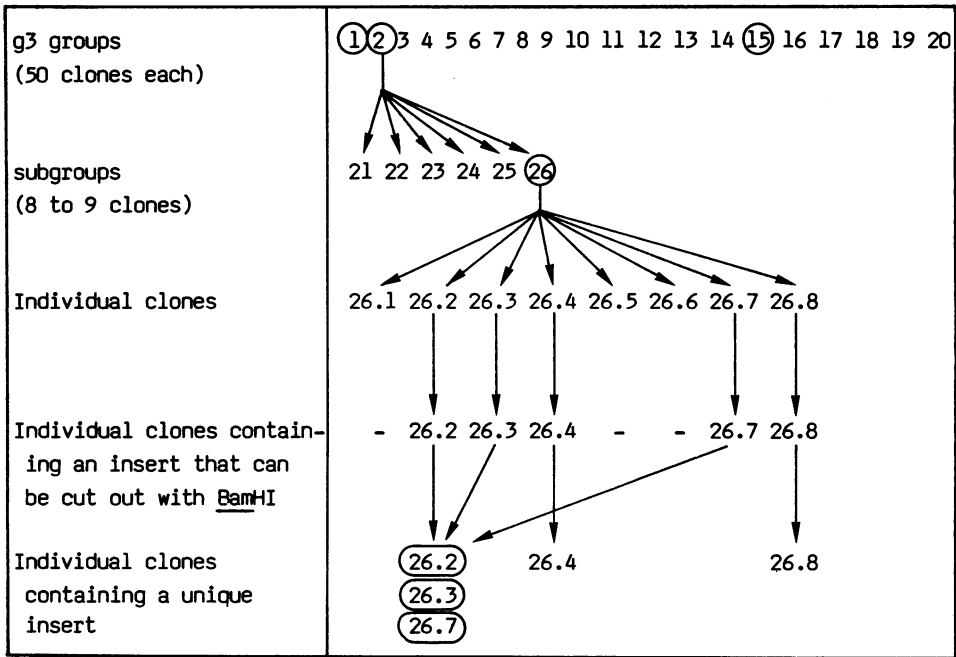


Fig. 1 Purification of *Bam*HI excised "insert DNA" derived from mixtures of 50 g3 clones by sucrose gradient centrifugation as outlined in Materials and Methods. Aliquots were analysed on 4% polyacrylamide gels before (left) and after (right) centrifugation. Isolated "insert DNA" was ethanol precipitated and bound on nitrocellulose filters.

nitrocellulose filters. The hybridization-elution-translation assay of 20 filters each containing "insert DNA" derived from 50 g3 clones with SEA-induced splenocyte mRNA (purified by sucrose gradient centrifugation) yielded three groups (1, 2 and 15) which gave a clear positive signal (see Table 1). Subsequent hybridization of filters containing "insert DNA" derived from subgroups (each containing 8 to 9 clones) of group 2 yielded one subgroup (2-6) which showed a positive signal. The subgroup 2-6 consisted of eight clones of which only five contained an insert which could be cut out with *Bam*HI. Moreover, restriction enzyme analysis (not shown) revealed three of the five clones (2-6.2, 2-6.3 and 2-6.7) to be identical (undoubtedly they had been picked from the same transformant). Finally, after hybridization with filters containing individual "insert DNAs" only the clones 2-6.2, 2-6.3 and 2-6.7 gave a strong, reproducible signal. As the three were identical, one of them was chosen for further use and was designated as pHIIF-SV-70.

Colony hybridization of 2300 g3 colonies and 880 g2 colonies with a <sup>32</sup>P-labeled internal *Dde*I fragment (620 bp) derived from pHIIF-SV-70 resulted in the identification of seven positive clones (pHIIF-SV-71, 2,

Table 1 Identification of an IFN- $\gamma$  cDNA clone



Circled numbers indicate filters which showed a positive signal in the hybridization-elution translation assay.

3, 5, 6, 8, 9) in the g3 group (1 in 300) and one positive clone (pHIIF-SV- $\gamma$ 10) in the g2 group (1 in 900). All but one of these clones (pHIIF-SV- $\gamma$ 9) contained inserts which could be cut out with BamHI. The size of the "insert DNA" ranged from 1000 to 1250 bp. Such a difference in the frequency with which positive clones were found in the g3 and g2 clones was not unexpected since it is a reflection of the number of full length cDNA copies in each group. Both group 1 and group 15 of the g3 clones which gave a positive signal in the first hybridization-elution translation screening assay turned out to contain a positive clone.

The BamHI excised "insert" DNA derived from the pHIIF-SV- $\gamma$  clones was purified by a sucrose gradient centrifugation and analysed by digestion with the restriction enzymes DdeI and HinfI. From the pattern obtained after gel electrophoresis (data not shown), we were able to deduce that there had not been any major rearrangements such as occurred during the cloning of IFN- $\beta$  cDNA (37, 38). It was also clear that most of the DNA preparations were heterogenous mixtures of molecules due to variation in the length of the dG:dC tails. Such a heterogeneity may result from slippage during replication of the plasmids.

### DNA sequence analysis

The complete nucleotide sequence of the cDNA insert of pHIIF-SV- $\gamma$ 0 and most of pHIIF-SV- $\gamma$ 1 was determined by the chemical degradation method of Maxam and Gilbert (34). The sequences of the ends of the insert cDNA of pHIIF-SV- $\gamma$ 5 and pHIIF-SV- $\gamma$ 10 were also determined and extended the length of the known DNA sequence. Fig. 2 shows the strategy for the sequence analysis together with the deduced and the experimentally verified restriction sites. The results obtained from the series of pHIIF-SV- $\gamma$  clones enabled us to establish the primary structure of most of the human IFN- $\gamma$  mRNA as presented in Fig. 3 in the form of a double stranded DNA sequence. A continuous reading frame of 498 nucleotides is present starting with the first AUG (at position 92) near the 5'-end of the mRNA and ending with a stop codon UAA (at position 590). This continuous reading frame together with the hexanucleotide AAUAAA found in the clone pHIIF-SV- $\gamma$ 10 before the site of polyadenylation (39) enabled us to determine the orientation of the coding strand in the pHIIF-SV- $\gamma$  clones. It may be noted that all clones analysed lacked an oligo dA:oligo dT sequence at their 3'-end; it was presumably removed by SI-nuclease during the synthesis of ds-cDNA. The use of the first AUG as an initiator codon is a property common to most eukaryotic mRNAs (40). The 3'-untranslated region is 600 nucleotides long and thus accounts for almost half the nucleotide sequence of the mRNA; although remarkably long, this is certainly not a unique example (41).

The amino acid sequence of human IFN- $\gamma$  deduced from the nucleotide sequence of the coding region, is presented in Fig. 4. The 20 NH<sub>2</sub>-terminal amino acids of this sequence presumably comprise a signal peptide which is removed upon secretion. Indeed, as is commonly observed for secretory proteins (42), this sequence contains mainly hydrophobic amino acid residues and residues 18-21, Ser-Leu-Gly-Cys, are also found in a similar position in human IFN- $\alpha$ , (43), (and other IFN- $\alpha$ s (44)) and, by analogy, one might suppose that cleavage of the IFN- $\gamma$  signal peptide occurs also between the glycine and cysteine residues. The deduced protein sequence points to a mature human IFN- $\gamma$  that contains 146 amino acids, and which has a calculated molecular weight of 17 147. It has been shown that under denaturing conditions, IFN- $\gamma$  can give rise to two subspecies of 20 000 dalton and 25 000 dalton but their possible relationship is not clear (23, 45). Considering the glycosylation of natural IFN- $\gamma$  (46), these estimates are in reasonable agreement with the value deduced from the gene sequence. However, authentic IFN- $\gamma$  secreted by lymphocytes has a molecular weight of approximately 45 000 dalton (47, 48, and our own unpublished observations). Even allowing for glycosylation, this value is hard to reconcile with the molecular weight of the polypeptide, and therefore it seems likely that the mature

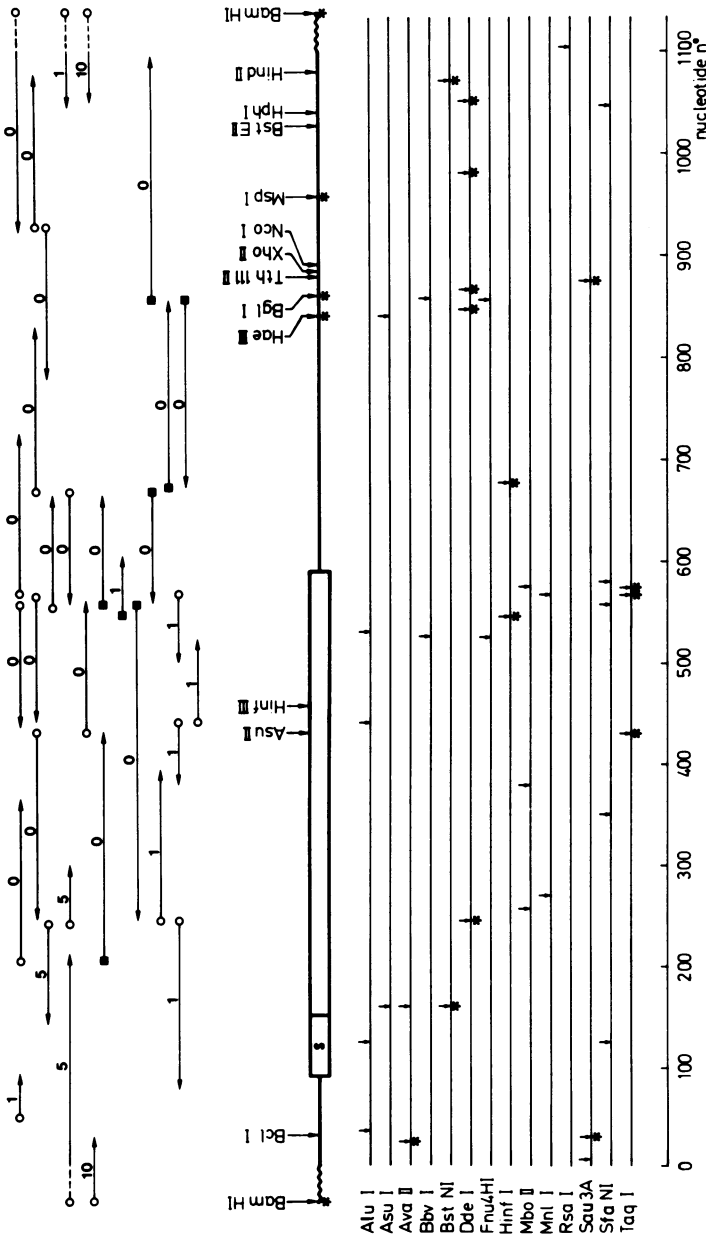


Fig. 2. Restriction map and sequencing strategy for the human IFN- $\gamma$  cDNA gene. The central diagram shows the organization of the mRNA for human IFN- $\gamma$ , with the 5'-untranslated region, the boxed translated sequence with the presumed signal peptide and the 3'-untranslated segment. Wavy lines represent dG:dC tails. The positions of the cleavage sites of the restriction enzymes that cut the ds-cDNA only once are indicated. The regions covered by sequencing are indicated at the top. The open circles correspond to the  $^{32}$ P-labeled 5'-ends and the black squares to  $^{32}$ P-labeled 3'-ends. The numbers above the arrows refer to the different clones analysed. Below is a restriction endonuclease map constructed from a computer search made on the complete nucleotide sequence. Experimentally verified sites are indicated by asterisks.



|   |      |
|---|------|
| AAGAGAAGATCAGTTAAGTCCTTTGGACC TGATCAGCTTGATACAAGAAC TACTGATTTCAACTTCTTTGGCTT<br>TTC TCTTTCTAGTCAATTCAGGAAACCTGGACTAGTCTGAAC TATGTTCTTGATGACTAAAGTTGAAGAACCAGGAA | 75   |
| AATTCCTCGGAAACCTAATAATATACAAGTTATATCTTTGGCTTTTCAGCTCTGCATCGTTTTGGGTCTCTTGG<br>TTAAGAGAGCCTTTTCTACTTTTATATGTTCAATATAGAACC GAAAAGTCGAGACGTAGCAAAACCCAAGAGAACC     | 150  |
| CTGTTACTGCCAGGACCCATATGTAAAAGAAGCAGAAAACCTTAAGAAATATTTTAAATGCAGGTCAATTCAGATGT<br>GACAATGACGGTCTTGGGTATACATTTTCTTCGTCTTTTGGAAATCTTTATAAAAATTACGTCCAGTAAGTCTACA   | 225  |
| AGCGGATAAATGGAAC TCTTTTCTTAGGCATTTTGAAGAATTGGAAGAGGAGAGTGACAGAAAAAT AATGCAGAG<br>TCGCCTATTACCTTGAGAAAAGAATCCGTA AAC TCTTAACCTTCTCCCTCACTGTCTTTTATTACGTCTC       | 300  |
| CCAAATTGTC TCC TTTTACTTCAAAC TTTTAAAAAC TTTAAAGATGACCAGAGCATCCAAAAGAGTGTGGAGAC<br>GGTTTAACAGAGGAAAATGAAGTTTGA AAAATTTTGA AATTTCTACTGGTCTCGTAGGTTTTCTCACACCTCTG  | 375  |
| CATCAAGGAAGACATGAATGTCAAGTTTTCAATAGCAACAAAAGAAACGAGATGACTTCGAAAAGCTGACTAA<br>GTAGTTCCTCTGTACTTACAGTTCAAAAAGTTATCGTTGTTTTCTTTGCTCTACTGAAGCTTTTCGACTGATT          | 450  |
| TTATTCGGTAACTGACTTGAATGTCCAACGCAAGCAATACATGAAC TCAATCAAGTGATGGCTGAAC TGTCCGC<br>AATAAGCCATTGACTGAAC TTAACAGTTGCGTTTCGTTATGTACTTGAGTAGGTTCACTACCGACTTGACAGCGG    | 525  |
| AGCAGCTAAAACAGGGAAGCGAAAAGGAGTCAGATGCTGTTTCGAGGTCGAAGAGCATCCCACTAAATGGTTGTC<br>TCGTCGATTTGTCCTTCGCTTTTCTCAGCTACGACAAGCTCCAGCTTCGTCGAGGTCATTACCAACAG             | 600  |
| CTGCCTGCAATATTTGAATTTTAAATCTAAATCTATTTTATAATATTTAACATTATTTATATGGGGAATATATTT<br>GACGGACGTTATAA ACTTAA AATTTAGATTTAGATAAATAATTATAAATTTGAATAAATATACCCCTTATATAAA    | 675  |
| TTAGACTCATCAATCAAATAAGTATTTATAATAGCAACTTTTGTGTAATGAAAATGAATATCTATTAATATATGT<br>AATCTGAGTAGTTAGTTTATTCATAAATATTATCGTTGAAAACACATTACTTTTACTTATAGATAATTATATACA      | 750  |
| ATTATTTATAATTCCTATATCCTGTGACTGTCTCACTTAATCCTTTGTTTTCTGACTAATTAGCCAAGGCTATGT<br>TAATAAATATTAAGGATATAGGACACTGACAGAGTGAATTAGGAAACAAAAGACTGATTAATCCGTTCCGATACA      | 825  |
| GATTACAAGGCTTTATCTCAGGGGCCAACTAGGCAGCCAACCTAAGCAAGATCCCATGGGTTGTGTGTTTATTTCT<br>CTAATGTTCCGAAATAGAGTCCCGGTTGATCCGTCGGTTGGATTCGTTCTAGGGTACCCAACACACAATAAAG       | 900  |
| ACTTGATGATACAATGAACACTTATAAGTGAAGTGATACTATCCAGTTACTGCCGGTTTGA AAAATATGCTTGCAA<br>TGA ACTACTATGTTACTTGTAATATTTCACTTCACTATGATAGGTCAATGACGGCCAACCTTTTATACGGACGTT   | 975  |
| TCTGAGCCAGTCTTAAATGCATGTCAGACAGA ACTTGAATGTGTCAGGTGACCTGATGAAAACATAGCATCT<br>AGACTCGGTCACGAAATACCGTACAGCTGTCTTGAAC TTAACAGTCCACTGGGACTACTTTGTATCGTAGA           | 1050 |
| CAGGAGATTTTACGCTGGTGCTTCCAATATTGTTGACA ACTGTGACTGTACCCAAAATGGAAGTAACTCATTT<br>GTCCCTAAAAGTACGGACCAGAAAGTTTATAACA ACTGTTGACACTGACATGGGTTTACCTTTATTGAGTAAA        | 1125 |
| GTAAAATTATCAATATCTAATATATATGATAAAGTGTGAAGTTC<br>CAATTTTAATAGTTATAGATTATATATACTTATTTACATTCAAG  | 1200 |

Fig. 3 Nucleotide sequence of human IFN- $\gamma$  cDNA

The nucleotide sequence was determined by the Maxam and Gilbert technique (35). The initiation and termination codons are shown in full outlined boxes. The AATAAA sequence near the polyadenylation signal is shown in a dashed box. The coding region is indicated by a heavy line above the nucleotide sequence; the vertical arrow indicates the position where the presumed signal peptide is cleaved.

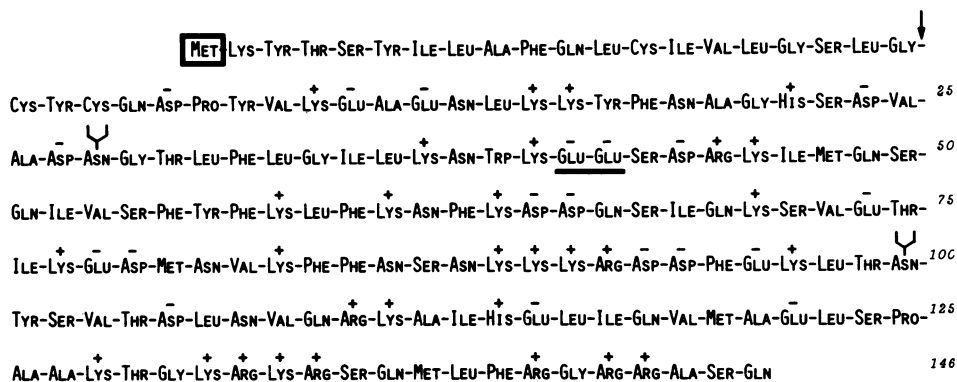


Fig. 4 Amino acid sequence of human IFN- $\gamma$   
 The amino acid sequence consists of a presumed 20-amino acid signal peptide, starting from the boxed methionine up to the vertical arrow, plus a mature polypeptide. The underlined sequence Glu-Glu occurs in all three forms of IFN at approximately the same position.  $\Psi$  indicates the potential N-glycosylation sites; + and - indicate basic and acidic amino acid residues respectively.

molecule exists as a complex of subunits, most probably as a dimer.

The amino acid sequence of IFN- $\gamma$  also reveals two potential N-glycosylation sites at positions 28 and 100. Different degrees of glycosylation at these sites could result in the formation of multiple subspecies of denatured IFN- $\gamma$  polypeptides as observed by Yip et al. (23) and by Chen et al. (45). The amino acid sequence shows a remarkably high content of basic residues which would explain the high pI value observed by Yip et al. (22). More particularly, there are two clusters of four consecutive basic residues, viz. Lys-Lys-Lys-Arg (at position 89-92) and Lys-Arg-Lys-Arg (at position 131-134). The basic nature of the mature protein may be related to the acid lability of its biological activity. Since the only cysteine residues in the amino acid sequence occur at the NH<sub>2</sub>-terminal end (positions 1 and 3), it is unlikely that disulphide bridges are essential for maintaining the conformation of the mature protein. This is in agreement with the finding that the antiviral activity is retained after treatment with  $\beta$ -mercaptoethanol (22, 23, 45, 49) and with the observation that there is sulphhydryl specific binding of human IFN- $\gamma$  on an Affigel 501 matrix (50). The nucleotide sequence that we have determined for the IFN- $\gamma$  mRNA and the amino acid sequence deduced for IFN- $\gamma$  are in agreement with the data recently obtained by Gray et al. (29), except for the amino acid residue at position 140 which we determined to be an arginine residue as opposed to a glutamine residue (CGA codon versus CAA). The correctness of our nucleotide sequence was

further demonstrated by the presence of two consecutive TaqI sites at these positions (Fig. 2). It remains to be shown whether this could be an example of allelic variation. The homology between the amino acid sequences of IFN- $\gamma$  and several recombinant forms of IFN- $\alpha$  was examined by Epstein L.B. (51) who found that there are 18 identical amino acids or very conservative replacements in precise sequence order. Moreover, the sequence Glu-Glu occurs in all three forms of IFN at approximately the same position (residues 41 and 42 in IFN- $\alpha$  and IFN- $\gamma$ , residues 42 and 43 in IFN- $\beta$ ). The conservation of these two residues is remarkable in view of the experimental finding that the receptor for IFN- $\gamma$  is different from that of IFN- $\alpha$  and IFN- $\beta$  (21) and that IFN exerts its action without internalization (52, 53).

#### Transfection of AP8 monkey cells with pHIIF-SV- $\gamma$ DNA

In order to prove that the clone that we had isolated did actually code for human IFN- $\gamma$ , it was necessary to express the genetic information and to characterize the resulting product. From the results obtained by restriction analysis, it was apparent that only clones pHIIF-SV- $\gamma$ 1, pHIIF-SV- $\gamma$ 5 and pHIIF-SV- $\gamma$ 10 contained the complete coding region of human IFN- $\gamma$ . These clones were therefore the only candidates for expression of IFN- $\gamma$  in monkey cells transfected with these plasmids. Furthermore, analysis based on locating the AvaII site (which is present

Table 2 IFN production by monkey cells (AP8) transfected with plasmid DNA

| <u>Plasmid DNA</u> (a) (0.1 $\mu$ g/20 mm well) | <u>Laboratory units</u> (b) |
|---|-----------------------------|
| pHIIF-SV- $\gamma$ 1                            | 100                         |
| pHIIF-SV- $\gamma$ 5                            | < 3                         |
| pHIIF-SV- $\gamma$ 10                           | < 3                         |
| pSV529-HFIF-1                                   | 10,000                      |
| pHIIF-SV- $\gamma$ 1<br>pH2 treated (c)         | < 3                         |

a pHIIF-SV- $\gamma$ 1 contained the IFN- $\gamma$  information in the sense orientation relative to SV40 late transcription; pHIIF-SV- $\gamma$ 5 and pHIIF-SV- $\gamma$ 10 contained the information in an antisense orientation.

b One leucocyte I.U./ml equals 50 laboratory units/ml.

c For pH2 treatment the sample was dialysed overnight against 0.01M glycine.HCl, pH 2.0 buffer, and followed by dialysis against phosphate buffered saline.

Conditions for culturing the AP8 cells and transfection with plasmid DNA are as described by Gheysen, D. and Fiers, W. (1).

Table 3 Serological characterization of cloned pHIIF-SV- $\gamma$ 1 interferon

| IFN           |                                    | Laboratory units/ml |
|---------------|------------------------------------|---------------------|
| IFN- $\alpha$ | standard                           | 30                  |
|               | id. + anti $\alpha$ -serum (S)     | <3                  |
| IFN- $\beta$  | standard                           | 300                 |
|               | id. + anti $\beta$ -serum (G)      | <3                  |
| IFN- $\gamma$ | standard                           | 100                 |
|               | id. + anti $\gamma$ -serum (R)     | <3                  |
|               | id. + anti $\gamma$ -serum (M)     | <3                  |
| IFN           | (AP8 cells + pHIIF-SV- $\gamma$ 1) | 100                 |
|               | id. + anti $\alpha$ -serum (S)     | 100                 |
|               | id. + anti $\beta$ -serum (G)      | 160                 |
|               | id. + anti $\gamma$ -serum (R)     | <3                  |
|               | id. + anti $\gamma$ -serum (M)     | <3                  |

Sera used: anti $\alpha$  (sheep - K. Cantell); anti $\beta$  (goat - E. De Clercq & A. Billiau); anti $\gamma$  (rabbit - Langford & Stanton); anti $\gamma$  (mouse - this laboratory).

at the 5'-end of the IFN- $\gamma$  cDNA) showed that the cDNA in the plasmids pHIIF-SV- $\gamma$ 5 and pHIIF-SV- $\gamma$ 10 was inserted in an opposite orientation relative to the SV40 late promoter. Since the expression of human IFN- $\beta$  in monkey cells transfected with the chimaeric plasmid-SV40 containing IFN- $\beta$  cDNA required the IFN- $\beta$  cDNA insert to be correctly orientated (1), pHIIF-SV- $\gamma$ 1 was the only clone which could possibly lead to expression of IFN- $\gamma$  after transfection of cultured monkey cells. Table 2 shows that pHIIF-SV- $\gamma$ 1 was indeed the only clone that resulted in the appearance of an antiviral activity after transfection of AP8 cells. This IFN activity was neutralized by an anti-human IFN- $\gamma$  serum but not by anti IFN- $\alpha$  or anti IFN- $\beta$  serum and is therefore immune IFN specific (Table 3). The antiviral activity was acid labile (Table 2), and showed no virus protection on bovine, murine or feline cells (not shown). However, the antiviral activity of IFN- $\beta$ , produced after transfection of AP8 cells under similar conditions with an IFN- $\beta$  cDNA containing plasmid (pSV529-HFIF) was about 100 times higher. Clearly, this high level of expression is necessary for detection of an IFN cDNA containing clone in a mixture of randomly cloned cDNA. The low level of expression of IFN- $\gamma$  in AP8 cells transfected with pHIIF-SV- $\gamma$ 1 DNA could explain our inability to detect this clone using our direct screening method (see above). The reason for the difference in expression level between IFN- $\gamma$  and IFN- $\beta$  containing chimaeras is not clear at present and needs further study.

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Abbreviations: IFN, interferon; SEA, Staphylococcal enterotoxin A; dsDNA, double-stranded DNA; LB medium, Luria broth medium.

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