Taylor & Francis
Taylor & Francis Group

Experimental Lung Research

ISSN: 0190-2148 (Print) 1521-0499 (Online) Journal homepage: https://www.tandfonline.com/loi/ielu20

Interferon Production in Rat Type II Pneumocytes and Alveolar Macrophages

Nicholas Hahon & Vincent Castranova

To cite this article: Nicholas Hahon & Vincent Castranova (1989) Interferon Production in Rat Type II Pneumocytes and Alveolar Macrophages, Experimental Lung Research, 15:3, 429-445, DOI: 10.3109/01902148909087869

To link to this article: https://doi.org/10.3109/01902148909087869

Interferon Production in Rat Type II Pneumocytes and Alveolar Macrophages

Nicholas Hahon and Vincent Castranova

ABSTRACT: *The time course and magnitude of interferon production induced by influenza virus were determined in type II pneumocytes and alveolar macrophages isolated from rats (Sprague-Dawley). Although the peak of interferon production was approximately 20 h in both alveolar cell types, it was more than three- to fourfold higher in type II cells than in macrophages. Dose-response relationships were noted between the virus multiplicity of induction as well as population numbers of either alveolar cell type and interferon yields. The viral-induced cytokines produced by rat type II cells and alveolar macrophages exhibit physiochemical and biological properties characteristic of interferons and, with respect to type II pneumocytes, mark their heretofore unrecognized capability to produce interferon. The best cross-species antiviral protection afforded by these rat interferons to cells of different origin, expressed as percentage of homologous species (100%), was as follows: guinea pig 50%, mouse 40%, and both human and monkey 0%. The heterologous antiviral activity by interferons from either rat alveolar macrophages or type II cells on α interferon-sensitive guinea pig cells suggests that these cytokines may be more appreciably related to the α-like interferon species. The growth of influenza and Sendai viruses was precluded in both rat alveolar macrophages and type II pneumocytes. The findings herein suggest that type II cells may be a major source of alveolar interferon for activating the antiviral state and modulating alveolar cell functions requisite for lung integrity.*

INTRODUCTION

Since the first reported isolation of type II pneumocytes [1], the granular cells located in the alveolar epithelial lining, extensive studies have characterized their physiologic attributes and functional activities, with the totality as yet incomplete [2–7]. Type II cells are now known to be the primary source of

From the Division of Respiratory Disease Studies, National Institute for Occupational Safety and Health, and the Departments of Pediatrics and Physiology, West Virginia University, School of Medicine, Morgantown, West Virginia.

Address all correspondence to Nicholas Hahon, NIOSH, 944 Chestnut Ridge Road, Morgantown, WV 26505-2888.

Received 1 May 1988; accepted 28 September 1988.

Experimental Lung Research 15:429–445 (1989)

pulmonary surfactant [8–10], progenitors of type I pneumocytes for repair of alveolar epithelium [11, 12], capable of xenobiotic transformation [13], and synthesizers of various collagenous proteins [14, 15]. Alveolar macrophages, being mobile and functionally different, may be found either interstitially, superposed on, or as part of the alveolar surface epithelium [16, 17]. Alveolar macrophages play an integral role in maintaining pulmonary health and, as predominant inflammatory and immune effector cells, they are active participants in both acute and chronic pulmonary diseases [18].

Interferon, a cellular induced protein possessing defensive properties and capable of biological response modification, that is, antiviral, anticellular, and immunoregulatory [19], has been reported to be produced and secreted by alveolar macrophages of both animal and human origin [20–22]. Interferon, by enhancing cell surface Fc receptors, can increase macrophage phagocytic and cytotoxic capacity [23]. While these defensive activities of macrophages may be modulated by interferon, there is evidence that endothelial cell proliferation and lung fibroblast replication [24, 25] along with inflammatory and other immune responses within the lung may also be influenced by secreted interferon [26, 27]. The broad range of biologic activities affected by interferon together with the selective and high sensitivity of the interferon induction process to insidious agents, which are capable of affecting the lungs adversely [28–30], presignify an accessory role for interferon in maintaining pulmonary integrity. In keeping with investigative efforts to understand more fully the relationships between different lung cells and the interferon system, the capability of rat type II pneumocytes to produce interferon was examined.

This report describes the viral induction in rat type II pneumocytes of an interferon-like protein, heretofore unreported. The time course and magnitude of its production by type II cells are compared to those of rat interferon produced by alveolar macrophages.

MATERIALS AND METHODS

Isolation of Lung Cells

Male Sprague-Dawley rats (200–300 g) were anesthetized with pentobarbital sodium (65 mg/kg body weight). The trachea was clamped to prevent aspiration of blood into the lungs, and the heart and lungs were removed en bloc. Blood was removed from the lungs by perfusing phosphate-buffered medium through the pulmonary artery and veins until the lungs blanched. The composition of phosphate-buffered medium (PBM) was (mM) 145 NaCl, 5 KCl, 9.35 Na_2HPO_4, 1.9 NaH_2PO_4, and 5.5 glucose (pH 7.4). The heart, adipose tissue, blood vessels, and loose connective tissue were then removed. Free alveolar macrophages were collected by lavaging the lung 10 times with ice-cold PBM. Lungs were lavaged once with 8 ml of enzyme solution—40 U/ml

type I, elastase, 0.1% collagenase, and 0.018% type II DNase (bovine pancreas) (Sigma, St. Louis, MO)—in PBM, then filled with 11 ml of enzyme solution, and incubated at 37°C for 30 min to free lung cells from the pulmonary epithelium. Thereafter, large airways and connective tissue were removed and the lungs were finely minced with a tissue chopper adjusted for a slice thickness of 0.5 mm. Digestion was arrested by suspending minced lung tissue in 20 ml of inhibitor solution (0.018% DNase and 25% fetal calf serum in PBM). This suspension was incubated at 37°C for 10 min with agitation to free lung cells from the tissue mass. The resultant suspension was strained through nylon mesh of successively smaller pore size (150, 330, and 440 mesh; New York Silk Stencil, New York, NY). Isolated cells in this whole-lung digest were then concentrated by centrifugation (1000 × g at 2°C) for 5 min and resuspended in PBM containing DNase (0.018%).

Type II pneumocytes were purified by centrifugal elutriation [4, 31]. This technique employs an elutriation rotor (model JE-6 developed by Beckman Instrument, Fullerton, CA) that allows sedimentation of cells under centrifugal force while simultaneously exposing the cells to a flow of buffered medium counter to the centrifugal force [32]. Because the sedimentation rate depends on cell size, different cell types can be separated by altering either rotor speed or flow rate [33].

Suspensions of isolated lung cells were loaded into the elutriation rotor at a flow rate of 10 ml/min and a rotor speed of 2000 rpm. Then 200 ml of PBM containing 0.5% bovine serum albumin was infused through the elutriator at a flow rate of 10 ml/min to remove cell fragments and small cells (mainly lymphocytes and leukocytes). Type II cells were then recovered at a flow rate of 18.9 ml/min. The type II cell-enriched fraction was then centrifuged (1000 × g at 2°C) for 5 min and the supernatant fluid was removed by aspiration. The cells were then washed once by resuspension in HEPES-buffered medium [(mM) 145 NaCl, 5 KCl, 1 $CaCl_2$, 10 Na HEPES, 5.5 glucose (pH 7.4)], and centrifuged. The cell pellet was again resuspended in HEPES-buffered medium. A Coulter model ZB electronic cell counter (Coulter Instrument, Hialeah, FL) was used to estimate yields of lung cells that were 19.0 ± 4.0 × 10^6 type II pneumocytes/rat with purity of 90.3 ± 1.3%.

Alveolar type II cells were routinely identified using a Coulter electronic cell sizing attachment as described previously [4]. We have verified their identify by the presence of lamellar bodies observed under transmission electron microscopy and by fluorescent microscopy using the lipophilic fluorescent dye phosphine 3R [4]. In addition, our laboratory has shown that type II cells isolated as described above synthesize surfactant materials as judged by the incorporation of [^{3}H]palmitate into disaturated phosphatidylcholines [5].

Alveolar macrophages were harvested from rat lungs by pulmonary lavage with PBM as described above. This procedure yielded 7.1 ± 0.5 × 10^6 alveolar macrophages/rat with purity of 91.2 ± 1.1%. Cellular viability was esti-

mated by trypan blue dye exclusion [34]. Viability exceeded 90% for both alveolar type II cells and macrophages.

Viruses and Cell Cultures

Virus strains and cell lines used in this study were obtained from the American Type Culture Collection (Rockville, MD). The Ao/PR/8/34 influenza and parainfluenza (Sendai) viruses, used for interferon induction and assay, respectively, were prepared from embryonated chicken eggs and assayed for virus infectivity by the immunofluorescent cell-counting technique [35]. The rat fetal lung fibroblast (RFL-6) cell line was used for assay of interferon, while cell lines of rhesus monkey kidney (LLC-MK_2), mouse connective tissue (L-929), and fetal guinea pig (104C1) were used in species specifity determinations of interferon. Cell lines were propagated in plastic tissue culture flasks (75 cm^2) with Eagle's minimum essential medium fortified with 100% Essential Vitamin Mixture (10 ml/l), 200 μM solution L-glutamine (10 ml/l), to which was added sodium bicarbonate (2.2 g/l), and fetal bovine serum to 10%. Cells were maintained with the aforementioned medium containing 0.5% fetal bovine serum.

Interferon Induction and Production

Experiments were duplicated using different lots of cell preparations. The procedure generally used for viral induction of interferon in rat alveolar cells and to monitor interferon production was carried out as follows: type II pneumocytes or alveolar macrophages, in concentrations ranging from 1.0×10^6 to 7.0×10^6 suspended in 5 ml growth medium, were seeded onto plastic flasks (25 cm^2). Influenza virus, which had been inactivated by ultraviolet irradiation for 45 s at a distance of 76.2 mm and wavelength of 253.7 nm, was added in a 1-ml volume in flasks. The multiplicity of induction (MOI), the ratio of virus to cells, ranged from 1.0 to 10 depending on the experimental design. Flasks were then incubated at 35°C for 2 h with gentle shaking on a platform rocker (Bellco Glass, Inc., Vineland NJ). Thereafter, the fluid content of flasks was decanted into sterile tubes and centrifuged at 170 × g for 5 min. The supernatant fluid, which contained residual virus, was decanted. The cells were suspended again in 5 ml growth medium and returned to the original flasks for incubation at 35°C for 20 h. To follow interferon production, flasks were removed at various time intervals inclusive from 0 to 48 h and stored at −80°C. Later, the content of flasks was thawed, and the fluids centrifuged at 1000 × g for 1 h, to sediment cellular debris. Supernatant fluids were passed through Millex filters, GV, 0.22 μm (Millipore Corp., Bedford, MA) to obtain sterile preparations, which were then stored at −80°C until they were assayed for interferon activity.

Interferon assay. An immunofluorescent cell-counting assay of interferon that had been described previously was used to determine the interferon potency of test samples [36]. Interferon-treated RFL—6 cell monolayers were challenged with 10^4 cell-infecting units of Sendai virus, and infected cells were visualized by direct fluorescent-antibody staining. The reciprocal of the interferon dilution that reduced the number of infected cells to 50% of the control served as the measure of interferon activity, that is, 50% infected cell-depressing dilution (ICDD50). Although there is no currently available rat reference interferon standard for use, 0.89 primate interferon units corresponded to 1.0 unit of National Institutes of Health reference standard Hu interferon β (G-023-902-527) using this assay system with human cell cultures (1-5c-4). A twofold decrease (50%) or increase (100%) of interferon production from the control, which exceeds 98% confidence limits of the assay [37], was considered significant.

Interferon Characterization

Virus-induced interferons from alveolar macrophages and type II cells were assayed for antiviral activity using RFL-6 cell monolayers grown on 15-mm coverslips. Cells were incubated with interferon preparations at 35°C for 20 h and then challenged with 10^4 cell-infecting units of either Sendai or influenza viruses. Infected cells were visualized by direct fluorescent-antibody staining with interferon activity indicated by reduction in the number of infected cells [36].

Physicochemical determinations made on interferon-like preparations included the following: stability at pH 2.0 for 24 h (4°C) as well as at 56°C (pH 7.1) for 1 h, solubility (nonsedimentation upon centrifugation at 100,000 × g for 1 h, nondissolution (dialyzed against HCl-KCl buffer pH 2.0 and then PBS, pH 7.1, at 4°C for 24 h).

Protein nature of rat interferons induced in both alveolar-type cells was determined by mixing 1 ml of interferon preparation with 5 mg trypsin in solution (2.5% trypsin, GIBCO Labs, Grand Island, NY) or with PBS (control) and incubating mixtures at 35°C for 1 h. Thereafter, test preparations received 4 mg trypsin (soybean) inhibitor (Sigma). After additional incubation (35°C, ½ h), text mixtures were assayed for interferon activity.

The dependency of virus-induced interferon on RNA synthesis, characteristic of interferons, was determined by using the inhibitor actinomycin-D (AD). Alveolar macrophage (5×10^6) cells in plastic flasks (25 cm^2) were first incubated with UV-irradiated influenza virus at 35°C for 1 h, and then with 4 μg AD (Sigma) for 2 h. After removal of virus and AD, 5 ml growth medium was added to cell cultures, which were incubated at 35°C for 20 h. Interferon content of medium and controls (no AD treatment) was assayed in the usual manner.

Priming, an additional activity trait of interferons, was determined by pre-

treating both rat type II pneumocytes (1.7×10^6) and alveolar macrophages (1.0×10^6) in 75-cm^2 flasks with 40 ICDD50 of rat interferon at 35°C for 2 h. Cells were then exposed to UV-irradiated influenza virus with MOI of 10 and incubated at 35°C for 1 h. Controls consisted of cell cultures treated similarly but without interferon priming. Interferon production at 35°C was followed for 48 h, during which time, at designated intervals, flasks were removed and stored at −80°C. Flask contents were processed as described earlier and assayed for interferon activity.

Rat anti-interferon globulin prepared in rabbits (Lee Biomolecular Research Laboratories, San Diego, CA) was used for neutralization of rat interferon activity employing constant serum and varied interferon concentrations [38]. Assessment of interferon neutralization was made by pretreating RFL-6 cell monolayers with interferon-serum mixtures for 20 h followed by challenge with Sendai virus via the procedure for interferon assay. The anti-interferon serum did not discriminate as to the α or β content of rat interferon.

Virus Multiplication

The growth of either influenza or Sendai viruses concomitant with interferon production was determined in both type II and alveolar macrophage cells (3.5×10^6) seeded separately onto 25-cm^2 flasks. Following adsorption of virus to cells (MOI of 1.0) at 35°C for 1 h on a platform rocker, the fluid content of flasks was decanted into tubes and centrifuged at $170 \times g$ for 5 min. The supernatant fluid, which contained residual virus, was decanted, and the cells were suspended again in 5 ml growth medium and returned to the original flasks for incubation at 35°C. At designated time intervals, from 0 to 48 h, flasks were removed and stored at −80°C. Thereafter, each flask was thawed (25°C) and frozen (−80°C) twice to disrupt cells, lightly centrifuged ($40 \times g$, 10 min) to sediment debris, and the supernatant fluid was divided in aliquots. One portion was assayed for virus content on 1-5c-4 cell monolayers [35], and the other was processed for interferon assessment using RFL-6 cell monolayers as described earlier.

RESULTS

The in vitro production of interferon in both rat type II pneumocytes and alveolar macrophages ($\sim 2.0 \times 10^6$ cells of each) after viral induction (MOI of 5.0) was followed for 48 h (Fig. 1). The time dependence of interferon production in both type II cells and macrophages was somewhat similar, with the peak interferon production occurring at 16–20 h. Throughout the time course the role of interferon production in type II cells slightly exceeded that of alveolar macrophages but, most significantly, the magnitude of interferon production was more than threefold higher in type II cells than in the latter

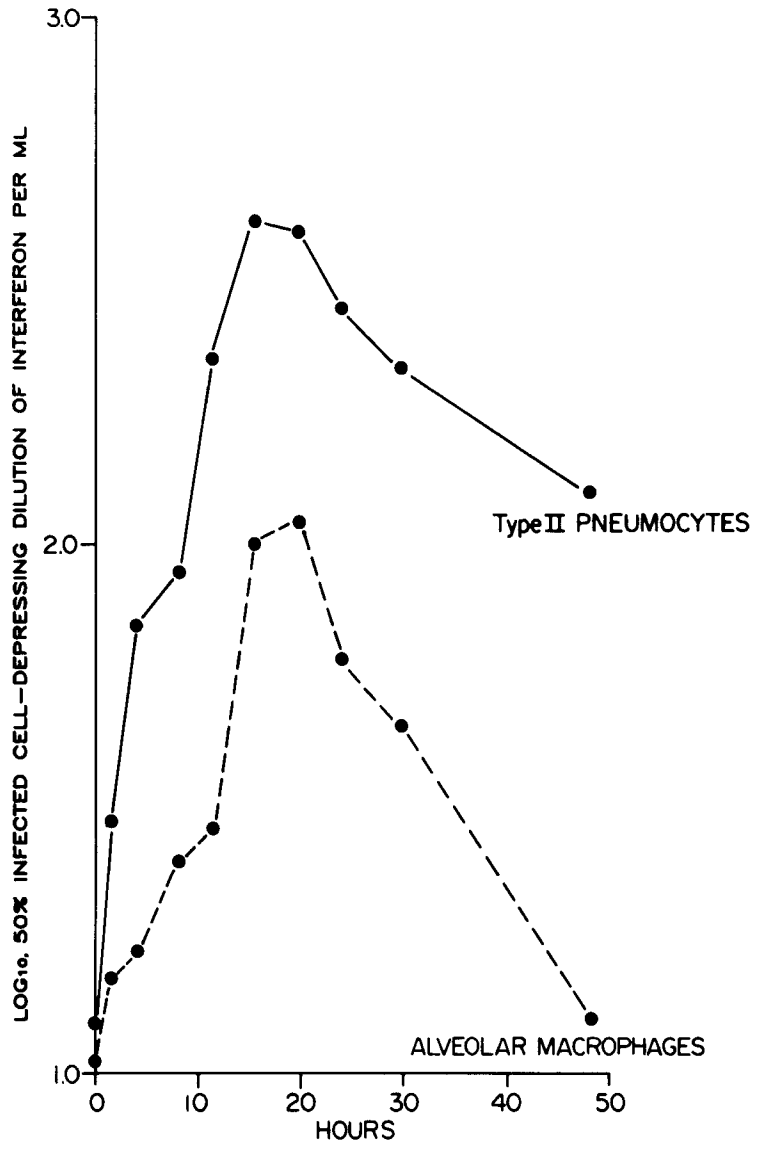

Figure 1 Influenza virus- (UV-irradiated Ao/PR/8/34) induced interferon production in rat alveolar type II (1.9×10^6) and macrophage (1.5×10^6) cells incubated at 35°C at multiplicity of induction of 5.0. Representative data of three determinations.

during the peak period. Thereafter, interferon production rapidly declined in both alveolar type cells.

To ascertain that the cytokines produced by rat type II pneumocytes and alveolar macrophages were interferons, the viral-induced substances were examined for properties characteristic of interferons, for example, antiviral activity, nonsedimentation, protein nature, stability at pH 2.0 and at 56°C, nondissolution, neutralization by anti-interferon serum, and sensitivity of the

Table 1 Properties of Rat Alveolar Cell-Derived Interferons

Interferon characteristics	Pre- → postinterferon titer[a] for rat interferon cell origin	
	Type II	Macrophage
Solubility: 100,000 × g, 1 h	110 → 105	78 → 78
Trypsin sensitive: 35°C, 1 h	70 → 5	19 → <5
Stability: pH 2, 4°C, 24 h	120 → 122	82 → 80
Stability: 56°C, 1 h	82 → 80	80 → 78
Nondialyzable	110 → 108	78 → 80
Neutralized by rat anti-interferon serum	120 → 20	82 → <5
Production inhibited by actinomycin-D	140 → <5	80 → <5
Antiviral activity[b]		
Sendai virus	85%	82%
Influenza virus	81%	86%

[a]Reciprocal of interferon titer expressed as infected cell-depressing dilution/ml.

[b]Expressed as percent reduction of virus from control.

interferon induction phase (RNA synthesis) to actinomycin-D (Table 1). The cytokines induced in both these alveolar cell types exhibited all the preceding characteristics associated with α/β interferons.

An additional criterion for acceptance as an interferon is priming. This non-antiviral function of interferon involves the exposure of cells to small amounts of interferon prior to administration of a viral or nonviral inducer. As a consequence, interferon is produced more quickly and in greater quantity. Type II pneumocytes primed with a small amount of rat interferon exhibited both features with respect to rapidity (at least 4 h earlier) and enhanced (threefold greater) interferon produced in comparison to control (nonprimed) cells (Fig. 2). Although not depicted graphically, and similar to that shown with type II cells, priming of alveolar macrophages also enhanced interferon production relative to time (4 h earlier) and magnitude (threefold greater) than in control (nonprimed) macrophages.

To define the cellular host range for rat interferons derived from type II cells and alveolar macrophages, their heterologous antiviral activity was examined in established cell lines of the following origins: guinea pig, mouse, human, and monkey. Rat fibroblast lung cells (RFL-6) represented the homologous (control) cell line. Results (Table 2) show that interferons from the two rat alveolar cell types exhibited the most antiviral protection when tested on the rat cell line. The best cross-species antiviral activity of these rat derived interferons was exhibited in guinea pig cells (50%) and was succeeded by

mouse cells (40%). There was no antiviral protection observed by these interferons to either human or monkey cell cultures.

The relationship between multiplicity of induction (MOI) and interferon yield was appraised by interacting various concentrations of UV-irradiated influenza virus with a constant number of type II pneumocytes (1.5×10^7 cells) in the manner prescribed for interferon induction. Results (Fig. 3) show

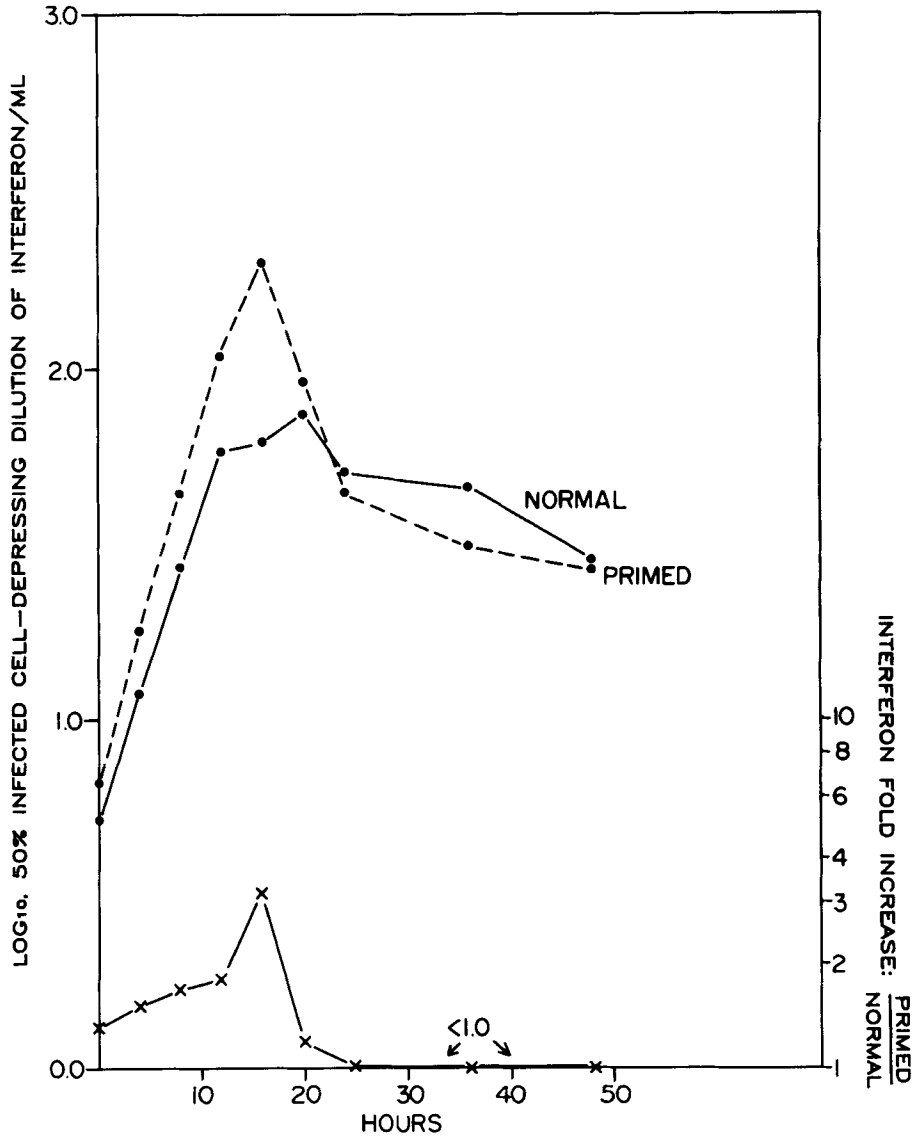

Figure 2 Priming of rat type II pneumocytes (1.7×10^6), pretreated with 40 ICDD50 of rat interferon for 2 h, before influenza virus- (UV-irradiated Ao/PR/8/34) induced interferon production at 35°C with multiplicity of induction of 10. Control cell cultures treated similarly with the exception of priming treatment. Representative data of three determinations.

Table 2 Defined Cellular Host Ranges for Antiviral Activity of Rat Interferons from Type II Pneumocytes and Alveolar Macrophages

Host cells and species origin	Percent homologous antiviral activity of alveolar cell-derived interferon	
	Type II	Macrophage
RFL-6 (rat)	100[a]	100[a]
104C1 (guinea pig)	52.3 ± 3.8[b]	49.3 ± 7.3
L-929 (mouse)	40.8 ± 5.3	40.6 ± 9.9
1-5c-4 (human)	0.0 ± 0.0	0.0 ± 0.0
LLC-MK_2 (monkey)	3.2 ± 1.6	0.0 ± 0.0

[a]Homologous titers of rat alveolar interferons representative of 100% antiviral activity as derived from type II and macrophage cells were 260 and 150, respectively, and expressed as 50% infected cell-depressing dilution of interferon/ml.

[b]Mean of two determinations expressed as percent of homologous titer ± standard error of mean.

a dose-response relationship over an MOI range from 0.002 to 1.3 with direct increase of interferon yields.

The magnitude and comparative relationship between cell numbers of alveolar macrophages and type II pneumocytes and interferon yields was investigated. The cell numbers tested ranged from 5.2×10^3 to 1.0×10^7 with a constant MOI of 1.0. The interferon yield increased linearly with increased numbers of either alveolar cell types (Fig. 4). However, on a per cell basis, the interferon yield from type II cells was at least fourfold more than that from alveolar macrophages. This difference in magnitude was exhibited throughout the range of cell populations tested and reaffirmed the higher interferon production capability of type II cells over that of alveolar macrophages.

The in vitro growth of influenza (Ao/PR/8/34) and Sendai viruses in either rat alveolar macrophages or type II pneumocytes was determined concomitant with interferon production for a duration of 48 h. The multiplicity of infection was 1.0. Results (Fig. 5) show that in neither alveolar macrophages nor type II cells was the multiplication of either influenza or Sendai viruses supported as evident by the precipitous decline in virus throughout the interval of 48 h. Interferon production, after a brief lag period, reached a peak level in both alveolar cell types at 20 h. At this time, it was again fourfold higher in type II cells than in macrophages. While it appears that the corresponding increase in interferon production was responsible for the preclusion of virus growth, the sharp initial decline of virus at a time when interferon production was inchoate indicates that other factors are involved.

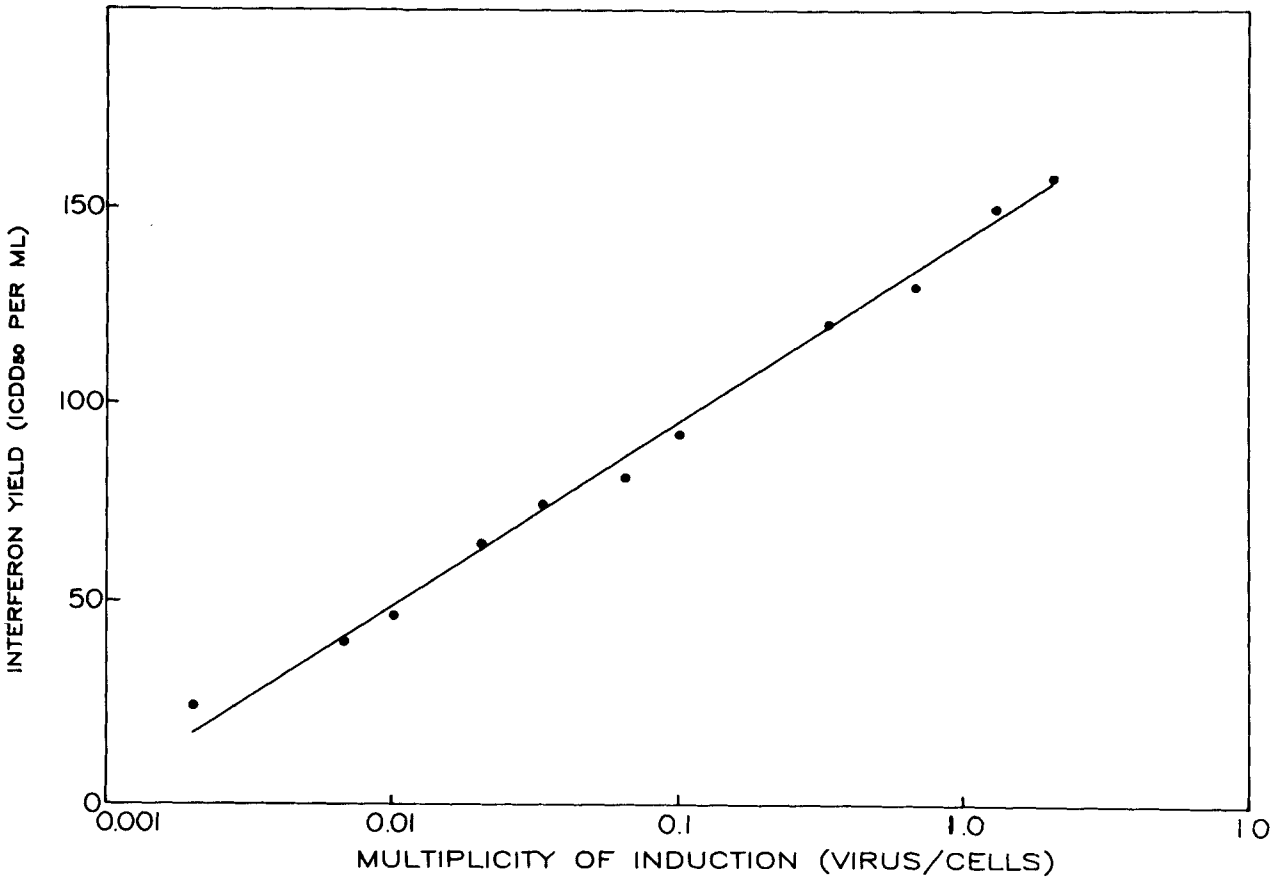

Figure 3 Relationship between multiplicity of induction (UV-irradiated influenza Ao/RP/8/34 virus/type II rat pneumocytes) and interferon yield (50% infected cell-depressing dilution of interferon/ml), with incubation at 35°C for 20 h. Representative data of three determinations.

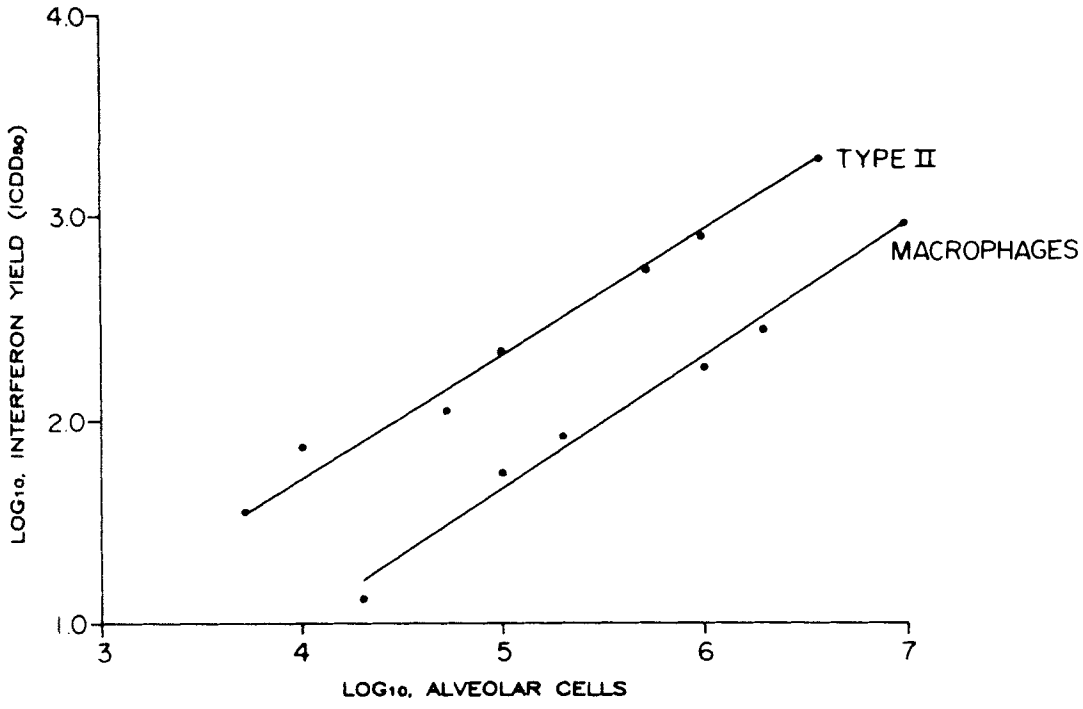

Figure 4 Comparative relationship between numbers of rat alveolar macrophages and type II pneumocytes and interferon yield (50% infected cell-depressing dilution of interferon/5 ml of cell culture medium) with incubation at 35°C for 20 h and multiplicity of induction of 1.0. Representative data of three determinations.

DISCUSSION

The findings reported herein demonstrate that rat type II pneumocytes, in response to a viral inducer, are active producers of interferon. In comparison to rat alveolar macrophages, interferon production by type II pneumocytes exceeded that of the former by at least three- to fourfold in magnitude (Figs. 1, 4, and 5). This capability, more often associated with cellular defense potential, constitutes another physiologic activity attributable to type II pneumocytes added to those already known [8–15].

The viral-induced cytokines produced by both rat alveolar macrophages and type II pneumocytes exhibited (Table 1) many of the biological and physiochemical properties characteristic of interferons [39, 40]. Among these rat alveolar interferons was "priming" (Fig. 2), a non-antiviral function [41]. DNA-dependent RNA synthesis, a requirement of virus-induced interferon synthesis, was also inhibited by actinomycin-D in both alveolar cell types [42]. Additionally, the antiviral activity of rat interferon from both alveolar cell types was neutralized by rat anti-interferon serum. The unavailability of specific α, β, or γ rat anti-interferon serum precluded a distinction of interferon species. The best cross-species antiviral protection of note was afforded

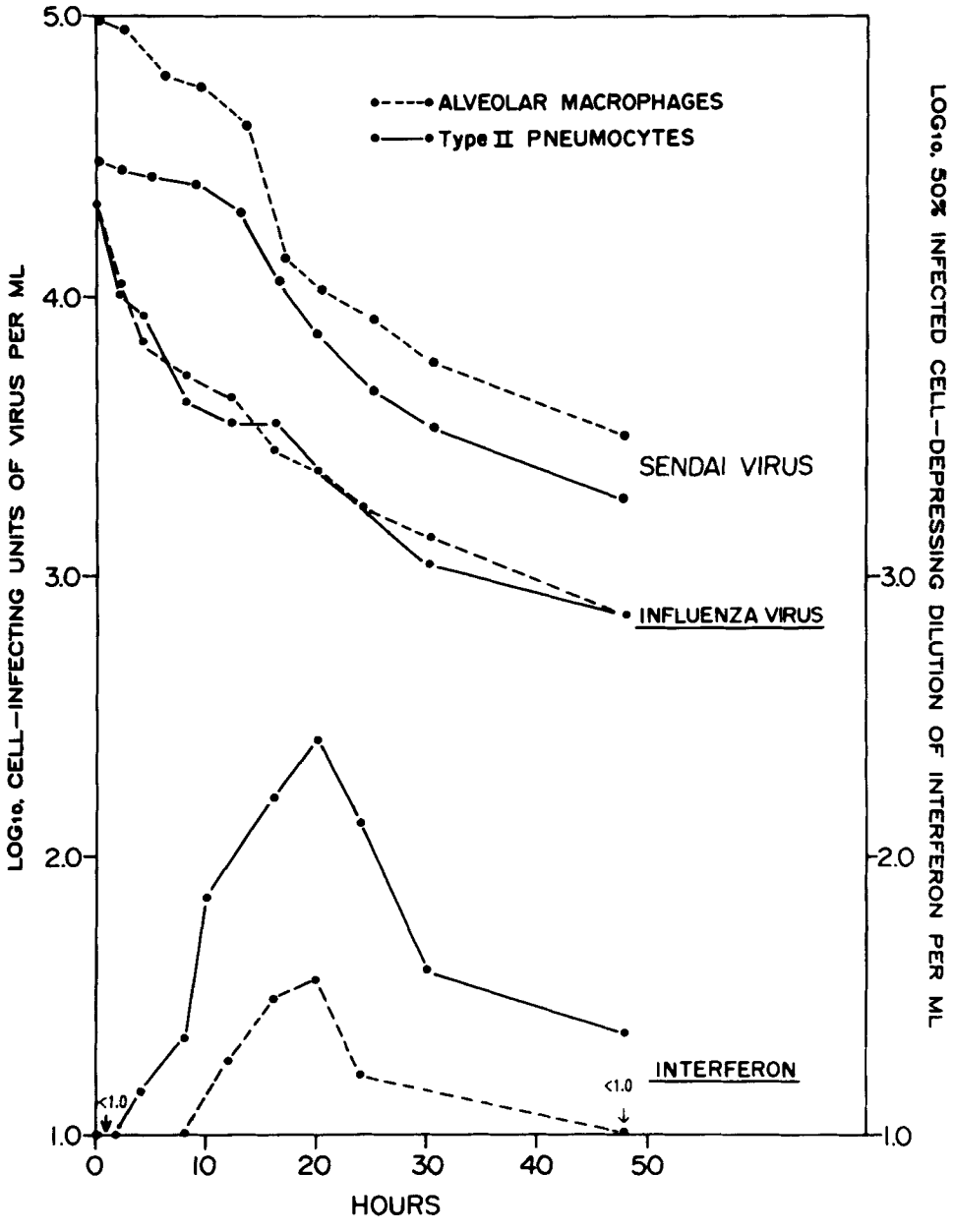

Figure 5 Decline of influenza Ao/PR/8/34 and Sendai viruses concomitant with interferon production in rat alveolar macrophages (3.0×10^6) and type II pneumocytes (3.5×10^6 incubated at 35°C with multiplicity of induction of 1.0. Mean of two determinations.

by both rat alveolar interferons on guinea pig cells, ~50% protection. This correlates with the antiviral protection of guinea pig cells attained with interferon derived from rat embryo fibroblasts [43]. Because guinea pig cells are particularly sensitive to the antiviral activity of α interferon [44], it would appear, although not exclusive, that the rat interferons produced by alveolar macrophages and type II pneumocytes are closely related to α-like species of interferon. These interferons exhibited activities and properties concordant to those reported for rat interferons that were virus-induced in a rat tumor glial cell line C6 [45].

A direct dose-response relationship was observed between the virus multiplicity of induction (from 0.002 to 1.3) and interferon production; a linear relationship was noted between cell population numbers (from 5.2×10^3 to 1.5×10^7) of either alveolar cell types and yields of interferon. Ideally, an MOI of 1.0 will ensure maximal interferon yields under efficient conditions for virus-cell adsorption. The dose-response relationship is obviously self-limiting with excessive MOI. To achieve high interferon yields, cell population numbers are a significant factor. As evident in Fig. 4, rat interferon yields from either alveolar cell types with a constant MOI of 1.0 increased linearly as the cell population numbers increased. Rat type II cells were highly efficient producers of interferon. With equivalent cell numbers, approximately fourfold more interferon was produced by type II cells than by alveolar macrophages.

The ability of rat alveolar macrophages and type II pneumocytes to support the growth of either influenza or Sendai viruses was not in evidence. Factors other than interferon alone may account for the preclusion of virus growth in these cells, despite evidence of concomitant production of interferon (Fig. 5). This is suggested by the following: (1) the initial virus decline occurred when interferon production was minimal, and (2) the similar patterns of virus decline occurred in both alveolar cell types despite their different magnitudes of interferon production. The lack of either cell surface receptors conducive for virus attachment or ineffective virus-cellular intrareactions at the molecular level requisite for virus replication and assembly are other considerations that may account for the nonviral susceptibility of both alveolar macrophages and type II cells. The rat is not normally susceptible to influenza virus infection but is infectable by Sendai virus [46]. The foci of Sendai virus multiplication in the respiratory tract of rats most likely occurs in parenchymal lung cells other than alveolar macrophages and type II cells.

Although type II cells comprise approximately 15% of the total lung cell population and account for a small part (4%) of total alveolar epithelial surface area, they make up from 60 to 66% of alveolar epithelial cells by number [47, 48]. In view of this dominance and the magnitude of interferon production therein, type II cells could be one of the major sources of alveolar interferon for activating the antiviral state, activating and enhancing phagocytosis,

and modulating immune mechanisms and accessory alveolar cell functions requisite for lung integrity.

We thank James A. Booth and William H. Pailes for their excellent technical assistance.

REFERENCES

1. Kikkawa Y, Yaneda K: The type II epithelial cell of the lung. I. Method of isolation. Lab Invest 30:76, 1974.
2. Weibel ER, Gehr P, Haies D, Gil J, Bachofen M: The cell population of the normal lung. In: Bouhuys A, ed., Lung Cell in Disease. Amsterdam, Elsevier/North Holland, 1976, p. 3.
3. Gail DB, Lenfant CJM: Cells of the lung: Biology and clinical applications. Am Rev Respir Dis 127:366, 1983.
4. Jones GS, Miles PR, Lantz RC, Hinton DE, Castranova V: Ionic content and regulation of cellular volume in rat alveolar type II cells. J Appl Physiol 53:258, 1982.
5. Miles PR, Wright JR, Bowman L, Castranova V: Incorporation of [^{3}H]palmitate into disaturated phosphatidylcholines in alveolar type II cells isolated by centrifugal elutriation. Biochim Biophys Acta 753:107, 1983.
6. Dobbs LG, Geppert EF, Williams MC, Greenleaf RD, Mason RJ: Metabolic properties and ultrastructure of alveolar type II cells isolated with elastase. Biochim Biophys Acta 618:510, 1980.
7. Batenburg JJ: Isolated type II cells from fetal lung as model in studies on synthesis and secretion of pulmonary surfactant. Lung 158:177, 1980.
8. Askin FB, Kuhn CK: The cellular origin of pulmonary surfactant. Lab Invest 25:260, 1971.
9. Goerke J: Lung surfactant. Biochim Biophys Acta 344:241, 1974.
10. King RJ: Utilization of alveolar epithelial type II for the study of pulmonary surfactant. Fed Proc 38:2637, 1979.
11. Adamson IYR, Bowden DH: Derivation of type 1 epithelium from type 2 cells in the developing rat lung. Lab Invest 32:736, 1975.
12. Adamson IYR, Bowden DH: The type II cell as a progenitor of alveolar epithelial regeneration. Lab Invest 30:35, 1974.
13. Devereux TR, Fouts JR: Xenobiotic metabolism by alveolar type II cells isolated from rabbit lung. Biochem Pharmacol 30:1231, 1981.
14. Sage H, Farin FM, Striker GE, Fisher AB: Granular pneumocytes in primary culture secrete several major components of the extracellular matrix. Biochemistry 22:2148, 1983.
15. Crouch EC, Moxley MA, Longmore W: Synthesis of collagenous proteins by pulmonary type II epithelial cells. Am Rev Respir Dis 135:1118, 1987.
16. Karrer HE: The ultrastructure of mouse lung: The alveolar macrophage. J Biophys Biochem Cytol 4:693, 1958.
17. Sorokin S: Properties of alveolar cells and tissues that strengthen alveolar defenses: Symposium on pulmonary response to inhaled material. Arch Intern Med 126:450, 1970.

18. Hunninghake GW, Gadek JE, Kawanami O, Ferrans VJ, Crystal RG: Inflammatory and immune processes in the human lung in health and in disease. Evaluation by bronchoalveolar lavage. Am J Pathol 97:149, 1979.
19. Taylor-Papadimitriou J: Effects of interferon on cell growth and function. In: Gresser I, ed., Interferon, Vol. 2. New York, Academic Press, 1980, p. 13.
20. Acton JD, Myrvik QN: Production of interferon by alveolar macrophages. J Bacteriol 91:2300, 1966.
21. Kolot FM, Baron S, Yeager H Jr, Schwartz SL: Comparative production of interferon by explanted lymphoreticular tissue and alveolar macrophages from rabbits and humans. Infect Immun 13:63, 1976.
22. Nugent KM, Glazier J, Monick MM, Hunninghake GW: Stimulated human alveolar macrophages secrete interferon. Am Rev Respir Dis 131:714, 1985.
23. Balkwill FR: The regulatory role of interferons in the human immune response. In: Taylor-Papadimitriou J, ed., Interferons, Their Impact in Biology and Medicine. New York, Oxford University Press, 1985, p. 61.
24. Garrett KC, Brady MS, Hemken CL, Hampson F, Shasby SS, Shasby DM, Hunninghake GW: Regulation of endothelial cell proliferation by interleukin-1 and immune interferon (Abstract). Clin Res 32:346A, 1984.
25. Hunninghake GW, Nugent K, Hemken C, Brady M, Hampson F: Mediators released by human lung cells are potent growth factors for human lung fibroblasts (Abstract). Clin Res 32:563, 1984.
26. Hammer SP, Winterbauer RH, Bockess D, Remington F, Sale GE, Meyers JD: Endothelial cell damage and tubuloreticular structures in interstitial lung disease associated with collagen vascular disease and viral pneumonia. Am Rev Respir Dis 127:77, 1983.
27. Gresser I, Maury C, Tovery M, Morel-Maroger L, Pontillon F: Progressive glomerulonephritis in mice treated with interferon preparations at birth. Nature (Lond) 263:420, 1976.
28. Hahon N: Effect of coal rank on the interferon system. Environ Res 30:72, 1983.
29. Hahon N, Booth JA: Coinhibition of viral interferon induction by benzo[a]pyrene and chrysotile asbestos. Environ Res 40:103, 1986.
30. Hahon N, Booth JA: Silicate minerals and the interferon system. Environ Res 43:395, 1987.
31. Jones GS, Miles PR, Lantz RC, Hinton DE, Shreve JM, Castranova V: Effects of metallic ions on the oxygen consumption of isolated type II pneumocytes. In: Mahlum DD, Gray EH, Felix WD, eds., Proceedings 20th Annual Hanford Life Sciences Symposium: Coal Conversion and the Environment—Chemical, Medical and Ecological Considerations. Washington, DC, Department of Energy, 1981, p. 352.
32. Grabske RJ: Separating cell populations by elutriation. Fractions (Ab. 1). Palo Alto, CA, Beckman Instruments, 1978, p. 1.
33. Lindahl PE: Principle of a counter-streaming centrifuge for the separation of particles of different sizes. Nature (Lond) 161:645, 1948.
34. Phillips HJ, Terryberry JE: Counting actively metabolizing tissue cultured cells. Exp Cell Res 13:341, 1957.
35. Hahon N, Booth JA, Eckert HL: Cell attachment and penetration by influenza virus. Infect Immun 7:341, 1973.

36. Hahon N: Hemadsorption and fluorescence determinations for assay of virus-yield induction of interferon. In: Pestka S, ed., Methods in Enzymology, Vol. 78, Interferons Pt A. New York, Academic Press, 1981, p. 373.
37. Hahon N, Booth JA, Eckert HL: Interferon assessment by the immunofluorescent, immunoperoxidase, and hemadsorption cell-counting techniques. Arch Virol 48:239, 1975.
38. WHO Expert Committee: Standardizatoin of interferons. Antiviral Res 14:75, 1984.
39. Lockart RZ Jr: Criteria for acceptance of a viral inhibitor as an interferon and a general description of the biological properties of known interferons. In: Finter NB, ed., Interferons and Interferon Inducers. Amsterdam, North Holland, 1973, p. 11.
40. Stewart WE II: Interferons: Their purification and characterization. In: The Interferon System. New York, Springer-Verlag, 1981, p. 134.
41. Stewart WE II: Non-antiviral actions of interferons. In: The Interferon System. New York, Springer-Verlag, 1981, p. 223.
42. Burke DC: The production of interferons by viruses: Mechanisms. In: Finter NB, ed., Interferons. Amsterdam, North Holland, 1966, p. 55.
43. Davis, CT, Rusckowski M, Murasko DM: Biological similarities of rat fibroblast interferon to human and mouse alpha interferons. J Gen Virol 66:1961, 1985.
44. Borecky L, Fuchsburger N, Hajrecka V: Electrophoretic profiles and activities of human interferons in heterologous cells. Intervirology 3:369, 1974.
45. Poindron P, Coupin G, Selinger D, Fauconnier B: Induction of rat interferon in vivo and in vitro—Physiochemical and biological characterization. In: Pestka S, ed., Methods in Enzymology, Vol. 78, Interferons Pt A. New York, Academic Press, 1981, p. 165.
46. Jacoby RO, Bhatt PN, Jonas AM: Viral diseases. In: Baker HJ, Lindsey JR, Weisbroth SH, eds., The Laboratory Rat, Vol. I. New York, Academic Press, 1979–1980, p. 294.
47. Haies DM, Gil J, Weibel ER: Morphometric study of rat lung cells. I. Numerical and dimensional characteristics of parenchymal cell population. Am Rev Respir Dis 123:533, 1981.
48. Harwood JL, Desai R, Hext P, Tetley T, Richards R: Characterization of pulmonary surfactant from ox, rabbit, rat, and sheep. Biochem J 151:707, 1975.