PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **181**, 298–304 (1986)

# Limited Efficacy of Aerosolized Recombinant Alpha Interferon against Virulent Influenza A/HK Infection in Mice (42257)

CHONG-SON SUN, SAMUEL Z. WILSON, AND PHILIP R. WYDE

*Department of Microbiology and Immunology, Baylor College of Medicine, Houston, Texas 77030*

*Abstract.* Hybrid recombinant human alpha interferon A/D (rIFN A/D) is unusual in that it has equivalent antiviral activity in *in vitro* assays using mouse or human tissue cells. This interferon was delivered to outbred Swiss mice in small particle aerosols before and/or after these mice were inoculated with virulent influenza A/HK/68 virus. Although rIFN A/D was effective in inhibiting influenza virus replication *in vitro* in primary mouse embryo cells, it had only a limited degree of effectiveness in the *in vivo* tests; only animals exposed to rIFN A/D for 4 hr and inoculated with influenza virus 4 hr later exhibited a significant decrease in mortality (approximately 25% reduction in deaths; $P < 0.025$) compared with that of the untreated control animals. The limited effectiveness of rIFN A/D seen in these studies indicated that the use of this or similar recombinant alpha interferons alone to prevent or treat influenza virus infection in humans is not promising.


The potential use of exogenously administered interferons to treat respiratory viral infections of man has long been recognized, but, to date, no interferon preparation has been licensed for use against such infections. This failure can be attributed to the high costs of producing purified interferons, to side effects associated with the use of human interferon therapy (1–4) and to the mixed results of clinical trials testing interferons (5–14). The recent development of recombinant interferons produced by *Escherichia coli* implanted with human alpha interferon genes (15, 16) has led to production of relatively large amounts of homogenous, concentrated preparations, making the use of interferons to treat respiratory viral diseases less costly, more feasible, and likely to yield desirable results (17–19) than heretofore.

Delivery of interferons directly to the respiratory tract by small particle aerosol offers another potential means of improving the efficacy of exogenously administered interferons against respiratory virus infections. We have performed a number of studies testing this concept in mice (20–22) and cotton rats (*Sigmoden hispidus*) (23). Recombinant interferon A/D (rIFN A/D), a hybrid human leukocyte recombinant interferon that is highly active in mouse and human tissue cells and which has some antiviral activity in cotton rat cells, was used in these studies. It was found that rIFN A/D reached significant levels in lungs of animals exposed to small particle aerosols (SPA) of this material and inhibited vesicular stomatitis virus (VSV)-induced pulmonary infection in both rodent species. The protective effect in mice lasted up to 7 days after aerosolization and was significantly greater than the protective effects of the same interferon preparation given intraperitoneally (22). In the present study, rIFN A/D was tested for its ability to inhibit influenza A/HK/68 virus infection in mice. It was found that groups of animals exposed to rIFN A/D prior to virus inoculation generally had a more reduced mortality than untreated control mice. However, only the group of animals exposed for 4 hr to a small particle aerosol of rIFN A/D and inoculated with virus 4 hr later had significantly lower mortality ($P < 0.025$) than untreated control mice.

**Materials and Methods.** *Animals.* Six to eight-week-old Swiss mice of either sex were obtained from Harlan Breeding Laboratories, Houston, Texas. These mice were housed in cages covered with barrier filters and fed water and food *ad libitum.*

*Virus.* The isolation, adaption to mice, and characterization of the virulent influenza A/HK/68 (H3N2) (mouse passage 9) virus used in these experiments have been described in detail previously (24). Each lung pool was homogenized using Hanks' balanced salt solution containing 2% gelatin as a diluent, clarified using centrifugation, filtered through a 0.45-



0037-9727/86 $1.50

$\mu$m filter (Acrodisc; No. 4181; Gelman Sciences, Inc., Ann Arbor, Mich.), portioned, and stored at −70°C.

*Infection of animals.* Mice were lightly anesthetized with ether and inoculated intranasally (in) with two to four median lethal doses of mouse-adapted influenza A/HK/68 virus in a 0.05-ml volume.

*Tissue culture.* Primary mouse embryo (PME) cells were prepared as described previously (25). Briefly, mouse embryos were cut into small pieces and then covered with neutral protease (Dispase; grade II; 2.4 U/ml; Boehringer-Mannheim Biochemicals, Indianapolis, Ind.). The dissociated cells and remaining fragments were washed, pelleted, resuspended in 20% fetal calf serum (FCS)–MEM and added to 150-cm$^2$ flasks. The resulting monolayers of PME cells were passaged when confluent, using 20% FCS–MEM as the medium. Passage levels 2 and 3 of the PME cells were used in these studies.

Madin Darby canine kidney (MDCK) tissue cells were used to titer influenza virus pools or suspensions of lungs from inoculated mice. The MDCK cells were obtained originally from Flow Laboratories (Bethesda, Md.) and passaged continuously in the laboratory using 10% FCS–MEM as a growth medium.

*Virus isolation and titration.* Lungs were aseptically removed from randomly selected animals taken from each test group. One lobe from each removed lung was processed for histologic studies; the remaining lungs were washed in sterile medium, homogenized, and centrifuged. The supernatant fluids were serially diluted using serial 3.33-fold dilutions in minimal essential medium with Earle's salt containing 2 $\mu$g of trypsin/ml (Worthington Biochemicals Corp., Freehold, N.J.) and immediately inoculated onto MDCK monolayers. Just prior to inoculation, the growth media on these monolayers was removed and the monolayers were rinsed to remove residual protein. After a 90-min adsorbtion period, additional MEM with trypsin was added to the monolayers. After incubation for 5 days at 36°C, suspensions of 0.5% chicken erythrocytes were added to each monolayer. Cultures exhibiting hemadsorbtion were considered to be infected with influenza virus. Titers were expressed as the reciprocal of the last dilution of test suspension that exhibited hemadsorbtion. Hemadsorbing cultures were spot-checked for virus specificity using fluorescein isothiocyanate (FITC)-conjugate antiserum (Cappel Laboratories, Inc., Downingtown, Pa.) specific to type A influenza virus.

*Histological methods.* Organs were prepared for histological studies by fixation in buffered Formalin, embedding in low-melting-point paraffin, and sectioning at 5-$\mu$m thickness. Sections were stained with hematoxylin and eosin.

*Interferons.* rIFN$\alpha$A and rIFN$\alpha$ A/D were obtained from the Department of Immunotherapy, Hoffmann–La Roche, Inc. The construction and specific molecular activities of these interferons have been described in detail previously (26). Mouse alpha interferon (No. 22051) was obtained from Lee Biomolecular Research Laboratories, Inc., San Diego, California. Mouse type 1 (No. G-002-904-511) interferon reference reagent was obtained from Dr. June K. Dunnick and Dr. John R. LaMontagne of the Antiviral Substance Program, National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH), Bethesda, Maryland.

*Measurement of antiviral activity.* Assays were carried out in triplicate using PME cells and 96-well round-bottom plates (No. 76-013-05; Flow Laboratories, McLean, Va.). All dilutions and tissue culture suspensions were prepared with 5% FCS–MEM. Briefly, 0.05 ml of medium, test sample, or interferon reference standard was added to the first well of each row and serially diluted (twofold), using microtiter loops (Rotatiter, No. 002-961-0100; Dynatech Laboratories, Inc., Alexandria, Va.). PME tissue cells were added next in 0.5-ml amounts (ca. $2 \times 10^4$ cells/well), and the plates were incubated at 37°C overnight. The next morning, the medium was removed with a suction device and about 100 median tissue culture infective doses of influenza virus was added to each well in 0.1 ml. Five days later the medium in every well was tested for the presence of hemagglutinating virus using chicken erythrocytes (0.5% suspension) as indicator cells. The presence of interferon was indicated by the inhibition of hemagglutination, and interferon titers (per 0.05 ml) were expressed as the geometric mean ($\log_2$) of the reciprocal of the last dilutions which completely inhibited hemagglutination. Titers of

the interferon reference standards used in these assays rarely varied by more than twofold from assay to assay. All titers were adjusted to be relative to the stated titer of the mouse NIH interferon reference standard.

*Aerosol treatments.* Aerosol machines containing Collison nebulizers modeled after a design by K. R. May (27, 28) were used in these experiments. These machines and their use to deliver antivirals have been described in detail previously (27). The preparation of rIFN A/D used in this study had a titer of $6 \times 10^7$ U/ml and was diluted in aerosol machine reservoirs with 350 ml of sterile, normal saline (cat. No. 2B1322, Travenol Lab, Inc., Derrfield, Ill.) so that the starting reservoir concentration was $1.7 \times 10^5$ U/ml. The rIFN A/D was delivered at an approximate rate of 12.5 liter/min to the animals kept in plastic cages covered tightly with plastic tops. Under these conditions the estimated total deposition of rIFN A/D per mouse was 5000 units (20, 22).

Schedules for administrating small particle aerosols of rIFN A/D to mice fell into three basic groups: prophylactic (P), treatment (T), and combined prophylactic and treatment (PT) groups. As shown in Table I, groups P1 to P6 were exposed to aerosols of rIFN A/D for 4 hr and inoculated with influenza virus 1 to 48 hr after ceasing delivery of interferon. Groups P7 to P9 were exposed to rIFN A/D aerosols 4 hr/day for 2–4 days and inoculated with virus 24 hr after ceasing the last aerosol. Groups T1 to T3 were exposed to rIFN A/D aerosols 16 hr/day for 2–4 days beginning 8 hr after virus inoculation. Groups PT1 and PT2 were exposed to 24-hr intervals of rIFN A/D aerosols, inoculated 16 hr after ceasing the last pretreatment and then exposed to 24 hr of continuous interferon aerosols for 1–4 days starting 8 hr after virus challenge.

*Statistics.* Statistical significance of differences in mortalities between groups was determined by comparing the number of deaths and survivors using the $\chi^2$ $2 \times 2$ contingency test with one degree of freedom (29). Geometric mean titers (GMT) and standard deviations were calculated using a programmed Texas Instruments TI-55 calculator.

**Results.** *In vitro anti-influenza virus activity of rIFN A/D.* Table II compares the antiviral activity of rIFN A/D against influenza A/HK/68 virus in PME tissue cells with that of rIFN

TABLE I. SCHEDULES OF ADMINISTRATION OF RECOMBINANT INTERFERON A/D AEROSOL USED IN THESE EXPERIMENTS[a]

| Treatment group[b] | Duration of aerosol (hr) | Number of exposures | Total exposure (hr) | Interval between virus challenge and aerosolization (hr)[c] |
|---|---|---|---|---|
| P1 | 4 | 1 | 4 | +48 |
| P2 | 4 | 1 | 4 | +40 |
| P3 | 4 | 1 | 4 | +24 |
| P4 | 4 | 1 | 4 | +16 |
| P5 | 4 | 1 | 4 | +4 |
| P6 | 4 | 1 | 4 | +1 |
| P7 | 4 | 4 | 16 | +24 |
| P8 | 4 | 3 | 12 | +24 |
| P9 | 4 | 2 | 8 | +24 |
| T1 | 23.5 | 4 | 96 | −8 |
| T2 | 23.5 | 3 | 72 | −8 |
| T3 | 23.5 | 2 | 40 | −8 |
| PT1 | 23.5 | 5 | 118 | +16, 8 |
| PT2 | 23.5 | 2 | 26 | +16, 8 |
| v.c. | 0 | 0 | 0 | — |

[a] The reservoir concentration of rIFN A/D used in all experiments was $1.7 \times 10^5$ units/ml. The estimated deposition of rIFN A/D per mouse per 4 hr, was 5000 units.

[b] P indicates groups which received prophylactic regimens of rIFN A/D aerosol; T indicates groups which received aerosols after inoculation; and PT indicates groups that were exposed to aerosols both before and after virus inoculation.

[c] A plus symbol indicates that animals were inoculated with virus after ceasing the last interval of aerosolization; negative symbols indicate that animals were inoculated with virus before the first aerosol exposure.

TABLE II. COMPARISON OF THE ANTI-INFLUENZA VIRUS ACTIVITY OF HYBRID RECOMBINANT LEUKOCYTE INTERFERON A/D WITH HYBRID RECOMBINANT LEUKOCYTE INTERFERON (rIFN A), NATURAL HUMAN ALPHA INTERFERON, AND NATURAL MOUSE ALPHA INTERFERON IN PRIMARY MOUSE EMBRYO (PME) TISSUE CELLS[a]

| Interferon tested | Interferon titer (GMT $\log_2$ + SD)[b] | |
|---|---|---|
| | Expt 1 | Expt 2 |
| Human alpha | 0 | 0 |
| Mouse alpha | 7.0 ± 1.0 | 8.7 ± 0.6 |
| rIFN A | 0 | 0 |
| rIFN A/D | 11.3 ± 0.6 | 12.0 ± 0 |

[a] Values were calculated relative to a mouse reference interferon reagent (NIH Cat. No. G-002-902-511).

[b] To test for virus presence a 0.5% suspension of chicken erythrocytes was added to each well 5 days after the addition of challenge virus (approximately 100 $TCID_{50}$ of influenza A/HK/well). Titers = the reciprocal of the last dilution of interferon in wells in which no hemagglutinating was observed. All values are expressed as the geometric mean titer (GMT) $\log_2$ ± SD; 0 = undetectable activity; $n$ = 3 for each experiment.

A, natural mouse alpha interferon and natural human alpha interferon. As indicated, natural mouse alpha and rIFN A/D interferon, but not rIFN A or natural human interferon, significantly reduced influenza virus replication in this system. The titers obtained for rIFN A/D and natural mouse alpha interferon in these experiments were eight- to 16-fold less than titers obtained in assays using vesicular stomatitis virus and L929 mouse fibroblast cells (data not shown). However, the values obtained in the two test systems were not directly comparable since not only were the challenge viruses different, but also the tissue cells and endpoints.

*Efficacy of aerosolized recombinant interferon.* The cumulative mortality of animals exposed to rIFN A/D aerosols and inoculated with influenza A/HK/68 virus is shown in Table III. As indicated, all groups of mice exposed to rIFN A/D prior to virus inoculation had reduced mortality compared to untreated control mice. Reductions ranged from 5 to 22% (mean reduction 13%). However, only group P5, the group of mice which was inoculated with virus 4 hr after stopping rIFN A/D aerosol, had statistically significant protection when cumulative mortalities in these groups were compared to the number of deaths which occurred in untreated control mice. The $P$ value for group P5 was $>0.025$ but $<0.01$ ($\chi^2 = 5.47$). In contrast, maximum reduction in mortality in groups of mice treated with aerosols of rIFN A/D after virus inoculation was 7%.

Virus titers in lungs of randomly selected mice taken from each of the experimental groups 4 days after virus inoculation were determined; none of the mean lung virus titers in groups given interferons exogenously were statistically different from the mean lung virus titer of the virus control group when compared using the $t$ test. However, as shown in Table IV which presents pulmonary virus titers in lungs of mice from groups P3, P5, and the untreated control group, pulmonary virus titers varied greatly in individual mice exposed to interferon aerosols. In some instances 100- to 1000-fold less virus was detected in lungs of mice in groups exposed to interferon than in groups of unexposed animals. However, even in group P5, the GMT (5.7 ± 1.7 $\log_{10}$) was not statistically significant compared to the virus titers in lungs of virus control mice (GMT = 7.1 ± 0.3 $\log_{10}$). Pulmonary titers in other groups (of which group P3 is representative) fell between those seen in the virus control and group P5 (e.g., the GMT for group P3 = 6.6 ± 0.8 $\log_{10}$).

All stained sections of lungs from the different groups revealed marked peribronchial and peribronchiolar leukocytic infiltrate with scattered microareas of interstitial pneumonitis. Generally, however, lungs from groups of mice having higher geometric mean pulmonary virus titers exhibited greater histopathologic changes than lungs from groups with the lowest geometric mean pulmonary virus titers.

**Discussion.** In these studies the efficacy of rIFN A/D delivered to mice as a small particle aerosol before and/or after inoculation of lethal doses of influenza virus was studied. Generally mice exposed to rIFN A/D before virus exhibited reduced numbers of deaths than untreated control mice. However, although a number of administration schedules were used (Table I), only mice which received aerosols of rIFN A/D for 4 hr, 4 hr before inoculation, had significantly lower mortality (22%) than untreated virus control mice (Table III).

TABLE III. CUMULATIVE MORTALITY AND $\chi^2$ ANALYSIS OF MICE EXPOSED TO RECOMBINANT INTERFERON A/D AEROSOL AND INOCULATED WITH INFLUENZA A/HK VIRUS

| Exptl[a] group | Total IFN exposure (hr) | Cumulative mortality | | $\chi^2$ values[b] |
|---|---|---|---|---|
| | | No. dead/ No. inoculated | Percentage | |
| V.C. | None | 39/50 | 78 | — |
| P1 | 4 | 20/30 | 67 | 1.24 |
| P2 | 4 | 32/50 | 64 | 2.38 |
| P3 | 4 | 20/30 | 67 | 1.24 |
| P4 | 4 | 22/30 | 73 | 0.24 |
| P5 | 4 | 28/50 | 56 | 5.47[c] |
| P6 | 4 | 22/30 | 73 | 0.24 |
| P7 | 16 | 18/20 | 60 | 2.97 |
| P8 | 12 | 19/30 | 63 | 2.02 |
| P9 | 8 | 19/30 | 63 | 2.02 |
| V.C. | None | 44/55 | 80 | — |
| T1 | 96 | 17/20 | 85 | 0.24 |
| T3 | 72 | 29/40 | 73 | 0.73 |
| T3 | 48 | 32/40 | 80 | 0.00 |
| PT1 | 118 | 27/35 | 77 | 0.11 |
| PT2 | 26 | 27/35 | 77 | 0.11 |

[a] Abbreviations: Exptl, experimental; IFN, interferon; V.C., virus control.

[b] $\chi^2$ values were obtained by comparing mortality between experimental groups with the appropriate virus control group using a two by two contingency test (28). The values were not corrected with continuity factor ($n = >30$).

[c] The $P$ value for this group was $0.025 < P < 0.01$; for all the other groups the $P$ values were greater than 0.05 ($P = 0.05$ when $\chi^2 = 3.84$).

Overall, pulmonary virus titers and inflammation in lungs from this group were only marginally reduced compared to untreated control mice (Table IV). However, individual animals in the group had remarkably lower pulmonary virus titers and inflammation. These results were similar to those reported by Takano, Jensen, and Warren in 1963 (30) and by Blach-Olszewska, Magur, and Skurska (31) in 1972. In the Takano studies, mice were exposed to aerosols of interferon prepared from the allantoic fluid of infected eggs or mouse lungs; each preparation contained less than 400 units of interferon/ml. Although many prophylactic and therapeutic schedules of application of interferon were tried in their studies, protection against lethal influenza B/GL virus infection was observed only when aerosols of interferon were given 1 hr before or for 4-hr intervals on 4 consecutive days before virus challenge; the maximum reductions in deaths compared to controls that they observed was less than 50% and required relatively large numbers of mice in each group to be seen. In the 1972 studies, mice were given alpha interferon intranasally or intravenously 24 hr before and every 48 hr after they were challenged intranasally with lethal doses of influenza A/PR/8 virus. Only mice given interferon intranasally were protected, and protection compared to control mice was only 40%. Differences in the precise administration schedules that were effective and the percentage of protection observed in the three studies could be attributable to differences in aerosol machines, challenge viruses, and interferon preparations.

The rIFN A/D used in the present studies was of much greater homogeneity and concentration ($1.7 \times 10^5$ U/ml) than the interferon preparations used in either the 1963 or 1972 studies. Moreover, in *in vitro* assays (Table II) the rIFN A/D was as effective as natural mouse alpha interferon in protecting mouse tissue cells from infection with influenza A/HK/68 virus (the virus used to challenge mice in succeeding aerosol studies), and in earlier studies, rIFN A/D was shown both to protect mice (21) and cotton rats (23) from VSV-induced pneumonia and to be significantly more protective than the same preparations delivered intraperitoneally (22). For all of these

TABLE IV. PULMONARY TITERS OF INFLUENZA VIRUS IN LUNGS OF MICE EXPOSED TO SMALL PARTICLE AEROSOLS OF rIFN A/D 24 OR 4 hr BEFORE VIRUS CHALLENGE[a]

| Group | Duration of exposure (hr) | Interval between IFN and virus inoculation (hr) | Pulmonary virus titers ($log_{10}$/lung) | GMT ± SD[b] |
|---|---|---|---|---|
| Virus control (no interferon) | — | — | 6.8, 7.3, 7.3, 6.8, 7.3 | 7.1 ± 0.3 |
| rIFN A/D | 4 | 24 | 6.8, 7.3, 7.3, 5.3, 6.3 | 6.6 ± 0.8 |
| rIFN A/D | 4 | 4 | 7.3, 7.3, 5.3, 3.3, 5.3 | 5.7 ± 1.7 |

[a] All mice were inoculated intranasally with approximately two median lethal doses ($LD_{50}$) of influenza A/HK/68 virus.

[b] Geometric mean titer ± SD.

reasons better protection against influenza virus lung infection was expected in our studies than was seen. We do not know why exogenously administered recombinant alpha interferon did not have more of an effect on influenza virus infection in these studies. Although we did observe 100- to 1000-fold reductions in lung virus titers in individual mice within groups exposed to rIFN A/D as compared to untreated control animals, our general failure to improve upon earlier trials despite the use of better materials and aerosol machines suggests that the use of exogenously administered alpha interferons alone will not be effective in reducing influenza virus-induced respiratory disease in humans. Use of alpha interferons in conjunction with a second interferon (e.g., gamma interferon), or with another anti-viral may prove more effective against influenza virus lung infections than use of these materials alone. It has been reported by Czarniecki *et al.* (32) that a combination of alpha or beta interferon with gamma interferon had higher antiviral and immune modulatory effects than alpha interferons alone, and Luscri has reported that human interferons and ribavirin, amantadine, or rimantadine have synergistic antiviral effects against influenza virus infection *in vitro* [B. J. Luscri, Synergy between human beta interferon (IFN) and ribavirin (RVN). Abstr. Annual Meeting, American Society of Microbiology P15 (A84), 1982. B. J. Luscri, Synergistic effects of human interferons with either amantadine, or rimantadine. Abstr. Annual Meeting, American Society of Microbiology P22 (A130). 1983.] Testing of aerosols containing both human recombinant alpha and recombinant mouse gamma interferons against influenza virus lung infections is currently in progress.

This work was supported by a grant from Hoffman–La Roche, Inc., Nutley, N.J.

1. Scott GM, Butler JK, Cartwright T, Richards BM, Kingham JG, Wright R, Tyrrell DAJ. Interferon skin reactivity and pyrexial reactions. Lancet **2**:402–403, 1977.
2. Billiau A, De Somer P, Edy VG, DeClercq E, Heremans H. Human fibroblast interferon for clinical trials: Pharmacokinetics and tolerability in experimental animals and humans. Antimicrob Agents Chemother **16**:56–63, 1979.
3. Scott GM, Wallace J, Tyrrell DAJ, Cantell K, Secher DS, Stewart WE II. Interim report on studies on "toxic" effects of human leukocyte-derived interferon-alpha (HuIFN-$\alpha$). J Interferon Res **2**:127–130, 1982.
4. Adam F, Queseda JR, Gutterman JV. Neuropsychiatric manifestations of human leukocyte interferon therapy in patients with cancer. JAMA **252**:938–941, 1984.
5. Merigan TC, Reed SE, Hall TS, Tyrrell DAJ. Inhibition of respiratory virus infection by locally applied interferon. Lancet **1**:563–567, 1973.
6. Greenberg SB, Harmon MW, Couch RB, Johnson PE, Wilson SZ, Dacso CC, Bloom K, Quarles J. Prophylactic effect of low doses of human leukocyte interferon against infection with rhinovirus. J Infect Dis **145**:542–546, 1982.
7. Arnaoudova V. Treatment and prevention of acute respiratory virus infection in children with leukocytic interferon. Virologie **27**:83–88, 1976.
8. Dunnick JR, Galasso GJ. Clinical trials with exogenous interferon: Summary of a meeting. J Infect Dis **139**:109–123, 1979.
9. Finter NB. Of mice and men: Studies with interferon. In: Wolsteinholme GEW, ed. Ciba Foundation Symposium, London, Churchill, p204, 1982.

10. Finter NB. The assay and standardization of interferon and interferon inducers. In: Finter NB, ed. Interferons and Interferon Inducers. New York, Amer. Elsevier, 2nd ed, pp135–361, 1973.
11. Greenberg SB, Harmon MW, Johnson PE, Couch RB. Antiviral activity of intranasally applied human leukocyte interferon. Antimicrob Agents Chemother **14**: 596–600, 1978.
12. Solov'ev VD. The result of controlled observations on the prophylaxis of influenza with interferon. Bull WHO **41**:683–688, 1969.
13. Imanishi J, Karaki T, Sasaki O, Matsuo A, Oishi K, Pak CB, Kishida T, Toda S, Nagata H. The preventive effect of human interferon-alpha preparation on upper respiratory disease. J Interferon Res **1**:169–178, 1980.
14. Scott GM, Reed S, Cartwright T, Tyrrell D. Failure of human fibroblast interferon to protect against rhinovirus infection. Arch Virol **65**:135–139, 1980.
15. Goedel DV, Yelverton VE, Ullich A, Heyneker HL, Miozzari G, Holmes W, Seeburg P, Dull T, May L, Stebbing N, Crea R, Maeda S, McCandliss R, Sloma A, Tabor JM, Gross M, Familletti PC, Pestka S. Human leukocyte interferon produced by *E. coli* is biologically active. Nature (London) **287**:411–416, 1980.
16. Pestka S. The purification and manufacture of human interferons. Sci Amer **249**:37–43, 1983.
17. Krasikova VA, Poddubnaia AE, Ritova VV, Schastnyi EI, Kuznetsov VP, Shenderova YA, Frolova MI, Menshova AA. Interferogenesis and efficacy of refined leukocyte interferon use in acute respiratory viral infections in premature children. Pediatriya (Moscow) **5**:42–48, 1977. [in Russian]
18. Scott GM, Phillpotts RJ, Wallace J, Secher DS, Cantell K, Tyrrell DAJ. Purified interferon as protection against rhinovirus infection. Brit Med J **284**:1822–1825, 1982.
19. Scott GM, Phillpotts RJ, Wallace J, Gauci CL, Greiner J, Tyrrell DAJ. Prevention of rhinovirus colds by human interferon alpha-2 from *Escherichia coli*. Lancet **2**:186–188, 1982.
20. Wyde PR, Wilson SZ, Krammer MJ, Sun C-S, Knight V. Pulmonary deposition and clearance of aerosolized interferon. Antimicrob Agents Chemother **25**:729–734, 1984.
21. Wyde PR, Wilson SZ, Sun C-S, Knight V. Interferon aerosol suppression of vesicular stomatitis virus replication in lungs of infected mice. Antimicrob Agents Chemother **26**:450–454, 1984.
22. Wyde PR, Sun C-S, Wilson SZ, Knight V. Duration of effect of interferon aerosol prophylaxis of vesicular stomatitis virus infection in mice. Antimicrob Agents Chemother **27**:6–64, 1985.
23. Sun C-S, Wyde PR, Wilson SZ, Knight V. Efficacy of aerosolized recombinant interferon against vesicular stomatitis virus-induced lung infection in cotton rats. J Interferon Res **4**:449–459, 1984.
24. Wyde PR, Couch RB, Mackler BF, Cate TR, Levy BM. Effects of low- and high-passage influenza virus infection in normal and nude mice. Infect Immun **15**: 221–229, 1977.
25. Wyde PR, Wilson MR, Cate TR. Interferon production by leukocytes infiltrating the lungs of mice during primary influenza virus infection. Infect Immun **38**: 1249–1255, 1982.
26. Rehberg E, Kelder B, Hoal EG, Pestka S. Specific molecular activities of recombinant interferon and hybrid leukocyte interferons. J Biol Chem **257**:11497–11503, 1982.
27. Wilson SZ, Knight V, Moore R, Larson EW. Amantadine small-particle aerosol: Generation and delivery to man. Proc Soc Exp Biol Med **161**:350–354, 1979.
28. May KR. The collison nebulizer: Description, performance and application. Aerosol Sci Technol **4**:235–243, 1973.
29. Sokal RR, Rohlf EJ. Biometry. San Francisco, Freeman, pp40–57, 1981.
30. Takano K, Jensen KE, Warren J. Passive interferon protection in mouse influenza. Proc Soc Exp Biol Med **114**:472–475, 1963.
31. Blach-Olszewska Z, Magur R, Skurska Z. The influence of route of administration of interferon on its therapeutic effect. Arch Immunol Ther Exp **20**:491–496, 1972.
32. Czarniecki CW, Fennie CW, Powers DB, Estell DA. Synergistic antiviral and anti-proliferative activities of *Escherichia coli*-derived human alpha, beta, and gamma interferons. J Virol **49**:490–496, 1984.

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Received April 4, 1985. P.S.E.B.M. 1986, Vol. 181.
Accepted October 28, 1985.