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, α1, α1β, and α1, 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-α1, with the remaining activity being due to tro-IFN-α1 and tro-IFN-α1β, 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-α1 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-α1 and -α1β 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 & Swetly (1985) have reported that a new type of IFN, IFN-ω1, 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 1 (IFN-α).

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 10000 g 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 Töth 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.

**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 × 7.5 mm internal diameter; Tessek A/S).

**IgG 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 (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 in biocompatible PEEK columns (50 mm × 4.6 mm internal diameter).

**Chromatographic procedures**

(i) High performance dye-ligand affinity chromatography (HP-DLAC). Concentrated crude tro-IFN (2.05 × 10⁶ 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 restore 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 1D/2D scanner to determine the Mr, by comparison with Bio-Rad protein standards. To determine the IFN antiviral activity on unstained gels, 2 mm slides of the gel were extracted at 4 °C for 24 h into 0.5 ml volumes of PBS pH 7.4 containing 0.1% SDS and 0.02% Na3. Aliquots (100 μl) were then taken and assayed for IFN antiviral activity.

**Protein determination.** The concentration of protein in crude tro-IFN preparations was determined by the dye binding assay (Bradford, 1976) using bovine serum albumin (BSA) as a standard. The concentration of purified IFN was measured by absorbance at 280 nm, or derivatization with fluorescamine and injecting the samples into a fluorescence 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

Fig. 1. HP-DLAC of crude tro-IFN on HEMA-BIO 1000 VS 3GA. IFN (2.05 × 10⁶ 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 A₂₈₀ 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>0.076</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>γ</td>
<td>100</td>
</tr>
<tr>
<td>α + β</td>
<td>ND†</td>
</tr>
<tr>
<td>α₈₁ + β</td>
<td>100</td>
</tr>
<tr>
<td>α + α₈₁</td>
<td>0</td>
</tr>
</tbody>
</table>

*Neutralization tests were performed with a 10-fold excess of the respective antiserum.
†ND, Not determined.

IU/ml, 200 IU/ml after treatment), whereas the anti-IFN-α antibodies neutralized 25% of the activity (initially 800 IU/ml, 600 IU/ml after treatment) (Table 2). Fractions 21 to 40 (see Fig. 1), eluted from the column with NaCl (0.6 to 0.8 M), were completely neutralized by polyclonal anti-IFN-α antibodies, but not with anti-IFN-α₁, anti-IFN-β or anti-IFN-γ antibodies. This showed that the five IFN peaks consisted of IFN-α₁. However, the IFN peak fractions (50 to 53) that were eluted from the column using ethylene glycol concentrations between 40 and 50% were partially neutralized by polyclonal anti-IFN-β antibodies, and to the same extent by polyclonal anti-IFN-α and anti-IFN-α₁ antibodies. Surprisingly, these IFN fractions were completely neutralized by a combination of anti-IFN-α₁ and anti-IFN-β antibodies, and polyclonal anti-IFN-α and anti-IFN-β antibodies, but not polyclonal anti-IFN-α and anti-IFN-α₁ antibodies. This suggested that fractions 50 to 53 were a mixture of tro-IFN-α₁ and tro-IFN-β.

Fractions 50 to 53 (see Fig. 1) were further characterized by passage through a HEMA-BIO 1000 VS-anti-IFN-β (polyclonal) antibody column. The antiviral activity of the flowthrough fractions and the bound IFN eluted from the column at pH 2.4 was characterized further using antiserum to human IFN-α, IFN-β and recombinant human IFN-α₁ (Table 3). These assays showed that the antiviral activity of the flowthrough
Table 3. Neutralization of the antiviral activity of fractions 50 to 53 after passage through an anti-IFN-β antibody column

<table>
<thead>
<tr>
<th>Anti-human IFN serum*</th>
<th>Residual antiviral activity (%a)</th>
<th>Flowthrough</th>
<th>Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>α1</td>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>β</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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

fractions was not neutralized by antiserum to IFN-β but was completely neutralized by anti-IFN-α1 and polyclonal anti-IFN-α antisera. However, the bound IFN was completely neutralized by human anti-IFN-β 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-α1 component (fractions 21 to 40 from Fig. 1) on a HEMA-BIO 1000 VS-anti-IFN-α (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-α1 and tro-IFN-α1I activities corresponded to the protein bands with Mₙs 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-β (from fractions 56 to 58 in Fig. 1); tro-IFN-β 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-α1</th>
<th>tro-IFN-α1I</th>
<th>tro-IFN-β</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-α1 and tro-IFN-β bound to the Con A affinity column (data not shown), whereas tro-IFN-α1 did not bind. This suggested that tro-IFN-α1 and tro-IFN-β 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-α1 and tro-IFN-α1I 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-α1I was twice that conferred by tro-IFN-α1. In contrast, tro-IFN-β 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-β exclusively. The results presented here show that trophoblast cultures infected with Sendai virus produce a mixture of IFN-α1, IFN-α1I and IFN-β; 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 (α1, α1I and β) are antigenically distinct and this may suggest that they are structurally different. Antiserum to recombinant human IFN-α1I could not neutralize the tro-IFN-α1 component (fractions 21 to 40
in Fig. 1) or tro-IFN-β, but completely neutralized tro-IFN-α1 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-α1 can be explained by the fact that IFN-α1 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-α1, IFN-α11, and IFN-β.

The response of different cell lines to the antiviral effects of tro-IFNs when tested by inhibition of plaque formation varied. The results presented in Table 4 show that the tro-IFNs differ from one another in their ability to confer protection against VSV infection of different human and bovine cell species. Tro-IFN-β shows a degree of species specificity, protecting human cells but not bovine (MDBK) cells. However, tro-IFN-α and -α1 protect both human and bovine cells. The variations in the relative antiviral activities of tro-IFNs in the different cells may indicate that they differentially affect biochemical pathways induced by IFNs, such as phosphorylation of the eukaryotic initiation factor 2 by the dsRNA-dependent protein kinase or activation of the 2'-5'-oligoadenylate system (Marié et al., 1990; Baglioni, 1979). That IFNs differentially induce biochemical pathways which correlate with particular specific antiviral activities in various lines of cells is indicated by studies with different IFN preparations (Rosenblum et al., 1990). Differences between distinct IFNs or between distinct cell types may be of importance for the therapeutic application of IFNs, particularly when such differences occur within the same organism.

The trophoblast layer of the human placenta constitutes the maternal–foetal interface and acts as a barrier to the transmission of infection from mother to foetus. The ability of trophoblast cells to produce IFNs may represent a system for the protection of the foetus from viral infection. This is shown by the fact that, in some cases, maternal infection may spread to the placenta but fail to progress to the foetus (Yamauchi et al., 1974; Klein et al., 1976; Remington & Desmonts, 1976). In addition to the antiviral activity of IFNs, there is increasing evidence that IFNs are also involved in normal physiological and regulatory processes such as cell proliferation and differentiation (Zullo et al., 1985; Fisher & Grant, 1985). Cells of the cytotrophoblast have been shown (Bulmer et al., 1988) to be highly proliferative, as measured by the proliferative marker Ki67 or the presence of mitoses, but lose their proliferative activity and begin to differentiate as they migrate into decidua during implantation. Although the factors that control such aspects of trophoblast behaviour are not known, IFNs may play a major role in the development of these differentiated states. Furthermore, IFN-α has been shown to be involved in prolongation of allograft survival (Hirsch et al., 1974; Mobratten et al., 1973) and suppression of graft-versus-host disease (Corettini et al., 1973). Such modulation of the immune response by IFN may be important in preventing the rejection of the allogenic foetus.

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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