**Biological Activities and Receptor Binding of Two Human Recombinant Interferons and their Hybrids**

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**SUMMARY**

Two human recombinant lymphoblastoid interferon-α subtypes, LyIFN-B (α8) and LyIFN-D (α1), and 10 hybrids generated therefrom were produced in *Escherichia coli* and purified. The antiviral and antiproliferative activities and the induction of (2′-5′)oligoadenylate synthetase were compared to their receptor binding affinities. The IFN subtypes and their hybrids had similar specific antiviral activities on bovine cells. On human cells both the specific antiviral and antiproliferative activities of LyIFN-B were about 30-fold higher than those of LyIFN-D. This difference in activity could be attributed partly to the N-terminal amino acids 1 to 60 and partly to amino acids 61 to 92. A third domain affecting the biological activities was found within the carboxy-proximal segment from amino acids 93 to 150. The differences in these activities were found to correlate with their ability to bind the receptor, suggesting that the differences in activity might be due to altered binding of the IFNs to the cellular receptors. In contrast, the induction of (2′-5′)oligoadenylate synthetase did not follow the same activity profile. On mouse cells, the efficiency of the hybrids was affected by at least four sites on the IFN protein. A hybrid with the N-terminal segment 1 to 60 from IFN-B and amino acids 61 to 166 from IFN-D had a specific antiviral activity on mouse cells as high as on human cells corresponding to a 500- and 5000-fold increase in specific activity compared to IFN-D and IFN-B, respectively. We suggest that on mouse cells the IFN activity may be more dependent on conformational differences than on human cells, which in turn might reflect a less precise fit to the mouse receptor than to the human receptor.

**INTRODUCTION**

Human interferon-α (IFN-α) comprises a group of structurally related proteins derived from a multigene family (Nagata *et al.*, 1980; Goeddel *et al.*, 1981). Interferons exhibit a number of biological activities including establishment of an antiviral state, inhibition of cell multiplication and enhancement of natural killer cell activity (Masucci *et al.*, 1980). At the biochemical level, IFN has been shown to induce the synthesis of HLA antigens and β2 microglobulin (Heron *et al.*, 1978; Fellous *et al.*, 1979) and to enhance a number of pre-existing enzyme activities including (2′-5′)oligoadenylate synthetase (Kerr & Brown, 1978), protein kinase (Zilberstein *et al.*, 1978), and 2′-phosphodiesterase (Schmidt *et al.*, 1979). Studies with purified recombinant IFN subtypes α1 (D) and α2 (A) and hybrids thereof have shown that IFN-α subtypes do show different antiviral and antiproliferative activities (Streuli *et al.*, 1981; Weck *et al.*, 1981; Rehberg *et al.*, 1982; Sen *et al.*, 1984) and that similar differences are

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seen in their binding to receptors (Uzé et al., 1985). It has been proposed that the relative antiviral activity of IFN-α is mediated by two binding regions, one in the amino-terminal and one in the carboxy-terminal half (Streuli et al., 1981).

The first step in the action of IFN is believed to involve binding of IFN to specific cell surface receptors (Aguet, 1980). It has been shown for a number of IFN-α subtypes that the antiviral activity can be correlated with their relative binding affinities (Aguet et al., 1984). In order to clarify whether the multiple biological effects of IFNs are triggered by the same determinants on the protein and to define in more detail the sites on the IFN molecule which mediate the biological effects, we compared the antiviral and antiproliferative activities, and the ability to increase (2'-5')oligoadenylate synthetase, of two recombinant lymphoblastoid IFNs [LyIFN-D (αD) and LyIFN-B (αB)] with the activities of ten hybrid molecules comprising determinants derived from both parental IFNs. In order to correlate these biological activities with receptor binding capacity, we measured the direct binding of some radioactively labelled IFNs to human and mouse cells.

METHODS

Cells. Daudi cells (Klein et al., 1968) were cultivated in RPMI 1640 medium with 15% foetal calf serum (Flow Laboratories) in static suspension culture (Dron & Tovey, 1983). HEp-2 cells are derived from a human carcinoma of the larynx (ATCC no. CCL 23). The WISH cell line is derived from a human amnion.

Enzymes and chemicals. Restriction enzymes, T4 kinase, T4 DNA ligase were from New England Biolabs, and were used as recommended by the supplier.

Construction of hybrid interferon expression plasmids. The cDNAs coding for the mature lymphoblastoid IFN-B and IFN-D were joined to the 3' end of the Escherichia coli tryptophan promoter, operator and leader ribosome binding site followed by the initiation codon ATG (Goeddel et al., 1980; Rink et al., 1984) to generate the expression vectors, pLyIFN-B and pLyIFN-D. LyIFN-B and LyIFN-D are allelic variants of LeIFN subtypes B (αB) and D (αD), respectively (F. Meyer, unpublished).

B/D hybrid interferons were constructed in vivo by standard procedures as outlined in Fig. 1. Briefly, the coding sequences of the parent expression vectors were cleaved at the common restriction sites S1 (Sau3AI), P (PvuII), S (Sau3AI) to generate DNA fragments comprising the coding sequences for the amino acids 1 to 60, 1 to 92, 61 to 92, 93 to 150, 92 to 166 and 151 to 166, respectively. These DNA fragments isolated from 6% polyacrylamide gels were used to generate the hybrid IFN genes upon ligation of the appropriate DNA segments.

For the expression of the recombinant genes, the HindIII-PstI DNA fragments were ligated into the large HindIII-PstI DNA fragment of pBR322 and the resulting expression vectors were transfected into E. coli. Restriction enzyme mapping and DNA sequence analysis by the method of Sanger & Coulson (1975) were performed on the ligation junctions of the recombinant plasmids to confirm the predicted constructions.

Interferon preparations. E. coli HT-2, a mutant derived from HB101, harbouring the indicated IFN expression plasmids was grown in 200 ml of modified M9 medium (Rink et al., 1984) to an optical density at 650 nm of 5 to 8. The cells were harvested and resuspended in 100 ml of Tris-HCl pH 8.0, 0.5 M NaCl, 100 μM phenylmethylsulphonyl fluoride (PMSF) (10 ml buffer per 1 g of cell pellet). Lysozyme was added to a final concentration of 1 mg/ml at 0 °C and the cells were disrupted in a Sorvall Omnimix (setting 6, 3 × 60 s). The resulting suspensions were clarified by centrifugation and the crude extracts were purified by a two-step procedure to define in more detail the sites on the IFN molecule which mediate the biological effects, we compared the antiviral and antiproliferative activities, and the ability to increase (2'-5')oligoadenylate synthetase, of two recombinant lymphoblastoid IFNs [LyIFN-D (αD) and LyIFN-B (αB)] with the activities of ten hybrid molecules comprising determinants derived from both parental IFNs. In order to correlate these biological activities with receptor binding capacity, we measured the direct binding of some radioactively labelled IFNs to human and mouse cells.

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The concentration of the purified proteins was determined by the method of Bradford (1976), using bovine serum albumin as a reference. The interferon concentration was adjusted to 0.1 mg/ml and aliquots were stored at −20 °C.

The purity of the preparations was confirmed by SDS–polyacrylamide gel electrophoresis (Fig. 2). The purity of the preparations was confirmed by SDS–polyacrylamide gel electrophoresis (Fig. 2).

Assay of the antiviral activity of IFN. Interferon titres were determined by a cytopathic inhibition assay on MDBK, HEp-2, WISH and secondary monolayer cell cultures of 17-day-old Swiss or A2G mouse embryos (Mogensen & Bandu, 1983) using vesicular stomatitis virus (VSV) as challenge virus. The titres were calibrated against the international human leukocyte IFN references MRC 69/19 or NIH G-023-907-527 or the NIH research reference reagent for mouse IFN-α/β G002-904-511.

Assay of the antiproliferative activity of interferon. Daudi cells were seeded in microtest III Falcon plates at a concentration of 5 × 10⁴ cells/ml in RPMI 1640 medium with 15% foetal calf serum. The serial dilutions of the IFN preparation to be assayed were added and the cells were counted at 48 and 72 h in a Coulter model 2BI counter. The results are expressed relative to IFN-B, taking a 10⁻⁷ dilution of IFN-B (ID₅₀) as 100% activity.
Biological activities of human hybrid IFNs

Induction and assay of the (2'-5')oligoadenylate synthetase. Daudi cells were seeded at 2 x 10^5 cells/ml in RPMI 1640 medium with 10% foetal calf serum containing 1 U/ml, 10 U/ml, 10^2 U/ml, 10^3 U/ml of a particular IFN. After 24 h treatment, 5 x 10^6 cells per sample were centrifuged (9000 r.p.m., 2 min), and resuspended in 500 μl of cold lysis buffer (20 mM-HEPES pH 7.5, 5 mM-MgCl₂, 120 mM-KCl, 7 mM-dithiothreitol, 10% glycerol, 0.5% NP40) and incubated for 2 min on ice. The samples were then centrifuged (9000 r.p.m., 8 min, 4°C) and the supernatants were recovered and assayed for (2'-5')oligoadenylate synthetase activity. Cell extracts were mixed with 50 μl poly(rI)-(rC)-agarose for 15 min at 30°C and the unadsorbed material was removed by centrifugation. The poly(rI)-(rC)-bound material was incubated with 2.5 mM-[α-32P]ATP (400 Ci/mmol; Amersham) for 20 h at 30°C, treated with bacterial alkaline phosphatase and then eluted from a column of acid alumina (300 μl) with 3 ml 1 M-glycine-HCl pH 2.0 as previously described (Merlin et al., 1981). Results are expressed as x-fold induction of activity as compared to untreated control cells.

Radiolabelling. The IFNs were radiolabelled with 125Iodide as described by Mogensen & Bandu (1983), except that 2.5 mCi 125Iodide per 14 μg of protein was used in the chloramine-T reaction.

Cell binding experiments. The procedure for binding experiments was carried out as described previously (Mogensen & Bandu, 1983). Briefly, 5 ml of exponentially growing Daudi cells at 1 x 10^5 cells/ml were incubated in RPMI 1640 medium containing 15% foetal calf serum, the day before the experiment. The average doubling time of the cultures was 21.6 h, so that IFNs were added to exponentially growing cells at an average of 2.35 x 10^5 cells/ml. Cells reached saturation density between 10^6 and 2 x 10^6 cells/ml. Therefore, the effects of IFN could be followed over at least two cycles of exponential growth. Separation of cell-bound from free IFN was achieved by centrifugation at 2 to 4°C, so that the cells could be washed twice without significant loss of specifically bound IFN. The amount of non-specifically bound radioactivity (about 0.01% of the input radioactivity) was estimated by incubating controls with a 100-fold excess of unlabelled IFN. All binding data are presented after subtraction of non-specific backgrounds.

RESULTS

Construction of plasmids containing IFN genes

The lymphoblastoid IFN genes LyIFN-D (α₁) and LyIFN-B (α₈) code for mature IFN molecules of 166 amino acids, which differ from each other by 35 amino acids. LyIFN-B differs from LeIFN-B at four positions (amino acids 98 to 101) (F. Meyer, unpublished). Both genes have been expressed in E. coli under the control of the E. coli trp promoter to yield the corresponding mature IFNs. By using restriction sites common to both IFN genes, and re-ligating the appropriate DNA fragments in vitro, we constructed 10 genetic hybrids containing portions of each parental molecule (Fig. 1). The HindIII-PstI DNA fragments containing the hybrid IFN genes and the E. coli trp promoter element were ligated into the HindIII-PstI large fragment (3600 bp) of plasmid pBR322, so that direct expression of these hybrid genes would occur in E. coli.

Recombinant and hybrid IFNs

All the parental and hybrid IFNs were purified by a two-step procedure involving a monoclonal antibody column. LyIFN-B showed an aberrant mobility on SDS-polyacrylamide gels corresponding to an apparent mol. wt. of 26000 (Fig. 2). The segment of the protein conferring this aberrant mobility could be allocated to determinants within amino acids 61 to 92. Sequence analysis of the cyanogen bromide fragment from amino acid 61 to 101 confirmed the amino acid sequence deduced from the DNA, thus excluding the possibility of post-translational modification of the proteins in E. coli (M. Grutter, unpublished results). When electrophoresis was performed in 8 M-urea, the parental IFN-B migrated with an apparent mol. wt. of 20000 (data not shown).

Comparison of antiviral activities

The specific antiviral activities were determined by the cytopathic effect inhibition assay, which has a standard error of about 50%. All 12 IFNs showed a similar activity on bovine MDBK cells varying from 0.5 x 10^8 to 2 x 10^8 U/mg (Table 1). On human cells the activities varied from 3 x 10^8 to 1 x 10^8 U/mg on WISH cells and 9 x 10^5 to 8 x 10^8 U/mg on HEP-2 cells (330- to 900-fold) (Table 1). The determinants modulating the effects appeared to be located within two domains: amino acids 1 to 60 and amino acids 61 to 92 (Fig. 3c). On WISH cells the
Fig. 1. Construction of B/D hybrid IFN expression plasmids. On the left, the plasmids coding for the parental IFNs, pLyIFN-D and pLyIFN-B and the DNA fragments isolated from them. On the right, 10 reconstructed HindIII-PstI fragments comprising the hybrid IFN genes and the tryptophan promoter segment (pBR322 sequences are omitted). The segments comprising the 3' extracistronic cDNA segment from IFN-B is shorter than the one from IFN-D. IFN-coding regions are drawn as open (IFN-D) and filled bars (IFN-B). The protein sections encoded by the DNA fragments are indicated under 'amino acids'. Open box: tryptophan promoter, operator and leader ribosome binding site including the initiation codon. Thin lines: pBR322 DNA. Bold lines: IFN 3'-extracistronic cDNA. H, HindIII; E, EcoRI; S1, S2, Sau3AI; Pv, PvuII; Ps, PstI.

Table 1. Specific antiviral activities of parental and hybrid interferons

<table>
<thead>
<tr>
<th>Specific antiviral activity (U/mg) in</th>
<th>MEF* (mouse)</th>
<th>MDBK† (bovine)</th>
<th>WISH‡ (human)</th>
<th>HEP-2§ (human)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>BBBB</td>
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<td>1.6 x 10^8</td>
<td>2.1 x 10^8</td>
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<td>1.2 x 10^8</td>
<td>1.3 x 10^8</td>
<td>2.6 x 10^8</td>
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<tr>
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<td>1.7 x 10^8</td>
<td>2.3 x 10^8</td>
<td>3.7 x 10^8</td>
</tr>
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<td>1.8 x 10^8</td>
<td>8.0 x 10^7</td>
<td>4.1 x 10^8</td>
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<tr>
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<td>4.5 x 10^7</td>
<td>6.7 x 10^6</td>
<td>1.0 x 10^7</td>
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<tr>
<td>DDBB</td>
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<td>1.7 x 10^8</td>
<td>8.0 x 10^6</td>
<td>1.6 x 10^7</td>
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<tr>
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<td>3.3 x 10^5</td>
<td>8.7 x 10^5</td>
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<td>1.3 x 10^7</td>
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<tr>
<td>DBBB</td>
<td>1.8 x 10^5</td>
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<td>1.0 x 10^8</td>
<td>7.0 x 10^5</td>
<td>1.5 x 10^6</td>
</tr>
</tbody>
</table>

* MEF, Mouse embryo fibroblasts corrected for internal reference IFN.
† Average of two independent experiments and duplicate assays, corrected for reference IFNs Hu LeIFN-α NIH G-023-907-527 and MRC 69/19, respectively.
‡ Corrected for reference, MRC 69/19.
§ Corrected for reference, G-023-907-527.

ratios of BDDD, DBDD, BBDD and IFN-B to IFN-D were 19, 3, 34 and 31, respectively. This finding was substantiated by the reciprocal recombinants: the efficacy of DDBB was seven- to 13-fold lower than that of DBBB and 370- to 640-fold lower than that of BBBB. Furthermore, a third domain interfering with the activity was identified by exchanging the segment 93 to 150 in
Fig. 2. SDS-PAGE of the purified parental and hybrid B/D IFNs. Approximately 2 μg of each IFN was loaded on 15% SDS-polyacrylamide gels and stained with Coomassie Brilliant Blue. Lanes 1, 5, 13 and 16 contain mol. wt. markers: phosphorylase B (92.5K), bovine serum albumin (66.2K), ovalbumin (45K), carbonic anhydrase (31K), soybean trypsin inhibitor (21.5K), lysozyme (14.4K). The structure of the IFNs is drawn schematically below each lane and indicated by the notation also used in the text. White bars, IFN-D sequences; black bars, IFN-B sequences.
Fig. 3. Specific biological activities of the purified B/D IFNs on human cells. (a) Induction of (2'-5')oligoadenylate synthetase activity in Daudi cells. For each IFN a dose response is shown using (from left to right) 1 U/ml, 10 U/ml, 10² U/ml and 10³ U/ml (MDBK units, see Table 1). Results are expressed as the x-fold increase in (2'-5')oligoadenylate synthetase in extracts from IFN-treated cells relative to untreated control cells. (b) Antiproliferative activities on Daudi cells. Serial 10-fold dilutions of each IFN were assayed for inhibition of Daudi cell multiplication, determined by counting the cells 48 h and 72 h after IFN treatment. The effect of the IFNs is analysed in reciprocal dilutions scoring 50% inhibition of cell count and growth rate. Results are expressed as values relative to the activity of IFN-B, which is taken as 100%. (c) Antiviral activities on WISH cells using VSV as a challenge virus. IFN-B has a 100% value. The structure of the IFNs is drawn schematically as in Fig. 2.

When tested on mouse cells the specific activities varied between 10⁴ to 10⁸ U/mg, with some hybrids having a higher activity on mouse cells than either of the parental IFNs (Table 1). It was found that B₁₋₆₀ caused an increase in the activity of up to 500-fold, as can be seen from the ratios: BBDB/BBBB = 2; BBDD/BBBB = 1 to 3; BDDD/BDBB = 1.

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following ratios: BBBB/DBBB \(\approx 1\) and BBDB/BBBB \(\approx 600\); BDBB/BBBB = 300; BBDD/BBBD > 100.

**Comparison of antiproliferative activities**

The IFNs were tested for their ability to inhibit cell growth on human Daudi cells. The results were analysed by percentage inhibition of growth rates and cell counts. The range of dilutions for 50% inhibition was from \(10^{-4}\) to \(10^{-7}\) for preparations of IFN having MDBK antiviral titres of about \(10^7\). The profile of the antiproliferative potency of the various IFNs (Fig. 3b) was found to correlate with the profile obtained for the specific antiviral activities (Fig. 3c).

**Induction of (2'-5')oligoadenylate synthetase**

The increase of (2'-5')oligoadenylate synthetase was measured in Daudi cells. The results are represented as the increase in activity as compared to untreated control cells (Fig. 3a). The induction of synthetase did not correlate with the specific antiviral and antiproliferative activities on human cells. Within the concentration range used (1 to \(10^3\) U/ml) the parent IFNs and five hybrids showed a low induction (four- to sixfold). Three hybrids (BBBD, BBDB and BDBB) showed a significantly higher potency (10-fold) and BDDD as well as DDDB increased the induction of synthetase by 15- to 17-fold.

**Binding of radioactive IFNs to Daudi cells**

The present IFNs as well as complementary hybrid pairs that showed large differences in their specific biological activities, e.g. BDDD/DBBB = 60; BBDB/DDDB = 300, were chosen to follow the kinetics of binding to Daudi cells (Fig. 4). Radioactivity was converted into MDBK units for reference. On the basis that binding would reflect the major differences in specific activity, the doses were chosen to cover the range around and above 50% saturation of binding sites on Daudi cells at 3 h (Mogensen & Bandu, 1983). At the doses used for hybrid IFNs containing the amino acids B_1-60, the hybrids having D_1-60 showed little or no detectable binding. At a 30-fold higher dose, specific binding of DDDD was comparable to that of the interferons containing B_1-60, yet specific binding of DDBD could hardly be distinguished above background and then only at early times; therefore, only two representative values are given (Fig. 4f).

These results are a preliminary comparative assay of receptor function in response to a set of novel ligands. For this purpose a study of paired dose kinetics on the cell line Daudi is particularly appropriate as the relationship between receptor function and biological activity has been established on this cell line both for IFN-A (\(\alpha_2\)), which has a specific activity similar to that of IFN-B, and for IFN-D (\(\alpha_1\)) (Uzé et al., 1985). A more detailed description of the binding functions for the hybrid molecules is currently in preparation.

Clearly, the N-terminal sequence 1 to 60 determined the apparent affinity of binding. Furthermore, substituting the section B_93-150 into IFNs possessing an N-terminal D_1-60 sequence reduced the specific binding as was observed for the biological activities. The highest ratios of bound ligand to free ligand obtained for each IFN are summarized in Table 2.

**Binding of radioactive BDDD to mouse L1210 cells**

The hybrid BDDD showed a high specific activity on mouse embryo fibroblasts (Table 1). We found a high affinity for mouse IFN receptors on the L1210 mouse lymphoma line as well (Fig. 5). Specific binding of the hybrid to mouse IFN receptors may be inferred from the fact that binding was displaced by mouse \(\alpha/\beta\) IFN and to a far lesser extent (1/30) with human LeIFN. Furthermore, no specific binding was seen on L1210-R, a strain of L1210 cells that lacks high affinity receptors for mouse IFN (Aguet, 1980).

**DISCUSSION**

The characteristic features of recombinant lymphoblastoid IFN-B and IFN-D reside in their different specific antiviral and antiproliferative activities. IFN-B is about 30 times more active on human cells than IFN-D, and about tenfold less active on mouse cells than IFN-D, whereas
Fig. 4. Receptor binding kinetics of $^{125}$I-labelled B/D IFNs to exponentially growing Daudi cells at 37 °C. One ml of culture contained an average of 235000 cells at the time of binding. (a) LyIFN-B: O, 20 U/ml; ▲, 100 U/ml. (b) LyIFN-D: O, 580 U/ml; ▲, 2900 U/ml. (c) IFN BDDD: O, 60 U/ml; ▲, 300 U/ml. (d) IFN DBBB: O, 510 U/ml; ▲, 2250 U/ml. (e) IFN BBDB: O, 24 U/ml; ▲, 120 U/ml. (f) IFN DDBD: O, 580 U/ml; ▲, 2400 U/ml.

Table 2. Percentage bound IFN/free IFN for different interferons binding to Daudi cells

<table>
<thead>
<tr>
<th>IFN*</th>
<th>%</th>
<th>IFN†</th>
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<tr>
<td>BBB</td>
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<tr>
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<td>DBBB</td>
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<tr>
<td>BBDB</td>
<td>14.64</td>
<td>DDBD</td>
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* Input IFN: 100 U/ml (MDBK).
† Input IFN: 3000 U/ml (MDBK).

Both IFNs have a comparable activity on bovine cells. The specific activity of IFN-D ($\alpha_1$) has been described previously (Weck et al., 1981; Streuli et al., 1981; Rehberg et al., 1982; Uzé et al., 1985). On a molecular basis LyIFN-B differs from LyIFN-D by 35 amino acids (Fig. 6). LyIFN-B is an allelic mutant of LeIFN-B from which it differs at four positions from amino acids 98 to 101 (Goeddel et al., 1981). LyIFN-D is identical to LeIFN-D. Several point mutations have been found in the extracistronic region of the cDNAs as well (F. Meyer, unpublished). LyIFN-B has an aberrant mobility on SDS–polyacrylamide gels and in the course of this work we found that the region from amino acids 61 to 92 confers a retarded mobility to the protein.
In order to define the domains which determine the efficiency of the IFN subtypes, we constructed genetic hybrids in vitro by making use of common restriction sites, such that IFN-B was converted progressively into IFN-D starting from either end and vice versa for IFN-D. The effects of defined sections were tested by generating double recombinants containing segments from either parent.

On human cells, we identified two sites within the NH₂-proximal half (amino acids 1 to 60 and amino acids 61 to 92), which affect both the antiviral and the antiproliferative activities. In addition, hybrids containing IFN-D₁₋₆₀ as well as IFN-B₉₃₋₁₅₀ showed a seven- to 21-fold drop in their antiviral and antiproliferative activities as compared to the hybrids having both segments from IFN-D. In hybrids with IFN-B₁₋₆₀, however, the exchange of segment 93 to 150 did not affect the activities. These data suggest a binding site within amino acids 93 to 150 with low affinity for the IFN-B sequences and higher affinity for the IFN-D sequences. We suggest the existence of long-range interactions between B₁₋₆₀ and B₉₃₋₁₅₀ which might be disrupted by inserting D₁₋₆₀ and thereby a reduced affinity might result, presumably due to conformational changes. Since the differences in activity were found to correlate with the binding of ¹²⁵I-labelled IFNs we conclude that these differences in activity may be due to the altered binding of these hybrids to the cellular IFN receptors.

The results obtained on mouse cells suggest that several sites affect the binding to the receptor.
In fact, it seems that the activity of human IFN on mouse cells is more susceptible to conformational changes than on human cells. This might reflect a narrow interacting domain, in contrast to what is found on bovine cells where the receptor binding appears to be relatively unaffected by structural changes. Whether the binding sites involved on human and on mouse cells are the same cannot be inferred from these data.

The induction of (2'-5')oligoadenylate synthetase by the various IFNs did not correlate with their specific antiproliferative and antiviral activities. Furthermore, while all IFNs showed induction, several showed no dose-dependent increase at IFN concentrations as low as 1 U/ml. Either the assay was not linear in these cases or a broader concentration range might be needed to obtain a better dose response. The level of synthetase activity was measured in vitro by the extent of (2'-5')oligonucleotide synthesis in cell extracts. Adsorption to agarose-poly(rI)-(rC) eliminates the interference by (2'-5')phosphodiesterase (Merlin et al., 1981). A lack of correlation of the induction of (2'-5')A synthetase with the antiviral and antiproliferative activities has been reported before for IFN-α, and IFN-α 26K (Goren et al., 1983).

Cells of the IFN-resistant L1210 subline were shown not to express specific IFN receptors (Aguet, 1980), and IFN did not increase the level of the synthetase (Marti et al., 1981). On the other hand, mouse embryonal carcinoma cells express specific IFN receptors that are apparently functional, as IFN increases the level of (2'-5')A synthetase, even though the cells are resistant to both the antiviral and the antiproliferative effects of IFN (Aguet et al., 1981). We therefore conclude that the level of (2'-5')A synthetase might be stimulated differently from the antiviral and antiproliferative activities. On the other hand, if the stimulation of (2'-5')A synthetase activity by IFNs were related to receptor activity, then the link might occur at threshold levels of IFN binding.

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REFERENCES

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