Characteristics of Human Lymphoblastoid (Namalva) Interferon

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SUMMARY

Interferon derived from the human lymphoblastoid cell line, Namalva, was fractionated by antibody affinity chromatography into two antigenically distinct interferon subspecies. At least 13% of the total Namalva interferon activity possessed the F antigenic determinant found on human interferon derived from fibroblast cultures, while the bulk of the Namalva interferon activity had the Le antigenic determinant characteristic for human leukocyte interferon. The separated Le and F subspecies of Namalva interferon differed in the degree of their heterospecific activities on bovine cells. The Le moiety resembled crude leukocyte interferon in that it was highly active in bovine cells. The F component of Namalva interferon showed a lower degree of activity in bovine cells, thus resembling crude fibroblast interferon. When analysed by sodium dodecyl sulphate–polyacrylamide gel electrophoresis and by isoelectric focusing, crude Namalva interferon qualitatively resembled crude leukocyte interferon.

INTRODUCTION

A number of differences exist between human interferon obtained from primary cultures of peripheral leukocytes and from fibroblast cells. The interferons derived from these two cell sources have been shown to differ antigenically (Havell et al. 1975a) and in the degree of their antiviral activities on cells of other species (Gresser et al. 1974). Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) of human leukocyte interferon resolved two components possessing antiviral activities: a faster and a slower migrating species, with apparent mol. wt. of about 15000 and 21000 respectively (Stewart & Desmyter, 1975). Fibroblast interferon was shown to migrate as a homogeneous entity in SDS–polyacrylamide gels, with an apparent mol. wt. of about 20000 (Knight, 1976). Isoelectric focusing of fibroblast interferon has shown that its activity focuses in a broad peak between pH 6·8 and 7·8 (Havell, Yamazaki & Vilček, 1977a), whereas the bulk of leukocyte interferon activity focuses in several distinct peaks between pH 5·5 and 6·6 (Fantes, 1970; Havell, Yip & Vilček, 1977b).

Lines of human lymphoblastoid cells derived from patients with Burkitt’s lymphoma represent another source of human interferon. One such cell line, Namalva (Klein, Dombos & Gothoskar, 1972), was shown to produce high levels of interferon on stimulation with Sendai virus (Strander, Mogensen & Cantell, 1975).

In this paper, we report our findings on the antigenic and biological characteristics and on certain physicochemical properties of Namalva cell-derived human interferon.
METHODS

Interferons. The Namalva interferon used in these studies was the kind gift of C. E. Buckler and K. Zoon, National Institutes of Health, Bethesda, Maryland. It was induced with Newcastle disease virus in suspension cultures of the Namalva line of lymphoblastoid cells (C. E. Buckler, K. Zoon, U. Ruegg and D. Gurari-Rotman, personal communication). After inactivation of the inducing virus by exposure to pH 2, the material was found to contain 6144 reference units/ml. The human leukocyte interferon (Strander & Cantell, 1966) was a generous gift of Dr Karl Cantell, State Serum Institute, Helsinki, Finland; this interferon preparation contained 65000 reference units/ml. Human fibroblast interferon was made in cultures of the FS-4 strain of foreskin fibroblast cells, induced with the synthetic polynucleotide, polynosinate-polyctydylate [poly (I). poly (C)], as described (Havell & Vilček, 1972; Vilček & Havell, 1973). This interferon preparation contained 20000 reference units/ml. All interferon preparations were used without prior purification or concentration.

Interferon assays were done by the micro-method of Armstrong (1971) as modified by Havell & Vilček (1972), using either the FS-7 or the GM-258 (Human Genetic Mutant Cell Repository, Camden, New Jersey) fibroblast strains and vesicular stomatitis virus. The GM-258 strain, containing three copies of chromosome 21, was shown by Tan & Greene (1976) to be more sensitive to the antiviral action of interferon than normal diploid skin fibroblast cells. In our hands, the titre of the human leukocyte interferon standard G-023-901-527 (obtained from the National Institute of Allergy and Infectious Diseases, Bethesda, Maryland) was about 20 times higher than its assigned value when titrated in GM-258 cells. Comparison of antiviral activities in human FS-7 and bovine MDBK cells (obtained from Dr P. B. Sehgal, Rockefeller University, New York) was done by simultaneous titration in pre-seeded cultures in MicroTest II plastic trays. Although an interferon standard was included with each assay, interferon titres are expressed as actual experimental values without correction to the standard.

Anti-interferon sera and affinity chromatography. The rabbit anti-human fibroblast (F) interferon serum was made and used in quantitative neutralization assays according to the procedures of Havell et al. (1975a). The titre of the antiserum is expressed as the reciprocal of the dilution of serum which is capable of neutralizing an equal volume of interferon containing 10 units/ml (final concentration). The sheep anti-human leukocyte (Le) interferon antibody used in these studies was the kind gift of Dr D. Gurari-Rotman and Dr C. B. Anfinsen. The coupling of the antibodies to CnBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) and the affinity chromatography procedures were essentially as described by Havell et al. (1975a). Both sera employed in these studies were monospecific, i.e. they did not react with human interferon of heterologous antigenic type.

SDS–polyacrylamide gel electrophoresis. A modification of the SDS–PAGE method of Stewart & Desmyter (1975) was employed. This procedure uses 10 % polyacrylamide and 0.02 M-sodium phosphate buffer, pH 7.2, with 0.1 % SDS. Prior to electrophoresis, the samples were made up to 1 % SDS, incubated at 36 °C for 1 h, and then dialysed in 0.02 M-sodium phosphate buffer, pH 7.2, with 0.1 % SDS. The dialysed samples were then made to contain 10 % sucrose and electrophoresed for 6 h. The current applied was 10 mA/gel. Myoglobin, chymotrypsinogen, ovalbumin, and cytochrome c were used as markers for mol. wt. determinations; the marker proteins were treated in a manner identical to the interferon samples prior to electrophoresis. Following SDS–PAGE the marker gels were treated with 20 % sulphosalicylic acid for 12 h, fixed in 12 % trichloroacetic acid, and then
Table 1. Antibody affinity chromatography fractionation of Namalva interferon

<table>
<thead>
<tr>
<th>Specific anti-interferon column</th>
<th>Total interferon units*</th>
<th>Reacted</th>
<th>Unretained</th>
<th>% unretained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Le†</td>
<td>12,288</td>
<td>1,600</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Anti-F‡</td>
<td>10,240</td>
<td>6,400</td>
<td>62</td>
<td></td>
</tr>
</tbody>
</table>

* Interferon units/ml ¥ total volume (ml) of sample (tested in FS-7 cells).
† Whole Namalva interferon (3.0 ml) in phosphate buffered saline (PBS), pH 7.4, was reacted at 4 °C with anti-leukocyte interferon (anti-Le) γ-globulin covalently coupled to Sepharose 4B beads (2 ml) as described (Havell et al. 1975a). The reacted mixture was poured into a column at room temperature, and the eluate plus five successive column washes with PBS were pooled (total volume 12.5 ml) and assayed to determine total interferon activity which was not specifically retained by the immobilized antibody.
‡ Namalva interferon (2.5 ml) was similarly reacted with anti-fibroblast (anti-F) γ-globulin covalently coupled to Sepharose 4B beads (10 ml). The eluate plus five successive column washes with PBS were pooled (total volume 55 ml) and assayed for interferon activity.

stained with 0.1 % Coomassie blue. Unstained sample gels electrophoresed in parallel were frozen and cut into 1 mm slices. Two adjacent slices (referred to as one fraction) were pooled, and interferon activity was eluted in 0.5 ml of phosphate buffered saline (PBS), containing 5 % foetal bovine serum, at room temperature overnight.

Isoelectric focusing in polyacrylamide gels. A modification of the isoelectric focusing procedure of Gainer (1973) was used to determine the isoelectric points of the interferons. Cylindrical gels (6 × 80 mm) consisted of 1.8 ml of 7.5 % polyacrylamide containing 2 % ampholytes (LKB, Hicksville, New York, pH range 5 to 7). Samples to be focused were dialysed against 0.05 M-NaCl, made up to 10 % sucrose, and 0.25 ml of this material was layered on the anode end of the gel. The sample was overlaid with 0.2 ml of 2 % ampholytes containing 5 % sucrose. The solutions in the electrode vessels were 1 % H₂SO₄ (anode) and 2 % ethanolamine (cathode). Isoelectric focusing was done at 4 °C over approximately a 5 h period, with an initial potential of 150 V. Fractionation and elution of interferon was the same as described for SDS–PAGE gels, except that elution was done at 4 °C. Determination of the pH gradient was done by eluting fractions of a blank gel overnight at 4 °C in distilled water and measuring the pH at 4 °C.

RESULTS

Fractionation of Namalva interferon by means of antibody affinity chromatography

The antiviral activities of interferons derived from leukocyte or fibroblast cell cultures are selectively neutralized by antisera specific for each interferon (Berg, Ogburn & Paucker, 1975; Havell et al. 1975a). Preliminary investigations carried out in this laboratory revealed that Namalva interferon is not completely neutralized by either specific anti-leukocyte or anti-fibroblast interferon sera; however, a mixture of the anti-leukocyte and anti-fibroblast interferon sera did cause complete neutralization of the antiviral activity of Namalva interferon (Cavalieri et al. 1977).

The finding that neither antiserum alone was capable of neutralizing the antiviral activity of Namalva interferon suggested that this preparation might contain a mixture of the two known antigenically distinct interferon species (Le and F). To test this idea, Namalva interferon was reacted with immobilized anti-Le or anti-F interferon antibodies covalently coupled to Sepharose 4B. Each of these two antisera are known to react only with homologous interferon (Berg et al. 1975; Havell et al. 1975a).
Table 2. Antigenic specificity of Namalva interferon components separated by affinity chromatography

<table>
<thead>
<tr>
<th>Cell source of interferon</th>
<th>Fraction</th>
<th>Anti-F</th>
<th>Anti-Le</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes</td>
<td>Unfractionated</td>
<td>&lt; 100</td>
<td>3300</td>
</tr>
<tr>
<td>Namalva</td>
<td>Unfractionated</td>
<td>&lt; 40</td>
<td>&lt; 40</td>
</tr>
<tr>
<td></td>
<td>Unretained*</td>
<td>600</td>
<td>&lt; 40</td>
</tr>
<tr>
<td></td>
<td>Retained†</td>
<td>&lt; 40</td>
<td>3700</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>Unfractionated</td>
<td>600</td>
<td>&lt; 40</td>
</tr>
</tbody>
</table>

* Portion of interferon activity which did not bind to anti-Le serum coupled to Sepharose 4B (see Table 1).
† Portion of interferon activity specifically bound to the same anti-Le column and recovered after elution with 0.1 M-acetic acid, pH 2.5 (Havell et al. 1975a).

Of the total Namalva interferon applied to the columns, 13% failed to bind to the anti-Le column, whereas 62% was recovered from the unretained fractions after contact with the immobilized anti-F antibody (Table 1). Whereas the original unfractionated Namalva interferon could not be completely neutralized with either of the two antisera alone (see also Table 2), the unretained fractions from the anti-Le or the anti-F columns were completely neutralized by the anti-F or anti-Le antisera, respectively (not shown). Taken together these results indicate that of the total Namalva interferon activity, at least 13% (which did not bind to the immobilized anti-Le serum) is the F species, and at least 62% (which did not react with the immobilized anti-F antibody) is Le interferon. Since the two isolated components only add up to 75% of the original activity, some non-specific binding or inactivation has apparently occurred, and the real contents of either one or both of the species is likely to be somewhat higher than indicated by the experimental results.

Nevertheless, we also had to consider the possibility that some of the interferon activity might be able to react with both antisera and that the existence of such a 'hybrid' antigenic species might account for the less than 100% recovery of activity in the preceding experiment. However, the results shown in Table 2 argue against the presence of hybrid interferon. In this experiment, Namalva interferon was reacted with immobilized anti-Le antibody. The unretained interferon was shown to be neutralized by anti-F antibody. After extensive washing at pH 7 the column was eluted with acetic acid, pH 2.5, to recover the interferon activity specifically retained by the column. This portion of the activity was completely neutralized by anti-Le antibody, but not by anti-F serum, indicating that this interferon possessed only the Le determinant.

Heterospecific activities of the Le and F subspecies of Namalva interferon

Gresser et al. (1974) reported that human leukocyte interferon exhibited a high degree of antiviral activity on bovine cells, whereas fibroblast interferon possessed considerably less activity on these cells. Unfractionated Namalva interferon and both Namalva subspecies separated by affinity chromatography as described above were assayed simultaneously in cultures of human fibroblasts (FS-7) and bovine (MDBK) cells (Table 3). The Namalva interferon Le subspecies demonstrated a ratio of human to bovine antiviral activity similar to that of leukocyte and unfractionated Namalva interferons. The F subspecies of Namalva interferon resembled fibroblast interferon in that it was considerably less active on the bovine cells.
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Table 3. Heterospecific activities of Namalva interferon components separated by affinity chromatography

<table>
<thead>
<tr>
<th>Cell source of interferon</th>
<th>Interferon titre on cells</th>
<th>Human/bovine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fraction</td>
<td>Human (FS-7)</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>Unfractionated</td>
<td>262 144</td>
</tr>
<tr>
<td>Namalva</td>
<td>Retained†</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Unfractionated</td>
<td>196 608</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>Retained†</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>Unfractionated</td>
<td>8</td>
</tr>
</tbody>
</table>

*, † See Table 2.

Fig. 1. SDS-polyacrylamide gel electrophoresis of (a) leukocyte and (b) Namalva interferons. Approximately 50% of the interferon activities applied to the gels were recovered, as determined by interferon assay of the eluted activity on GM-258 cells. Mol. wt. markers: OA, ovalbumin; CT, chymotrypsinogen; MG, myoglobin; and CC, cytochrome c.

SDS-PAGE of Namalva interferon

Stewart & Desmyter (1975) showed that human leukocyte interferon can be resolved into two active mol. wt. components by SDS-PAGE. The interferon activity profiles after simultaneous SDS-PAGE analysis of leukocyte and Namalva interferons are shown in Fig. 1. Two distinct mol. wt. species were seen in each of the two interferon preparations.
The bulk of activity found in the Namalva interferon was associated with the faster migrating component (Le₅), whereas the activity of leukocyte interferon was equally distributed between Le₅ and the slower (Le₆) migrating species.

The calculated mol. wt., based on migratory distances relative to the standard protein markers used in this experiment (Fig. 1), were 23500 for Le₆ and 17500 for the Le₅ subspecies.

Since the slower migrating interferon species has an apparent mol. wt. within the range reported by Knight (1976) for fibroblast interferon, it is likely that at least some of the Namalva interferon activity present in the Le₆ peak is due to the presence of the F interferon moiety.

Isoelectric focusing of Namalva and leukocyte interferons

The technique of isoelectric focusing enables molecular separation based on differences in electrostatic charge. Recently, we have reported (Havell et al. 1977b) that leukocyte interferon preparations could be resolved into several distinct peaks by isoelectric focusing in polyacrylamide gels containing 2% ampholytes (pH gradient 5 to 7). Leukocyte and Namalva interferon were compared using the same method of isoelectric focusing. The resulting distribution of interferon activities recovered from the ampholyte-containing gels is shown in Fig. 2.

The majority of interferon activity for both leukocyte and Namalva interferons was recovered from the acidic regions of the gels. A distinct minor component in each of the two interferons was found to focus just past neutrality. Previous studies (Havell et al. 1977a, b) have shown that the major portion of fibroblast interferon activity focuses in a single broad
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peak (pH 6.8 to 7.8), whereas leukocyte interferon forms at last three distinct bands in the pH range 5.5 to 6.6 and a minor band near pH 7.0. Three components of Namalva interferon, focusing between pH 5.4 and 6.3, were also observed by K. H. Fantes, C. J. Burman and M. J. Johnston (personal communication).

Both the Namalva and leukocyte interferons formed several bands in the acidic regions of the pH gradient (between pH 5.0 and 6.5) and a single peak at pH 7.2 to 7.5. The position of the latter activity is similar to that seen with fibroblast interferon (Havell et al. 1977a). Preparations of leukocyte interferon are known to contain a very small quantity (~1% of total activity) of F interferon (Berg et al. 1975; Havell, Berman & Vilček, 1975b), and we showed earlier that the component of leukocyte interferon preparations focusing at the higher pH contains interferon with the F antigenic specificity (Havell et al. 1977a). Although we did not examine the properties of the interferon focusing at pH 7.2 to 7.5 in this experimental series, it is likely that it represents the F interferon moiety.

DISCUSSION

The present studies were initiated when it was observed that interferon mRNA extracted from induced Namalva cells, on injection and translation in Xenopus laevis oocytes, gave rise to an interferon product which could only be neutralized by a mixture of both specific anti-leukocyte and anti-fibroblast interferon sera, and not by either antiserum alone (Cavaliere et al. 1977). Using antibody affinity chromatography techniques, we have now demonstrated in Namalva interferon preparations the existence of a considerable amount of interferon activity possessing the F antigenic specificity. Previous studies (Berg et al. 1975; Havell et al. 1975b) have shown that ~1% of the total activity of leukocyte interferon preparations consists of F interferon. The proportion of F interferon found in the Namalva interferon preparation used in our present experiments was about 13% of the total interferon activity. (Since the total recovery of interferon activity was less than 100%, the proportion of the F interferon moiety in native Namalva interferon preparations could be somewhat higher.) This interferon not only possessed the antigenic determinant(s) of F interferon but also exhibited a degree of heterospecific antiviral activity on bovine cells similar to that of fibroblast-derived interferon.

Strander et al. (1975) reported that a rabbit antiserum raised against whole leukocyte interferon was capable of neutralizing the antiviral activity of both Namalva and leukocyte interferon to the same degree. This antiserum was also capable of neutralizing the antiviral activity of fibroblast interferon, albeit to a lower degree than that of leukocyte or Namalva interferons. These authors concluded that Namalva interferon resembled antigenically leukocyte interferon. In a previous study (Havell et al. 1975b), we reported that the ability of certain rabbit anti-leukocyte interferon sera to neutralize both leukocyte and fibroblast interferons was due to the presence of two neutralizing antibody populations, one specific for leukocyte and the other specific for fibroblast interferon. The finding of Strander et al. (1975) that Namalva interferon was completely neutralized by the antiserum possessing activity against fibroblast interferon was probably due to the neutralization of both the Le and F subspecies present in Namalva interferon by the two distinct neutralizing antibody populations present in the antiserum. This possible interpretation is supported by the fact that neither our monospecific anti-leukocyte serum nor our anti-fibroblast interferon serum completely neutralized the antiviral activity of Namalva interferon (Table 2), whereas its antiviral activity was abolished when both antisera were added (Cavaliere et al. 1977). In addition, the Namalva interferon preparation used by Strander et al. (1975) might have
contained a smaller proportion of F interferon than the preparation used in our studies which would then remain undetectable in the neutralization assay.

The use of SDS–PAGE enabled the resolution of two molecular size species of interferons present in the Namalva interferon which had migratory properties similar to those observed for leukocyte interferon. The Namalva interferon subspecies with the smaller mol. wt. (Le6) was the dominant species in this preparation. Isoelectric focusing demonstrated similar patterns for both leukocyte and Namalva interferons, except that the amount of Namalva activity focusing in the pH 6-6 region was less than in the leukocyte preparation. We have determined that the leukocyte activity focusing in this pH range contains the bulk of the slower migrating (Le8) species of leukocyte interferon isolated on SDS–PAGE (Havell et al. 1977b). The smaller amount of Namalva activity focusing in the pH 6-6 range may be due to a lower concentration of the higher mol. wt. interferon species in Namalva interferon. It is conceivable, however, that the proportions of individual subspecies vary between preparations.

The origin of the F interferon in both leukocyte and Namalva interferon preparations remains unknown. Leukocyte interferon is made in cultures of buffy coat cells which represent a collage of different cell types. Lymphocytes are probably the major interferon source in these cultures. We had speculated that a non-lymphoid cell type present in these cultures (macrophages?) was possibly responsible for the synthesis of the interferon bearing the F antigenic determinant (Havell et al. 1975a). However, as far as is known, the Namalva line is composed of a homogeneous cell population; hence in this instance a single cell type is apparently producing both the Le and F interferons.

We have recently demonstrated that mRNAs for Le and F interferons are translated with fidelity in Xenopus laevis oocytes, with the products of translation having the same distinct characteristics as conventional Le and F interferons. We concluded that two separate structural genes are likely to exist for these two antigenic species of human interferon (Cavalieri et al. 1977). These earlier results and the present data suggest that both interferon genes are expressed during the induction of Namalva cells.

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