Characterization of the Heterogeneous Molecules of Human Interferons: Differences in the Cross-Species Antiviral Activities of Various Molecular Populations in Human Leukocyte Interferons

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SUMMARY

Human leukocyte interferon (HuLeIF) preparations were separated into populations of molecules with different sizes, by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), and with different charges, by isoelectric focusing. These populations with different sizes and charges were analysed for their antiviral activity on homologous cells and on heterologous (bovine) cells.

The distribution of interferon activity into two broad peaks by SDS-PAGE was similar whether assayed on human or bovine cells. However, within these peaks, the relative ratio of the activity in human cells and bovine cells varied significantly: while most of the size components had similar human/bovine cell activities, the fastest migrating component (apparent mol. wt. ~ 13500) was more than 100 times more active on bovine cells than on human cells.

The peaks of activity in isoelectric focusing were distributed from pH 5.5 to 7.0. There was generally correspondence between human and bovine cell activities, but while the more neutral pH range peaks were consistently slightly more active on human cells than on bovine cells, the more acid range peaks were always slightly more active on bovine cells than on human cells. However, with the most acidic peak, there was more than 100 times greater activity on bovine cells than on human cells.

These data show that the heterogeneity of HuLeIFs is greater than merely two size populations, and data confirm that different forms of human leukocyte interferon can vary markedly in biological activity.

INTRODUCTION

Human leukocyte interferon (HuLeIF) preparations have been shown to contain distinct molecular populations. Dissociating conditions in SDS-polyacrylamide gels allow resolution of two broad bands of activity with peaks of apparent mol. wt. of approx. 21000 and 15000 (Stewart II & Desmyter, 1975; Desmyter & Stewart II, 1976; Stewart II et al. 1976; Torma & Paucker, 1976; Havell et al. 1977; Lin et al. 1977; Paucker et al. 1977; Vilcek et al. 1977). It has also been demonstrated that the distinct molecular populations in HuLeIFs differ in their antiviral activities in rabbit cells (Stewart II & Desmyter, 1975) and cat cells (Desmyter & Stewart II, 1976). However, Paucker et al. (1977) have recently reported
that the two peaks of human leukocyte interferon activity separated by SDS-hydroxylapatite chromatography and corresponding to the 21,000 and 15,000 mol. wt. peaks isolated by SDS-PAGE were equally active in human cells and in heterologous (bovine) cell cultures; they concluded that the different molecular species of HuLeIFs are biologically essentially similar.

In view of this apparent discrepancy concerning the relative activities of the various HuLeIF forms in the cells of other animals, we have undertaken to determine whether rabbit and cat cells were peculiar in distinguishing between the human leukocyte interferon forms. Our findings with bovine cells are presented here and support our contention that HuLeIF preparations are composed of molecular populations with drastically different cross-species antiviral activities.

METHODS

Cells and virus. Human fibroblast cells trisomic for chromosome-21, designated GM258, were obtained from the Mammalian Genetic Mutant Cell Repository (Camden, New Jersey). A stable line of bovine kidney cells, designated MDBK, was provided by Dr P. Sehgal (Rockefeller University). All cells were grown in Eagle's minimal essential medium (MEM) supplemented with 10% foetal calf serum. Vesicular stomatitis virus (VSV) stocks were prepared as previously described (Stewart II et al. 1977a).

Interferons and interferon assays. A HuLeIF preparation, designated P-IF, was obtained from Dr K. Cantell (Helsinki, Finland) and had a titre of approx. 6 × 10⁶ units/ml and a sp. act. of about 10⁶ units/mg of protein. Interferon samples were assayed on GM-258 cells and on MDBK cells in a microtitration assay involving protection against the cytopathogenic effect of VSV. In each assay system, the human leukocyte reference reagent G023-901-527 (National Institutes of Health, Bethesda) was used for calibration and all results are expressed in units based on this reagent.

Isoelectric focusing. A modification of the isoelectric focusing procedure described by O'Farrell (1975) was used (Stewart II et al. 1977b). Gels consisted of a mixture of 4% acrylamide, 9 M-urea, 2% Nonidet P40, and 2% Ampholine. Cylindrical gels (2.5 × 240 mm) were used, and 0.02 ml of an interferon sample in focusing buffer was applied to each gel. Isoelectric focusing was performed at room temperature with a potential of 400 V, for approx. 16 h, until the current reached a minimum of 0.3 mA. The potential was then raised to 800 V for 1 h. Gels were sliced into 2-2 mm segments and each segment was eluted into 1 ml MEM containing 10% foetal calf serum. Eluates were stored at 4 °C for interferon assays. The pH gradient of focusing gels was determined by eluting 10 mm segments of a control gel in 1 ml 0.0375 M-NaCl and measuring the pH at room temperature.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Interferon samples in 0.01 M-sodium phosphate buffer, pH 7.1, were constituted to contain 5 M-urea and 1% SDS. Bromophenol blue in sucrose was added to make samples 6% with respect to sucrose, and samples were heated to 100 °C for 1 min. A 0.1 ml sample was electrophoresed in a 200 mm cylindrical SDS-polyacrylamide gel, as previous described (Stewart II, 1974). When the dye front had migrated about 180 mm into the gel, the gel was sliced into 2-2 mm segments. Two adjacent slices were pooled to form each fraction and eluted overnight at 4 °C into 1 ml of MEM containing 10% foetal calf serum. Eluates were stored at 4 °C for interferon assays.
**RESULTS**

*Comparison of size heterogeneities of human leukocyte interferons in homologous and heterologous cells*

HuLeIF preparations were subjected to SDS-PAGE and each gel fraction was assayed for activity on human cells and on bovine cells. Activity on cells of both species was distributed within a size range from about 23000 to 13000 in two broad, overlapping bands with peaks at 21000 and 15000 (Fig. 1). Interestingly, the interferons in all the more slowly migrating fractions (apparent mol. wt. \( \geq 18000 \)) were slightly, but consistently, more active on human cells than on bovine cells and we have invariably observed this difference with several other preparations of human leukocyte interferons obtained from Dr Cantell’s laboratories, having various specific activities.

In contrast, the interferons in the more rapidly migrating fractions had similar activity on both human and bovine cells, while the most rapidly migrating interferon (apparent mol. wt. 13500) was at least 100 times more active on bovine cells than on human cells. We have also invariably observed this disparity in activities in the material isolated at this apparent size in SDS-PAGE with several other human leukocyte interferon preparations with different degrees of purification, and with a human lymphoblastoid (‘Namalva’) interferon preparation (unpublished data).

*Comparison of charge heterogeneities of human leukocyte interferons in homologous and heterologous cells*

HuLeIF preparations were subjected to isoelectric focusing and each gel fraction was assayed for activity on human fibroblast cells and on bovine cells. Activity on cells of
both species was distributed in several peaks with a pH range from about 7.0 to 5.5 (Fig. 2).

The interferons focusing toward the more nearly neutral pH range in the gels were slightly but consistently more active on human cells than on bovine cells. However, toward the more acidic pH range of the gel the interferons had similar activity levels on both human and bovine cells, while the interferon isolated at the lowest pH value, 5.5, was at least 100 times more active on bovine cells than on human cells.

Stability of the homologous and heterologous cell activities of the isolated molecular populations of human leukocyte interferons

It seemed possible that the disparate ratios of activity of the interferons might reflect different stabilities of some structures of the molecules which are required for recognition of the interferons by human cells, but not by bovine cells, or vice versa. We therefore determined the activity ratios of various freshly isolated fractions from focusing gels and SDS gels by simultaneous assays on human and bovine cells and repeated these assays on isolates at monthly intervals. As shown in Table 1, the original activity levels of each isolate were maintained on both cell types for several months; the larger interferons always exerted slightly higher activities on human cells than on bovine cells, the smaller forms gave similar homologous and heterologous titres, and the smallest interferons were always much more active on bovine than on human cells. Similarly, the interferons focusing at the different pH values maintained their original human/bovine ratios, ranging from about 3/1 (near the neutral pH range) to about 1/100 (at pH 5.5).

It is noteworthy that the interferons maintained all their activities on both human and bovine cells over several months of storage at 4 °C, for, while it would be impossible to detect minor losses of activity in samples titrating several hundreds of units/ml, even those samples titrating only about 10 to 30 units/ml retained their original titres.
Specificity of human leukocyte interferons

Table 1. Stabilities of the antiviral activities of human leukocyte interferon populations isolated by SDS–PAGE or isoelectric focusing in homologous and heterologous cells

<table>
<thead>
<tr>
<th>Interferon population by SDS-PAGE:</th>
<th>Interferon titre (log₁₀ units/ml)</th>
<th>Human</th>
<th>Bovine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1*</td>
<td>2</td>
</tr>
<tr>
<td>21000 mol. wt.</td>
<td></td>
<td>3.5</td>
<td>3.3</td>
</tr>
<tr>
<td>15000 mol. wt.</td>
<td></td>
<td>3.5</td>
<td>3.3</td>
</tr>
<tr>
<td>13500 mol. wt.</td>
<td></td>
<td>1.5</td>
<td>1.0</td>
</tr>
<tr>
<td>by Focusing:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pl 6.8</td>
<td></td>
<td>1.8</td>
<td>1.5</td>
</tr>
<tr>
<td>pl 6.5</td>
<td></td>
<td>3.3</td>
<td>3.3</td>
</tr>
<tr>
<td>pl 6.2</td>
<td></td>
<td>2.0</td>
<td>2.0</td>
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<tr>
<td>pl 6.0</td>
<td></td>
<td>1.5</td>
<td>1.0</td>
</tr>
<tr>
<td>pl 5.7</td>
<td></td>
<td>3.3</td>
<td>3.0</td>
</tr>
<tr>
<td>pl 5.5</td>
<td></td>
<td>1.0</td>
<td>1.0</td>
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</tbody>
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* Storage times in months of samples at 4 °C.

DISCUSSION

These data clearly demonstrate that human leukocyte interferon preparations can be separated into a number of distinguishable populations in SDS–polyacrylamide gels and in isoelectric focusing gels; thus the extent of the heterogeneity of human leukocyte interferons is more than simply the two peaks originally interpreted from SDS–PAGE profiles by Stewart II & Desmyter (1975).

The present studies support the original interpretation of Desmyter et al. (1970) that HuLeIF preparations are composed of interferons with different homologous and heterologous activities. It was previously shown (Desmyter & Stewart II, 1976) that SDS–PAGE of HuLeIF preparations isolated an interferon at 13500 daltons which was about 100 times more active on cat cells than on human cells. However, other authors have recently observed that two components of HuLeIFs resolved by SDS–hydroxylapatite chromatography were equally active in homologous and heterologous (bovine) cells (Paucker et al. 1977), and concluded that the different molecular species of HuLeIFs are biologically similar. Their inability to discern the differences in biological activity which are reported here probably results from the fact that in their chromatography technique all the components were put in two pools.

The disparate cross-species activities of the HuLeIFs raises the question of whether the 13500 mol. wt. component, with a significantly higher activity on bovine cells than on human cells, represents a homogeneous interferon population with relatively lower affinity for human cells than for bovine cells, or represents a mixture of a minor component with normal human cell affinity and a majority of separate interferon molecules active exclusively on bovine cells. Studies are under way with neutralizing anti-interferon antisera which may reveal which of these possibilities applies.

A number of laboratories are presently involved in large-scale production and purification of human interferons destined for clinical evaluations; because of the relative ease of growing and maintaining such cells as MDBK (bovine) or Vero (monkey) cells as compared to human fibroblasts, several of these laboratories are presently using non-human cells for routine assays of human leukocyte interferons. If it were presumed that all HuLeIF
forms attainable were essentially biologically similar in terms of cross-species antiviral activity (as has been advocated by Paucker and his associates, 1977), and if non-human (bovine) cells were used to monitor purification procedures, one could conceivably end up with a HuLeIF preparation with the character of the 13500 mol. wt. form or the pI 5.5 population; such preparations though having titres in bovine cells similar to interferons that had been proved to be clinically efficacious, could yield surprisingly disappointing clinical results.

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