Comparison of Antigenic Glycoproteins and Glycoprotein Receptors of Concanavalin A Isolated from Duck Embryo Cells Infected with Marek's Disease Virus and a Herpes Virus of Turkeys (Strain FC126)

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SUMMARY

Surface changes were demonstrated in duck embryo cells following infection with both Marek's disease virus (MDV) and a herpes virus of turkeys (HVT) by agglutination with concanavalin A (con A). Materials labelled with fucose were extracted from the surface of cells with Nonidet. Antigens and con A receptors were isolated from these extracts by affinity chromatography and were compared using double labelling and electrophoresis in polyacrylamide gels. Eight or nine glycoproteins containing fucose were detected in cell surface extracts. Major con A receptors (average mol. wt. 95,000 and 130,000) detected in MDV and in HVT infected preparations were also present in uninfected cells. Antigens isolated from MDV and HVT infected preparations by immunoadsorption using MDV antiserum were different from the major con A receptors. The relationship between con A binding glycoproteins and antigenic glycoproteins is discussed. Two antigens (average mol. wt. 30,000 and 43,000) associated with the surface of MDV infected cells were MDV specific but the antigens precipitated from culture fluids of MDV and HVT infected cells with MDV antiserum appear to be identical.

INTRODUCTION

Marek's disease virus (MDV) and the herpes virus of turkeys (HVT, strain FC126) are antigenically related viruses which differ in pathogenicity (Witter et al. 1970) and in growth properties in vivo (Phillips & Biggs, 1972) and in vitro (Witter et al. 1970). Lymphocytes obtained from chickens infected with MDV and HVT contain infectious virus but only those derived from MDV infected chickens are capable of synthesizing host DNA, as shown by uptake of thymidine in vitro (Lee, 1972). There is also evidence of host DNA synthesis and of cell proliferation when duck embryo (DE) cells are infected with the virulent HPRS-16 strain of MDV but not with attenuated virus (Newton & Ross, 1973). Since these findings suggest that there might be in vitro correlates of oncogenicity, it was of interest to study surface changes in DE cells infected with virulent and attenuated strains and to relate these to virus specific products.

METHODS

Cells and media. DE cells obtained from 12 to 14 day old embryos were grown in roller bottles (64 × 320 mm) in 199 medium, as previously described (Ross, Biggs & Newton,
Cultures were initiated by seeding bottles with $10^7$ cells in 80 ml medium containing 5% calf serum. The monolayers were infected when confluent and the cultures maintained in medium containing 2% calf serum.

**Virus.** A stock of pathogenic MDV (HPRS-16) grown in DE cells (Ross et al. 1973) was used throughout the experiments. HVT (strain FC126) (Witter et al. 1970) was passed three times in DE cells before use. Cell-associated virus was used to initiate infection in all cases. The proportion of cells acting as infective centres in the stock preparations was 2% and 1% for MDV and HVT, respectively, when assayed in chicken kidney cells.

**Antiserum.** Sera collected from a group of brown leghorns infected by natural exposure to Marek's disease virus were pooled and used throughout the experiments. This serum neutralized both MDV and HVT. Multiple precipitin lines were obtained when the serum was tested against extracts of MDV and HVT infected cells in immunodiffusion tests, but MDV specific antigens were apparent when cell-associated extracts were tested. However, the main antigen present in culture fluids (the 'A' antigen) was common to both MDV and HVT. The immunodiffusion results will be published elsewhere.

**Preparation of adsorbents.** Immunoabsorbent was prepared essentially as described previously (Ross et al. 1973) using the method of Cuatrecasas & Anfisen (1971). 50 mg of globulin obtained by ammonium sulphate precipitation of MDV antiserum was attached to 8 ml cyanogen bromide activated Sepharose 4B (Pharmacia). Eighty-five per cent of the added globulin attached. Remaining active groups were blocked with 1 M-ethanolamine and the gel was stored at 4°C in the presence of 0.02% sodium azide.

Concanavalin A (con A) (Calbiochem) was conjugated to Sepharose 4B, as described by Allan, Auger & Crumpton (1972), using 50 mg of lectin and 5 ml of activated Sepharose 4B. Seventy-five per cent of the added con A attached. Remaining active groups were blocked with 1 M-ethanolamine and the gel stored at 4°C in 0.1 M-acetate, pH 6, +1 M-NaCl + $10^{-3}$ M-CaCl$_2$ + $10^{-3}$ M-MnCl$_2$ + 0.02% sodium azide.

**Infection and labelling of cells.** Monolayers of $10^7$ to $10^8$ cells were infected by adding $10^5$ infective centres in maintenance medium. Cultures received either 60 μCi of [1-3H]-L-fucose (2.8 Ci/mmol) (Radiochemical Centre, Amersham) or 15 μCi of [1-14C]-L-fucose (56.2 mCi/mmole) (NEN Chemicals Ltd). The times of labelling are given in the Results section.

**Concentration of antigens present in culture fluids.** Culture fluids were clarified by sedimentation at 10000 g for 1 h. Solid ammonium sulphate was added gradually, with gentle stirring, to a final concentration equivalent to 70% saturation. The precipitate was sedi-

**Extraction of cell membrane glycoproteins.** Cells were washed once in PBS, detached with 0.02% versene in PBS, and washed again with PBS. The versenate and the PBS washings were combined with culture fluids for precipitation with ammonium sulphate. The cells were sedimented, washed once more with PBS and cell surface materials were solubilized by treating the intact cells with 0.5% Nonidet P40 (Shell Limited) for 10 min at 4°C according to the procedure used by Schwartz & Nathenson (1971) for the extraction of histocompatibility antigens. The cells which were intact at the end of the Nonidet treatment were sedimented at 5000 g for 10 min and the supernatant fluid was collected and used for isolation of antigens and receptors of con A.

**Isolation of antigens and con A receptors.** Batch procedures were used throughout. Immunoabsorbent was washed three times (30 min for each wash) using 10 vol. of 2.5% Nonidet + 3.5 M-sodium thiocyanate in 0.2 M-phosphate buffer, pH 6.8. Thiocyanate was
removed before using the gel by washing several times with 2.5 % Nonidet in buffer. Samples of 1 ml of packed gel (5 mg of immune globulin) were mixed with 0.5 ml of Nonidet extract (1 to 2 mg protein) and the mixture stirred at room temperature for 2 h. Unadsorbed material was then washed away by gentle stirring with 10 vol. of 2.5 % Nonidet in buffer for periods of 30 min at a time. Supernatant fluids were decanted after allowing the gels to settle for 10 min. Three washes were sufficient for complete removal of unadsorbed radioactivity. Elution of adsorbed material was then carried out by washing three times with 2 ml vol. of 3.5 M-sodium thiocyanate + 2.5 % Nonidet in phosphate buffer, pH 6-8. The first two elutions were done at room temperature for 1 h with constant stirring. The final step was carried out overnight at room temperature.

The procedure for attachment of con A receptors and their elution from con A-adsorbent was essentially as described above. The gels were washed with methyl a-D glucoside + methyl a-D mannoside in 2.5 % Nonidet + 10^-4 M-CaCl₂ + 10^-3 M-MnCl₂ in acetate buffer, pH 6, and washed further overnight with Nonidet, CaCl₂ + MnCl₂ in buffer but omitting glucoside and mannoside. Samples of 1 ml of gel (10 mg con A) were mixed with 0.5 ml Nonidet extract and unadsorbed material washed away. Adsorbed material was eluted by two treatments with glucoside followed by a final treatment overnight with mannoside.

Precipitation of antigens with antiserum. Antigens obtained by ammonium sulphate precipitation of culture fluids were precipitated with MDV antiserum in the presence of 8 % NaCl. Optimal concentrations of antigen and antibody for maximum precipitation were determined by preliminary immunodiffusion tests. Antigens and serum were centrifuged at 80000 g for 1 h before use. Precipitation was carried out in plastic tubes at 37 °C for 1 h and precipitates were allowed to stand overnight at 4 °C. They were then sedimented (40000 g/h) and washed three times with 8 % NaCl in PBS.

Cell agglutination with con A. Agglutination of cells by con A was assayed by the method of Inbar & Sachs (1969) using plastic agglutination trays with flat bottom wells. Monolayers of DE cells in 10 cm plates were infected with MDV and with HVT at an input multiplicity of 1 p.f.u./100 cells. After 4 days’ incubation at 37 °C, the monolayers were washed with PBS and the cells were detached by treatment with 0.02 % versene in PBS, washed once in PBS and resuspended in PBS at a concentration of 10^6 cells/ml. Agglutination was initiated by mixing 0.5 ml of cells and 0.5 ml of con A (0.1 to 1 mg/ml in PBS) for 30 min at room temperature. The plates were then cooled to 4 °C to stop further reaction and the proportion of cells agglutinated were determined microscopically without delay. Cells found in groups of three or more were considered to be agglutinated.

Polyacrylamide gel electrophoresis. Electrophoresis was performed in 6 mm × 9 cm tubes using essentially the discontinuous pH SDS system described by Dimmock & Watson (1969). Samples for electrophoresis were precipitated with 10 vol. of acetone overnight at 4 °C. Precipitates were sedimented, evaporated to dryness and were dissolved by boiling for 2 to 3 min in 2 % SDS + 2 % mercaptoethanol + 0.5 M-urea + 0.06 M-tris-phosphate, pH 6.9, +10 % sucrose + phenol red. Electrophoresis was carried out at 4 mA/tube and was stopped when phenol red had travelled 5 to 6 cm in the separation gel. Gels were extruded, measured and were either stained with Coomassie blue or were frozen at -70 °C and sliced in 1 mm fractions for estimation of radioactivity. An attempt was made to estimate mol. wt. since the mobility of the following standard proteins relative to phenol red was proportional to the logarithm of their mol. wt. Relative mobilities in 8.5 % gels were as follows: ovalbumin (mol. wt. 43000) 0.525, bovine serum albumin (mol. wt. 67000) 0.39, β-galactosidase (mol. wt. 130000) 0.21. The mobilities of chymotrypsinogen (mol. wt. 25700), ovalbumin and bovine serum albumin in 10 % gels were 0.74, 0.48 and 0.29, respectively.
Gels stained with Coomassie blue were scanned using a modified Gilford spectrophotometer and scanner.

Assay of radioactivity. Samples were counted for 10 min at a constant temperature of 24 °C using a liquid scintillation counter (Corumatic 200, Tracer Lab. Ltd). Two methods previously described (Ross et al. 1973) were used: (1) TCA-insoluble material was determined by the filter paper method using toluene based scintillation fluid. Counting efficiency was 10 % and 30 % for [3H] and [14C], respectively. Background count rates were 30 ct/min and 15 ct/min in the [3H] and [14C] channels, respectively. (2) Acrylamide gel samples were treated with 0.5 ml of 0.1 % SDS at 37 °C overnight and the radioactivity eluted was determined by mixing 0.3 ml with 3 ml Triton X-100-toluene based scintillant. In double labelling experiments [3H] counts were corrected for [14C] spillover. The proportion of [14C] counts recovered in the [3H] channel was 25 %, and was the same for all fractions. The degree of quenching was constant for all fractions. Preliminary experiments showed that there was a good correlation between estimation of quenching by the channels ratio method and estimation of quenching using the external standard method. The latter procedure was therefore routinely used. The mixtures were clear one phase solutions of low viscosity at time of counting in all cases. The efficiency of counting [3H] and [14C] was 20 % and 50 %, respectively. The radioactivity recovered from the gels was over 90 %.

Protein estimations were made, following the method of Lowry et al. (1951).

RESULTS

Evaluation of the double labelling technique

The detection of peaks of radioactivity after separation of radioactive materials by electrophoresis in acrylamide gels and estimation of relative amounts of [3H] and [14C] in each peak has been used in this study to identify differences in glycoproteins synthesized in cells infected with two different viruses. Since the sp. act. of the materials used were low and count rates ranged from 50 to 100 ct/min after subtracting background, it was important to evaluate the significance of the differences observed. Pilot experiments showed that concordant patterns of radioactivity are obtained on co-electrophoresis of [3H] and [14C] labelled materials obtained from cells labelled separately with [3H] and [14C] fucose. It was also noted that the variation of [3H]/[14C] ratios could be accounted for purely by chance since the majority of the ratios were within two standard deviations on either side of the mean. However, excessively high ratios were obtained in the region of the refractile band observed in the discontinuous pH system used here, particularly with old preparations. The reasons for this anomaly are not known but it is possible that compounds containing [3H] labelled fucose are degraded more rapidly than [14C] labelled materials, possibly by alkaline hydrolysis resulting in the formation of [3H] labelled products of low mol. wt. which accumulate in the region of the refractile band on electrophoresis. Comparison of labelled materials was therefore restricted to fractions ranging from the cathodal end of the gels up to 2 mm away from the refractile band.

Incorporation of radioactive precursors and distribution of radioactive materials

Judged microscopically, HVT multiplied faster and was more cytolytic than MDV under the conditions of infection used in these experiments. Cultures infected with HVT contained fairly discrete areas of c.p.e, consisting of rounded cells and a few small syncytia 2 days after infection. These areas of c.p.e, expanded rapidly and infection had spread throughout the culture by the 4th day. In cultures infected with MDV however, dense foci consisting of
Glycoproteins of MDV and HVT

Table 1. Incorporation of radioactive fucose into TCA insoluble materials in cells and in culture fluids of control and of MDV and HVT infected DE cells

<table>
<thead>
<tr>
<th>Material and input radioactivity</th>
<th>Vol. (ml)</th>
<th>Protein (mg/ml)</th>
<th>Total (ct/min × 10⁻³)</th>
<th>Sp. act. (ct/min/µg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MDV Infected (62 µCi [³H])</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>10</td>
<td>5.4</td>
<td>71.2</td>
<td>1.3</td>
</tr>
<tr>
<td>Cells (sonicate)†</td>
<td>1</td>
<td>4.5</td>
<td>95.2</td>
<td>21</td>
</tr>
<tr>
<td>Cells (Nonidet)</td>
<td>1</td>
<td>3</td>
<td>168</td>
<td>56</td>
</tr>
<tr>
<td><strong>MDV Infected (16 µCi [¹⁴C])</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>12</td>
<td>4.8</td>
<td>32.8</td>
<td>0.56</td>
</tr>
<tr>
<td>Cells (sonicate)†</td>
<td>1</td>
<td>3.8</td>
<td>52.8</td>
<td>14</td>
</tr>
<tr>
<td>Cells (Nonidet)</td>
<td>1</td>
<td>2.5</td>
<td>102</td>
<td>41</td>
</tr>
<tr>
<td><strong>HVT Infected (62 µCi [³H])</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>10</td>
<td>4.8</td>
<td>42</td>
<td>0.88</td>
</tr>
<tr>
<td>Cells (sonicate)†</td>
<td>1</td>
<td>2.4</td>
<td>60</td>
<td>25</td>
</tr>
<tr>
<td>Cells (Nonidet)</td>
<td>1</td>
<td>2</td>
<td>196</td>
<td>98</td>
</tr>
<tr>
<td><strong>HVT Infected (16 µCi [¹⁴C])</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>12</td>
<td>4.8</td>
<td>23.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Cells (sonicate)†</td>
<td>1</td>
<td>2.2</td>
<td>32</td>
<td>14.5</td>
</tr>
<tr>
<td>Cells (Nonidet)</td>
<td>1</td>
<td>1.9</td>
<td>94</td>
<td>50</td>
</tr>
<tr>
<td><strong>Control (62 µCi [³H])</strong></td>
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<td></td>
</tr>
<tr>
<td>Medium</td>
<td>5</td>
<td>10</td>
<td>30</td>
<td>0.6</td>
</tr>
<tr>
<td>Cells (Nonidet)</td>
<td>1</td>
<td>6.8</td>
<td>150</td>
<td>22</td>
</tr>
</tbody>
</table>

* Estimated by filter paper method.
† Cells were detached with versene, counted and divided into two equal parts. One part was resuspended in water and disrupted by sonication. The other part was treated with Nonidet as described in Methods. Radioactivity was estimated after sedimenting cell debris at 5000 rev/min (5 min).

Table 2. Agglutination of DE cells with con A

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>HVT infected</th>
<th>MDV infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not treated (PBS)</td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Con A (1 mg/ml)</td>
<td>1</td>
<td>35</td>
<td>28</td>
</tr>
<tr>
<td>Con A (0.1 mg/ml)</td>
<td>0</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Con A (1 mg/ml+0.1 M-methyl-α-D-glucopyranoside)</td>
<td>0</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

* Cells in clumps of three or more were considered to be agglutinated.

Elongated refractile cells and few rounded cells were observed after 3 days. The number of rounded cells increased gradually so that by the 5th day the degree of c.p.e. judged by the presence of rounded cells was comparable to that in HVT infected cultures 3 days after infection. For these reasons, cultures infected with HVT were labelled from 2 to 4 days after infection and those infected with MDV from 3 to 5 days. The period of labelling was 48 h in all cases. The proportion of cells containing antigens, determined by immunofluorescence using MDV antiserum, was 30% to 35% at the time of harvesting the cells.

The distribution of radioactivity and the sp. act. of the radioactive materials used in these experiments are summarized in Table 1. Fucose was poorly utilized since on average only 2% of added radioactivity was incorporated into TCA-insoluble materials. It was noted that the radioactivity of Nonidet extracts was consistently higher than that of cell sonicates.
Fig. 1. Electrophoresis of fucose labelled receptors of con A and antigens isolated from Nonidet extracts of MDV infected cells by affinity chromatography. Results shown for electrophoresis in 8.5% gel. (a) Electrophoresis of Nonidet extract. Scan of Coomassie blue stained gel. (b) Electropherogram of Nonidet extract. (c) Electropherogram of Con A receptors eluted from Con A adsorbent (cf. eluates 1+2+3 Table 3). (d) Electropherogram of antigens eluted from immuno-adsorbent (cf. eluates 1+2+3 Table 4). • — •, [3H] counts; PR, phenol red; apparent mol. wt. x 10^-3 are indicated above peaks.

suggesting that the detergent was more effective in solubilizing bound radioactivity than ultrasonic disruption in distilled water.

Changes at the surface of infected cells revealed by agglutination with con A

Experiments have consistently shown that DE cells are agglutinated by con A after infection with both MDV and HVT. The results of one experiment are shown in Table 2. The effect was specific since the lectin did not agglutinate control cells and agglutination of infected cells was inhibited in the presence of methyl-α-D-glucopyranoside (Sigma). Since the
Fig. 2. Electrophoresis of fucose labelled receptors of con A and antigens isolated from Nonidet extracts of HVT infected cells by affinity chromatography. Results shown for electrophoresis in 8.5% gel. (a) Electrophoresis of Nonidet extract. Scan of Coomassie blue stained gel. (b) Electropherogram of Nonidet extract. (c) Electropherogram of receptors of con A eluted from con A adsorbent (cf. eluates 1 + 2 + 3 Table 3). (d) Electropherogram of antigens eluted from immuno-adsorbent (eluates 1 + 2 + 3 Table 4). ○——○, [14C] counts; apparent mol. wt. × 10^-1 are indicated above peaks; PR, phenol red.

proportion of cells containing MDV antigens, as shown by immunofluorescence tests, was approx. 30% in both MDV and HVT infected preparations and was equal to the proportion of cells that were agglutinated, it is likely that the cells that were agglutinated were the infected ones.

Isolation of antigens and receptors of con A

Although the above experiment showed that receptors of con A are exposed at the surface of MDV and HVT infected cells, it is not known whether the receptors are identical or whether there are receptors specific to pathogenic virus. Furthermore, the relationship
between con A receptors and virus specific antigens detected at the cell surface by Ahmed & Schidlovsky (1972) is also unknown. In order to resolve these points, antigens and materials binding to con A were extracted from the cell surface and were compared by electrophoresis.

**Extraction of cell surface glycoproteins**

Materials present at the cell surface were extracted by brief treatment of intact cells with Nonidet, as described by Schwartz & Nathenson (1971), for extraction of histocompatibility antigens. Fifty million cells were treated with 0.5% Nonidet in PBS at 4 °C for 10 min as described in Methods. Radioactivity profiles of fucose labelled materials extracted with Nonidet from MDV and HVT infected cells are shown in Fig. 1b and 2b, respectively and scans of duplicate gels stained with Coomassie blue are shown in Fig. 1a and 2a, respectively. Eight or nine glycoproteins containing fucose were resolved in both MDV and HVT preparations and these corresponded fairly well with the Coomassie blue stained bands.

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**Fig. 3.** Electrophoresis of solubilized MDV infected cells in 8.5% gel. (a) Scan of Coomassie blue stained gel. (b) Electropherogram of fucose labelled cells. ●●●, [3H] counts; PR, phenol red.
Glycoproteins of MDV and HVT

Table 3. Adsorption of fucose labelled materials in Nonidet extracts of MDV and HVT infected DE cells to con A-Sepharose adsorbent

<table>
<thead>
<tr>
<th>Material</th>
<th>Input radioactivity (ct/min x 10^{-2})</th>
<th>ct/min specifically eluted x 10^{-3}</th>
<th>Total radioactivity eluted (% input)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st†</td>
<td>2nd‡</td>
</tr>
<tr>
<td>HVT infected</td>
<td>[^{14}C] 23.5*</td>
<td>3.776</td>
<td>2.042</td>
</tr>
<tr>
<td>MDV infected</td>
<td>[^{3}H] 25.2</td>
<td>3.718</td>
<td>1.382</td>
</tr>
<tr>
<td>Control</td>
<td>[^{3}H] 15</td>
<td>1.92</td>
<td>0.956</td>
</tr>
</tbody>
</table>

* Radioactivity of TCA insoluble material was estimated by the filter paper method.
† Eluted with methyl-a-D-glucopyranoside.
‡ Eluted with methyl-a-D-mannoside.

Table 4. Adsorption of fucose labelled materials in Nonidet extracts of MDV and HVT infected DE cells to MDV antiserum-Sepharose absorbent

<table>
<thead>
<tr>
<th>Material</th>
<th>Input radioactivity (ct/min x 10^{-2})</th>
<th>ct/min specifically eluted x 10^{-3}</th>
<th>Total radioactivity eluted (% input)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st†</td>
<td>2nd‡</td>
</tr>
<tr>
<td>HVT infected</td>
<td>[^{14}C] 23.5*</td>
<td>2.27</td>
<td>0.866</td>
</tr>
<tr>
<td>MDV infected</td>
<td>[^{3}H] 25.2</td>
<td>1.988</td>
<td>0.872</td>
</tr>
<tr>
<td>Control</td>
<td>[^{3}H] 15</td>
<td>0.11</td>
<td>0.025</td>
</tr>
</tbody>
</table>

* Radioactivity of TCA insoluble materials was estimated by the filter paper method.
† Eluted with 3.5 M-sodium thiocyanate.

There were a few large proteins, particularly in HVT preparations, which remained at the top of the gel and which contained relatively little fucose. The radioactive pattern obtained on analysis of solubilized whole cells (Fig. 3) was similar to those obtained with Nonidet extracts (Fig. 1 and 2). This is not surprising since fucose has been reported to be a specific precursor of plasma membrane glycoproteins (Atkinson & Summers, 1971). However, the extinction profiles of Coomassie blue stained gels were clearly different (Fig. 1a and 3a). Plainly, extraction with Nonidet was selective but it is possible that the materials extracted are not exclusively surface glycoproteins.

Comparison of con A receptors and antigens isolated from Nonidet extracts

The results of attachment and of specific elution of fucose labelled con A receptors and antigens are summarized in Tables 3 and 4, respectively. For equal inputs of radioactivity, approximately twice as much materials attached to con A adsorbent compared to immunoadsorbent. This was observed for both MDV and HVT preparations. It is unlikely that this effect is due to saturation of the immunoabsorbent since preliminary experiments showed that comparable amounts of immunoabsorbent can adsorb twice as much homologous antigenic material. The recovery of radioactivity (excluding losses in pipettes and containers) was 80% to 85% for con A adsorption and 75% to 80% for immunoabsorption. These results are consistent with those of electrophoresis (Fig. 1 and 2) and suggest that approx. half of the fucose labelled materials binding to con A is not antigenic and probably represents host glycoproteins. It was noted that the proportion of added radioactivity in HVT preparations that adsorbed to immunoabsorbent was marginally higher than that of homologous material (Table 4). This unexpected result may be due to the low specificity
Fig. 4. (a) Co-electrophoresis of MDV and HVT antigens eluted from immunoadsorbent (same materials as for Fig. 1 d and 2 d). — , \([^{3}H]\) counts MDV infected; – – – , \([^{14}C]\) counts HVT infected. (b) Electropherogram of material eluted from immunoadsorbent when a mixture of fucose labelled materials extracted from MDV infected and uninfected cells was applied. — , \([^{3}H]\) counts MDV infected; – – – , \([^{14}C]\) counts uninfected. (c) Co-electrophoresis of MDV and HVT materials eluted from con A adsorbent (same materials as for Fig. 1 c and 2 c). — , \([^{3}H]\) counts MDV infected; – – – , \([^{14}C]\) counts HVT infected. (d) Electrophoresis of material eluted from con A adsorbent when a mixture of fucose labelled materials (1:1, protein basis) extracted from MDV infected and from uninfected cells was applied. — , \([^{3}H]\) counts MDV infected; – – – , \([^{14}C]\) counts uninfected. Results shown for a and b and for c and d are for electrophoresis in 10% and in 8.5% gels, respectively. Apparent mol. wt. \(\times 10^{-3}\) are indicated above peaks; PR, phenol red.

Fig. 5. Agar gel immunodiffusion test showing presence of antigens in con-A eluates. Centre well = MD serum. O and O/2, original Nonidet extract of MDV infected cells and 1/2 dilution, respectively; S, supernatant fluid after adsorption with con A; E and E/2, glucoside eluate and 1/2 dilution, respectively.
Fig. 6. Co-electrophoresis of fucose labelled materials and antigens in culture fluids of MDV and HVT infected cultures. Results shown for electrophoresis in 8·5 % gel. (a) Electropherogram of materials before precipitation with antiserum. • — , [3H] counts MDV infected; ○ -- ○, [14C] counts HVT infected. (b) Electropherogram of precipitate obtained by adding MDV antiserum to a mixture of culture medium of MDV infected and uninfected cultures (1:1 mixture, protein basis). • — , [3H] counts MDV infected; △ -- △, [14C] counts uninfected. (c) Antigens present in culture fluids of MDV and HVT infected cultures were separately precipitated with MDV antiserum. The precipitates were combined and analysed. • — , [3H] counts MDV infected; ○ -- ○, [14C] counts HVT infected; PR, phenol red; apparent mol. wt. × 10^{-3} are indicated above peaks.

of the MDV antiserum used and to the presence of larger amounts of cross-reacting antigens in HVT preparations.

Comparison of the radioactive patterns of Nonidet extracts and those of the eluates from con A and immunoadsorbents (Fig. 1 and 2) shows that selective isolation of con A receptors and antigens was achieved. Major con A receptors isolated from MDV and HVT preparations were in the mol. wt. range 95,000 to 105,000 and were also present in uninfected preparations (Fig. 4d). Additional con A binding materials mol. wt. 24,000 to 26,000 and 35,000 to 38,000 in MDV preparations (Fig. 1c and 4d) and 28,000 in extracts from HVT infected cells (Fig. 2c) appear to be virus specific.

The main antigens isolated from MDV infected preparations using immunoadsorbent were smaller than the major con A receptors (Fig. 1c and 1d). Differences between MDV and HVT antigens eluted from immunoadsorbent were demonstrated by co-electrophoresis
in 10% gel (Fig. 4a). Antigenic materials (mol. wt. 33,000 to 43,000) present in MDV preparations were not detected in HVT preparations. Paradoxically antigens were detected in HVT materials (mol. wt. 46,000 and 59,000) that were not resolved in MDV preparations. This is discussed later.

It is obvious from the results of immunodiffusion tests (Fig. 5) that con A eluates contain precipitating MDV antigens. Similar results were obtained with HVT con A eluates. Clearly, some con A binding materials are antigenic but it is not known whether they are identical to the fucose labelled antigenic materials isolated by immunoadsorption (Fig. 1d and 2d).

**Comparison of glycoproteins and antigens present in culture fluids**

Since the 'A' antigen, a major precipitating antigen associated with MDV infections is secreted in culture medium of MDV and HVT infected cells, it was of interest to compare the glycoproteins present in the medium, particularly the precipitating antigenic glycoproteins. Similar patterns of radioactivity were obtained on co-electrophoresis of immune precipitates (Fig. 6c). Although the two glycoproteins resolved are in the mol. wt. range reported previously for the 'A' antigen (79,000 to 89,000) (Ross *et al.* 1973), it is possible that they are immunologically distinct since culture medium may contain other precipitating antigens in addition to the 'A' antigen. More information on this may be obtained using antiserum specific to the 'A' antigen.

**DISCUSSION**

Surface changes were demonstrated in both MDV and in HVT infected cells by agglutination with con A. Plainly, the capacity of infected cells to be agglutinated by this lectin is not a specific property of virulent virus. No attempt was made to compare the degree and time course of agglutination since meaningful comparisons can only be made under conditions of one step growth and the use of cell associated virus precludes this. However, evidence was obtained for the presence of MDV specific antigenic glycoproteins in infected cells. Since these antigens were extracted from intact cells with Nonidet and contained fucose, it is highly probable that they are antigens associated with the cell surface and may correspond to the cell surface antigens reported by Ahmed & Schidlovsky (1972).

Comparison of virus specific products in cells infected with MDV and HVT by electrophoresis in acrylamide gels was hampered by the low specific activity of the materials available for analysis. This may be attributed to the fact that only 30% of cells were infected when cell associated virus was used to initiate infection and to the poor utilization of fucose. However, the use of fucose is desirable because it has been reported to be a specific precursor of plasma membrane glycoproteins (Atkinson & Summers, 1971). Although the radioactive counts were low, the essential findings reported here were confirmed in a separate experiment in which labels were reversed.

The relationship between cell surface antigens and con A receptors is interesting since con A has been reported to bind to envelope glycoproteins of a number of viruses (Oram *et al.* 1971; Becht, Rott & Klenk, 1972). The presence of antigens in con A eluates was clearly shown by immunodiffusion (Fig. 5) and work is in progress to determine whether these are identical to the fucose labelled antigens eluted from immunoadsorbent. It is possible that the small con A receptors (mol. wt. 24,000 and 35,000) found in MDV preparations are identical to the MDV specific antigens (Fig. 1), since their mobility is comparable. However a large proportion of the con A binding materials (mol. wt. 95,000 to 125,000) is not antigenic and is probably host material. Alternatively, these large glycoproteins could be antigenic but of lower affinity for antiserum than the small mol. wt. glycoproteins.
Glycoproteins of MDV and HVT

The results reported here suggest that antigenic glycoproteins mol. wt. 30000 and 43000 approx. (Fig. 4) are MDV type specific (not antigenic in HVT preparations). It is of interest that Savage, Roizman & Heine (1972), using cross-linked antisera, were unable to relate type specific differences between herpes simplex 1 and 2 to type specific glycoproteins differing in size. It is therefore likely that the isolation of type specific materials depends on technique, on specificity of antiserum and on the degree of relatedness of the virus strains. Differences in the mobility of the antigens isolated from MDV and HVT preparations may be due to antigenic determinants unique to MDV or to the covalent linkage of identical antigenic determinants to other molecules differing in size. This could explain for example, the detection of antigens isolated from HVT preparations with MDV antiserum that are apparently not present in MDV preparations (Fig. 4a). However, a more likely explanation for this anomaly may be that convalescent MD antiserum contains antibodies against cross-reacting antigens that are synthesized in HVT infected cells but not in MDV infected cells in vitro. With respect to this last point it is worth noting that cell free infectious MDV particles can be obtained from feather follicles of infected chickens but not from infected cell cultures, whereas cell free infectious HVT particles can readily be obtained in vitro. Thus it is likely that convalescent MD antiserum contains antibodies to some components of mature particles that may not be synthesized in vitro. However, the results presented here are consistent with those of immunodiffusion studies (to be published) which have shown that MDV type specific antigens are associated with cell material and that culture fluids contain mainly cross-reacting antigens.

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REFERENCES


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