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Spontaneous production of human interferon

(interferon heterogeneity/cross-species antiviral activity/sodium dodecyl sulfate/polyacrylamide gel electrophoresis/antigenic heterogeneities/lymphoblastoid cells)

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ABSTRACT **Several established lines of human lymphoblastoid cells were evaluated for abilities to produce interferons. Some cell lines were able to produce interferon when induced with either Newcastle disease virus or Sendai virus, whereas others failed to produce detectable interferon when so induced. However, several cell lines were able to spontaneously produce interferon without induction. Spontaneously produced interferon was liberated by cells only during logarithmic growth phase, reaching levels ranging from about 10 reference units/ml of growth medium for some cell lines to 1000 reference units/ml for others. The interferons produced by induced lymphoblastoid cells and the spontaneously produced interferons were all characterized as type I human leukocyte interferon by high levels of cross-species antiviral activities on bovine cells and by neutralizations by antiserum to human leukocyte interferon but not by antiserum to human fibroblast interferon. However, analysis by electrophoresis in sodium dodecyl sulfate/polyacrylamide gels revealed that spontaneously produced interferon was less size heterogeneous than human leukocyte interferon, migrating as a single band of activity with a peak at 20,000 daltons, whereas human leukocyte interferon contained peaks of major activity at 23,000 and 18,000 daltons and virus-induced Namalva lymphoblastoid cell interferon migrated predominantly as the 18,000-dalton form. Also, although neither virus-induced primary leukocyte interferon nor any of the virus-induced lymphoblastoid cell interferons were neutralized by antiserum to mouse interferon, all of the spontaneously produced interferons were neutralized by antiserum to mouse interferon. These data suggest significant structural similarities between the active cores of certain interferons from phylogenetically diverse animal species.**

Human interferons are presently being clinically evaluated for both antiviral and antitumor efficacies; such trials have provided preliminary evidence of benefit to patients (1). Consequently, large amounts of human interferon are in demand both for expanded clinical use and for purification and characterization studies, including amino acid sequence determinations. Present sources of human interferons are suspensions of virus-induced human peripheral blood leukocytes (2), monolayer cultures of human diploid fibroblasts induced with double-stranded polyribonucleotides (3), and virus-induced human lymphoblastoid cells (4–6). The interferon produced by human leukocyte suspensions appears to have pharmacologically advantageous properties in comparison with that produced by human fibroblast cultures (7–9), but its supply is predictably restricted by the availability of fresh human blood. Virus-induced human lymphoblastoid (Namalva) cell interferon is predominantly leukocyte type in terms of its antigenicity (10, 11), cross-species activity (12), and pharmacologic properties (unpublished data). Therefore, interferons with pharmacologically desirable properties could be produced in large quantities from unrestricted supplies of established human lymphoblastoid cell lines.

A number of reports have demonstrated that certain cells can spontaneously produce interferons, though the levels of interferons produced were only a small percentage of the interferon yields obtained from induced cells, often even requiring concentration of the culture medium in order to be detectable (13–33). However, apparently no systematic approach has been undertaken to look for better spontaneous interferon producers or to characterize the interferons they produce. Therefore, as part of our program to identify alternative sources for human interferons, we have developed methods to systematically evaluate the abilities of various established human lymphoblastoid cell lines to produce interferons, either spontaneously or when induced with virus, and have characterized the interferons so produced. These studies have revealed sources of human interferons with properties distinct from other known human interferons.

MATERIALS AND METHODS

Cells and Viruses. The human lymphoblastoid cell lines evaluated for ability to produce interferons were designated as received from the donors, with the one exception indicated below. Namalva cells, derived from a Burkitt lymphoma patient (34), were kindly provided by K. Zoon (National Institutes of Health). Raji cells (35) and Daudi cells (36), also originating from Burkitt tumors, were gifts, respectively, from M. deSousa and J. Fogh (Sloan-Kettering Institute, New York). Cell lines derived from an IgG-type myeloma patient, designated ARH-77, and from an IgM myeloma patient, designated BM, were provided by P. Ralph (Sloan-Kettering Institute), who also generously supplied an IgG plasmacytoma cell line (SULTAN), two lines of normal adult peripheral leukocytes transformed *in vitro* by Epstein–Barr virus (RPMI-1788 and EP-2; ref. 37), a line of cells from an acute myelogenous leukemia patient (CESS), one line of human lymphoblastoid cells of undetermined origin (NBRL AG57), and two lines of cells from patients with diffuse histiocytic lymphoma (MANCA and SKW-4). Five cell lines of normal adult peripheral blood leukocytes transformed *in vitro* by Epstein–Barr virus and designated ER-1, CAH, RMcG, FS-2, and SP0-3 (38) were provided by Nancy Collins (Sloan-Kettering Institute). P. Ralph also provided a line of cells originally derived from a patient with acute myelogenous leukemia and now designated clone 8866.7. Another human lymphoblastoid cell line, thought to have been derived initially from the RPMI-8866 strain, was obtained in 1972 from M. Goulian (University of California, San Diego). However, the line was recently characterized by G. Moore (University of

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Abbreviation: SpIF, spontaneously produced interferon.

Colorado Medical School, Denver), who, based on differences in phosphoglucomutase-1 isozyme fingerprints, determined that these cells are not valid RPMI-8866 cells (G. Moore, personal communication). Therefore, we have redesignated these cells LuKII-0. Clones subsequently derived in our laboratories from this culture have been designated LuKII-1, -2, etc. All lymphoblastoid cells were grown in RPMI-1640 medium supplemented with 10% (vol/vol) fetal calf serum plus 40 μg of gentamycin sulfate per ml.

Human fibroblasts trisomic for chromosome 21 (GM258) were obtained from the Mammalian Genetic Mutant Cell Repository (Camden, NJ), and a stable bovine kidney cell line, MDBK, was obtained through the generosity of P. Sehgal (Rockefeller University, New York). Mouse L cells were obtained from G. Bekesi (Mount Sinai Hospital, New York).

Vesicular stomatitis and Newcastle disease viruses were prepared as described (39, 40). Sendai virus from a stock originally provided by K. Cantell (Helsinki, Finland) was prepared in 11-day-old embryonated chicken eggs and titrated approximately 2500 hemagglutinating units/ml.

Interferon Assays and Neutralizations by Antisera. Interferon assays were performed by microtitration assays of cytopathology of vesicular stomatitis virus as described (39). Results are expressed in units/ml adjusted to human leukocyte interferon reference reagent G023-901-527 obtained from the National Institutes of Health, which gave values approximately 6 times higher than the assigned reference titer on both GM258 and MDBK cells. Interferons were neutralized by the method described by Havell *et al.* (10). Antiserum to human leukocyte interferon was a gift from K. Cantell (Helsinki, Finland); antiserum to human fibroblast interferon and antiserum to mouse L-cell interferon were provided by the National Institute of Allergy and Infectious Diseases (Bethesda, MD). Namalva cell interferon and human fibroblast interferon were also provided by the National Institute of Allergy and Infectious Diseases.

Electrophoresis. Samples of interferon preparations in 10 mM sodium phosphate (pH 7.1) were made 1% in $NaDodSO_4$ and 5 M in urea and were boiled for 1 min before application to cylindrical polyacrylamide gels. Electrophoresis conditions were identical to those previously described (41). After electrophoresis, gels were sliced into 2.1-mm segments, which were eluted into 1 ml of growth medium overnight at 4°C and assayed for interferon activity.

RESULTS

Development of Screening Procedures for Spontaneous and Induced Interferon Production. Several studies have demonstrated that interferon is produced by virus-induced primary leukocytes or lymphoblastoid cells within a few hours after induction and is completed within only a few hours. However, the kinetics of spontaneous interferon production by cell cultures has not been elucidated. Some investigators have reported interferon to be present in media from lymphoblastoid cultures incubated for various intervals; they usually harvested samples of "spent" media after several days. Others have assumed that spontaneously produced interferon (SpIF) was elaborated continuously and have seeded cells into fresh medium at nearly saturation density and harvested samples of medium after incubations of a day or two.

It was not known, however, whether SpIF was elaborated continuously from lymphoblastoid cells in a resting state either before they entered logarithmic growth or after they reached saturation density. Therefore, we devised a screening method to determine the abilities of lymphoblastoid cells to spontaneously produce interferon at each stage of culture growth.

As each cell line was received, the cells were resuspended in fresh medium and the cell density was adjusted to 0.5–3 × 10^5 cells per ml. At daily intervals aliquots were removed for interferon assays and cell counts. Depending upon the cell line, saturation density occurred anywhere from 5 × 10^5 cells per ml to >2 × 10^6 cells per ml. Cells that had previously reached saturation density before resuspension typically had a lag phase lasting 1–2 days (Fig. 1*A*) before beginning to grow. Cells that had been growing exponentially before resuspension had a very short lag (<24 hr). During this lag phase, little if any interferon was produced (Fig. 1*B*). During the next several days, cell numbers and interferon titers both increased logarithmically and interferon production reached plateau level in each culture at approximately the same time cells reached saturation densities.

These data suggested that interferon was produced only during cell growth. To test whether the lag before interferon production was due to subdetectable production of interferon per cell at the low cell densities, we seeded cultures at nearly saturation density with freshly washed cells. Under these conditions, there was again a slight lag in cell growth and in interferon production. Also, once in the logarithmic phase, the cells had a lower growth rate and the interferon levels obtained were generally much lower than those achieved in rapidly growing cultures. Presumably the large number of cells per ml quickly depleted the medium of nutrients required to maintain rapid cell growth and interferon production.

Each cell line was also screened for the ability to produce interferon when induced with virus. For this screening procedure, cells were grown to subsaturation densities. These exponentially growing cells were then induced with 100 hemagglutinating units of Sendai virus per ml or 5 plaque-forming units of Newcastle disease virus per cell. In all cases, Sendai virus was a much better inducer than Newcastle disease virus. After 1 hr at 37°C, cultures in duplicate were left undiluted or were diluted 1:2, 1:3, and 1:4 with fresh medium containing 2% fetal calf serum. Uninduced controls were treated similarly to determine the amount of interferon due to spontaneous rather

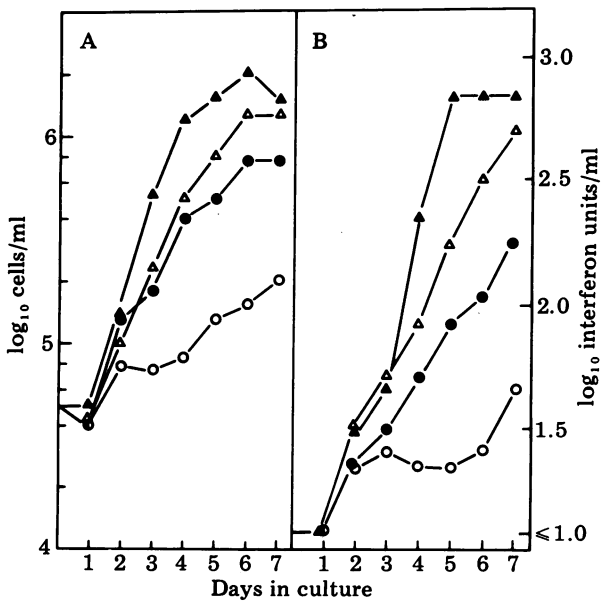

FIG. 1. Kinetics of cell growth and spontaneous production of interferon by human LuKII lymphoblastoid cells. Cells were suspended at 5 × 10^4 cells per ml in RPMI-1640 medium supplemented with: ○, 0.5% fetal calf serum; ●, 1% fetal calf serum; △, 2% fetal calf serum; or ▲, 10% fetal calf serum. At daily intervals after incubation at 37°C in 5% CO_2, aliquots were removed for cell counts (*A*) and interferon assays (*B*).

than induced production. Cells were harvested after 24–48 hr and supernatants were adjusted to pH 2.0 to inactivate any residual virus. Prior to being assayed, the supernatants were readjusted to pH 7.0. By using this procedure, exponentially growing cells produced maximal interferon titers in the undiluted cultures. Saturation-density cells, however, produced lower overall levels of interferon and had maximal interferon titers in the 1:3 or 1:4 dilutions. These dilutions resulted in lower cell densities and also supplied nutrients needed for cell growth and interferon production. The above data indicate that cell growth is essential for the lymphoblastoid cells to spontaneously produce interferon as well as to produce interferon when induced by virus.

Each of the cell lines listed in Table 1 was tested for ability to produce interferon either when induced with Sendai virus or spontaneously when grown in this screening procedure from low density to saturation density. In our hands the Namalva cell line was a poorer producer when induced than were some of the uninduced cell lines. However, workers in other laboratories have been able to induce significantly higher levels of interferon in the Namalva cells (5, 6), possibly owing to technical subtleties. Of considerable interest is the finding that, of 19 randomly obtained lymphoblastoid cell lines tested by this procedure, 10 were found to spontaneously produce interferon. The final levels of SpIF obtained in these cultures varied from about 10 units/ml up to 1000 interferon reference units/ml. Of the nine cell lines that did not produce detectable levels of interferon spontaneously, five lines were able to produce interferon when induced with Sendai virus but four cell lines failed to produce interferon either spontaneously or when induced with viruses.

Characterization of Induced and Spontaneously Produced Lymphoblastoid Interferons. The virus inhibitors produced by the lymphoblastoid cells, either spontaneously or when induced by viruses, were characterized as interferons as follows: the ability to induce virus-nonspecific resistance in human fibroblast cultures but not in actinomycin D-treated cultures; the inhibitors were destroyed by trypsin but not by DNase or RNase and were not sedimented by ultracertrifugation; and all SpIFs and virus-induced leukocyte and lymphoblastoid interferons were stable at pH 2.

Several different human interferons were assayed for degrees of cross-species antiviral activity or bovine cells (Table 2): human leukocyte interferon is known to be highly active on bovine cells whereas human fibroblast interferon is not (1, 42). The lymphoblastoid cell interferons, whether virus-induced or spontaneously produced, were all highly active on bovine cells.

All the lymphoid cell interferons were neutralized by antiserum prepared against human leukocyte interferon, and none of those tested was neutralized by antiserum against human fibroblast interferon (Table 3).

Thus, all these lymphoblastoid interferons, either spontaneously produced or virus-induced, can be classified as type I human leukocyte interferons on the bases of acid stability, antigenic properties, and cross-species antiviral activity.

Of particular interest was the observation that, although neither the virus-induced human leukocyte interferon nor any of the virus-induced lymphoblastoid interferon preparations were neutralized by antiserum to mouse interferon, each of the 10 different SpIFs was neutralized by antiserum to mouse interferon (Table 3). Although the SpIFs were neutralized by antisera to either mouse or human leukocyte interferon, mouse interferon was not neutralized by the latter antiserum.

Size Heterogeneities of Virus-Induced Leukocyte and Lymphoblastoid Interferons and Spontaneously Produced Lymphoblastoid Interferons. Workers in several laboratories have established that human leukocyte interferon and Namalva lymphoblastoid interferon migrate as size-heterogeneous molecular forms when electrophoresed in $NaDodSO_4$/polyacrylamide gels with major peaks at 23,000–21,000 daltons and 18,000–15,000 daltons (1). A batch of SpIF from cultures of LuKII cells was partially purified by ammonium sulfate precipitation at pH 2.0, and the interferon precipitated between 20% and 40% saturation was dialyzed against 30 mM NH_4HCO_3 (pH 7.6), lyophilized, resuspended in 10 mM sodium phosphate (pH 7.1), and electrophoresed in $NaDodSO_4$/polyacrylamide gels. Parallel gels were electrophoresed with human leukocyte interferon or virus-induced Namalva lymphoblastoid interferon. Interestingly, whereas the human leukocyte interferon distributed into two bands of activity with

Table 1. Production of interferon by human lymphoblastoid cell lines

Cell line	Interferon production* Spontaneous†	Virus-induced‡
Raji	<10	30–100
CAH	<10	60–200
ER-1	<10	60–200
Namalva	<10	100–300
SPO-3	10	100–600
RPMI-1788	10	10–30
BM	10	30–100
ARH-77	10–30	—§
CESS	30–100	—
EP-2	30–100	—
FS-2	30–100	—
RMcG	30–100	—
NBRL-AG57	60–200	—
8866.7	300–1000	—
LuKII	300–1000	—
Daudi	<10	<10
MANCA	<10	<10
SKW-4	<10	<10
SULTAN	<10	<10

* Interferon titers are expressed as reference units/ml of culture medium. Ranges indicate low-to-high determinations from repeated experiments.

† Spontaneous interferon production was determined as the maximal level produced by cultures grown in 10% fetal calf serum, as described for the screening procedure.

‡ Virus-induced interferon production was determined as 24-hr production from cultures induced with Sendai virus.

§ Interferon titers were not significantly increased above spontaneously produced levels when cultures were induced with virus.

Table 2. Cross-species antiviral activities of human interferon on bovine cells

Interferon source	Induced	Crossactivity,* % homologous titer
Primary human leukocytes	+	≈100
Lymphoblastoid (Raji, Namalva, CAH, SPO-3, ER-1)	+	≈100
Lymphoblastoid (RPMI-1788, BM, ARH-77, CESS, EP-2, FS-2, RMcG, NBRL-AG57, 8866.7, LuKII)	−	≈30–100
Diploid human fibroblasts	+	<1

* Interferon preparations from indicated cells were simultaneously assayed for antiviral activity on human and bovine cells.

Table 3. Antigenic dissimilarities among human leukocyte, fibroblast, and lymphoblastoid interferons

Interferon	Induced	Neutralization titer of indicated antiserum*		
		Anti-leukocyte interferon	Anti-fibroblast interferon	Anti-mouse interferon
Human leukocyte	+	53,000	–	–
Human fibroblast	+	–	1000	–
Namalva	+	80,000	–	–
Raji	+	64,000	ND	–
SPO-3	+	133,000	ND	–
ER-1	+	80,000	ND	–
CAH	+	80,000	ND	–
LuKII	–	107,000	–	2670
8866.7	–	160,000	–	4000
NRBL-AG57	–	16,000	ND	2670
CESS	–	3,000	ND	530
ARH-77	–	11,000	ND	270
RMcG	–	5,000	ND	270
EP-2	–	21,000	–	+†
FS-2	–	ND	ND	270
BM	–	3,000	ND	80
RPMI-1788	–	6,000	ND	640

* Interferon preparations were tested for neutralization against indicated antiserum. –, No neutralizing activity detectable; ND, not determined.

† Positive neutralization, but endpoint value not obtained.

peaks at 23,000 and 18,000 daltons (Fig. 2*A*) and the Namalva lymphoblastoid interferon migrated predominantly as the smaller (18,000-dalton) species (Fig. 2*B*), the SpIF migrated in a single peak at 20,000 daltons (Fig. 2*C*).

DISCUSSION

Several isolated reports over the last 15 years have indicated that an occasional human lymphoblastoid cell line can spontaneously produce interferon, albeit at very low levels (13–33). The present studies demonstrate that spontaneous production of interferon may be a common occurrence in human lymphoblastoid cell lines, being detectable in more than 50% of the randomly procured lines examined in this study; in many of these cultures, SpIF titers were higher than those obtained from virus-induced lymphoblastoid cells. The key to demonstration of SpIF at significant levels seems to be the screening method developed for these studies: lymphoblastoid cells produce interferon spontaneously only when in logarithmic growth phase.

All the interferons from lymphoblastoid cells were characterized as type I (acid-stable) interferons and were antigenically identified as the leukocyte subtype. It is interesting that each of the SpIFs in the present studies was also neutralized by antiserum to mouse interferon. Mouse interferon was not detectably neutralized by antiserum to human leukocyte interferon, and neither virus-induced human leukocyte nor any of the virus-induced lymphoblastoid interferons were neutralized by antiserum to mouse interferon. In this regard, a minor low molecular weight component of mouse interferon has been identified that is antigenically recognized by human leukocyte interferon antiserum and significantly crossreactive on human cells (43, 44). The lower molecular weight form of mouse interferon has significant amino acid sequence homologies with one of the molecular species of human lymphoblastoid interferon (45). These data clearly indicate that much of the interferon molecule has been preserved throughout evolution.

The molecular heterogeneity observed in preparations of

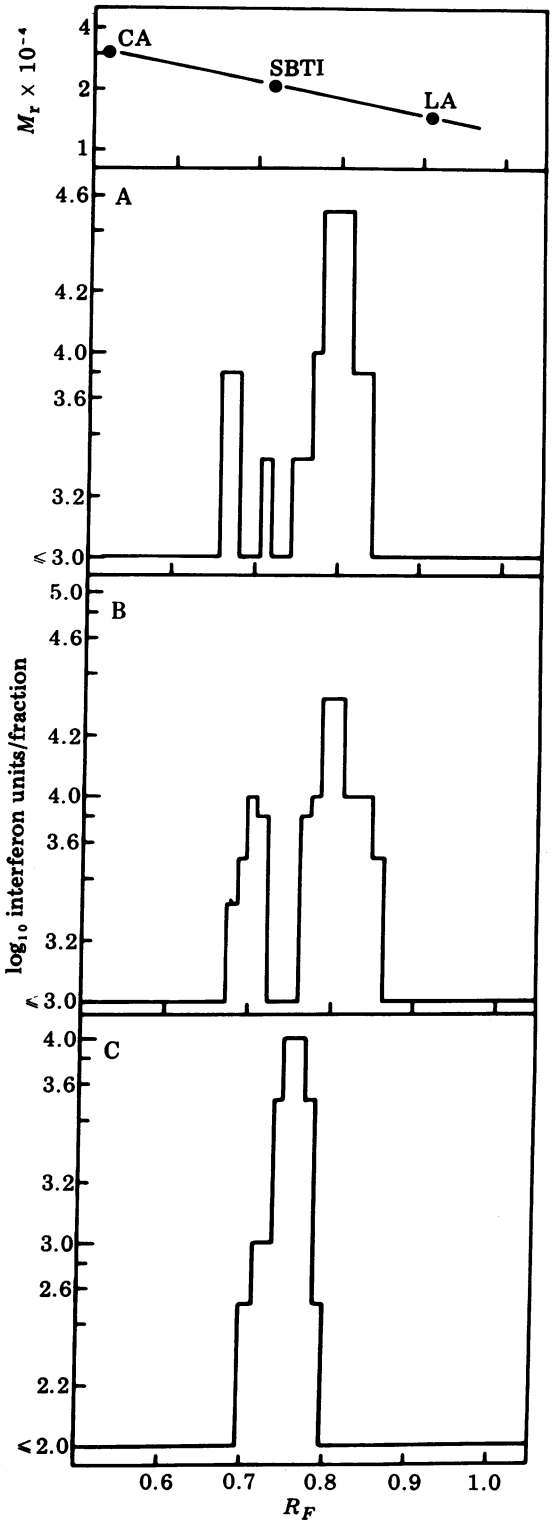

FIG. 2. Comparisons of the molecular weight heterogeneities of human lymphoid interferons. Interferon preparations were dialyzed against 10 mM phosphate buffer (pH 7.1) and an aliquot of 0.1 ml was applied to each gel. (*A*) Human leukocyte interferon derived from Sendai virus-induced peripheral blood leukocytes; (*B*) human lymphoblastoid cell interferon derived from Sendai virus-induced Namalva cells; (*C*) human lymphoblastoid cell interferon spontaneously produced by LuKII cells. Approximately 100% of the applied activity of each sample was recovered from the gels. Molecular weight standards were carbonic anhydride (CA), soybean trypsin inhibitor (SBTI), and lactalbumin (LA).

human leukocyte interferon seems to be contributed largely by carbohydrate moieties (39, 46). The degree of glycosylation could be distinct for different cell types in the leukocyte "buffy coat" suspensions; thus, the Namalva lymphoblastoid cells produce predominantly the smaller molecular weight form, whereas the buffy-coat interferon contains a significant amount of the larger form as well as the smaller species. The finding that SpIF from LuKII cells contained only the larger interferon form suggests that either these cells can glycosylate the interferon more extensively than can Namalva cells or that the virus-induced interferons from lymphoblastoid cells are processed differently from SpIFs. Because each of the SpIFs was recognized by antiserum to mouse interferon and none of the virus-induced human lymphoid interferons was neutralized by the heterologous antiserum, it is tempting to speculate that the virus-induction process initiates a distinct pathway for lymphoid interferon production. Further characterizations of each of the virus-induced and SpIFs should resolve these alternatives.

These studies have identified sources of human lymphoid interferons with properties distinct from those of virus-induced lymphoid interferons. In view of the marked pharmacokinetic distinctions observed between human leukocyte and fibroblast interferons (7–9), it is tempting to speculate that such distinct forms of lymphoid interferons could have importantly different pharmacologic properties. Therefore, it seems worthwhile to produce and evaluate each of the distinct molecular forms of human interferons.

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1. Stewart, W. E., II (1979) *The Interferon System* (Springer, Vienna).
2. Cantell, K. & Hirvonen, S. (1978) *J. Gen. Virol.* **39,** 541–542.
3. Havell, E. A. & Vilcek, J. (1972) *Antimicrob. Agents Chemother.* **2,** 476–484.
4. Strander, H., Mogensen, K. E. & Cantell, K. (1975) *J. Clin. Microbiol.* **1,** 116–124.
5. Zoon, K. C., Buckler, C. E., Bridgen, P. J. & Gurari-Rotman, D. (1978) *J. Clin. Microbiol.* **7,** 44–51.
6. Johnson, M. D., Christofinis, G., Ball, G. D., Fantes, K. & Finter, N. B. (1979) *Dev. Biol. Stand.* **42,** 189–192.
7. Hanley, D. F., Wiranowska-Stewart, M. & Stewart, W. E., II (1979) *Int. J. Immunopharmacol.* **1,** 219–226.
8. Edy, V. G., Billiau, A. & Desomer, P. (1978) *Lancet* **i,** 451–452.
9. Billiau, A., Desomer, P., Edy, V. G., Decpercquie, E. & Heremans, H. (1979) *Antimicrob. Agents Chemother.* **16,** 56–63.
10. Havell, E. A., Yip, Y. K. & Vilcek, J. (1978) *J. Gen. Virol.* **38,** 51–60.
11. Zoon, K. C., Smith, M. E., Bridgen, P. J., ZurNedden, D. & Anfinsen, C. B. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 5601–5605.
12. Cavalieri, R. L., Havell, E. A., Vilcek, J. & Pestka, S. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 3287–3291.
13. Henle, G. & Henle, W. (1965) *J. Bacteriol.* **89,** 252–260.
14. Smith, T. J. & Wagner, R. R. (1967) *J. Exp. Med.* **125,** 559–578.
15. Smith, T. J. & Wagner, R. R. (1967) *J. Exp. Med.* **125,** 579–593.
16. Talas, M., Weiszfeiler, G. & Batkai, L. (1968) *Acta Virol.* **12,** 378–380.
17. Talas, M., Szolgay, E. & Rozsa, K. (1972) *Arch. Gesamte Virusforsch.* **38,** 149–158.
18. Northrop, R. L. & Deinhardt, F. (1967) *J. Natl. Cancer Inst.* **39,** 685–689.
19. Deinhardt, F. & Burnside, J. (1967) *J. Natl. Cancer Inst.* **39,** 681–684.
20. Haase, A. T., Johnson, J. S., Kasel, J. A., Margolis, S. & Levy, H. B. (1970) *Proc. Soc. Exp. Biol. Med.* **133,** 1076–1083.
21. Minnefor, A. B., Halsted, C. C., Seto, D. S., Glade, P. R., Moore, G. E. & Carver, D. H. (1970) *J. Infect. Dis.* **121,** 442–451.
22. Morgan, M. J., Colby, C. & Hulse, J. L. N. (1973) *J. Gen. Virol.* **20,** 377–388.
23. Archer, D. L. & Young, B. G. (1974) *Infect. Immun.* **9,** 684–689.
24. Epstein, L. B. & Salmon, S. E. (1974) *J. Immunol.* **112,** 1131–1138.
25. Zajac, B. A., Henle, W. & Henle, T. (1969) *Cancer Res.* **29,** 1467–1475.
26. Swart, B. E. & Young, B. G. (1969) *J. Natl. Cancer Inst.* **42,** 941–944.
27. Adams, A., Lidin, B., Strander, H. & Cantell, K. (1975) *J. Gen. Virol.* **28,** 219–225.
28. Adams, A., Strander, H. & Cantell, K. (1975) *J. Gen. Virol.* **28,** 207–214.
29. Lidin, B. & Adams, S. (1975) *Intervirology* **5,** 205–218.
30. Tovey, M. G., Begon-Lours, J., Gresser, I. & Morris, A. G. (1977) *Nature (London)* **267,** 455–456.
31. Jarvis, A. & Colby, C. (1978) *Cell* **14,** 355–364.
32. Dalton, B. & Paucker, K. (1979) *Infect. Immun.* **23,** 244–248.
33. Klein, G. & Vilcek, J. (1980) *J. Gen. Virol.* **46,** 111–117.
34. Klein, G., Dombros, L. & Gothoskar, B. (1972) *Int. J. Cancer* **10,** 44–57.
35. Pulvertaft, R. J. V. (1975) *J. Clin. Pathol.* **18,** 261–273.
36. Klein, E., Klein, G., Nadkarni, J. S., Nadkarni, J. J., Wigzell, H. & Clifford, P. (1968) *Cancer Res.* **28,** 1300–1310.
37. Steinitz, M., Klein, G., Koskimies, S. & Makel, O. (1977) *Nature (London)* **269,** 420–422.
38. Hansen, J. A., Fu, S. M., Antonelli, P., Kamoun, M., Hurley, J. N., Winchester, R. J., Dupont, B. & Kunkel, H. G. (1979) *Immunogenetics* **8,** 51–64.
39. Stewart, W. E., II, Lin, L. S., Wiranowska-Stewart, M. & Cantell, K. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 4200–4204.
40. Stewart, W. E., II, Chudzio, T., Lin, L. S. & Wirandowska-Stewart, M. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 4814–4818.
41. Stewart, W. E. II (1974) *Virology* **61,** 80–86.
42. Gresser, I., Bandu, M. T., Brouty-Boye, D. & Tovey, M. (1974) *Nature (London)* **251,** 543–545.
43. Havell, E. A. (1979) *Virology* **92,** 324–330.
44. Stewart, W. E., II & Havell, E. A. (1980) *Virology* **101,** 315–318.
45. Taira, H., Broeze, R. J., Jayaram, B. M., Lengyel, P., Hunkapiller, M. W. & Hood, L. E. (1980) *Science* **207,** 528–530.
46. Stewart, W. E., II, Wiranowska-Stewart, M., Koistinen, V. & Cantell, K. (1979) *Virology* **97,** 473–476.