Protection of Mice Against Aerosol-transmitted Influenza A2 Virus Infection by Stimulation of Interferon

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There are many reports in the literature indicating that the administration of exogenous interferon or induction of endogenous interferon can protect experimental animals against certain virus infections (reviewed by Finter, 1966, and Vilcek, 1969). In most of these studies, challenge was by parenteral inoculation of relatively large quantities of virus and the protective effects of interferon were assessed in terms of decreased mortality or less severe disease. However, naturally acquired virus infection is generally the consequence of exposure to much smaller quantities of virus, and with respiratory viruses, infection is initiated by deposition of virus in appropriate areas of the respiratory tract and not by parenteral inoculation. Accordingly, experiments were designed to determine whether interferon induced by Newcastle disease virus (NDV) could protect mice from infection by influenza virus transmitted by other mice. This experimental system was described in detail by Schulman & Kilbourne (1963) and was used to study the effects on transmission of infection of other modifications of the host such as immunization (Schulman, 1967). We believe that this model closely approximates the natural spread of influenza in man.

Male MF-1 pathogen-free mice 9 to 16 weeks old (Manor Farms, Staatsburg, N.Y.) were used. These mice are susceptible to influenza (Schulman & Kilbourne, 1963) and also produce high concentrations of serum interferon after intravenous administration of NDV (Rytel & Kilbourne, 1966). Intravenous injection of mice with NDV, in the doses employed in these experiments, has not been associated with any discernible effect, other than the induction of interferon (Rytel & Kilbourne, 1966). Interferon was titrated in clone L-929 mouse fibroblasts with vesicular stomatitis virus as the indicator virus. The end-point in interferon titrations was taken as the highest dilution giving 50% reduction in plaque number (Rytel & Kilbourne, 1966).

Mice were inoculated intravenously with approximately 10⁴ p.f.u. of NDV or with saline. Three hours later they were placed in contact (one from each group in a series of cages) with two mice which had been infected 24 hr earlier by exposure to an aerosol of influenza A2/JAP.305 virus. After a 24 hr contact period the contact mice were removed, separated, and quarantined for 48 hr. Ground lung suspensions from individual contact mice were then inoculated into chick embryos to test for the presence of virus. Twenty-four hours after inoculation with NDV or saline, lungs and sera of five cohort mice from each group were pooled for interferon assay. Six experiments were made (Table 1). In each experiment mice which were given NDV 3 hr before exposure to infected animals were less susceptible to transmitted infection. Lungs of positive contacts in both contact groups were tested by inoculation of serial dilutions into chick embryos to determine whether intravenous inoculation of NDV resulted in lower pulmonary virus concentrations in positive contacts as well as fewer positive contacts. The mean titre was 10⁴⁻⁸ EID₅₀ in control contact mice and 10⁴⁻⁸ EID₅₀ in NDV inoculated contacts.
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Table 1. Effect of intravenous inoculation of NDV on titres of interferon in lungs and serum and on susceptibility of mice to transmitted influenza A2 virus infection

<table>
<thead>
<tr>
<th>Treatment group*</th>
<th>Interferon titre†</th>
<th>Incidence of transmitted infection‡</th>
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<tbody>
<tr>
<td></td>
<td>Serum</td>
<td>Lung</td>
</tr>
<tr>
<td>NDV</td>
<td>4000</td>
<td>776</td>
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<tr>
<td>Saline</td>
<td>&lt; 10</td>
<td>&lt; 10</td>
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* Intravenous inoculation of 10^6 p.f.u. of NDV in 0.2 ml phosphate-buffered saline, or phosphate-buffered saline alone, 3 hr before exposure to infected mice.
† Units/2 ml., 24 hr after inoculation; specimens consisted of pooled serum and lung homogenates of five cohort mice from a representative experiment.
‡ Numerator = infected mice; denominator = mice in the experimental group; combined results of six experiments.

Fig. 1. Comparison of influenza A2 virus titres in nebulizer fluid necessary to infect 50% of exposed mice pretreated with NDV (○—○) and control mice (●—●) which received an equal volume of phosphate-buffered saline.

The reason for this small difference may be explained by the time factors in this model. Pulmonary virus titres were determined in contact mice 48 hr after the end of the contact period—72 hr after NDV inoculation. In our previous studies (Rytel & Kilbourne, 1966) we found that interferon titres reached a peak 6 to 12 hr after NDV inoculation, declined by 24 hr, and were undetectable by 48 hr. Youngner (1968) has reported similar interferon titres and kinetics of response following administration of NDV to mice. Therefore, influenza A2 virus replication in the lungs of infected contacts inoculated with NDV occurred in the absence of appreciable titres of interferon for more than 24 hr before titration.

In a separate series of experiments groups of ten mice inoculated intravenously with either NDV or saline were challenged 3 hr later by exposure to aerosols of serial four-
fold dilutions of influenza A2 virus. Two days later the percentage of mice infected in each group was determined by inoculation of ground lung suspensions into chick embryos. The EID 50 in mice inoculated 3 hr earlier with NDV was eight times greater than the EID 50 in untreated mice (Fig. 1).

Previous studies have indicated that interferon induced by NDV inoculation protects animals against death following parenteral challenge with high concentrations of virus. The present experiments indicated that interferon induced by NDV increased resistance of mice to infection after exposure to concentrations of airborne influenza A2 virus approximating those associated with the natural disease. That this protection was mediated by interferon is likely in view of the findings summarized by Finter (1967) indicating that growth of influenza virus in organ cultures of mouse or human respiratory epithelium was suppressed by addition of interferon. These data raise hope that eventually an effective prophylactic and therapeutic approach, utilizing induction of endogenous interferon, may be developed against respiratory virus infections in man.

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REFERENCES


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