Characterization of Sendai virus-induced human placental trophoblast interferons

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Human placental trophoblast cultures produce a mixture of interferons (IFNs) when challenged with Sendai virus. High-performance dye-ligand and immunoaffinity chromatography of a trophoblast IFN (tro-IFN) preparation enabled the isolation of three antigenically distinct IFNs, α, αII and β, with Mr's of 16K, 22K and 24K respectively, by reducing and non-reducing SDS-PAGE. The major IFN, responsible for 75% of the total antiviral activity, was tro-IFN-α, with the remaining activity being due to tro-IFN-αII and tro-IFN-βII, as determined by an antiviral neutralization test using specific anti-human IFN antibodies. The antiviral activities of the tro-IFNs were stable at pH 2.0 for 24 h and tro-IFN-αII and -β were shown to be glycoproteins. The three tro-IFNs showed different antiviral activities when assayed on human and bovine cell species; tro-IFN-α and -αII protected both human and bovine (MDBK) cells from virus infection, whereas tro-IFN-β showed a high degree of species specificity, protecting only the human cell types tested.

Introduction

Interferons (IFNs) are a family of proteins produced by vertebrate cells after exposure to certain inducers, e.g. viruses, synthetic dsRNA or mitogens. They exert a broad spectrum of biological activities, the most prominent being the ability to impair virus replication. In humans, three functionally related but antigenically distinct IFNs (α, β and γ) have been identified and are recognized by the Committee on the Nomenclature of Interferons. However, other types of human IFN have been reported by other workers (Wilkinson & Morris, 1983; Dianzani et al., 1986), but these have not been characterized fully. In addition, Adolf (1987) and Hauptmann & Swety (1985) have reported that a new type of IFN, IFN-ω, is released from cells challenged with Sendai virus. This unique IFN (designated IFN-ω1 by Adolf, 1987) has 60% sequence identity with human IFN-α and 26% with IFN-β, and is different antigenically from human IFN-α, -β and -γ. IFN-ω1 was therefore considered to belong to a subfamily of IFN-α (Capon et al., 1985) and was termed IFN-α subclass II (tro-IFN-αII), in contrast to the classical IFN-α in IFN-α subclass I (IFN-αI).

Several workers (Chard et al., 1986; Duc-Goiran et al., 1985; Bocci et al., 1985) have reported the presence of IFN-α and -β in amniotic fluids and specimens from placentae, however the cellular origins of these IFNs have not been identified. Besides IFN-α and -β, Chin et al. (1986) have reported that IFN-γ is produced by placental lymphocytes, monocytes and granulocytes after phytohaemagglutinin and interleukin 2 stimulation. Recently, Tóth et al. (1990a) demonstrated that IFN-β is produced by human placental trophoblasts in vitro after stimulation with poly(rI).poly(rC). Further studies by Tóth et al. (1990b) showed that challenge of trophoblast cultures with Sendai virus resulted in the production of a mixture of IFN-α and -β. In this paper we describe the isolation and characterization of the Sendai virus-induced human placental tro-IFN components.

Methods

Cell culture and IFN induction. Human placental trophoblast cells were isolated using immunomagnetic microspheres as described by Douglas & King (1989). IFN was produced by challenge of trophoblast cultures with Sendai virus as described by Tóth et al. (1990b). The supernatants containing IFN were centrifuged at 10000g for 1 h, adjusted to pH 2.0 by addition of concentrated HCl and stored at 4 °C for 24 h to inactivate the virus. The crude IFN preparation was neutralized with concentrated NaOH and precipitated by adding 1 g ammonium sulphate per 2 ml IFN preparation. The precipitate was redissolved in 0.02 M-sodium phosphate buffer pH 7.2 and dialysed against two changes of a 500-fold excess of the same buffer for 10 h at 4 °C.
**IFN bioassay.** The IFN antiviral assay was performed as described by Tóth et al. (1990a). Results were standardized in international units (IU) by comparison with the National Institute of Health standards for human IFN-β (G-023-902-527) and IFN-α (GA-023-902-530). IFN titres, using human trisomic 21 fibroblast cell lines GM 2504 and GM 2767 (Cell Repository, Camden, N.J., U.S.A.), and bovine (MDBK) cells, were determined similarly using vesicular stomatitis virus (VSV) as the challenge; results were expressed as the highest dilution giving 50% protection.

**Antigenic characterization of IFN components.** The antigenic specificities of the crude and isolated tro-IFN components were determined by antibody neutralization tests. Polyclonal anti-human IFN-α, anti-human IFN-β, anti-IFN-γ and anti-recombinant human IFN-α1 antibodies were used for the assay and preparation of HPLC sorbents. Polyclonal anti-IFN-α antibodies obtained from Boehringer Mannheim (produced in BALB/c mice using human IFN-α purified from Sendai virus-stimulated human lymphoblastoid cells) neutralize human IFN-α, and recombinant human IFN-α, and IFN-α1, but do not react with human IFN-β or γ. Polyclonal human anti-IFN-β antibodies, produced in horses immunized with human IFN-β (fibroblast), were obtained from Boehringer Mannheim; they do not react with human IFN-α, IFN-α1 or IFN-γ. Antirecombinant human IFN-α1 antibodies were supplied by Günther R. Adolf (Department of Biotechnology, Dr Boehringer-Gasse, A-1121 Vienna, Austria) and do not react with IFN-α, IFN-α1 or IFN-γ. Anti-recombinant human IFN-α1 antibodies are neutralized 100% by IFN-α1 or anti-IFN-α1 antibodies; they do not react with IFN-α1 or IFN-γ. Serial dilutions of IFN and a fixed dilution of antibody in 10-fold excess were incubated for 1 h at 37 °C and residual antiviral activity was determined as described by Tóth et al. (1990a).

**Preparation of HPLC sorbents.** HEMA-BIO 1000 VS 3GA was prepared by covalently attaching Cibacron Blue 3GA by a spacer arm, 1,4-diaminobutane, to vinyl sulphonate-activated HEMA-BIO 1000 (10 µm particle size) as described previously (Aboagye-Mathiesen et al., 1991). The prepared gel was then packed into a biocompatible PEEK (Poly Ether Ether Ketone) column (250 mm x 7.5 mm internal diameter; Tessek A/S).

Immunoadsorbents (anti-IFN-α and anti-IFN-β antibodies) and concanavalin A (Con A) columns were prepared by adsorption-promoted enhanced covalent-immobilization of polyclonal anti-IFN-α and anti-IFN-β antibodies, and Con A on macroporous HEMA-BIO 1000 VS. Briefly, 0.5 g dry HEMA-BIO 1000 VS was swollen with immobilization buffer (0.1 M-sodium borate, 0.75 M-ammonium sulphate buffer pH 8.0) and 5 mg antibodies or 10 mg of Con A dissolved in 4 ml or 15 ml (for Con A) of immobilization buffer was added to the wetted gels. Immobilization was allowed to proceed under gentle rotation for 16 to 20 h at 4 °C. The gels were then settled by centrifugation, the supernatants were removed, the gels were washed eight times with distilled water and residual reactive groups were blocked by incubation with 3 ml 0.1 M-ethanolamine in 0.1 M-sodium borate buffer pH 9.0 for 6 h. The gels were then packed into biocompatible PEEK columns (50 mm x 4.6 mm internal diameter).

**Chromatographic procedures**

(i) **High performance dye-ligand affinity chromatography (HP-DLAC).** Concentrated crude tro-IFN (2.05 x 10^9 IU) was filtered through a 0.22 µm Millipore filter and applied to a HEMA-BIO 1000 VS 3GA column equilibrated with column buffer (0.02 M-sodium phosphate buffer pH 7.2). After the column had been washed with the same buffer containing 0.2 M-NaCl, proteins were eluted with a linear gradient of 0.2 to 1.0 M-NaCl for 35 min at a flow rate of 1.5 ml/min; elution from the column was monitored continuously by measuring the absorbance at 280 nm. The column was washed further with 1.0 M-NaCl in column buffer for 20 min until the absorbance was almost zero. Protein was then eluted from the column with increasing concentrations of ethylene glycol in 0.02 M-sodium phosphate buffer pH 7.2 containing 1.0 M-NaCl, elution was with 0 to 50% ethylene glycol for 10 min and 50% for 35 min at a flow rate of 1.5 ml/min. Fractions of 1.5 ml were collected and assayed for IFN antiviral activity.

(ii) **High performance immunoaffinity chromatography (HP-IAC).** Crude tro-IFN preparations and the fractions containing IFN antiviral activity from the HEMA-BIO 1000 VS 3GA column were applied separately to HEMA-BIO 1000 VS-anti-IFN-α (polyclonal) antibody or -anti-IFN-β antibody columns. In each case, the columns were washed for 10 min with PBS pH 7.4 and proteins were eluted with 0.1 M-glycine-HCl pH 2.4. Fractions of 0.5 ml were collected and dialysed against PBS for 16 h at 4 °C to remove the pH to neutral, and then assayed for IFN antiviral activity.

**PAGE.** Slab SDS-PAGE was carried out according to Laemmli (1970). Freeze-dried IFN samples were prepared with or without 5% 2-mercaptoethanol. After electrophoresis, the gels were silver-stained using a Bio-Rad silver staining kit and scanned on a Biomed SLR HPLC monitor, using known concentrations of BSA as a standard.

**Glycoprotein analysis.** The presence of sugar residues in the tro-IFNs was determined by their binding to a Con A affinity column as described previously (Aboagye-Mathiesen et al., 1990).

**Results**

**Purification of tro-IFNs**

Fig. 1 illustrates the HP-DLAC of crude tro-IFN on HEMA-BIO 1000 VS 3GA. The tro-IFNs bound completely when applied in 0.02 M-sodium phosphate buffer pH 7.2 at low ionic strength (0.2 M-NaCl). Development of the column with a linear concentration gradient of NaCl separated 20% of the total IFN activity applied into five peaks (fractions 21 to 40 eluted between 0-6 and 0-8 M-NaCl). Further development of the column with a linear concentration gradient of the hydrophobic solute ethylene glycol produced two IFN peaks (fractions 50 to 53 and 54 to 70), eluted from the column at ethylene glycol concentrations of 40 to 50% and 50%. A summary of the HP-DLAC of the tro-IFNs is presented in Table 1.

**Characterization of tro-IFNs**

The tro-IFN components were identified by antiviral neutralization tests using polyclonal antisera to human IFN-α, -β and -γ and anti-IFN-α1 antibodies. Polyclonal antiserum to human IFN-β neutralized 75% of the antiviral activity of crude tro-IFN samples (initially 800...
Sendai virus-induced trophoblast IFNs

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Fig. 1. HP-DLAC of crude tro-IFN on HEMA-BIO 1000 VS 3GA. IFN (2.05 x 10^6 IU) was applied to a column and IFN activity (■) was eluted with a 60 min linear gradient of 0.2 m- to 1.0 m-NaCl (—) for 60 min. The column was further eluted with increasing concentrations of ethylene glycol (— —) from 0 to 50% for 10 min and 50% for 35 min. The A_280 is shown by the continuous line.

Table 1. Purification of placental tro-IFN components by HP-DLAC

<table>
<thead>
<tr>
<th></th>
<th>Total activity (IU x 10^-4)</th>
<th>Total protein (mg)</th>
<th>Specific activity (IU/mg x 10^-3)</th>
<th>Purification (fold)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude tro-IFN</td>
<td>205</td>
<td>401.9</td>
<td>5.10</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Fractions 21-40</td>
<td>41</td>
<td>0.328</td>
<td>1250</td>
<td>245-0</td>
<td>20</td>
</tr>
<tr>
<td>Fractions 50-53</td>
<td>742</td>
<td>0.070</td>
<td>1060</td>
<td>207-8</td>
<td>3-6</td>
</tr>
<tr>
<td>Fractions 54-70</td>
<td>138</td>
<td>0.076</td>
<td>18200</td>
<td>3568-6</td>
<td>67-32</td>
</tr>
</tbody>
</table>

Table 2. Antibody neutralization of tro-IFN components isolated by HP-DLAC*

<table>
<thead>
<tr>
<th>Anti-human IFN sera</th>
<th>Residual antiviral activity after incubation with antisera (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fractions 21-40</td>
</tr>
<tr>
<td>α</td>
<td>0</td>
</tr>
<tr>
<td>β</td>
<td>100</td>
</tr>
<tr>
<td>α_1</td>
<td>100</td>
</tr>
<tr>
<td>γ</td>
<td>100</td>
</tr>
<tr>
<td>α + β</td>
<td>ND†</td>
</tr>
<tr>
<td>α_1 + β</td>
<td>100</td>
</tr>
<tr>
<td>α + α_1</td>
<td>0</td>
</tr>
</tbody>
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* Neutralization tests were performed with a 10-fold excess of the respective antiserum.
† ND, Not determined.
Fig. 2. Silver-stained SDS-polyacrylamide gel containing purified tro-IFNs. (a) Lane 1, standard protein markers; lane 2, tro-IFN-\(\alpha_{1}\) and tro-IFN-\(\alpha_{1}1\) purified from a crude tro-IFN preparation on a HEMA-BIO 1000 VS-anti-IFN-\(\alpha\) antibody column; lanes 3 and 4, HP-IAC-purified tro-IFN-\(\alpha\) under reducing and non-reducing conditions respectively. (b) Lane 1, standard protein markers; lanes 2 and 3, HP-DLAC-purified tro-IFN-\(\beta\) (from fractions 56 to 58 in Fig. 1) under reducing and non-reducing conditions respectively.

Table 3. Neutralization of the antiviral activity of fractions 50 to 53 after passage through an anti-IFN-\(\beta\) antibody column

<table>
<thead>
<tr>
<th>Anti-human IFN serum*</th>
<th>Residual antiviral activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flowthrough</td>
</tr>
<tr>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td>(\alpha_{1})</td>
<td>0</td>
</tr>
<tr>
<td>(\alpha)</td>
<td>0</td>
</tr>
<tr>
<td>(\beta)</td>
<td>100</td>
</tr>
</tbody>
</table>

* The anti-IFN immune sera were used in 10-fold excess.

fractions was not neutralized by antiserum to IFN-\(\beta\) but was completely neutralized by anti-IFN-\(\alpha_{1}1\) and polyclonal anti-IFN-\(\alpha\) antisera. However, the bound IFN was completely neutralized by human anti-IFN-\(\beta\) antiserum.

Fig. 2 shows the patterns obtained by SDS-PAGE of fractions from HP-IAC of crude tro-IFN preparations and the HP-DLAC-purified tro-IFN-\(\alpha\) component (fractions 21 to 40 from Fig. 1) on a HEMA-BIO 1000 VS-anti-IFN-\(\alpha\) (polyclonal) antibody column. As shown in Fig. 2(a) and by antiviral activity neutralization assays of IFNs extracted from unstained gels [similar to the gel in Fig. 2(a) under reducing and non-reducing conditions], tro-IFN-\(\alpha_{1}\) and tro-IFN-\(\alpha_{1}1\) activities corresponded to the protein bands with \(M_s\) of 16K and 22K respectively; no IFN activity was associated with the 67K protein band. Fig. 2(b) shows an SDS-polyacrylamide gel of tro-IFN-\(\beta\) (from fractions 56 to 58 in Fig. 1); tro-IFN-\(\beta\) migrated as a 24K protein on reducing and non-reducing SDS-polyacrylamide gels (Fig. 2b, lanes 2 and 3 respectively).

Table 4. Protection of cell lines from different species against VSV infection

<table>
<thead>
<tr>
<th>Species</th>
<th>Cell line</th>
<th>tro-IFN-(\alpha_{1})</th>
<th>tro-IFN-(\alpha_{1}1)</th>
<th>tro-IFN-(\beta)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>WISH</td>
<td>64</td>
<td>64</td>
<td>256</td>
</tr>
<tr>
<td>Human</td>
<td>GM 2504</td>
<td>192</td>
<td>224</td>
<td>768</td>
</tr>
<tr>
<td>Human</td>
<td>GM 2767</td>
<td>192</td>
<td>224</td>
<td>768</td>
</tr>
<tr>
<td>Bovine</td>
<td>MDBK</td>
<td>128</td>
<td>256</td>
<td>2</td>
</tr>
</tbody>
</table>

* Antiviral activity is expressed as the highest dilution giving 50% protection against VSV.

The antiviral activity of the crude tro-IFN preparation and the purified IFN components were stable after 24 h incubation at pH 2. Tro-IFN-\(\alpha_{1}1\) and tro-IFN-\(\beta\) bound to the Con A affinity column (data not shown), whereas tro-IFN-\(\alpha_{1}\) did not bind. This suggested that tro-IFN-\(\alpha_{1}1\) and tro-IFN-\(\beta\) are glycoproteins.

Antiviral specificity of tro-IFNs

Table 4 shows the antiviral activities of the tro-IFN components on different human and bovine cell lines. They exhibited a broad spectrum of antiviral activities on the human cells (WISH, GM 2504 and GM 2767) tested, but tro-IFN-\(\alpha_{1}\) and tro-IFN-\(\alpha_{1}1\) protected bovine MDBK cells better than human cells (twofold and fourfold, respectively, relative to human WISH cells). More interestingly, the protection of MDBK cells by tro-IFN-\(\alpha_{1}1\) was twice that conferred by tro-IFN-\(\alpha_{1}\). In contrast, tro-IFN-\(\beta\) did not protect bovine MDBK cells but did protect all the human cell lines tested.

Discussion

Previous work (Tóth et al., 1990a; Aboagye-Mathiesen et al., 1990) in our laboratory has shown that human placental trophoblast cultures stimulated with poly(rI).poly(rC) produce IFN-\(\beta\) exclusively. The results presented here show that trophoblast cultures infected with Sendai virus produce a mixture of IFN-\(\alpha_{1}\), IFN-\(\alpha_{1}1\) and IFN-\(\beta\); IFN production increased after differentiation of the cytotrophoblast to syncytiotrophoblast in vitro (Tóth et al., 1990a). Similarly, Burke et al. (1978) have reported that the production of IFN and IFN sensitivity change during differentiation of mouse embryonal carcinoma cells in vitro.

The tro-IFNs (\(\alpha_{1}\), \(\alpha_{1}1\) and \(\beta\)) are antigenically distinct and this may suggest that they are structurally different. Antiserum to recombinant human IFN-\(\alpha_{1}1\) could not neutralize the tro-IFN-\(\alpha_{1}\) component (fractions 21 to 40
in Fig. 1) or tro-IFN-β, but completely neutralized tro-IFN-ζ1 purified by HP-IAC. The ability of polyclonal anti-human IFN-α (lymphoblastoid) antiserum to neutralize both the antiviral activities of tro-IFN-α and tro-IFN-ζ1 can be explained by the fact that IFN-α and IFN-ζ1 is a component of natural mixtures of lymphoblastoid and leukocyte IFN preparations (Adolf, 1987) used to prepare polyclonal antibodies. The neutralization results support the suggestion (Adolf, 1987) that human type 1 IFN is made up of three antigenically distinct proteins, IFN-α, IFN-ζ1 and IFN-β.

The results presented in this paper have raised several questions concerning the trophoblast IFN system. Will different viruses (or non-viral inducers) induce different levels of the tro-IFNs already identified?; how many tro-IFNs can be expected to exist?; is there a molecular basis for the observed biological dissimilarities of the tro-IFNs?; and do the biological differences between the tro-IFNs reflect structural dissimilarities of the molecules? Our present work is directed towards answering these questions and we believe that the results will be biochemically and virologically important for understanding the mechanism of action and involvement of tro-IFNs in foetal development, as suggested by the presence of apparently constitutive IFNs in human placental blood (Duc-Goiran et al., 1985) and the proven role of IFNs in maternal recognition of pregnancy in sheep (Cross & Roberts, 1989).

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References


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