Interferon Production in Mouse Spleen Cells and Mouse Fibroblasts (L Cells) Stimulated by Various Strains of Newcastle Disease Virus

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SUMMARY

Interferon production in mouse spleen cells and mouse fibroblasts (L cells) stimulated by three strains of Newcastle disease virus (NDV), Italian, La Sota and Ulster, was investigated. Strain Italian was fully infectious and highly virulent; strain Ulster exhibited very low infectivity and very low virulence; strain La Sota was between these extremes. All of these strains of MDBK cell-grown NDV could induce interferon in mouse spleen cells, and it was concluded that proteolytic cleavage of F₀ protein of NDV and, consequently, virus penetration are not necessary for interferon induction in these cells. On the other hand, NDV with uncleaved F₀, which was characterized by an apparent lack of haemolytic and cell fusion activity and infectivity for tissue culture cells, had no interferon-inducing ability in L cells. The cleavage of F₀ protein was paralleled by an appearance of interferon-inducing activity in L cells.

Myxoviruses and paramyxoviruses have been found to be good inducers of interferon (Stewart, 1979). When fibroblasts were infected with these viruses, functional virus RNA was needed for interferon induction in fibroblasts and the actual inducer of interferon in fibroblasts was considered to be virus nucleic acid, probably double-stranded RNA (Stewart, 1979). On the other hand, we have recently reported that simple contact of the virus glycoprotein(s) with the cell surface appears to be sufficient for interferon induction in mouse spleen cells stimulated with Sendai virus (Ito et al., 1978).

Nagai et al. (1976) found that the virions of Newcastle disease virus (NDV) strain Ulster contain two precursor glycoproteins, HN₀ and F₀, while virions of NDV strain La Sota have only one precursor glycoprotein, F₀. The other glycoprotein had been cleaved. These precursor glycoproteins (HN₀ and F₀) are converted by proteolytic cleavage into functional glycoproteins (HN and F respectively) (Nagai et al., 1976; Nagai & Klenk, 1977). In this study, interferon production in mouse spleen cells and mouse fibroblasts (L cells) stimulated by these strains of NDV was investigated.

Mouse L fibroblasts were grown in Eagle’s minimum essential medium (MEM) supplemented with 10% bovine serum, 10% tryptose–phosphate broth and antibiotics. MDBK cells were grown in Dulbecco’s MEM containing 5% foetal calf serum. The maintenance medium contained no bovine serum supplement. Male C57BL/6 mice weighing 25 to 30 g were used for the preparation of mouse spleen cell suspension. Whole spleens were aseptically removed and teased on a steel mesh in chilled Eagle’s MEM in a plastic dish. The cells passed through were then washed twice with the medium and resuspended in Eagle’s MEM supplemented with 10% foetal calf serum.

Strains Italian, La Sota and Ulster of NDV were used. Seed stock virus was grown in the allantoic cavity of 11-day-old embryonated eggs inoculated with a 10⁻³ dilution of infected allantoic fluid. The allantoic fluid was harvested at 48 h after infection and stored at −80 °C. For use in this study, MDBK cells were infected with egg-grown seed stock virus at an input multiplicity of infection (m.o.i.) of 50. After a 60 min adsorption period, the inoculum was removed and the cells were washed three times with Dulbecco’s MEM. The cells were
incubated for 12 h and the culture fluid was collected. The culture fluids were centrifuged at 3000 rev/min, and the supernatants were recentrifuged at 30 000 rev/min. The virus pellets obtained were suspended in phosphate-buffered saline (PBS) and used for interferon induction. The haemagglutination titrations were done in microplastic trays with 0.7% suspension of chicken erythrocytes. For haemolysis assay, the virus samples in 1 ml PBS were distributed in test tubes, and 2 ml of a 1% suspension of chicken erythrocytes in PBS were added. The test tubes were kept at 4 °C for 1 h and then transferred to a 37 °C water bath for 1 h with occasional shaking. The erythrocytes were then pelleted at 1500 rev/min for 15 min and the absorbance was measured at 540 nm against a blank obtained by incubating erythrocytes with PBS alone.

Interferon was assayed by the c.p.e. inhibition microassay method with mouse L cells and vesicular stomatitis virus as the challenge virus (Ito & Montagnier, 1977). The highest dilution of the titrated sample causing at least 50% protection was considered as the endpoint. One interferon unit was equivalent to two reference units of mouse interferon. NDV contained in the samples was neutralized by an anti-NDV serum before interferon titration. Trypsin (2 × crystallized) was obtained from Sigma and thermolysin from Boehringer, Mannheim.

A 0.1 ml amount of the NDV preparation was added on to L cell monolayers (Falcon 3001; cell numbers about 3 × 10⁶) in 1 ml Eagle’s MEM and 1 × 10⁷ mouse spleen cells suspended in 1 ml of Eagle’s MEM with 10% foetal calf serum (Falcon 3001). After a 20 h incubation of these cells at 35 °C, the culture fluids were collected and assayed for interferon. The MDBK cell-grown Italian strain of NDV (glycoprotein types HN and F) could induce interferon in both L cells and mouse spleen cells (Table 1). The MDBK cell-grown La Sota strain of NDV (glycoprotein types HN and Fo) was characterized by an apparent lack of haemolysis (Table 1) and cell fusion activity and infectivity for tissue culture cells, but it exhibited infectivity for eggs and haemagglutinating and neuraminidase activity (Nagai et al., 1976). The lack of infectivity for tissue culture cells is thought to be due to the inability of the virus to penetrate (Nagai et al., 1976). The interferon-inducing activity of MDBK cell-grown La Sota was studied. Table 1 shows that MDBK cell-grown La Sota was found to stimulate interferon production in mouse spleen cells, but no interferon production was observed in L cells. The haemolytic activity and interferon induction capability of strain La Sota in L cells was not affected by thermolysin treatment. In contrast, when MDBK cell-grown La Sota strain was treated with trypsin, the haemolytic activity and interferon-inducing activity in L cells were restored. When L cells and mouse spleen cells were infected with the least virulent strain of NDV, strain Ulster, grown in MDBK cells (glycoprotein types HN, HN₀ and Fo), interferon production was observed in mouse spleen cells, but not in L cells. Thermolysin converted HN₀ into HN, but the enzyme had no effect on Fo. The haemagglutinin (HA) titre increased fourfold, a relatively low factor because of the already significant level of haemagglutinating activity. Thermolysin treatment did not influence the interferon-inducing activity in either L cells or mouse spleen cells. On the other hand, trypsin treatment drastically enhanced haemolytic activity, and was accompanied by an appearance of interferon-inducing activity in L cells.

This study showed that the proteolytic cleavage of Fo was essential for interferon induction in L cells, whereas this cleavage was not necessary for interferon induction in mouse spleen cells.

NDV comprises a wide range of strains which differ markedly in virulence for their natural host, the chicken. The NDV strains Italian, La Sota and Ulster used in this study were representative of the virulent, less virulent and the least virulent strains respectively. Italian strain has a mean death time (MDT) of chick embryos of 50 h, and La Sota strain has an MDT greater than 100 h. The MDT of Ulster strain is infinite (Nagai et al., 1976).
Table 1. Interferon induction by three strains of NDV

<table>
<thead>
<tr>
<th>Strain*</th>
<th>Glycoprotein type</th>
<th>Treatment†</th>
<th>HA titre</th>
<th>Haemolysis (A₄₅₀)</th>
<th>L cell</th>
<th>Spleen cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Italian</td>
<td>HN, F</td>
<td>–</td>
<td>32</td>
<td>0.222</td>
<td>40</td>
<td>960</td>
</tr>
<tr>
<td>La Sota</td>
<td>HN, F₀</td>
<td>–</td>
<td>128</td>
<td>0.000</td>
<td>&lt;2</td>
<td>960</td>
</tr>
<tr>
<td></td>
<td>Thermolysin</td>
<td>128</td>
<td>0.030</td>
<td>&lt;2</td>
<td>960</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trypsin</td>
<td>128</td>
<td>0.760</td>
<td>120</td>
<td>1920</td>
<td></td>
</tr>
<tr>
<td>Ulster</td>
<td>HN, HN₀, F₀</td>
<td>–</td>
<td>32</td>
<td>0.060</td>
<td>2</td>
<td>1920</td>
</tr>
<tr>
<td></td>
<td>Thermolysin</td>
<td>128</td>
<td>0.070</td>
<td>4</td>
<td>1920</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trypsin</td>
<td>128</td>
<td>0.940</td>
<td>40</td>
<td>1920</td>
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</tr>
</tbody>
</table>

* MDBK cells were infected with egg-grown seed stock virus at an input m.o.i. of about 50. After 60 min adsorption, the inoculum was removed and the cells then washed three times with medium. The culture fluids were centrifuged at 3000 rev/min, and then the supernatants were suspended in PBS. These viruses were used for interferon induction.

† The virus preparation suspended in PBS was incubated with the indicated proteases (5 μg/ml) for 8 min at 37°C.

‡ L cells or mouse spleen cells were incubated with untreated NDV or protease-treated NDV for 20 h, and the culture fluids were assayed for interferon (IFN) activity.

Nagai et al. (1976) reported that with the virulent strain cleavage of the glycoprotein occurs in all host systems analysed, and the virions formed contain HN and F. With avirulent strains, however, this is the case only in the embryonated egg and in cultures of chorioallantoic membrane cells. All other cells produce particles containing uncleaved glycoproteins. With the least virulent strain of NDV they identified a precursor glycoprotein, HN₀. There is a striking difference between glycoprotein HN and F with respect to their susceptibility to proteolytic enzymes. Cleavage and activation of HN can be accomplished by a variety of proteases, such as chymotrypsin, elastase, thermolysin and trypsin. In contrast, F₀ shows a specific requirement for trypsin.

All three strains of MDBK cell-grown NDV used in this experiment, strains Italian, La Sota and Ulster, could induce interferon in mouse spleen cells. It was therefore concluded that the proteolytic cleavage of F₀ of NDV and, consequently, virus penetration are not necessary for interferon induction in mouse spleen cells, and that simple contact of glycoprotein(s) of NDV with the cell surface is enough to induce interferon in mouse spleen cells. A similar conclusion was obtained from our previous experiments using Sendai virus (Ito et al., 1978). Since Sendai virus is known to be widely prevalent in conventional mice or to be transmitted transplacentally, the possibility could not be ruled out completely that the spleen cell response was immunologically mediated due to prior infection of our mouse colony with Sendai virus in the previous experiment using Sendai virus. Since poultry (including chickens and ducks) is the natural host of NDV it is unlikely that recognition by mouse spleen cells of NDV is a specific immune recognition. Interferon produced in mouse spleen cells stimulated by NDV was acid-stable and was neutralized by anti-L cell-NDV interferon antiserum, that is, type I interferon. Interferon inducibility by HN₀ protein in mouse spleen cells could not be ruled out completely.

The interferon induction mechanism in mouse fibroblasts is different from that in mouse spleen cells. NDV with uncleaved F₀ characterized by an apparent lack of haemolytic and cell fusion activity and infectivity for tissue culture cells (Nagai et al., 1976), had no interferon-inducing ability in L cells. The cleavage of F₀ protein was paralleled by an appearance of interferon-inducing activity in L cells. Thus, it is inferred that the process of virus penetration into cells is essential for interferon induction in mouse fibroblasts. This speculation is in agreement with the conclusion of Burke's (1973) review that the initial interaction of viruses with cells is apparently insufficient to stimulate the interferon induction;
rather, some event(s) subsequent to adsorption, penetration and uncoating is usually required (Burke, 1973).

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REFERENCES


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