Inhibition by Exogenous Interferon of Replication of Poliovirus Ribonucleic Acid in Chick Brain

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ABSTRACT

Youngner, Julius S. (University of Pittsburgh, Pittsburgh, Pa.), and Marion E. Kelly. Inhibition by exogenous interferon of replication of poliovirus ribonucleic acid in chick brain. J. Bacteriol. 90:443-445. 1965.—The replication of poliovirus was studied after the intracerebral inoculation of infectious ribonucleic acid (RNA) into the brains of 2-day-old chicks; these animals are not susceptible to intact virus. Single-cycle replication of virus, which reached a peak in about 12 hr, was completely inhibited by prior intraperitoneal injection of interferon prepared from the allantoic fluid of chick embryos infected with influenza virus. A single dose of as little as 900 units of interferon, measured by a plaque-reduction method, completely suppressed viral replication when injected 24 hr prior to infectious RNA. This system provides a model for the study of the protection of target organs by passively transferred interferon injected at a distance.

The production of poliovirus in naturally resistant animals injected intracerebrally with ribonucleic acid (RNA) prepared from this agent was reported first by Holland, McLaren, and Syverton (1959). Infection of chick embryos with RNA from poliovirus also has been described (Mountain and Alexander, 1959; De Somer, Prinzie, and Schonne, 1959). Hermodsson and Philipson (1963) showed that the inhibition of virus yield in calf kidney cell cultures infected with poliovirus RNA can be used as a sensitive method for assay of interferon.

In the present study, the chick, an animal naturally unsusceptible to poliovirus infection, was utilized to determine both the rate of formation of complete poliovirus after intracerebral inoculation of RNA and the effect of exogenous interferon, injected intraperitoneally, on viral replication.

MATERIALS AND METHODS

Interferon. Preparation of interferon in chick embryos was done with the WS strain of influenza virus according to the method of Wagner (1961). Embryonated eggs (10 days old) were inoculated into the allantoic cavity with about $10^8 EID_50$ of virus. After incubation for 72 hr at 36 C, the allantoic fluids were harvested, pooled, clarified by centrifugation at 500 $\times g$ for 20 min, and stored at -60 C. Before use, the infected allantoic fluid was heated at 60 C for 1 hr to destroy the virus. The viral inhibitor was heat-stable, non-sedimentable at 100,000 $\times g$ for 2 hr, resistant to inactivation at pH 2 for 24 hr, trypsin-sensitive, effective in chick cells but not in mouse cells, and did not neutralize vesicular stomatitis virus (VSV), the test virus in the assay for interferon activity. These properties are compatible with those of chick-embryo interferon reported by others (Lampson et al., 1963).

Assay. Interferon assay was performed by use of a plaque-reduction method (Wagner, 1961) in primary chick-embryo cultures, as described previously (Youngner and Stonebring, 1964). Interferon units per milliliter of allantoic fluid were expressed as the reciprocal of the dilution which reduced the VSV plaque count to 50% of that of the controls.

RNA. Infectious RNA was prepared from the Mahoney strain of type 1 poliovirus by use of cold phenol extraction (Holland et al., 1960). Intracerebral injections of 0.05 ml of RNA in 1 M NaCl were made into 2-day-old white Leghorn chicks which were kept in an electrically heated brooder before and after inoculation. At different times after inoculation, the chick brains were removed and ground in an all glass homogenizer; 10% brain suspensions were prepared with tris-phosphate buffer (pH 7.2) as diluent and were clarified by use of low speed centrifugation. The brains of at least three chicks were pooled for each determination. It is important to note that repeated tests have failed to show any measurable production of interferon in the brains or in the circulation of chicks injected intracerebrally with infectious RNA.

Intact poliovirus content of the brain suspensions was determined by use of plaque assays in primary monkey kidney cell cultures.
TABLE 1. Effect of intraperitoneal injection of interferon on replication of poliovirus in 2-day-old chicks inoculated intracerebrally with infectious RNA

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Avg PFU per brain of chicks pretreated with interferon (5,000 units)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal allantoic fluid</td>
<td>Interferon (5,000 units)</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>6.4 x 10^2</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>4.0 x 10^2</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>2.4 x 10^2</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>1.2 x 10^2</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>1.9 x 10^2</td>
<td>0</td>
</tr>
</tbody>
</table>

* Inoculum: 7.2 x 10^6 PFU of RNA per brain.
† Three chicks per determination.
‡ A 2,000-unit amount of interferon in 2.0 ml was injected intraperitoneally 24 hr prior to RNA; 2,000 units were injected at the same time as RNA.

TABLE 2. Influence of amount of interferon inoculated intraperitoneally on inhibition of poliovirus replication in chicks inoculated intracerebrally with infectious RNA

<table>
<thead>
<tr>
<th>Amt of interferon injected 24 hr prior to RNA (units)</th>
<th>Avg PFU per brain at 12 hr</th>
<th>Per cent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control†</td>
<td>4.9 x 10^4</td>
<td>100</td>
</tr>
<tr>
<td>2,000‡</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>500‡</td>
<td>1.7 x 10^4</td>
<td>65</td>
</tr>
<tr>
<td>125‡</td>
<td>2.3 x 10^4</td>
<td>53</td>
</tr>
</tbody>
</table>

* Inoculum: 3.0 x 10^6 PFU of RNA per brain.
† Three chicks per determination.
‡ Normal allantoic fluid.

RESULTS

Preliminary studies were made of the appearance of complete poliovirus after the injection of infectious RNA into the brains of chicks. The results showed that plaque-forming units (PFU) resistant to ribonuclease and neutralizable by type 1 poliovirus antiserum appeared in the brains between 4 and 6 hr after injection and reached peak levels at 10 to 12 hr.

To test the influence of exogenous interferon given intraperitoneally on the appearance of intact poliovirus after RNA injection, the following experiment was carried out. Groups of 2-day-old chicks were injected intraperitoneally with either normal allantoic fluid (heated at 60 C for 1 hr) or interferon (allantoic fluid infected with WS virus and heated as described above). Two doses (2.0 ml) of normal or interferon-containing allantoic fluid were injected 24 hr apart; a total of 4,000 units of interferon were given to each chick. Immediately after the second dose of control allantoic fluid or interferon, 7.2 x 10^6 PFU of Mahoney virus RNA in 1 ml NaCl were injected intracerebrally into both groups of chicks. At different times after the injection of RNA, three chicks from each group were sacrificed; brains were removed, processed as described above, and assayed for plaque formation in monkey kidney cell cultures (Table 1). In chicks pretreated with normal allantoic fluid, intact poliovirus appeared in the brain 6 hr after RNA injection and reached a peak titer of 1.2 x 10^6 PFU per brain at 12 hr. In chicks pretreated with interferon, the replication of poliovirus RNA was completely suppressed.

The influence of the amount of exogenous interferon injected on the replication of poliovirus RNA in chick brain was studied as follows. A single injection of heated, infected allantoic fluid, diluted to contain different amounts of interferon, was given intraperitoneally 24 hr prior to the intracerebral inoculation of Mahoney RNA. Control animals were given a single dose of heated, normal allantoic fluid. Brains were harvested 12 hr after RNA injection, and tissue suspensions were assayed for infective poliovirus by use of the plaque technique. RNA replication was completely inhibited by as little as 500 units of interferon, and 31 and 125 units produced a 53 and 65% inhibition of virus yield, respectively (Table 2). Under the conditions of the test, only inhibition greater than 50% was considered significant.

DISCUSSION

The protective effects of interferon in animals usually have been demonstrated when the inhibitor was inoculated at the same site as the challenge virus (Nagano and Kojima, 1958; Isaacs and Westwood, 1959; Cantell and Tommila, 1960; Andrews, 1961; Scientific Committee on Interferon, 1962). In discussing the question of the antiviral action of circulating interferon, Baron (1964) presented evidence that circulating inhibitor may reach and protect cells in the target organ of the virus in advance of virus spread. Mice injected intravenously with mouse serum containing 2,000 units of interferon were protected against a simultaneous 60% lethal dose of vesicular stomatitis virus given intracerebrally. Protection was observed when interferon was given 4 hr before or 2 hr after intracerebral challenge with as many as 30 lethal doses of vesicular stomatitis virus. In addition, it was shown that active induction of circulating interferon by the intravenous injection of mice with Newcastle dis-
ease virus (Baron and Buckler, 1963) was followed by increased protection against vesicular stomatitis and encephalomyocarditis viruses injected intracerebrally. These findings are interpreted to indicate that circulating interferon can be protective against virus as it spreads to target organs and secondary peripheral sites.

Baron also showed that as little as 133 units of mouse-serum interferon given intravenously significantly protected mice from the lethal effects of Germiston virus given intraperitoneally. In this connection, Finter (1964) reported that mice are protected against intraperitoneal injection with encephalomyocarditis and Semliki Forest viruses by prior subcutaneous or intramuscular inoculation of 3,000 to 5,000 units of mouse interferon. These results are in essential agreement with those of Baron and the results in the present report which showed that as little as 500 units of interferon given intraperitoneally completely inhibited poliovirus RNA replication in the brain. The lack of standard interferon preparations and the variation in assay methods make it difficult to compare exactly the amounts of interferon necessary to protect in vivo in the different studies.

The inhibition of replication of poliovirus in the brains of chicks injected intracerebrally with infectious RNA by small amounts of exogenous interferon injected intraperitoneally provides favorable conditions for the evaluation of protection of target organs by passively transferred interferon injected at a distance. It is possible to study only one cycle of viral replication since the chick is not susceptible to intact poliovirus. This procedure, by eliminating the complicating circumstance of local production of interferon by the infecting virus, provides a sensitive tool for the investigation of interferon action in vivo.

ACKNOWLEDGMENT

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