Modification of Newcastle Disease Virus Release and Cytopathogenicity in Cells treated with Plant Lectins

By G. POSTE

Department of Experimental Pathology, Roswell Park Memorial Institute, Buffalo, N.Y. 14203, U.S.A.

D. J. ALEXANDER

Central Veterinary Laboratory, Weybridge, Surrey, England

AND P. REEVE AND G. HEWLETT

Department of Bacteriology, University College Hospital Medical School, London W.C. 1, England

(Accepted 21 January 1974)

SUMMARY

Binding of the plant lectins, concanavalin A (con A), wheat germ agglutinin, soybean agglutinin and Lens culinaris agglutinin to the surface of cells infected with different Newcastle disease virus (NDV) strains inhibited virus release. Binding of succinyl-con A with a lower valency than native tetrameric con A to the surface of infected cells did not inhibit virus release. It was proposed that binding of multivalent lectin molecules to the surface of infected cells impairs assembly of the virus envelope and the budding of new virus particles by the formation of cross-linkages and lattice formation between protein molecules on the cell surface. Binding of the same lectins to NDV-infected cells also caused significant inhibition of virus-induced cell fusion. In contrast, treatment of cells with a phytohaemagglutinin preparation from Phaseolus vulgaris enhanced NDV-induced cell fusion. A new form of NDV-induced c.p.e. was observed in lectin-treated cells involving extensive damage and fragmentation of cell nuclei. This c.p.e. was also found in lectin-treated cells infected with the avirulent NDV strains F and Queensland which normally induce only limited cell damage. The possible mechanisms underlying the varied effects of different lectins on NDV release and cytopathogenicity were discussed.

INTRODUCTION

A wide variety of lectins that bind specifically to carbohydrate-containing receptors on the surface of mammalian cells have now been isolated from plants and a number of invertebrates (Lis & Sharon, 1973; Nicolson, 1974). The interaction of these molecules with the cell surface has emerged as a potent experimental technique for studying the organization of the cell surface and for detecting alterations in cell surface properties. Several studies have shown that normal cells infected with non-oncogenic enveloped viruses that are released by budding from the plasma membrane show an increased susceptibility to agglutination by lectins (Becht, Rott & Klenk, 1972; Poste, 1972a; Poste et al. 1972a; Birdwell & Strauss, 1973), due to changes in the plasma membrane which allow greater mobility and clustering of lectin receptors on the cell surface (Poste & Reeve, 1974). In addition to the
detection of virus-induced alterations in the cell surface, lectins have also been used experimentally to modify virus replication. Binding of the lectin concanavalin A (con A) to the cell surface prevents the entry of certain viruses into the cell (Okada & Kim, 1972; Reeve, Poste & Alexander, 1974) and Rott et al. (1972) have shown that binding of con A to chick fibroblasts infected with fowl plague virus inhibited virus release. In this communication we confirm and extend these earlier observations and describe in detail the effect of a range of lectins with different receptor specificities on the cytopathogenicity and release of NDV strains grown in chick, hamster and bovine cells.

METHODS

Viruses. The origin and properties of the different NDV strains, methods of infection of cell cultures, and methods for the measurement of virus infectivity, haemagglutination, haemadsorption and virus RNA synthesis have been described (Alexander, Reeve & Allan, 1970; Reeve & Poste, 1971; Reeve et al., 1971, 1972; Alexander, Reeve & Poste, 1973a).

Cell cultures. Baby hamster kidney (BHK) and Madin–Darby bovine kidney (MDBK) cells were grown in reinforced Eagle’s medium (Dulbecco modification) supplemented with 10% foetal calf serum. Primary chick embryo (CE) cells were grown in Eagle’s basal medium supplemented with 10% calf serum. Unless stated otherwise, cells were grown in 60 mm plastic Petri dishes (Falcon Plastics) at 37°C in air + 5% CO₂.

Cell fusion. The terms cell fusion from without (FFWO) and cell fusion from within (FFWI) will be used here to describe cell fusion observed in cell cultures infected with NDV at high (1500 to 5000 EID₅₀/cell) or low (20 to 50 EID₅₀/cell) multiplicities, respectively. The characteristics of these two types of NDV-induced cell fusion have been described in detail elsewhere (Poste, 1972b; Poste et al., 1972b). The extent of virus-induced cell fusion, expressed as the percentage polykaryocytosis, was estimated from stained coverslip cultures by counting the number of nuclei present in polykaryocytes and expressing this as a percentage of the total number of nuclei present (Reeve & Poste, 1971). The extent of FFWO was measured 3 h after virus infection and FFWI was measured at intervals from 8 h after infection as described in the text.

Lectins. Concanavalin A (con A), which combines specifically with α-D-glucopyranosyl, α-D-mannopyranosyl and β-D-fructofuranosyl residues (Goldstein, Hollermann & Merrick, 1965; Goldstein & So, 1965), was purchased as a twice-crystallized preparation (Miles-Yeda, Kankakee, Illinois) and purified further by affinity chromatography (Poste & Reeve, 1974). Succinyl-con A (S-con A) was prepared from purified con A by the method of Gunther et al. (1973). Two sequences of succinylation of con A by incubation with succinic anhydride were used to obtain maximum biological activity. The binding of S-con A to Sephadex and its reactivity with rabbit anti-concanavalin A anti-serum were similar to that of native con A. Titration of the binding of con A and S-con A to several cell types indicated that both forms bound to the cell surface to a similar extent. In agreement with the observations of Gunther et al. (1973) S-con A was significantly less effective than native con A in causing cell agglutination. This disagrees with the results of Noonan & Burger (1973) who found S-con A to be as effective as native con A in producing cell agglutination.

Wheat germ agglutinin (WGA), which reacts with N-acetyl-D-glucosamine (D-GlcNAc), di-N-acetylchitobiose [(D-GlcNAc)₂] and sialic acid residues (Burger & Goldberg, 1967; Greenaway & Levine, 1973; Levine, Kaplan & Greenaway, 1973) was purified from commercial wheat germ lipase (Miles-Yeda) by the method of Nagata & Burger (1972) or purchased from Miles-Yeda purified by the same method.
Effect of lectins on virus release and c.p.e.

Soybean agglutinin (SBA), which combines with N-acetyl-D-galactosamine (D-GalNAc) and D-galactose-like residues (Lis et al. 1970) was purified as described by Sela et al. (1970). 

Lens culinaris agglutinin (LCA), which binds to D-GlcNAc and mannose-like residues (Kornfeld, Rogers & Gregory, 1971; Young et al. 1971) was isolated from a crude saline extract of lentils by specific absorption to Sephadex G-100 followed by elution with D-glucose and extensive dialysis to remove the glucose (Howard et al. 1971). The final preparation is a mixture of approximately equal amounts of two proteins but for descriptive convenience will be referred to as a single agglutinin.

Lotus agglutinin, which combines specifically with L-fucose-like sites (Kalb, 1968), was purchased from Miles-Yeda as an extract of Lotus tetragonolobus seeds purified by affinity chromatography using agarose-epsilon-amino caproyl fucosamine and used without further purification.

Phytohaemagglutinin (PHA), which combines with D-GalNAc residues (Borberg et al. 1966) was purchased as a freeze-dried extract of Phaseolus vulgaris from Burroughs Wellcome, Beckenham, Kent, England and Difco Laboratories, Detroit, Michigan. For experiments, stock freeze-dried PHA was prepared as serial tenfold dilutions in PBS without further purification.

Radioactively-labelled lectins. [3H]-con A and [3H]-lotus agglutinin were prepared from purified lectins using [3H]-acetic anhydride as described elsewhere (Poste & Reeve, 1974). [14C]-succinyl con A was prepared by the same method except that [14C]-succinic anhydride was used instead. The binding of radioactively-labelled lectins to cells was measured at room temperature or 4 °C as described by Poste & Reeve (1974). The amount of specific binding of each lectin was determined by measuring the amount of labelled-lectin bound in the presence of a specific haptenic inhibitor for the appropriate lectin and subtracting this from the amount bound in the absence of the inhibitor.

Reagents. D-galactose, N-acetyl-D-galactose, N-acetyl-glucosamine, α-methyl-D-glucopyranoside, α-methyl-mannoside, L-fucose and soybean trypsin inhibitor were obtained from the Sigma Chemical Company, St Louis, Missouri, and actinomycin D from P-L Biochemicals, Inc., Milwaukee, Wisconsin.

RESULTS

The effect of lectin binding to the cell periphery on virus release from NDV-infected cells

Treatment of BHK, CE and MDBK cells with 50 μg/ml con A following infection with NDV strain Herts caused complete inhibition of virus release (Table 1). Measurement of virus haemagglutinin (HA) released into the culture medium in Table 1 is a valid assay for virus release since more than 90% of the HA of strain Herts grown in these cell types is associated with infective particles (Alexander et al. 1973 b). The rate of virus RNA synthesis in con A-treated cells was similar to that in infected cells without con A (Fig. 1).

Incubation of NDV-infected cells with con A (50 μg/ml) for as little as one h after infection was sufficient to inhibit virus release (Table 1). At con A concentrations below 50 μg/ml, inhibition of virus release was proportional to lectin concentration, suggesting that saturation of con A receptors on the cell surface was required to achieve complete inhibition of virus release. This observation accords with previous studies of [3H]-con A binding to the same cell types which demonstrated saturation of cellular receptors at con A doses of approx. 50 μg/ml (Reeve et al. 1974).
Table 1. The release of virus haemagglutinin from BHK, CE and MDBK cells infected with Newcastle disease virus strain Herts (20 EID₅₀/cell) after treatment with con A (50 µg/ml)

<table>
<thead>
<tr>
<th>Time of removal of con A (h after infection)*</th>
<th>Released haemagglutinin (H.A.U./0·2 ml)†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BHK</td>
</tr>
<tr>
<td>Untreated control</td>
<td>16</td>
</tr>
<tr>
<td>1</td>
<td>&lt;2</td>
</tr>
<tr>
<td>2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>3</td>
<td>&lt;2</td>
</tr>
<tr>
<td>4</td>
<td>&lt;2</td>
</tr>
<tr>
<td>5</td>
<td>&lt;2</td>
</tr>
<tr>
<td>6</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Con A + 0·1 M α-methyl-D-glucopyranoside</td>
<td>16</td>
</tr>
<tr>
<td>Con A + 0·1 M α-methyl-mannoside‡</td>
<td>16</td>
</tr>
</tbody>
</table>

* Con A was added at the end of the virus adsorption period and removed at the times shown.
† Measured 16 h after infection.
‡ Con A was pre-incubated with inhibitor for 1 h at 37 °C, added to cells at the end of virus adsorption and removed after 2 h.

Incubation of con A with its specific haptenic inhibitors, α-methyl-D-glucopyranoside (0·1 M) or α-methyl-mannoside (0·1 M), for one h before addition to NDV-infected cells eliminated its ability to inhibit virus release (Table 1). However, addition of these inhibitors to NDV-infected CE or BHK cells that had been incubated with con A (50 µg/ml) for one h or longer failed to reverse the inhibition of virus release. Previous experiments on [³H]-con A binding to NDV-infected CE and BHK cells have shown that only 30 to 40% of the con A molecules bound to the cell can be eluted by these inhibitors (Reeve et al. 1974), and it would appear therefore that the con A molecules that fail to elute are sufficient to maintain effective inhibition of virus release.

Similar results to those in Table 1 have also been obtained in con A-treated CE and BHK cells infected with NDV strains Texas, Beaudette C and F.

Rott et al. (1972) reported that incubation of chick cells with con A for up to two h before infection with fowl plague virus prevented virus release without impairing virus replication. We have not been able to confirm this finding with NDV-infected cells. Incubation of CE or BHK cells with 50 µg/ml con A for only 30 min before infection with strain Herts prevented infection by inhibiting virus attachment.

Inhibition of virus release from CE and BHK cells infected with NDV strain Herts was also produced by treatment with WGA, SBA and Lens culinaris agglutinin (Table 2). Pre-incubation of these lectins with their specific inhibitors before addition to infected cells eliminated their capacity to inhibit virus release. In agreement with the results obtained earlier with con A-treated cells, binding of these lectins to the surface of infected cells did not alter the rate of virus RNA synthesis (Fig. 1).

Treatment of BHK or CE cells infected with strain Herts with up to 1500 µg/ml of Lotus agglutinin produced no detectable alteration of virus release. However, studies with [³H]-lotus agglutinin revealed only limited binding to both infected and uninfected cells, though the former did bind significantly more. However, the low binding affinity of this lectin for both infected and uninfected cells indicates that there are few available fucose-like residues on the surfaces of these cells.
Effect of lectins on virus release and c.p.e.

The accumulative incorporation of \([3H]\)-uridine into an acid-insoluble fraction in the presence of actinomycin D in BHK cells infected with Newcastle disease virus strain Herts (20 EID\(_{50}\)/cell) and similarly infected cells treated with 50 \(\mu\)g/ml con A, 150 \(\mu\)g/ml WGA, 350 \(\mu\)g/ml SBA or 500 \(\mu\)g/ml LCA. Actinomycin D (5 \(\mu\)g/ml) and \([3H]\)-uridine (sp. act. 1 \(\mu\)Ci/ml, 30.8 Ci/mmol) were added 30 min after infection and the various lectins added 1 h after infection. ●—●, specific incorporation into lectin-treated cells; ○—○, virus-infected cells without lectins; and ▲—▲, uninfected control cells.

**Fig. 1.** The accumulative incorporation of \([3H]\)-uridine into an acid-insoluble fraction in the presence of actinomycin D in BHK cells infected with Newcastle disease virus strain Herts (20 EID\(_{50}\)/cell) and similarly infected cells treated with 50 \(\mu\)g/ml con A, 150 \(\mu\)g/ml WGA, 350 \(\mu\)g/ml SBA or 500 \(\mu\)g/ml LCA. Actinomycin D (5 \(\mu\)g/ml) and \([3H]\)-uridine (sp. act. 1 \(\mu\)Ci/ml, 30.8 Ci/mmol) were added 30 min after infection and the various lectins added 1 h after infection. ●—●, specific incorporation into lectin-treated cells; ○—○, virus-infected cells without lectins; and ▲—▲, uninfected control cells.

**The effect of the valency of lectin molecules on their ability to inhibit virus release**

The highly different binding specificities of the lectins that inhibit virus release suggests that binding to a single type of saccharide residue on the cell surface is not responsible for this effect. A more likely possibility is that binding of multivalent lectin molecules to the cell surface impairs virus release by causing cross-linking and immobilization of protein molecules on the cell surface. At pH 7.0, con A is a tetramer with four saccharide binding sites (Edelman, Yahara & Wang, 1973). Because of their multivalent properties, con A molecules can form cross-linkages between adjacent receptor-bearing proteins on the cell surface. However, the ability of divalent con A molecules, such as succinyl-con A (S-con A), to produce this effect is significantly lower than that of native tetrameric con A (Yahara & Edelman, 1973). Similarly, divalent S-con A (Gunther et al. 1973; Reeve et al. 1974) has a
Table 2. The release of virus haemagglutinin from BHK and CE cells infected with Newcastle disease virus strain Herts (20 EID$_{50}$/cell) after treatment with wheat germ, soybean, Lens culinaris and Lotus agglutinins

<table>
<thead>
<tr>
<th>Lectin*</th>
<th>Inhibitor†</th>
<th>Released haemagglutinin (H.A.U./0.2 ml)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>None</td>
<td>16</td>
</tr>
<tr>
<td>Wheat germ (150 µg/ml)</td>
<td>0.2 M-N-acetyl-D-glucosamine</td>
<td>16</td>
</tr>
<tr>
<td>Soybean (350 µg/ml)</td>
<td>None</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Lens culinaris (500 µg/ml)</td>
<td>0.5 M-N-acetyl-D-galactosamine</td>
<td>16</td>
</tr>
<tr>
<td>Lotus (1500 µg/ml)</td>
<td>None</td>
<td>&lt;2</td>
</tr>
<tr>
<td></td>
<td>0.2 M-fucose</td>
<td>16</td>
</tr>
</tbody>
</table>

* Added 1 h after infection and removed 2 h later.
† Lectins were incubated with appropriate inhibitors for 1 h at 37 °C, added to cells 1 h after infection and removed 2 h later.
‡ Measured 16 h after infection.

Table 3. The release of virus haemagglutinin from BHK and CE cells infected with Newcastle disease virus strain Herts (20 EID$_{50}$/cell) after treatment with S-con A

<table>
<thead>
<tr>
<th>Lectin*</th>
<th>Released haemagglutinin (H.A.U./0.2 ml)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>16</td>
</tr>
<tr>
<td>Succinyl-con A</td>
<td></td>
</tr>
<tr>
<td>100 µg/ml</td>
<td>16</td>
</tr>
<tr>
<td>200 µg/ml</td>
<td>16</td>
</tr>
<tr>
<td>500 µg/ml</td>
<td>16</td>
</tr>
<tr>
<td>1500 µg/ml</td>
<td>16</td>
</tr>
</tbody>
</table>

* Added 1 h after infection and removed 5 h later.
† Measured 16 h after infection.

reduced ability to produce cell agglutination compared to tetrameric con A. In the light of these observations, we investigated the effect of S-con A on virus release.

Treatment of NDV-infected CE or BHK cells with divalent S-con A at concentrations up to 1500 µg/ml failed to inhibit virus release (Table 3). The possibility that the failure of S-con A to alter virus release was due to its inability to bind to the cell surface was excluded by the finding that binding of [³H]-con A to cells treated previously with S-con A was reduced to less than 10% of that bound to untreated cells. Since [³H]-con A and S-con A compete for the same receptors, the reduction in [³H]-con A binding to treated cells indicates that more than 90% of the receptors were already occupied by S-con A molecules.
Effect of lectins on virus release and c.p.e.

Table 4. The effect of con A (50 μg/ml) and S-con A (200 μg/ml) on cell fusion from within induced by Newcastle disease virus strains Herts, Texas, Warwick and Beaudette C

<table>
<thead>
<tr>
<th>Time of con A addition (h after infection)*</th>
<th>Uninfected control</th>
<th>% polykaryocytosis†</th>
<th>Beaudette C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Herts</td>
<td>Texas</td>
<td>Warwick</td>
</tr>
<tr>
<td>Untreated control</td>
<td>2.5</td>
<td>75.6</td>
<td>62.7</td>
</tr>
<tr>
<td></td>
<td>68.7</td>
<td>65.2</td>
<td>43.9</td>
</tr>
<tr>
<td>1</td>
<td>3.7</td>
<td>2.8</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>3.3</td>
<td>2.9</td>
</tr>
<tr>
<td>2</td>
<td>2.6</td>
<td>3.4</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>3.4</td>
<td>3.2</td>
<td>3.2</td>
</tr>
<tr>
<td>6</td>
<td>11.8</td>
<td>9.3</td>
<td>13.8</td>
</tr>
<tr>
<td>8</td>
<td>39.7</td>
<td>46.3</td>
<td>29.9</td>
</tr>
<tr>
<td>12</td>
<td>70.4</td>
<td>60.3</td>
<td>45.7</td>
</tr>
<tr>
<td>Con A + 0.1 α-methyl-D-glucopyranoside‡</td>
<td>1.6</td>
<td>65.7</td>
<td>45.9</td>
</tr>
<tr>
<td>Succinyl-con A§</td>
<td>2.6</td>
<td>73.5</td>
<td>62.8</td>
</tr>
<tr>
<td></td>
<td>69.3</td>
<td>40.7</td>
<td></td>
</tr>
</tbody>
</table>

* Con A was removed after 2 h.
† Measured 16 h after infection.
‡ Con A was pre-incubated with inhibitor for 1 h at 37 °C, added to cells 1 h after infection and removed after 2 h.
§ Added 1 h after infection and removed after 2 h.

Table 5. The effect of wheat germ agglutinin, Lens Culinaris agglutinin and Lotus agglutinin on cell fusion from within induced by Newcastle disease virus strains in HBK cell cultures

<table>
<thead>
<tr>
<th>Lectin*</th>
<th>Inhibitor†</th>
<th>Uninfected control</th>
<th>Herts</th>
<th>Texas</th>
<th>Warwick</th>
<th>Beaudette C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat germ agglutinin (150 μg/ml)</td>
<td>None</td>
<td>2.5</td>
<td>5.9</td>
<td>6.8</td>
<td>9.3</td>
<td>11.2</td>
</tr>
<tr>
<td></td>
<td>0.2 M-N-acetyl-D-glucosamine</td>
<td>3.1</td>
<td>69.3</td>
<td>72.4</td>
<td>60.5</td>
<td>48.6</td>
</tr>
<tr>
<td>Lens culinaris agglutinin (500 μg/ml)</td>
<td>None</td>
<td>3.8</td>
<td>8.2</td>
<td>7.4</td>
<td>6.2</td>
<td>9.1</td>
</tr>
<tr>
<td></td>
<td>0.2 M-N-acetyl-D-glucosamine</td>
<td>1.5</td>
<td>73.2</td>
<td>65.9</td>
<td>58.3</td>
<td>39.0</td>
</tr>
<tr>
<td>Lotus agglutinin (1500 μg/ml)</td>
<td>None</td>
<td>2.9</td>
<td>68.5</td>
<td>65.8</td>
<td>63.5</td>
<td>40.8</td>
</tr>
<tr>
<td></td>
<td>0.2 M-L-fucose</td>
<td>4.6</td>
<td>59.2</td>
<td>62.0</td>
<td>57.2</td>
<td>46.2</td>
</tr>
</tbody>
</table>

* Added 1 h after infection and removed 2 h later.
† Lectins were pre-incubated with inhibitors for 1 h at 37 °C, added to cells 1 h after infection and removed 2 h later.
‡ Measured 16 h after infection.

Inhibition of NDV-induced cell fusion by lectins

Treatment of BHK cells infected with the virulent NDV strains Herts, Texas and Warwick and the mesogenic strain Beaudette C with con A (50 μg/ml) up to 6 h after infection caused marked inhibition of the cell fusion (FFWI) which normally accompanies cellular infection by these strains (Table 4). When added later than 6 h after infection con A was increasingly ineffective in inhibiting FFWI (Table 4). Incubation of con A with 0.1 α-methyl-mannoside before addition to virus infected cells eliminated in its ability to inhibit FFWI (Table 4). Similar inhibition of NDV-induced FFWI by con A has also been observed in CE cells infected with these virus strains. Divalent S-con A did not inhibit FFWI in BHK cells under the same conditions (Table 4).

Significant inhibition of FFWI induced by NDV strains was also produced by treating cells immediately after infection with WGA or Lens culinaris agglutinin (Table 5). Treat-
ment of infected cells with Lotus agglutinin caused no detectable change in FFWI (Table 5). However, as outlined earlier, only very small amounts of this agglutinin bind to the cell surface.

Treatment of CE or BHK with con A (50 μg/ml), WGA (150 μg/ml) and Lens culinaris agglutinin (500 μg/ml) for up to two h before infection with high multiplicities (1500 EID₅₀/cell) of NDV strains Herts or Texas caused complete inhibition of virus-induced FFWO. However, as outlined earlier, pre-incubation of cells with these lectins limits virus attachment and this alone probably dictates that FFWO cannot occur, since this type of cell fusion results from fusion of the virus envelope with the plasma membrane of the cell.

Enhancement of NDV-induced cell fusion by phytohaemagglutinin

In contrast to lectin-mediated inhibition of NDV-induced FFWI described in the last section, observations on NDV-infected BHK cells treated with PHA revealed enhancement of FFWI (Fig. 2). The effect of PHA on NDV-induced FFWI involves an increase in the rate of cell fusion rather than an absolute increase in the number of cells undergoing fusion. For example, in BHK cell cultures treated with PHA before or immediately after infection with strain Herts between 65 and 70% of the cells had fused by 8 h after infection, compared to only 25 to 35% of the cells in control infected cultures without PHA. However, between 65 and 70% of cell in the latter will have fused by 16 h after infection (see, Poste et al. 1972b).

The time of addition of PHA in relation to virus infection was important in determining the increased rate of FFWI (Fig. 3). However, prolonged contact between PHA and cell surface receptors was not necessary to achieve maximum enhancement of FFWI, since cell cultures to which PHA was added 1 h after infection and removed 90 min later showed 70% polykaryocytosis by 8 h after infection, i.e. similar to that in cultures exposed to PHA for 8 h.

Pre-treatment of BHK cells with PHA also enhanced NDV-induced FFWO (Fig. 4). Detectable enhancement of FFWO occurred if the cells were incubated with PHA for as little as 15 min before exposure to virus, but maximum enhancement of FFWO required incubation of the cells with PHA for 90 min or longer (Fig. 4).

Incubation of PHA with 0.2 M-N-acetyl-galactosamine for one h at 37°C before addition to the cells eliminated its ability to enhance FFWO and FFWI. Similarly, pre-treatment of cells with 0.2 M-N-acetyl-galactosamine before exposure to PHA and subsequent virus infection eliminated PHA-induced enhancement of FFWI and FFWO, presumably as a result of competition between N-acetyl-galactosamine and PHA for common receptors on the cell surface.

We next investigated whether other lectins having the same carbohydrate-binding specificity as PHA could also enhance the rate of cell fusion. Unfortunately, the only well characterized lectin that binds to D-GalNAc residues is soybean agglutinin (SBA) (Lis et al. 1970), though two other exotic and less well studied lectins from Dolichos biflorus and Helix pomatia show similar binding specificities (Etzler & Kabat, 1968; Hammarstrom & Kabat, 1971).

Treatment of BHK cells with 350 μg/ml SBA immediately after infection with strain Herts produced a significant reduction in virus-induced FFWI (Fig. 3). In further contrast to the results obtained with PHA, pre-incubation of cells with 350 μg/ml SBA also inhibited NDV-induced FFWO (Fig. 4). These results indicate that SBA and PHA have opposite effects on virus-induced FFWI and FFWO which suggests that the enhancement of FFWI
Effect of lectins on virus release and c.p.e.

Fig. 2. The effect of different concentrations of phytohaemagglutinin (PHA) on the extent of cell FFWI in BHK cells infected with Newcastle disease virus strains Herts (25 EID_{50}/cell), Texas (20 EID_{50}/cell) and Beaudette C (B.C.) (50 EID_{50}/cell). Commercial PHA diluted at the indicated concentrations in maintenance medium was added 1 h after infection and the cells incubated in the presence of PHA until 8 h after infection when the extent of cell fusion was measured. The extent of FFWI in PHA-treated cell cultures is expressed as a percentage of that occurring in control cultures infected under the same conditions in the absence of PHA.

and FFWO by PHA is not determined simply by occupation of d-GalNAc-like sites on the cell surface.

Alteration of NDV-induced c.p.e. in lectin-treated cells

Under normal conditions of infection the ability of NDV strains to damage cells cultivated in vitro is related directly to their virulence for eggs and chickens in vivo (Reeve & Poste, 1971; Reeve et al. 1971, 1972). The normal c.p.e. induced by virulent and mesogenic strains in CE and BHK cell cultures involves the formation of polykaryocytes by cell fusion. However, as described in previous sections, treatment of cells with con A, WGA, SBA and Lens culinaris agglutinin modifies the normal c.p.e. by inhibiting cell fusion. Nonetheless, lectin-treated cells infected with virulent NDV strains showed extensive damage (Fig. 5, 6). As shown in Fig. 5 and 6 a high proportion of the cells were shrunken and distorted while others were vacuolated and enlarged and many cells showed extensive nuclear damage and
fragmentation. This type of c.p.e. developed rapidly and was advanced by only 8 h after infection. Importantly, this type of c.p.e. was not found in uninfected control cells exposed to identical concentrations of these lectins for the same time, or in control cells infected with virulent NDV strains in the absence of lectins. Also, the c.p.e. shown in Fig. 5 and 6 was not observed in NDV-infected cells treated with con A, WGA or SBA which had first been incubated with the appropriate haptenic inhibitor.

An even more striking modification of NDV-induced c.p.e. was found in cells treated with con A, WGA or SBA after infection with the avirulent strains Queensland and F. Under normal conditions, cells infected with strain F show only a limited degree of FFWI and little other cell damage, while cells infected with strain Queensland do not show any obvious damage. However, treatment of BHK cells with con A (50 µg/ml) for one h immediately after infection with these strains resulted in extensive cell damage (Fig. 7), similar to that...
Effect of lectins on virus release and c.p.e.

Fig. 5. Con A-treated BHK cells 16 h after infection with Newcastle disease virus strain Herts (30 EID_{50/cell}) showing extensive cell damage. Con A (50 μg/ml) was added 1 h after infection and removed 2 h later. There is considerable variation in cell size and many of the cells show extensive nuclear damage. Stain, May-Grunwald-Giemsa; magnification ×450.

found in lectin-treated cells infected with virulent NDV strains (Fig. 5, 6). Identical c.p.e. and extensive cell destruction following infection with the avirulent strains F and Queensland was also produced by treating BHK cells immediately after infection with WGA (200 μg/ml) or SBA (350 μg/ml). Uninfected control cells exposed to the same concentrations of these three lectins did not show significant damage or reduced viability and control cells infected with strains F and Queensland in the absence of lectins showed only the characteristic minimal c.p.e. Finally, the extensive c.p.e. shown in Fig. 7 was not found if the infected cells were exposed to lectins that had first been incubated with their appropriate inhibitor.

DISCUSSION

The present experiments have shown that binding of a variety of plant lectins to the surface of NDV-infected cells inhibits virus release. Our results extend the observations of Rott et al. (1972) who described an inhibitory effect of con A on fowl plague virus release from infected chick cells. The present experiments indicate that this effect is not confined merely to con A and is produced equally well by WGA, SBA and *Lens culinaris* agglutinin. Lectin-mediated inhibition of virus release has also been found by G. Nicolson (personal communication) in influenza virus and vesicular stomatitis virus-infected cells treated with *Ricinus communis* agglutinin.

Since the lectins that inhibit virus release display highly different carbohydrate-binding
specificities it seems unlikely that this effect is produced by specific binding to a single type of saccharide residue on the cell surface. However, binding of the different lectins to a single structural entity on the cell surface may well be occurring since single glycoproteins can carry binding sites for a number of lectins (Toyoshima, Fukada & Osawa, 1972).

It is considered that the ability of various lectins to inhibit virus release results from their multivalent properties and their ability to act as effective ligands by cross-linking adjacent receptor-bearing molecules on the cell surface (see, Edelman et al. 1973). The binding of multivalent lectin molecules to the surface of infected cells might impair virus release in two ways. Firstly, binding of lectin molecules to the cell surface causes cross-linking and lattice formation between adjacent molecules on the cell surface that carry appropriate receptors. This would reduce the mechanical flexibility and deformability of the membrane which, in turn, would probably hinder the budding process by which new virus particles are released. Secondly, binding of lectin molecules to the cell surface might interfere directly with the construction of the virus envelope. The envelope proteins of NDV and other paramyxoviruses are first synthesized in association with intracellular membranes (Compans, 1973; Stanley, Gandhi & White, 1973) and then inserted into the plasma membrane and concentrated in those membrane areas from which virus budding takes place. On the basis of the ability of protein molecules to diffuse and move laterally within the ‘fluid’ matrix of the plasma membrane (Singer & Nicolson, 1972), it has been proposed (Allison, 1971; Reeve et al. 1974) that this might provide one mechanism by which virus envelope proteins could be concentrated at specific areas of the plasma membrane during the assembly of the virus
Effect of lectins on virus release and c.p.e.

envelope. If such a mechanism is operating, then it is possible that binding of lectins to receptors on both virus and cellular protein molecules within the plasma membrane, by creating cross-linkages and lattice formation would frustrate the movement of virus envelope proteins within the membrane and hinder the assembly of the virus envelope even before the budding of new virus particles occurred. Circumstantial support for this proposal can be derived from the observation that lectins and other agents that induce cross-linking of proteins on the cell surface severely restrict the movement and clustering of protein molecules within the plasma membrane (Edelman et al. 1973; Inbar & Sachs, 1973).

The same lectins that inhibit the release of new virus particles from the cell surface also inhibited FFWI induced by NDV strains. Previous studies have shown that NDV-induced FFWI requires the insertion of virus-specified proteins into the plasma membrane of the cell (Reeve et al. 1972), and it is considered that con A and other lectins inhibit FFWI by impairing the correct association of these virus proteins within the plasma membrane due to cross-linking of surface proteins as outlined above. In the case of lectin-induced inhibition of FFWO, the significant reduction in the attachment of virus particles to lectin-treated cells is probably responsible, since this type of cell fusion requires attachment and subsequent fusion of large numbers of virus particles with the surfaces of apposed cells (Poste, 1972b).

The reduced attachment of virus particles to con A-treated cells is somewhat surprising since it might be expected that the binding of multivalent con A molecules to the cell surface

Fig. 7. Con A-treated BHK cells 16 h after infection with Newcastle disease virus strain Queensland (50 EID$_{50}$/cell) showing cellular vacuolation and extensive distortion and fragmentation of cell nuclei. The cells are not firmly attached to the glass and show a tendency to round up and form clumps. Con A (50 µg/ml) was added 1 h after infection and removed 2 h later. Stain, May-Grunwald-Giemsa; magnification ×450.
would provide a number of unoccupied carbohydrate binding sites to which virus particles could attach. On the other hand, if significant virus attachment was occurring on con A-treated cells then successful penetration should follow since endocytosis is unaffected by con A (Karsenti & Avrameas, 1973).

The increased rate of NDV-induced FFWI and FFWO in cells treated with PHA is more difficult to explain. Commercial PHA is a mixture of at least five glycoproteins which possess varying degrees of erythroagglutinating, leukoagglutinating and mitogenic activities (Miller et al. 1973). Numerous attempts to isolate and characterize these proteins has created a confusing and somewhat contradictory series of reports (Lis & Sharon, 1973). Consequently, identification of the specific PHA component(s) responsible for enhancement of virus-induced cell fusion may prove extremely difficult.

It is considered that the increased cytopathogenicity of avirulent NDV strains in cells treated with lectins can be explained by the action of lectins in inhibiting virus release which results in a compensatory increase in the intracellular titre of virus subunits. Previous studies on the replication of NDV strains have shown that differences in the ability of individual strains to damage cells is not determined simply by the rate of production of new infective virus particles. Rather, a more important factor is the balance between the rate at which virus subunits are synthesized and their rate of assembly and release as new virus particles (Reeve et al. 1971, 1972; Alexander et al. 1973a, b). Cellular infection by virulent strains which cause extensive c.p.e. is characterized by high titres of cell-associated virus subunits, because their rate of synthesis exceeds their rate of assembly and release from the cell surface as new virus particles. Conversely, cellular infection by avirulent strains under normal conditions is characterized by very low intracellular titres of virus subunits due to an efficient balance between the rate of synthesis of subunits and their release as new virus particles. These observations have prompted the proposal (Alexander et al. 1973a, b) that cell damage will be more likely to occur under conditions where intracellular accumulation of virus subunits occurs due to imbalance between synthesis and release. The increased cell damage found here in lectin-treated cells infected with avirulent NDV strains supports this view. The binding of lectin molecules to the cell surface inhibits the release of these strains without impairing intracellular virus replication and this creates an artificial intracellular accumulation of virus subunits resulting in cell damage and death.

This work was supported by grant number CA-13393 from the National Institutes of Health and a grant from the Agricultural Research Council of Great Britain. The excellent technical assistance of P. Newhouse, F. Dutton, A. MacKearnin, R. K. Eddy and H. Watkins is gratefully acknowledged. We thank Dr Garth Nicolson for permission to cite his unpublished observations.

REFERENCES


Effect of lectins on virus release and c.p.e. 269


(Received 6 November 1973)