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Passive Interferon Protection in Mouse Influenza. (28706)

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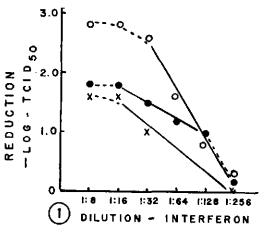
Since its discovery(1), the majority of reports on interferon have been concerned with the basic nature of this protein, the optimal conditions for its production and assay, and its mechanism of action(2). There have been comparatively few efforts to influence infection of the whole animal through administration of interferon before or after exposure to virus(3). The following communication describes experiments demonstrating that intranasal administration of interferon will enhance resistance of mice to infection with influenza virus.

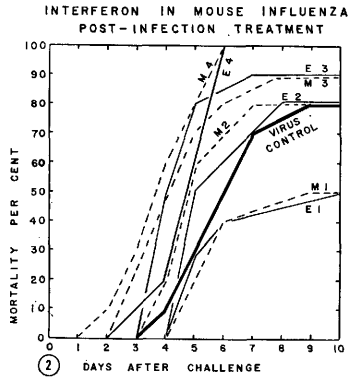
Methods. Interferon was prepared in eggs and mice using the GL strain of Type B influenza as infective virus. Allantoic fluids and mouse lung suspensions were clarified by low-speed centrifugation. Virus in these fluids was then absorbed with chick red blood cells following which the pH was adjusted to 2.0 to destroy any remaining virus. The fluids were next sterilized by filtration and stored at —70°C. The interferon from allantoic fluid was freeze-dried and concentrated by reconstituting in one-fifth original volume of distilled water, but mouse lung materials were not tested as concentrates. Interferon was assayed by titrating varying amounts of interferon against serial 10-fold dilutions of egg-adapted vaccinia virus in a tube culture system. Triplicate tubes were exposed to 0.3 ml of interferon for 18 hours at 37°C; the virus dilution was then added in 1 ml of Eagle's basal medium in Earle's saline (EBME). Interferon potency was established as the maximal dilution which would reduce the $TCID_{50}$ of vaccinia one log on the 6th day of observation. The standardized interferons were then used to treat mice experimentally infected with the B/GL strain of influenza virus.

Results. Tissue culture assays. In our first experiments interferon was assayed against vaccinia by a standard plaque reduction method(4). However, since titers were found to be 4- to 8-fold less than with the tube culture method, the latter was adopted for assays. That vaccinia virus infection of chick embryo tissue culture (CETC) provided an adequately sensitive system can be seen from dose-response data in Fig. 1. Although this interferon was most active in reducing the infective titer of the homologous B/GL virus, it was also operative against vaccinia and Sendai(5) and dilution endpoints were similar with the 3 viruses. The potency of that lot of egg interferon was calculated to be 380 units/ml since 0.3 ml was used to treat the cultures. It is of interest that within certain dilution ranges the relationship appears to be linear(6), and a similar dose response has been observed when this interferon was measured for its capacity to reduce the LD_{50} of mouse-adapted B/GL strain in mice. Interferon from mouse lung preparations was always at lower titer in the CETC-vaccinia assays and varied in the range of 6–24 units/ml. However, as shown below, their capacity to protect mice against influenzal infections was equal to that of the most active interferons from egg fluids.

Protective effect of interferons when ad-



① DILUTION - INTERFERON



② DAYS AFTER CHALLENGE

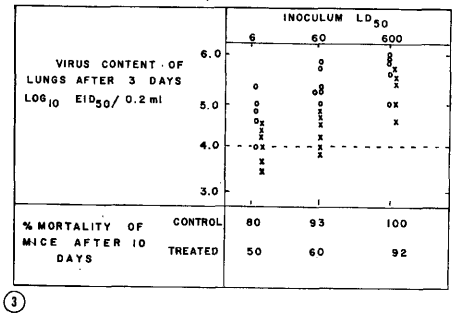


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FIG. 1. Dose response data from chick embryo tissue culture assays of concentrated interferon from B/GL infected egg fluids, titrated against vaccinia (●), B/GL influenza (○), and Sendai (×).

FIG. 2. Cumulative death rates in influenza-infected mice treated with interferon from egg fluids (E) or mouse lungs (M) once (1) before virus and daily (2, 3 and 4) for up to 3 times after infection.

FIG. 3. Comparison of B/GL influenza virus titer 3 days post-infection in lungs of individual mice, control (○) or egg interferon treated (×), and mortality incidence after 10 days' observation.

ministered to mice. Optimal dosage and timing of interferon administration have been explored in a large series of experiments employing groups of 10–40 mice per dilution. Using light ether anesthetization, 0.05 ml of undiluted interferon was instilled intranasally one hour before virus and at daily intervals one to 3 times after infection. Results from a typical experiment are plotted in Fig. 2. When interferons from either egg or mouse lung fluids were given in only one dose, a protective effect was found. In contrast, multiple treatments post-infection resulted in an increased mortality, a phenomenon probably due to the fluid effect described by Taylor (7) as it could be duplicated by repeated intranasal instillation of salt solutions into infected mice. When interferon was administered daily for 4 days prior to infection, the fluid effect was not evident although resistance was not markedly increased as anticipated by possibly building up the pulmonary concentration of interferon through repeated pre-treatment of the animals. Accumulated data from a series of experiments, in which 1130 mice were treated as indicated, are shown in Table I. Between 8 and 16 LD_{50} of virus in mouse lung suspension was used which produced a mortality of approximately 80% in controls and a survival time of 6.2 days. When a single dose of interferon was employed, mortality was reduced to 46%, although survival time of dying mice was not prolonged. With repeated treatments after infection, the fluid effect predominated so that the mortality rose with a decrease in survival time.

Aerosol treatment. When it was learned that intranasally administered interferon was effective when given once prior to infection while repeated instillations after virus inoculation only "drowned" the animals, aerosol inhalation was tested as a means of treatment. It was determined that aerosolization of 2 ml of interferon for 20 minutes with

TABLE I. Role of Dosage Schedule in Interferon Effect on Type B Influenza Infections of Mice.

Pre- and post-infection treatment schedule	Cumulative mortality (deaths/total)	%	Mean survival (days)
None—Virus only	435/540	81*	6.2
Prior, once	135/285	46*	6.1
Idem and once post daily	52/72	75	6.3
" and twice post daily	64/78	82	5.2
" and thrice post daily	156/156	100	4.7

* $P < .01$.

TABLE II. Relation of Pre-Infection Treatment to Influenza Virus Content of Mouse Lung Pools.

Treatment	Mortality	Days 1	2	3	4	5
None	16/20 (80%)	3.2*	6.0	9.0	8.7	6.7
Egg interferon, 19.2 units/.05 ml	8/20 (40%)	2.7	3.4	6.4	5.7	5.2
Mouse lung interferon, 1.2 units/.05 ml	9/18 (50%)	2.4	4.7	7.0	6.2	5.7
Normal mouse lung suspension	15/19 (79%)	4.0	7.3	8.2	6.2	6.1
Heated egg interferon	16/20 (80%)	3.7	8.3	8.0	6.0	4.7

* Log_{10} EID_{50}/ml. Challenge virus was 8 LD_{50} of mouse-adapted B/Gl strain.

groups of 10 mice in a container was as effective in decreasing mortality as instillation of 0.05 ml per mouse. The virus (16 LD_{50}) was given by the usual intranasal method. Variations in dosage schedule included (a) once a day for 4 days prior to virus, (b) once 24 hours before virus, (c) once 1 hour before virus, and (d) once 1 hour before and daily for 3 days after virus inoculation. Of these procedures, only (a) and (c) were effective and reduced mortality to 50% and 40%, respectively, while other mice, including the untreated groups, experienced 80–90% mortality. Thus, repeated daily pretreatments yielded better protection than did a single treatment 24 hours before virus. Confirming results obtained by fluid administration of interferon, the mice treated one hour before exposure to virus were most resistant and those to which interferon was administered daily post-infection were not protected.

Virus content of lungs. Lung tissue from 5 mice was pooled at daily intervals for 5 days after infection. As controls, some animals were treated with normal lung emulsion and others with egg interferon which had been heated 2 hours at 80°C to destroy activity. As shown in Table II, mortality for all control animals was approximately 80% compared with 40–50% in treated mice. In the latter, the virus titers of lung suspensions were significantly lower on the second and third days, but not so after 4 or 5 days. The question immediately arose as to whether a similar situation existed in lungs of individual mice. This was studied by giving virus at levels of 6, 60, and 600 LD_{50} and instillation of interferon one hour prior to infection. It was found that in each group the amount of virus in the lungs from interferon-treated mice was less than in the controls; however, this difference depended upon the challenge dose. As suggested by the data in Fig. 3, the significant role of interferon activity in this system is to restrict the amount of viral replication. The fate of each mouse is probably established by the concentration of virus in the lung on the third day. When the data were divided based on a titer of 10^4 EID_{50} as the critical point, the ratios for each group correlated well with the incidence of mortality of mice observed for 10 days.

Discussion. The finding that pre-infection treatment with exogenous interferon will protect mice against low challenge doses of influenza virus supports the thesis that the inhibitor must act upon cell metabolism before virus does in order to be effective. No protection was found when large doses of infective virus were used. As a possible explanation for this phenomenon, we suggest that with a low virus-cell multiplicity new interferon made during each replication cycle augments that given experimentally and protects an additional number of otherwise susceptible cells. In contrast, when lung tissue is exposed to large doses of virus, a major portion of the cells is immediately infected and damaged. Thus, there is little chance for interferon to play a significant role as few cells are protected by the relatively small amount of administered interferon and the endogenous interferon arrives too late.

These results are not easily reconciled with the report of Link *et al*(8) that interferon administered 4 hours prior to infection of mice with A/1 influenza virus enhanced virus multiplication, while doses given 4 hours prior to and 24 and 48 hours after infection re-

sulted in delayed viral replication. They also found no virus in the lungs of untreated control infected mice on days 3, 4, and 5, but virus was present in high titer on days 2 and 6 after infection. Unfortunately only 3 animals were sampled at a given interval.

In our experiments no mice were completely free of virus in lungs at autopsy, although there was often less in the tissues of the treated group. Despite use of concentrated interferon and multiple pre-infection treatments, protection was not complete even with small doses of challenge virus. As it seems unlikely that this is due to mechanical differences in handling animals, we must assume that the mice varied in sensitivity to interferon and its production. Possibly, resistance developed only in those animals whose cells quickly responded to interferon and also had the capacity to produce endogenous interferon as a result of viral replication.

Interferon produced in chick embryo cells is also active in cells of a heterologous species, mouse lung tissue. Thus, although interferon from mouse lungs was less active than egg interferon in chick embryo tissue cultures infected with vaccinia virus, essentially equal potency was measured in the influenza-mouse lung model. The species specificity previously reported(9) apparently is reflected only as a quantitative difference.

The work of Isaacs and Hitchcock(10) suggested that augmentation of endogenous interferon, produced as a result of infection, with externally added interferon would reduce the mortality of influenza virus infection in mice.

Summary. Interferon prepared from influenza virus infected eggs or mouse lungs when intranasally administered protected mice against a mouse-adapted Type B influenza challenge. To be effective, the interferons had to be given before virus, and treatments delayed 24 hours after virus exposure served only to aggravate the infection. The concentration of virus used in the challenge was critical. The effect was best observed when the dosage was approximately 10 LD_{50}.

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Angiotensin II and Erythropoiesis.* (28707)

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The relationship of the juxtaglomerular (JG) apparatus to erythropoietin remains to be defined. Several investigators have suggested a relationship of the JG apparatus to erythropoietin production(1-3). Further, Fisher and Crook(4) have reported that angiotensin, indirectly a product of JG cells, has erythropoietic stimulating activity in

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