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Aging In Vitro and Large-Scale Interferon Production by 15 New Strains of Human Diploid Fibroblasts

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To develop resources for large-scale production of human fibroblast interferon, we isolated, cryopreserved, and characterized 15 new strains of human diploid foreskin fibroblasts. Their life spans in vitro ranged from 52 to 72 population doublings. We based the selection of cell strains for mass interferon production on the number of population doublings during which consistently high yields of interferon were obtained after "superinduction" in roller bottles; our data show that aging in vitro leads to significant decline in amounts of interferon produced. In contrast, susceptibility to interferon remains largely unaffected by in vitro senescence. Karyotypic analysis indicated that the best interferon-producing strain, MLD (over 60,000 reference units/ml), has a translocation between chromosomes 5 and 15.

Interferon (IF) preparations have been effectively applied in treatment of both viral and neoplastic diseases of humans (2, 4, 11, 12, 14, 22, 27-29). Out of several forms of human IF, the human fibroblast interferon (HF-IF) molecule merits particular attention as a clinically useful drug because of its relative safety and ease of purification; it can be produced by strains of normal diploid cells that have been rigorously preselected by extensive testing and multifaceted characterization (17); in addition, efficient new techniques for HF-IF purification by affinity chromatography have been recently developed (9, 10, 19, 20, 30).

This paper presents information based on an 18-month experience in mass production (over 10^8 international reference units [ref. U] weekly) of HF-IF by human diploid cell strains isolated and characterized in this laboratory. Our data show that aging in vitro leads to a significant decline in the amount of HF-IF produced after the superinduction procedure with $rI_n \cdot rC_n$ and metabolic inhibitors (3, 15). Since the isolation (for therapeutic purposes) of products, specifically HF-IF, from human cell culture is still at a developmental stage, this overall study may serve as a valuable prototype. To that end, a specific quality control scheme for the IF product is introduced which insures the availability of an IF molecule with features of both biological potency and safety.

MATERIALS AND METHODS

Isolation of cell strains. Neonatal foreskins were obtained as fresh circumcision specimens from healthy males (1 to 3 days old) born at the Buffalo General Hospital, Buffalo, N.Y. The finely minced tissue was dispersed by treatment with CTC (0.1% collagenase [Worthington]-0.1% trypsin [GIBCO]-1% chicken serum [GIBCO] in Ca^{2+}- and Mg^{2+}-free Hanks balanced salt solution [21]) for 60 min at 37°C. After washing with minimal essential medium (MEM) (Eagle) with Earle salts and centrifugation (120 × *g*, 10 min), the cell pellet from each specimen was placed in a plastic 75-cm^2 flask (Falcon) containing MEM (GIBCO) supplemented with 10% (vol/vol) heat-inactivated fetal calf serum (FCS; KC Biological) and antibiotics (penicillin, 50 U/ml; streptomycin, 50 μg/ml). The cultures were incubated at 37°C in a 5% CO_2 atmosphere. To select cell strains with the lowest initial population doubling level (PDL), only cultures that produced a confluent monolayer within 7 days were further subcultured twice a week after dispersion by a trypsin (0.05%)-ethylenediaminetetraacetic acid (0.02%) solution (GIBCO). A 2:1 split ratio insured an average of one population doubling (PD) per passage. After the sixth passage, each cell strain (grown in 64 75-cm^2 plastic flasks with confluent monolayers) was trypsinized, pooled, and washed once, and portions (1 ml, containing 2 × 10^6 cells each) were dispensed into 96 borosilicate glass cryules (1.2 ml in size) (Wheaton). The cells were cryopreserved (10% glycerol in MEM with 10% [vol/vol] FCS) by controlled rate freezing (26) and stored in liquid nitrogen.

All diploid cell strains were periodically monitored for microbial (8) or mycoplasma (16) contaminants.

Large-scale IF production. Single ampoules of frozen cell stocks were removed from liquid nitrogen storage and rapidly thawed in a 37°C water bath. The content of each ampoule was placed in a single plastic 75-cm^2 flask containing MEM plus 10% FCS (vol/vol). As soon as cell cultures were confluent, they were subcultivated twice a week (2:1 split ratio) until the 11th PDL. Trypsinized cell suspensions from 10 75-

cm^2 plastic flasks (approximately 5×10^7 cells) were combined and inoculated into glass roller bottles with a 1,585-cm^2 growth surface area (Bellco Glass). The cell stocks, in 400 ml of growth medium per bottle, were cultivated at 37°C on a Rollacell apparatus (Bellco) rotating at a speed of 0.22 rpm. Subsequent passages (2:1 split ratio) were continued once a week. For large-scale production of crude human fibroblast IF, 2 to 30 roller bottle cultures (7 days old) of each strain, beginning at the 16th PDL, were superinduced at 34°C by a modification of published methods (3, 15): briefly, MEM (100 ml) containing $rI_n \cdot rC_n$ (75 μg/ml; Miles) and cycloheximide (60 μg/ml; Upjohn) was first added for 220 min; actinomycin D (0.75 μg/ml; Merck, Sharp and Dohme) was then added for an additional 110 min. The monolayers were then washed (3× Earle balanced salts solution; GIBCO), after which MEM (100 ml) containing either 5% FCS or 5% human serum (vol/vol) was added. Human serum was obtained from certified (according to Federal Drug Administration requirements [7]) blood donors. Crude HF-IF was harvested at 27 h, centrifuged ($5,000 \times g$ for 30 min), assayed for IF content, and stored at −90°C.

IF assay. IF was assayed in triplicate (FB-16-24 TC trays; Linbro), using monolayers of human diploid fibroblasts (strain 604, supplied by J. A. O'Malley). A modification of the colorimetric technique of Finter (5, 13), with bovine vesicular stomatitis virus as the challenge virus (multiplicity of infection, 0.15 plaque-forming units/cell), was used. An internal lyophilized laboratory HF-IF standard, calibrated against an international reference standard of human IF (no. G-023-901-527) (Research Resources Branch, National Institutes of Health), was included in each assay, and titers are expressed in international reference units.

Cell counts were performed on trypsin–ethylenediaminetetraacetic acid-dispersed cell suspensions, using an electronic particle counter (Coulter Counter model 2B1).

Sensitivity to IF was measured as an apparent titer of the internal laboratory standard (containing 19,800 ref. U/ml) determined in triplicate on monolayers prepared from each cell strain at various PDL during its life span.

The in vitro life span of each cell strain was determined by a rigid semiweekly schedule of subcultivation (cells were split in a ratio 2:1 in 75-cm^2 plastic flasks) until the time interval between PD became progressively greater, indicating the onset of cellular senescence (18) and a terminal period.

Statistics. The arithmetic means of IF present in crude HF-IF preparations produced during 5 (16 to 50 PDL) or 10 (over 51 PDL) consecutive PDL were calculated for each cell strain. As a measure of cell response to IF the arithmetic mean of the apparent titer of an internal laboratory standard was calculated for every consecutive 10 PDL for each cell strain.

Chromosome analysis. Actively dividing cultures (usually 48 h after seeding in a 75-cm^2 flask) were arrested in metaphase by colchicine treatment (0.05 μg/ml). After 60 min, cells were trypsinized, spread on glass slides, and air dried; Giemsa-stained chromosome preparations were counted, and quinacrine-stained preparations were used for detailed analysis of karyotype (23). In addition to establishing the normal karyology of a cell strain, these studies also serve to exclude the possibility of an accidental contamination by other cell types (24).

RESULTS

General characteristics of 15 cell strains from human foreskin. From 28 foreskin specimens, 15 cell strains were established and cryopreserved. Cultures of the remaining 13 specimens were discarded because confluent growth was not achieved within 7 days after initial seeding, indicating a low content of viable cells in the starting inoculum. Since our objective was to create a bank of human diploid cells sufficient for several years of continuous large-scale production of HF-IF, we selected only those cell strains that were established and cryopreserved at the lowest possible PDL. The established cell strains were designated BG-1, BG-2, BG-3, BG9, BG-10, BG-11, BG-14, BG-16, BG-18, BG-23, BG-24, BG-25, BG-26, BG-27, and MLD. At the 12th PDL, more than one distinct morphological type of cells was still evident in cell strains BG-1, BG-3, and BG-11; the remaining 12 cell strains produced typically smooth, contact-inhibited and uniform monolayers of fusiform cells, compatible with the morphology of human fibroblasts in culture (18) since the fourth PDL.

The saturation density of all cell strains between the 10th and 45th PDL both on glass (roller bottles) and on plastic (Falcon flasks) surfaces ranged from 5.0×10^4 to 7.8×10^4 cells/cm^2. The in vitro life span of these strains ranged from 52 for BG-9 to 72 for BG-10 and BG-23. Karyotypic analysis indicated that the distribution of the chromosome number in all strains was normal diploid for human male karyotype, i.e., 46, XY. Interestingly, strain MLD was found to have chromosomal abnormality (Fig. 1): distal parts of both long arms of one chromosome 5 were translocated to long arms of one chromosome 15 in more than 95% of the cells, resulting in the appearance of two marker chromosomes [46 XY,t($15q^-$,$15q^+$)]. This translocation was detected at all three (8, 22, and 52) PDL tested; this translocation was of particular interest (see below) since chromosome 5 has been implicated in control of human IF biosynthesis (32).

Periodic monitoring of the 15 established cell strains failed to reveal any microbial or mycoplasma contaminants.

Effect of human serum versus FCS on HF-IF production. Large-scale HF-IF production for eventual clinical application mandates the presence of human instead of calf serum in the production media. Therefore, IF yields of four cell strains (PDL from 16 to 30), using

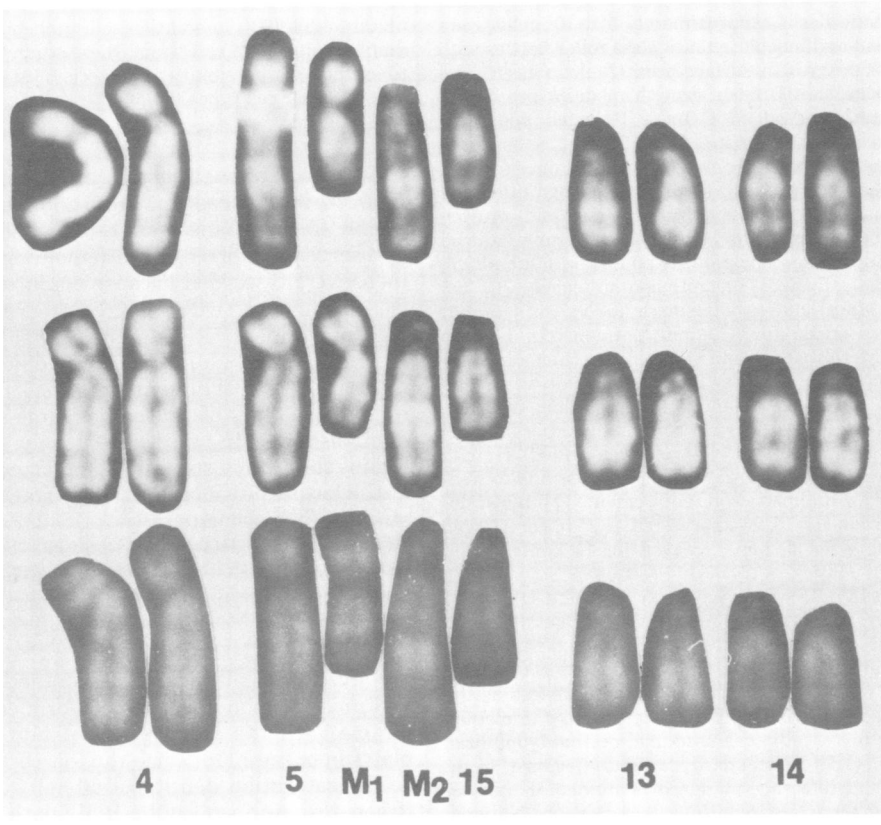

FIG. 1. *Three sets of chromosomes 4, 5, 15, 13, and 14 from strain MLD at 8 (top), 22 (middle), and 52 (bottom) PDL. Note two marker chromosomes (M_1 and M_2) resulting from translocation between chromosomes 5 and 15.*

production media (MEM) supplemented with heated serum (5%, vol/vol) of either fetal calf or human origin, were compared. The calculated average titers of HF-IF produced (pool of 2 to 14 roller cultures) at matched PDL are shown in Table 1; these data indicate that similar levels of IF are produced in the presence of human serum or FCS with these cell strains.

IF yields declined during the in vitro life span of diploid cells. Studies were also performed to determine whether the in vitro age of human diploid cells (as reflected by the PDL) affected the IF yield. All cell strains were superinduced under mass-culture conditions (from 2 to 14 bottles at each time) at consecutive PDL, beginning with passage 16. We calculated the average HF-IF yields obtained from individual cell strains, grouping together each five consecutive PDL (Fig. 2 and Table 2). Strain MLD (with the translocation at chromosome 5) was found to produce significantly higher amounts of IF; it consistently elaborated concentrations of this protein exceeding 25,000 ref. U/ml for at least 10 PDL longer than any of the other strains. The relative levels of IF produced by our cell strains between the 16th and 20th PDL were: four strains (BG-2, BG-16, BG-26, and MLD) produced over 40,000 ref. U/ml; four strains (BG-9, BG-23, BG-25, and BG-27) produced over 30,000 ref. U/ml; and four strains (BG-10, BG-14, BG-18, and BG-24) produced between 10,000 and 30,000 ref. U/ml. As mentioned, three strains (BG-1, BG-3, and BG-11) were excluded because of the presence of multiple-cell morphologies. Under our experimental conditions, all cell strains exhibited a progressive

TABLE 1. *Average HF-IF production in the presence of FCS and human serum between 16 and 30 PDL*

Medium	Interferon (ref. U/ml) produced by strain:			
	BG-9	BG-16	BG-23	MLD
MEM + 5% (vol/vol) FCS	27,200	30,200	25,800	46,500
MEM + 5% (vol/vol) human serum	26,600	28,200	24,200	46,700

decline in their ability to produce IF with increasing PDL (Fig. 2, with typical patterns). From a 4-fold to over a 500-fold drop in HF-IF titers occurred gradually between the relatively low (15 to 20) and high (51 to 60) PDL for each strain. To evaluate the possibility that the reduced IF yield was simply a consequence of a smaller total number of cells grown in production vessels at higher PDL, we determined the saturation densities of the cell strains at several PDL. We then calculated the average number of cells at the 16 to 20 PDL required to elaborate 1 ref. U of IF (Table 2) and compared values with those of older cells (51 to 60 PDL). Clearly, the average IF production per cell was substantially reduced in cultures at higher PDL.

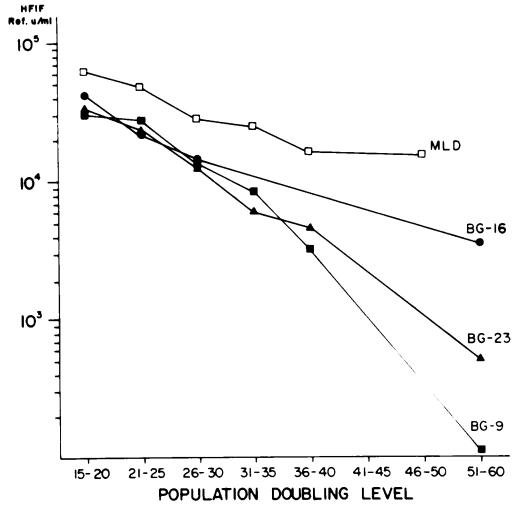

FIG. 2. *IF production after superinduction procedure during the life span of human diploid fibroblasts. Standard errors (not shown for clarity) were between 8 and 14% of the means.*

Sensitivity to IF is independent of senescence in vitro. As a measure of cellular sensitivity to exogenous IF we also determined the titers of a reference IF standard by its activity (assayed in triplicate) in each of eight cell strains at PDL ranging from 11 to over 50. Table 3 presents an average of the apparent titers calculated after first grouping results from each 10 consecutive PDL. Within the same strain, IF sensitivity fluctuated somewhat at different PDL, and there were also some differences from strain to strain; however, there was no suggestion of a trend toward modification of sensitivity to exogenous IF as a function of cell age. These data thus confirm and extend the observation (31) that the function(s) of a regulatory gene(s) controlling the response to exogenous IF is not significantly influenced by in vitro aging. Recent work suggests a role of C-21 in control of cell surface receptors of IF (25); our current observations imply that the number and function of these groups does not deteriorate with the onset of cell senescence.

DISCUSSION

Our results thus demonstrate that the synthesis of IF protein after superinduction gradually declines over the in vitro life span of human diploid fibroblasts. In contrast, sensitivity to exogenous IF molecules remains largely independent of the aging process, thus verifying an earlier report (31). These two cellular functions (biosynthesis of, and responsiveness to HF-IF) are apparently governed by genes located on different chromosomes (6, 25, 32), which may increase the possibility that one function can be differentially expressed at increased PDL. The difference in the levels of IF synthesis between young and old fibroblast strains suggests that, at least

TABLE 2. *Characteristics of IF production early and late in the life span of human diploid fibroblasts*

Strain	Young cells[a]			Old cells[b]		
	Avg IF harvest (ref. U/ml)	Cell saturation density ($\times 10^{-4}$/cm^2)	Avg no. of cells/1 ref. U of HF-IF	Avg IF harvest (ref. U/ml)	Cell saturation density ($\times 10^{-4}$/cm^2)	Avg no. of cells/1 ref. U of HF-IF
BG-2	40,500	6.92	27	120	3.64	4,808
BG-9	33,100	6.52	31	60	2.56	6,763
BG-10	25,200	5.30	33	300	4.24	2,240
BG-14	19,800	6.52	52	240	3.72	2,457
BG-16	42,900	7.06	26	3,500	4.33	196
BG-18	18,600	6.16	53	50	4.63	14,677
BG-23	34,800	7.53	34	470	5.34	1,801
BG-24	10,800	5.01	73	ND[c]		
BG-27	38,000	6.06	25	ND		
MLD	64,400	7.83	20	16,300	5.76	56

[a] 16 to 20 PDL.
[b] 51 to 60 PDL.
[c] ND, Not done.

TABLE 3. *Sensitivity to HF-IF of diploid cell strains during their life span in vitro*

PDL	Apparent avg titers of internal laboratory HF-IF standard assayed on cell strain:[a]							
	BG-2	BG-9	BG-10	BG-14	BG-16	BG-18	BG-23	MLD
11-20	12,600	14,800	8,400	9,300	9,000	12,200	10,500	7,100
21-30	ND[b]	12,400	9,600	7,300	7,800	7,800	11,500	9,700
31-40	9,400	7,900	10,400	7,100	6,800	6,800	8,250	7,500
41-50	7,100	9,600	6,500	5,700	7,100	7,100	4,800	4,300
Over 50	12,500	16,000	11,000	6,200	8,600	8,600	4,000	2,300

[a] Standard errors ranged between 5 and 10% of the means.
[b] Not done.

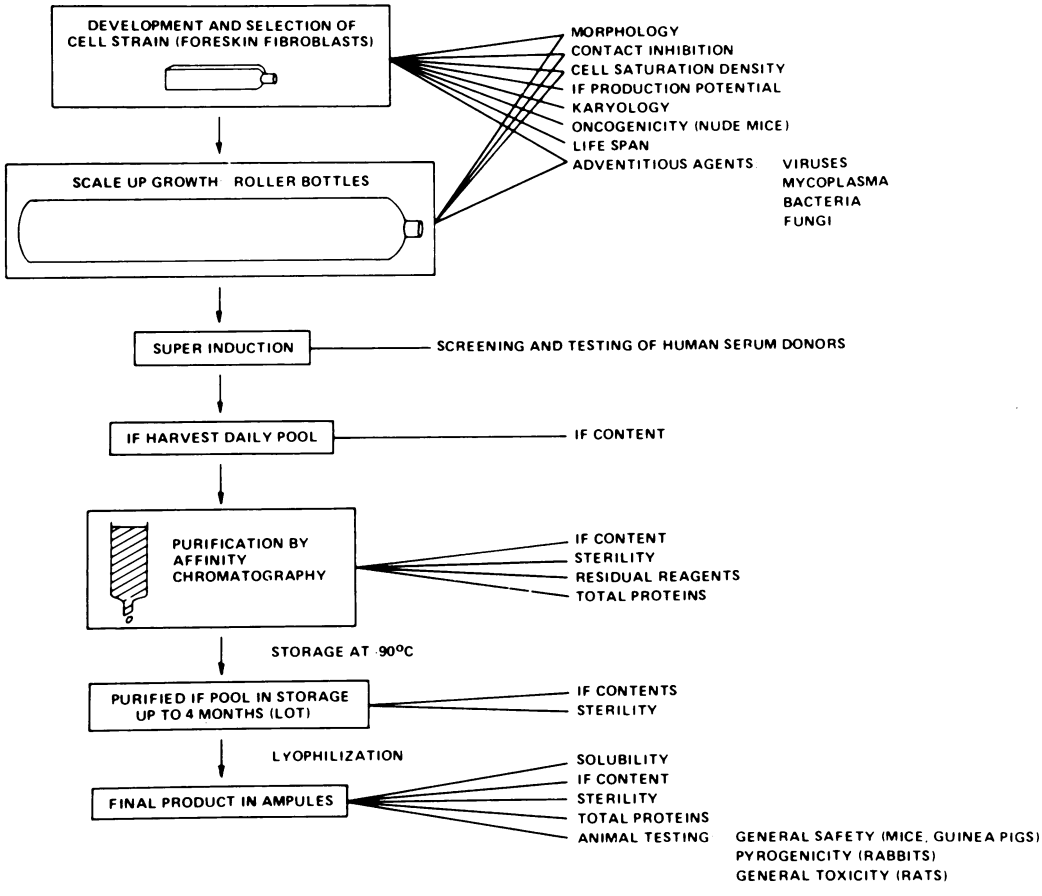

FIG. 3. *Quality control of HF-IF, based on Food and Drug Administration and British Medical Research Council guidelines for biological products.*

in vitro, the regulatory gene(s) controlling IF production might continuously and progressively be modified. Alternatively, one should consider the possibility of a relatively rapid selection in vitro of cells with low IF-producing potential, although this seems to be a less likely event.

Cell strain MLD merits special attention, since its further characterization might lead to new insights into the genetic control of human IF biosynthesis. After superinduction, strain MLD produced significantly greater amounts of IF (Fig. 1 and Table 2), and the high levels of production lasted over more passages than for any other strain studied. These unusual properties may be a consequence of the chromosomal translocation between long arms of chromosome 5 and chromosome 15, since chromosome 5 (and 2) apparently carries genes necessary for IF production (32).

Our current studies also bring into sharp focus a practical problem in mass production of HF-IF for clinical study. For example, economic considerations require that only cells capable of elaborating no less than 1,200 ref. U of HF-IF per cm^2 of confluent monolayer (or approximately 2×10^6 ref. U per roller bottle [growth surface, 1,585 cm^2]) be used. However, most human diploid fibroblasts strains do not fulfill these requirements beyond the 28th PDL (Fig. 2). Since it is not practical to introduce strains into the regular production cycle before the 18th PDL, the useful period of these cells in mass HF-IF production is limited to less than 10 PD. The majority of the approximately 60 PD in the life span of a typical cell strain clearly cannot be efficiently entered in the production cycle. For example, to build from one ampoule of cryopreserved cells (2×10^6) at the sixth PDL to the 8×10^9 cells in 80 roller bottles needed for weekly production of 10^8 ref. U of HF-IF will require 12 PD (18th PDL). To maintain weekly production, only half of the cell cultures is superinduced, while the other half is subcultured in preparation for subsequent weeks. Thus, it is essential to maintain adequate supplies of selected and well-characterized diploid cells that are cryopreserved at low (fifth to seventh) PDL.

The isolation and preparation of HF-IF for clinical use, while obviously fundamentally different from viral vaccine development, shares as a common denominator the requirement for the cell to elaborate the biological product of interest. However, unlike viral vaccine development programs, which function with well-established guidelines for product safety (7), similar strategies for human IF have not yet been advanced. It is important to bear in mind that leukocyte IF is produced from pools of leukocytes from thousands of individuals, some of which may carry slow viruses (33), and lymphoblastoid IF is produced in the presence of the Epstein-Barr virus genome (1). For these reasons, it is generally agreed that fibroblast IF has the best safety potential for man. Therefore, we feel it is timely to suggest an outline of a production/quality control scheme for HF-IF. To this end, Fig. 3 introduces a procedure, adapted from the Food and Drug Administration and British Medical Research Council guidelines, that has been used in this laboratory for 18 months; it identifies specific steps during production/purification/lyophilization of HF-IF at which the protein is evaluated for biological potency and various safety features. Since this scheme accommodates both safety as well as the new technologies for HF-IF purification (9, 10, 19, 20, 30), it may prove a useful prototype for a further refinement.

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LITERATURE CITED

1. **Adams, A., B. Lidin, H. Strander, and K. Cantell.** 1975. Spontaneous interferon production and Epstein-Barr virus antigen expression in human lymphoid cell lines. J. Gen. Virol. **28**:219–223.
2. **Arvin, A. M., A. S. Yeager, and T. C. Merigan.** 1976. Effect of leukocyte interferon on urinary excretion of cytomegalovirus by infants. J. Infect. Dis. **133**(Suppl):A205–A210.
3. **Billiau, A., M. Joniau, and P. DeSomer.** 1973. Mass production of human interferon in diploid cells stimulated by Poly I · C. J. Gen. Virol. **19**:1–8.
4. **Blomgren, H., K. Cantell, B. Johansson, C. Lagergren, U. Ringborg, and H. Strander.** 1976. Interferon therapy in Hodgkin's disease. Acta Med. Scand. **199**:527–532.
5. **Carter, W. A., P. M. Pitha, L. W. Marshall, I. Tazawa, S. Tazawa, and P. O. P. Ts'o.** 1972. Structural requirements of the $rI_n \cdot rC_n$ complex for induction of human interferon. J. Mol. Biol. **70**:567–587.
6. **Chany, C., M. Vignal, P. Couillin, N. Van Cong, J. Boue, and A. Boue.** 1975. Chromosomal localization of human genes governing the interferon-induced antiviral state. Proc. Natl. Acad. Sci. U.S.A. **72**:3129–3133.
7. **Code of Federal Regulations 21 (Food and Drugs).** 1976. Parts 600 to 1299. Office of the Federal Register National Archives and Records Service, General Services Administration, Washington, D.C.
8. **Coriell, L. L.** 1973. Detection, elimination, and prevention of bacteria and fungi in tissue cultures, p. 718–721. *In* P. F. Kruse, Jr., and M. K. Patterson, Jr. (ed.), Tissue culture methods and applications. Academic Press Inc., New York.
9. **Davey, M. W., J. W. Huang, E. Sulkowski, and W. A. Carter.** 1974. Hydrophobic interaction of human interferon with Concanavalin A-agarose. J. Biol. Chem. **249**:6354–6355.
10. **Davey, M. W., E. Sulkowski, and W. A. Carter.** 1976. Hydrophobic interaction of human, mouse and rabbit interferons with immobilized hydrocarbons. J. Biol. Chem. **251**:7620–7625.
11. **De Clercq, E., V. G. Edy, H. De Vlieger, and R. Eeckels.** 1975. Intrathecal administration of interferon in neonatal herpes. J. Pediatr. **86**:736–739.
12. **Desmyter, J., J. De Groote, V. J. Desmet, A. Billiau, M. B. Ray, A. F. Bradburne, V. G. Edy, P. De Somer, and J. Mortelmans.** 1976. Administration of human fibroblast interferon in chronic hepatitis-B infection. Lancet **ii**:645–647.
13. **Finter, N. B.** 1969. Dye uptake methods for assessing viral cytopathogenicity and their application to interferon assays. J. Gen. Virol. **5**:419–427.
14. **Greenberg, H. B., R. B. Pollard, L. I. Lutwick, P. B. Gregory, W. S. Robinson, and T. C. Merigan.** 1976. Effect of human leukocyte interferon on hepatitis B virus infection in patients with chronic active hepatitis. N. Engl. J. Med. **295**:517–522.
15. **Havell, E. A., and J. Vilcek.** 1972. Production of high-titered interferon in cultures of human diploid cells. Antimicrob. Agents Chemother. **2**:476–484.
16. **Hayflick, L.** 1973. Screening tissue cultures for mycoplasm infections, p. 722–728. *In* P. F. Kruse, Jr., and M. K. Patterson, Jr. (ed.), Tissue culture methods and applications. Academic Press Inc., New York.
17. **Hayflick, L.** 1974. Substrates for human interferon, p.

4-11. *In* C. Waymouth (ed.), The production and use of interferon for the treatment and prevention of human virus infections, Proceedings of a Tissue Culture Association Workshop. Tissue Culture Association, Rockville, Md.

18. **Hayflick, L., and P. S. Moorhead.** 1961. The serial cultivation of human diploid cell strains. Exp. Cell Res. **25**:585-621.
19. **Jankowski, W. J., M. W. Davey, J. A. O'Malley, E. Sulkowski, and W. A. Carter.** 1975. Molecular structure of human fibroblast and leukocyte interferons: probe by lectin and hydrophobic chromatography. J. Virol. **16**:1124-1130.
20. **Jankowski, W. J., W. von Muenchhausen, E. Sulkowski, and W. A. Carter.** 1976. Binding of human interferons to immobilized Cibacron Blue F3GA: the nature of molecular interaction. Biochemistry **15**:5182-5187.
21. **Kaighn, M. E., and A. M. Prince.** 1971. Production of albumin and other serum proteins by clonal cultures of normal human liver. Proc. Natl. Acad. Sci. U.S.A. **68**:2396-2400.
22. **Larsson, A., M. Forsgren, S. Hard Af Segerstad, H. Strander, and K. Cantell.** 1976. Administration of interferon to an infant with congenital rubella syndrome involving persistent viremia and cutaneous vasculitis. Acta Paediatr. Scand. **65**:105-110.
23. **Moorhead, P. S., W. W. Nicholls, F. T. Perkins, and L. Hayflick.** 1974. Standards of karyology for human diploid cells. J. Biol. Stand. **2**:95-101.
24. **Nelson-Rees, W. A., and R. R. Flandermeyer.** 1977. Inter- and intra-species contamination of human breast tumor cell lines HBC and BrCa5 and other cell cultures. Science **195**:1343-1344.
25. **Revel, M., D. Bash, and F. H. Ruddle.** 1976. Antibodies to a cell surface component coded by human chromosome 21 inhibit action of interferon. Nature (London) **260**:139-141.
26. **Shannon, J. E., and M. L. Macy.** 1973. Freezing storage and recovery of cell stocks, p. 712. *In* P. F. Kruse, Jr., and M. K. Patterson, Jr. (ed.), Tissue culture methods and applications. Academic Press Inc., New York.
27. **Strander, H., K. Cantell, G. Carlstrom, S. Ingimarsson, P. Jakobsson, and U. Nilsonne.** 1976. Acute infections in interferon-treated patients with osteosarcoma: preliminary report of a comparative study. J. Infect. Dis. **133**(Suppl.):A245-A248.
28. **Strander, H., K. Cantell, G. Carlstrom, and P. A. Jakobsson.** 1973. Clinical and laboratory investigations on man: systemic administration of potent interferon to man. J. Natl. Cancer Inst. **51**:733-742.
29. **Strander, H., K. Cantell, S. Ingimarsson, P. A. Jakobsson, U. Nilsonne, and G. Soderberg.** 1975. Interferon treatment of osteogenic sarcoma—a clinical trial, p. 377-381. *In* M. A. Chirigos (ed.), Proceedings of the Conference on Modulation of Host Immune Resistance in the Prevention or Treatment of Induced Neoplasias. Government Printing Office, Washington, D.C.
30. **Sulkowski, E., M. W. Davey, and W. A. Carter.** 1976. Interaction of human interferons with immobilized hydrophobic amino acids and dipeptides. J. Biol. Chem. **251**:5381-5385.
31. **Tan, Y. H., E. L. Chou, and N. Lundh.** 1975. Regulation of chromosome 21-directed antiviral gene(s) as a consequence of age. Nature (London) **257**:310-312.
32. **Tan, Y. H., R. P. Creagan, and F. H. Ruddle.** 1974. The somatic cell genetics of human interferon: assignment of human interferon loci to chromosomes 2 and 5. Proc. Natl. Acad. Sci. U.S.A. **71**:2251-2255.
33. **Wadell, G.** 1977. Hazards of human leukocyte interferon therapy. N. Engl. J. Med. **296**:1295.