Detection of Early Cell Surface Changes in Herpes Simplex Virus Infected Cells by Agglutination with Concanavalin A

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Changes in the cell membranes after infection or transformation of cells by oncogenic viruses have been demonstrated using a plant agglutinin, Concanavalin A (Con. A) isolated from jack beans. Upon infection or transformation of cells by oncogenic viruses, the cells agglutinate in the presence of Con. A (Inbar & Sachs, 1969; Ben-Bassat, Inbar & Sachs, 1970; Benjamin & Burger, 1970). Moore & Temin (1971) reported a lack of correlation between transformability by RNA tumor viruses and agglutinability of cells by Con. A. Recent evidence (Cline & Livingston, 1971; Ozanne & Sambrook, 1971; Arndt-Jovin & Berg, 1971; Inbar, Ben-Bassat & Sachs, 1971) has suggested that both normal and transformed cells have the same number of Con. A binding sites but agglutinate differentially to a given concentration of Con. A. Nicolson (1971) showed that single Con. A binding sites at the surface of normal cells are randomly distributed whereas in transformed cells the Con. A sites are present in clusters.

During infection of cells by herpes simplex virus (HSV), there are alterations of the cellular membranes which are reflected by changes in their antigenicity (Roane & Roizman, 1964; Watkins, 1964; Nii et al., 1968; Roizman & Spear, 1971; Lowry, Bronson & Rawls, 1971a). At least a portion of these new membrane antigens are similar to antigens present within the envelope of the virus particle (Nii et al., 1968; Roizman & Spear, 1971). Since surface changes specified by HSV involve the synthesis of new glycoproteins (Spear, Keller & Roizman, 1969) and since the action of Con. A with carbohydrates has been elucidated (Goldstein, Hollerman & Merrick, 1965a; Goldstein, Hollerman & Smith, 1965b; Goldstein & So, 1965), it was of interest to determine if HSV-induced cell surface changes could be detected by treating infected cells with Con. A.

The ch+ and ch− strains of HSV described previously (Lowry et al., 1971a; Lowry, Melnick & Rawls, 1971b) were grown and assayed in primary rabbit kidney cells by the plaque method (Rapp, 1963). For agglutination assays, chick embryo or Balb/3T3 cells growing in 60 mm Falcon plastic Petri dishes in Eagle's medium containing 10% foetal calf serum were infected with HSV at a m.o.i. of 1 or 2. After an adsorption period of 90 min. at 37°C, the cultures were washed 3 times with culture medium to remove the unadsorbed virus and flooded with 4 ml. of fresh medium. At different times after infection, the cultures were washed with Ca2+ and Mg2+ free phosphate buffered saline (PBS), pH 7.2, pre-warmed to 37°C and the cells were brought into single cell suspension with 0.02% disodium versenate solution. The cells were then washed with saline and suspended in PBS at a concentration of 1 to 3 × 106 cells per ml, and 0.3 ml. samples of cell suspension were transferred to 35-mm. Falcon dishes to which were added either 0.3 ml. of various concentrations of Con. A (100 µg/ml. to 250 µg./ml.) or 0.3 ml. of saline. The dishes were rotated at room temperature and the cells were examined for agglutination at various time intervals under a Zeiss inverted microscope. Control cultures which were not infected with the virus were treated in a similar fashion. The degree of agglutination was expressed as 1+(10% cells agglutinated) to 4+(80% to 90% cells agglutinated). The degree of agglutination also depended upon the size of the aggregates.
Preliminary experiments showed that chick embryo cells either derived from a local hatchery or from a leukosis-free stock (kindly provided by Dr Nazerian) and Balb/3T3 cells did not agglutinate spontaneously. When treated with 1000 μg./ml. of Con. A (Calbiochem, Los Angeles, Lot No. 010229), these cells either remained in a single cell state or were agglutinated to a very low degree (1+). Treatment of normal cells with 0.01% trypsin, however, resulted in a 4+ agglutination.

Chick embryo cells were infected with 1 to 3 p.f.u./cell of ch+ and ch− strains of HSV. Sixteen hr after infection, the cells were washed, monodispersed, and incubated with 1000 μg./ml. of Con. A. The results in Fig. 1 show that cells infected with ch+ strain of HSV were agglutinated by Con. A. Within 20 min. after incubation, a 3+ agglutination of infected cells with Con. A was achieved. Infected cells were not agglutinated by saline, indicating the absence of spontaneous agglutination. Cells infected with the ch− strain of HSV, which fails to replicate or synthesize virus DNA but infects the cells with an efficiency similar to that of the ch+ virus and produces virus antigen (Lowry et al. 1971a), did not
Table 1. Induction of Con. A agglutinin sites in Balb/3T3 cells by ch+ and ch− HSV

<table>
<thead>
<tr>
<th>Virus</th>
<th>Virus titre* (p.f.u./ml.)</th>
<th>Agglutination by Con. A (1000 µg/ml)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV(ch+)</td>
<td>3.2 x 10⁷</td>
<td>++ + + +</td>
</tr>
<tr>
<td>HSV(ch−)</td>
<td>8.2 x 10⁶</td>
<td>++ + + +</td>
</tr>
<tr>
<td>Uninfected</td>
<td>--</td>
<td>o or +</td>
</tr>
</tbody>
</table>

* Virus titres were determined 24 hr after virus infection.
† Agglutination assays were carried out 8 hr after virus infection.

Table 2. Effect of cycloheximide on the expression of Con. A agglutinin sites in HSV (ch+)-infected chick embryo cells

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Time of addition of inhibitor (hr)</th>
<th>Agglutination by Con. A* (500 µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>--</td>
<td>++ + + +</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>0</td>
<td>o</td>
</tr>
<tr>
<td>(25 µg./ml.)</td>
<td>1.5</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>+ +</td>
</tr>
<tr>
<td></td>
<td>9.5</td>
<td>+ + + +</td>
</tr>
</tbody>
</table>

* Cultures infected with an m.o.i. of 1 and 2 of ch+ strain of HSV were incubated for 16 hr with or without cycloheximide. Cells were washed with PBS, monodispersed, and tested for agglutination by Con. A.

agglutinate after interacting with Con. A, indicating that the expression of Con. A agglutinability by ch+ virus is a virus-determined function.

Results presented in Fig. 2 show the kinetics of induction by HSV of agglutinin sites and surface antigen. Infection of chick cells with the ch+ strain of HSV resulted in a 2+ agglutination by Con. A within 2 hr and a 4+ agglutination by 3 hr post-infection. The appearance of surface antigen was first detected by the immunofluorescence test described previously (Lowry et al. 1971a) at 6 hr after infection, and by 10 hr almost 80% of the cells were positive for surface antigen. It should be mentioned that the ch− strain of HSV, which failed to induce agglutination by Con. A, also failed to induce specific antigen at the cell surface detectable by immunofluorescence. These results indicated that the expression of Con. A agglutinin sites occurred early in the virus replicative cycle. Whether the agglutinin sites are similar to specific antigen is not known at this time.

Since the HSV (ch−) strain was defective in chick cells, the Balb/3T3 cell line was used to determine if ch− virus could induce Con. A agglutinin sites in these cells. The results in Table 1 show that both ch+ and ch− strains were able to induce Con. A agglutinin sites in Balb/3T3 cells and a 4+ agglutination was observed within 8 hr after infection. Normal 3T3 cells did not agglutinate by Con. A unless trypsinized. These results indicate that the strain HSV (ch−) which is defective in replication and in induction of Con. A agglutinin sites in chick embryo cells can replicate and induce Con. A agglutinin sites in 3T3 cells.

The results in Table 2 show that de novo protein synthesis is required for HSV to cause alterations in cell surfaces so that the cells can be agglutinated by Con. A. Cycloheximide (25 µg./ml.) added at the time of addition of virus completely prevented the expression of Con. A agglutinability. Addition of the drug up to 4 hr post-infection partially inhibited the agglutination by Con. A. The addition of the drug to control cultures did not lead to spontaneous agglutination of cells by Con. A.

The evidence presented in this study indicates that early in the replicative cycle HSV can
cause the induction of Con. A agglutinin sites on chick embryo and mouse cells. The surface change leading to the agglutination of infected cells is not a nonspecific change due to virus adsorption, penetration or virus cytopathic effects, since the ch− strain of HSV which adsorbs, penetrates, uncoats and induces cytopathic effects does not bring about this change. Results with ch− strain also indicate that the induction of Con. A agglutinin sites by HSV is a virus-determined function.

Mammalian cells infected with HSV develop virus-specific antigens at the cell surface which can be demonstrated by the inability of infected cells to replicate HSV after interaction with anti-HSV infected cell surface sera and complement (Roane & Roizman, 1964); mixed haemadsorption test (Watkins, 1964); indirect immunofluorescence test (Lowry et al., 1968); immunoferitin technique (Nii et al., 1968); and by a shift in the density of cell membranes after reaction with anti-HSV antibody (Roizman & Spear, 1971). All the evidence indicates that these techniques are measuring a virus-specific protein. The relationship between the specific surface antigens and the Con. A agglutinin sites induced by HSV early in its replicative cycle remains unsolved.

Oncogenic DNA viruses (polyoma and SV40) have been shown to bring about similar changes during infection or transformation in normal cells. With these viruses, a relationship between the induction of Con. A agglutinins and the transforming function has been suggested (Benjamin & Burger, 1970), since polyoma mutants have been isolated which lack the transforming ability and also fail to induce Con. A agglutinin sites. Whether these surface changes brought about by papovaviruses are similar to the surface changes induced by HSV reported here and by vaccinia virus (Zarling & Tevethia, 1971) is not known at this time.

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REFERENCES


Short communications


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