Complement-mediated Cytolysis of HSV-1 and HSV-2 Infected Cells: Plasma Membrane Antigens Reactive with Type-specific and Cross-reactive Antibody

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SUMMARY

Surface antigens of BHK-21 cells infected with HSV-1 or HSV-2 were radioiodinated with lactoperoxidase, immune precipitated and analysed by polyacrylamide gel electrophoresis (PAGE). Experiments using antisera to HSV-1 or HSV-2, absorbed with appropriate homotypic or heterotypic antigens, revealed that both type-specific (homotypic) and cross-reactive antibody combined with surface glycoproteins to form a single large radioactive peak. This peak, which constituted the major glycoprotein region (region a) observed in electropherograms, represented a range in mol. wt. from 115000 to 130000. Sensitization of cells to complement lysis, neutralization of infectious virus and immune precipitation of surface glycoproteins (region a) were found to be generally correlated properties of all the antibody preparations analysed, including antibody prepared specifically against region a antigens. These findings suggest a major immunological role for the surface glycoproteins migrating in PAGE region a.

INTRODUCTION

Surface antigens of cells infected with herpes simplex virus (HSV) and envelope antigens of infectious virions are thought to play a central role in immune regulation of herpetic infections (Hilleman, 1976; Watson & Honess, 1977). In a previous study (Glorioso & Smith, 1977), we showed that surface antigens of cells capable of reacting with specific antibody to HSV could be resolved by polyacrylamide gel electrophoresis (PAGE) into three regions (a, b and c), together comprising a total of about 11 proteins. Most of the precipitable radioactivity was found to be associated with glycoproteins in region a (115000 to 130000).

Both herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) can initiate human infection. In either case, two categories of antibody are evoked, namely, strictly homotypic (type-specific) antibody, which reacts with antigens of the primary infecting type of HSV, and heterotypic (cross-reactive) antibody, which reacts with antigens of both types (Watson & Honess, 1977; Smith, 1978). Evidence suggests that glycoproteins corresponding in mol. wt. to those in region a, apparently the major glycoprotein region

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induced by HSV in most systems, contain antigenic determinants that bind both type-specific and cross-reactive antibody (Savage et al. 1972; Spear, 1975). Although these glycoproteins are exposed at the surface of the HSV-infected cell (Glorioso & Smith, 1977), it has not yet been determined whether they can serve as antigenic targets for immune cytolysis. Furthermore, the consequences of interaction between virus-coded cellular antigens and type-specific or cross-reactive antibody have not been investigated.

Here we report results of efforts to identify the surface antigens that may be important for immune recognition and lysis of cells infected with either HSV-1 or HSV-2. The accessibility of membrane antigens to antibody has been demonstrated through the use of radioiodination in combination with immunoprecipitation and PAGE. By comparing results of experiments involving, on the one hand, complement-mediated immune lysis of infected cells and, on the other, neutralization of infectious virus, we can discern a major role for the membrane glycopeptides that migrate in region $a$ of electropherograms.

**METHODS**

**Cells and virus.** Continuous lines of baby hamster kidney (BHK-21) cells and African green monkey kidney (VERO) cells were prepared and maintained as previously described (Smith & Glorioso, 1976). Prototypic strains of HSV-I (strain KOS) and HSV-2 (strain 196) were used throughout the study (Rawls et al. 1968). Stocks were prepared and titered in VERO cells using the plaque count method (Smith et al. 1971).

**Radio-iodination and immunoprecipitation.** The conditions of radio-iodination and immunoprecipitation of HSV-induced surface antigens were similar to those described earlier (Glorioso & Smith, 1977). BHK-21 cells were infected for 2 h at 37 °C at a multiplicity of infection (m.o.i.) of 10 to 15 plaque-forming units (p.f.u.) and overlaid with Eagle’s minimal essential medium (EMEM) containing 2 % foetal calf serum (FCS), penicillin (100 units/ml) and streptomycin (100 µg/ml). At 10 h post infection, cells were harvested with tris-Versene solution (0.01 M-tris, 0.14 M-NaCl, 0.5 mM-EDTA), pH 7.2, and washed once with cold 0.1 M-tris buffered saline, pH 7.4, containing 0.5 M-KI. Suspensions were adjusted to contain 2 x 10$^7$ cells (> 95 % viable) in 1.5 ml of Earle’s balanced salts solution (EBSS), without phenol red, containing 10$^{-5}$ M-KI. Reactants were added to the suspension in the following order: lactoperoxidase (0.25 mg in 0.125 ml EBSS; Sigma Chemical Co., St Louis, Mo.), 0.125 ml of Na$^{125}$I (1 mCi; Amersham/Searle, Northbrook, Ill.) and 0.125 ml Na$_2$SO$_4$ (10 µM). Labelling was begun by addition of 25 µl of H$_2$O$_2$ (1.3 mm) and maintained by five more additions (25 µl) at 2 min intervals. Labelling was inhibited by addition of 1.5 ml (5 mM) of L-cysteine. Unincorporated iodine was removed by five washes in cold EBSS. Cells were lysed by addition of 1.5 ml of 2 % Triton X-100 in phosphate-buffered saline (PBS), pH 7.4. Nuclei and cell debris were removed from cell lysates by centrifuging at 80000 g for 30 min. Solubilized surface antigens were allowed to react with constant amounts of specific anti-HSV antibody and precipitated indirectly at equivalence using goat-anti-IgG as previously described (Glorioso & Smith, 1977).

**Polyacrylamide gel electrophoresis (PAGE) and autoradiography.** Immune precipitates were solubilized and subjected to SDS-PAGE using a 7 % resolving gel and a 3 % stacking gel, as previously described (Glorioso & Smith, 1977). After electrophoresis, gels were fixed in a solution containing 25 % methanol and 7 % acetic acid, dried and placed in contact with Kodak NS-2T X-ray film for 1 to 7 days. Absorbance tracings (550 nm) were produced from the resulting autoradiograms using a Gilford 240 spectrophotometer with gel-scanning device and recorder.
Antibody to HSV surface antigens

Table 1. Cross-reactive and type-specific antibody in rabbit antisera to HSV-1 and HSV-2

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Absorption cells*</th>
<th>Neutralization (log₁₀)†</th>
<th>Cytolysis‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HSV-1</td>
<td>HSV-2</td>
</tr>
<tr>
<td>HSV-1</td>
<td>None</td>
<td>2.62</td>
<td>1.65</td>
</tr>
<tr>
<td></td>
<td>Uninfected</td>
<td>2.55</td>
<td>1.60</td>
</tr>
<tr>
<td></td>
<td>HSV-1</td>
<td>&lt; 0.7</td>
<td>&lt; 0.7</td>
</tr>
<tr>
<td></td>
<td>HSV-2</td>
<td>2.38</td>
<td>&lt; 0.7</td>
</tr>
<tr>
<td>HSV-2</td>
<td>None</td>
<td>1.90</td>
<td>3.25</td>
</tr>
<tr>
<td></td>
<td>Uninfected</td>
<td>1.72</td>
<td>3.10</td>
</tr>
<tr>
<td></td>
<td>HSV-1</td>
<td>&lt; 0.7</td>
<td>&lt; 2.50</td>
</tr>
<tr>
<td></td>
<td>HSV-2</td>
<td>&lt; 0.7</td>
<td>&lt; 0.7</td>
</tr>
</tbody>
</table>

* Two ml portions of antisera were absorbed five times with 2 × 10⁸ lyophilized BHK-21 cells (infected or uninfected, as indicated).
† Antibody activity as determined by microneutralization assay (Rawls et al. 1970).
‡ Cytolytic antibody activity to surface antigens of HSV-infected target cells (% specific ⁵¹Cr-release), antibody diluted 1:5; values less than 5% were not considered significant based on repeat analysis of positive samples.

Absorption of antisera. Antisera to HSV-1 (KOS) and HSV-2 (196) were obtained by injecting rabbits with about 1 mg of partially purified HSV in complete Freund’s adjuvant, as reported earlier (Smith & Glorioso, 1976). Type-specific antibody was prepared by sequential absorption of 2 ml portions of antisera with 2 × 10⁸ (20 mg) lyophilized BHK-21 cells infected with heterotypic virus. Absorbing suspensions were incubated at 37 °C for 1 h and at 4 °C for 18 h. Cellular debris and virus antigens bound to cross-reactive antibody were removed by centrifuging at 2000 g for 10 min, followed by centrifuging at 30000 g for 30 min.

Antibody assays. Neutralizing antibody to HSV-1 and HSV-2 was assayed in VERO cells by the microneutralization technique of Rawls et al. (1968). Cytolytic antibody to surface antigens of HSV-infected cells was assayed by the complement-dependent ⁵¹Cr-release test previously described (Smith & Glorioso, 1976). Controls for each assay consisted of tubes containing only complement and ⁵¹Cr-labelled cells (spontaneous release) and tubes containing labelled cells that had been frozen and thawed twice (maximum release). Cytolytic antibody activity was expressed as percentage specific ⁵¹Cr-release according to the formula (Smith et al. 1972):

\[
\text{% of specific } ⁵¹\text{Cr-release} = \left( \frac{\text{⁵¹Cr-release in presence of test serum and complement}}{\text{maximium release}} \right) - \left( \frac{\text{spontaneous release}}{} \right) \times 100.
\]

RESULTS

Characterization of type-specific and cross-reactive antibody to HSV-1 and HSV-2

Detection of type-specific and cross-reactive surface antigens was based on the use of appropriately defined preparations of type-specific and cross-reactive antibody in procedures involving immunoprecipitation of radiolabelled proteins and PAGE analysis. Antisera prepared against either HSV-1 or HSV-2 usually contain both type-specific and cross-reactive antibody. The effect of type-specific antibody present in unabsorbed serum could be bypassed by using only heterotypic virus or heterotypically infected cells in test
Fig. 1. Absorbance tracings (550 nm) of autoradiograms derived from interaction of HSV-1 and HSV-2 induced surface antigens with antiserum to HSV-1. (a) HSV-1 infected cells reacted with unabsorbed antiserum to HSV-1 (cross-reactive and type-specific antibody). (b) HSV-1 infected cells reacted with antiserum to HSV-1 absorbed with HSV-1 antigens (cross-reactive and type-specific antibody removed). (c) HSV-1 infected cells reacted with antiserum to HSV-1 absorbed with HSV-2 antigens (type-specific antibody; cross-reactive antibody removed). (d) HSV-2 infected cells reacted with unabsorbed antiserum to HSV-1 (cross-reactive antibody). (e) HSV-2 infected cells reacted with antiserum to HSV-1 absorbed with HSV-1 antigens (cross-reactive and type-specific antibody removed). (f) HSV-2 infected cells reacted with antiserum to HSV-1 absorbed with HSV-2 antigens (cross-reactive antibody removed).

reactions (Fig. 1d and 2d). For experiments designed to detect the reaction of type-specific antibody with surface antigens, cross-reactive antibody had to be removed. The removal was accomplished by absorbing antisera with heterotypically infected cells, a process that leaves behind residual type-specific antibody (Smith & Glorioso, 1976).

Rabbit antisera to HSV-1 (rabbit KOS) or HSV-2 (rabbit 196) were used in most experiments. Table 1 shows the patterns of antibody activity before and after absorption. Before absorption, antisera from both rabbits contained cross-reactive antibody, as demonstrated
Antibody to HSV surface antigens

Fig. 2. Absorbance tracings (550 nm) of autoradiograms derived from interaction of HSV-1 and HSV-2 induced surface antigens with antiserum to HSV-2. (a) HSV-2 infected cells reacted with unabsorbed antiserum to HSV-2 (cross-reactive and type-specific antibody). (b) HSV-2 infected cells reacted with antiserum to HSV-2 absorbed with HSV-2 antigens (cross-reactive and type-specific antibody removed). (c) HSV-2 infected cells reacted with antiserum to HSV-2 absorbed with HSV-1 antigens (type-specific antibody and cross-reactive antibody removed). (d) HSV-1 infected cells reacted with unabsorbed antiserum to HSV-2 (cross-reactive antibody). (e) HSV-1 infected cells reacted with antiserum to HSV-2 absorbed with HSV-2 antigens (cross-reactive and type-specific antibody removed). (f) HSV-1 infected cells reacted with antiserum to HSV-2 absorbed with HSV-1 antigens (cross-reactive antibody removed).

Interaction of cross-reactive and type-specific antibody with surface antigens

Experiments were carried out to determine which membrane proteins were reactive with type-specific antibody and which with cross-reactive antibody (Fig. 1 and 2). Autoradiograms of polyacrylamide gels derived from reaction of unabsorbed antiserum to HSV-1 with surface antigens of viable HSV-1 infected cells (Fig. 1a) consistently showed three general regions of Ag-Ab interaction, arbitrarily designated a (115000 to 130000 daltons), b (60000 to 80000 daltons) and c (45000 to 50000 daltons). As was previously shown by both neutralization and cytolytic antibody assays. Cross-absorption removed antibody to heterotypic virus (neutralization), as well as to surface antigens (immune cytolysis), leaving antibody that was functionally type-specific. Absorption of antisera with uninfected cells, which served as controls, resulted in negligible loss of antibody activity. As would be expected, absorption of antisera with the virus antigens against which they were made (homotypic antigens) removed almost all antibody activity.
Fig. 3. Electropherograms derived from interaction of uninfected cells with non-immune sera. (a) HSV-1 infected cells reacted with unabsorbed pre-immune serum (HSV-2 rabbit). (b) HSV-1 infected cells reacted with pre-immune serum absorbed with uninfected cells. (c) HSV-1 infected cells reacted with pre-immune serum absorbed with HSV-1 infected cells. (d) Uninfected cells reacted with pre-immune serum. (e) HSV-1 infected cells reacted with commercial rabbit serum.

(Glorioso & Smith, 1977), regions a and b are composed largely of glycosylated proteins, whereas peptides in region c are largely unglycosylated.

When this same antiserum, containing cross-reactive antibody, was reacted with surface antigens of HSV-2 infected cells, all three regions were again detected (Fig. 1d). In both cases, however, most of the radioactivity that precipitated was associated with glycoproteins in region a. When a preparation containing type-specific antibody for HSV-1 was reacted with HSV-1 infected cells, the same pattern was observed (Fig. 1c). PAGE analysis of antibody preparations that displayed no cytolytic or neutralizing antibody activity (Table 1) revealed little or no radioactivity in region a, and only slight activity in regions b and c (Fig. 1b, e, f). The radioactivity observed in regions b and c in these instances, however, was less than that seen when cytolytic neutralizing antibody was present (Fig. 1a, c, d), suggesting that specific HSV antibody increased the amount of activity precipitated in these regions. When antiserum to HSV-2 was reacted with surface antigens of cells infected with either HSV-1 or HSV-2, patterns generally analogous to those produced by antiserum to HSV-1 were observed (Fig. 2). Region c was proportionately somewhat larger, however. Again, cross-reactive (Fig. 2d) and type-specific antibody (Fig. 2c) preparations combined with proteins in all three gel regions, giving reactions that corresponded in magnitude to the levels of cytolytic and neutralizing antibody shown in Table 1. The most striking feature of immune precipitates represented in both Fig. 1 and 2, however, was region a. In autoradiographic scans derived from antibody preparations that contained cytolytic and neutralizing antibody, the highest peak of radioactivity detected was associated with proteins ranging from 115,000 to 130,000 daltons.
Antibody to HSV surface antigens

Table 2. Cytolytic and neutralizing antibody to HSV present in normal rabbit sera

<table>
<thead>
<tr>
<th>Serum</th>
<th>Absorption cells*</th>
<th>Neutralization (log_{10})</th>
<th>Cytolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-immune (HSV-1 rabbit)</td>
<td>None</td>
<td>&lt; 0.7</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Uninfected</td>
<td>&lt; 0.7</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>HSV-1</td>
<td>&lt; 0.7</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>Normal (commercial)</td>
<td>None</td>
<td>&lt; 0.7</td>
<td>22</td>
</tr>
</tbody>
</table>

* See Table 1 for explanation of absorption procedures and antibody assays.

Reaction of normal sera with HSV-induced surface antigens

Besides the major amounts of activity demonstrated in region a with preparations containing cross-reactive or type-specific antibody, some activity was detected in regions b and c, leading us to suspect that these regions might also contain cross-reactive and type-specific determinants. Unlike the activity in region a, however, activity in regions b and c was evident even when no neutralizing or cytolytic antibody to HSV-1 or HSV-2 was detected (Table 1; Fig. 1 and 2). Earlier we showed that non-immune rabbit and human sera also demonstrate some activity in those regions, without corresponding activity in region a (Glorioso & Smith, 1977). The combination of these findings raises the possibility that proteins in regions b and c are the result of precipitation with normal immunoglobulin rather than with specific antibody to HSV. Further experiments were performed to determine the specificity of reactions involving regions b and c.

When pre-immune (rabbit 196) or normal rabbit serum from commercial sources was reacted with surface components of cells infected with HSV-1, regions b and c were again clearly detected (Fig. 3a, e), indicating the activity was not limited to individual normal sera. To determine whether this activity might be related to infection with HSV rather than to the nature of the sera, two types of experiments were performed. In the first, pre-immune serum was absorbed with both infected and uninfected cells and then tested for its ability to precipitate surface antigens that would end up in regions b and c (Fig. 3). The results show that uninfected cell antigens reduced the amount of activity slightly but that almost all the activity was removed with infected cell antigens. In the second type of experiment, pre-immune serum was reacted with surface components of uninfected cells. In the resulting immune precipitates, regions b and c were again evident, although much reduced in comparison with patterns seen with infected cells and pre-immune serum.

To determine whether the activity observed could have been the result of a specific reaction with antibody to HSV, sera were examined for neutralizing and cytolytic antibody (Table 2). No specific antibody was detectable by microneutralization. However, slight activity was detected in cytolytic antibody assays, roughly paralleling the activity observed in immune precipitation experiments (Fig. 3). Again, the larger amount of activity was observed when infected, rather than uninfected, cells were used.

Reaction of surface antigens with antisera prepared against HSV glycoproteins

If the reactions observed in regions b and c were due to the binding of HSV-specific antibody with surface antigens, then antisera prepared against polypeptides in region a might be expected to precipitate only proteins in region a. To test this question, we analysed
Fig. 4. Electropherogram of HSV-1 surface antigens precipitated with rabbit antiserum to the major glycoprotein gel region (VP 2/3; Powell & Watson, 1975) of HSV-1 infected cells.

Table 3. Rabbit antisera prepared against purified HSV glycoproteins corresponding to region a

<table>
<thead>
<tr>
<th>Glycoprotein immunizing preparation</th>
<th>Serum</th>
<th>Neutralization (log_{10})</th>
<th>Cytolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-1†</td>
<td>Pre-immune</td>
<td>&lt; 0.7</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Immune</td>
<td>1.6</td>
<td>50</td>
</tr>
<tr>
<td>HSV-2‡</td>
<td>Pre-immune</td>
<td>&lt; 0.7</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Immune</td>
<td>&lt; 0.7</td>
<td>44</td>
</tr>
</tbody>
</table>

* See Table 1 for explanation of antibody assays.
† Obtained from Drs D. H. Watson and K. L. Powell (University of Leeds) and prepared by injecting 2/3 region of polyacrylamide gel (corresponds to region a) into footpads of rabbits (Powell & Watson, 1975).
‡ Obtained from Dr R. J. Courtney of the Baylor College of Medicine, Houston, Texas, and prepared by injecting VP 123 region (corresponds to region a) of polyacrylamide gel into footpads of rabbits R. J. Courtney, personal communication.

Antisera prepared against the glycoproteins of region a. Antiserum to the HSV-1 glycoproteins (obtained from Drs D. H. Watson and K. L. Powell of the University of Leeds) was prepared by injecting sections of acrylamide gel containing HSV glycoproteins with mol. wt. similar to those of region a (called VP 2/3 region) into the footpads of rabbits (Powell & Watson, 1975). Reaction of the antiserum with HSV-1 infected cells resulted in a gel profile (Fig. 4) nearly identical to those given by antisera to whole, unfractionated HSV-1 and HSV-2 (Fig. 1 and 2). In addition to significant activity to region a, activity in regions b and c was also observed. To amplify this observation, we obtained from the same source a second specimen of antiserum to HSV-1 glycoproteins, along with the corresponding pre-immune specimen. In addition, a set of similarly prepared pre- and post-immuniz-
Antibody to HSV surface antigens

Fig. 5. Electropherograms of HSV-1 and HSV-2 surface antigens precipitated with pre-immune sera and antisera to the major HSV-1 and HSV-2 glycoprotein gel regions. (a) HSV-1 infected cells reacted with pre-immune rabbit serum (Powell & Watson, 1975). (b) HSV-1 cells reacted with antiserum to the major HSV-1 glycoprotein region (VP 2/3; Powell & Watson, 1975). (c) HSV-2 cells and pre-immune serum (Powell & Watson, 1975). (d) HSV-2 cells and antiserum to the major HSV-1 glycoprotein region (VP 2/3; Powell & Watson, 1975). (e) HSV-1 cells and pre-immune serum (R. J. Courtney, personal communication). (f) HSV-1 cells and antiserum to the major HSV-2 glycoprotein region (VP 123; R. J. Courtney, personal communication). (g) HSV-2 cells and pre-immune serum (R. J. Courtney, personal communication). (h) HSV-2 cells and antiserum to the major HSV-2 glycoprotein region (VP 123; R. J. Courtney, personal communication).

Post-immunization sera from both laboratories had cytolytic and neutralizing antibody activity, indicating that the major glycoproteins against which the antisera were made (corresponding to region a) participated in both of those immunological functions. The antiserum produced against HSV-1 glycoproteins (VP 2/3) was found to be sharply type-specific in both assays, reacting only with homotypically infected cells or homotypic virus. The antiserum prepared against HSV-2 glycoproteins, on the other hand, cross-reacted to some extent with heterotypically (HSV-2) infected cells in cytolytic antibody assays. Neutralizing antibody was also detected in this antiserum, but the levels were low and cross-reactive patterns could not be determined. Pre-immune sera displayed some cytolytic antibody activity, as determined by cytolytic antibody assay (51Cr-release), but no neutralizing antibody activity, as with the other normal sera listed in Table 2.

The results of PAGE analysis correlated well with the immunological assays with regard to region a. Increases in cytolytic and neutralizing antibody activity paralleled increases in the amount of radioactivity detected in this region, resulting in similar type-specific to
cross-reactive antibody profiles. Regions b and c were again observed with both pre-immune sera and antisera to region a, further suggesting that detection of activity associated with these proteins was not the result of interactions between surface proteins and specific antibody to HSV.

Additional rabbit antisera to HSV-1 and HSV-2, as well as human sera containing antibody to HSV-1 and HSV-2, were absorbed and examined in most of the experiments described above. The results were essentially the same, and therefore data are not shown.

DISCUSSION

In this study, we have shown that both type-specific and cross-reactive antibodies react with antigenic determinants associated with plasma membrane glycoproteins of region a. In addition, we have found that reaction of antibody with these surface glycoproteins sensitizes the infected cell to complement lysis. The same surface antigens appear to be present in the envelope of HSV virions, in accord with previous findings (Roizman & Furlong, 1974), and presumably serve as targets for the antibody that neutralizes infectivity.

Two kinds of evidence support these conclusions. First, we noted that the capacity of either cross-reactive or type-specific antibody to sensitize infected cells correlates directly with the capacity of the same sera to precipitate radio-iodinated glycoproteins identified in PAGE region a. Second, the same correlation of activities was found with antisera prepared to the isolated glycoproteins. These findings suggest that the glycoproteins in PAGE region a may have important functions relating to immune recognition, by infected cells, of antibody to HSV, as particularly expressed in cytolysis and neutralization of infectious virus.

It is probable that region a of the electropherogram comprises a heterogeneous assortment of glycoproteins, as reflected in the wide range of mol. wt. observed (115,000 to 130,000). Although on occasion we could detect more than one 125I-labelled peak, the consistent feature of region a using both type-specific and cross-reactive antibody preparations was a single strong band. With antisera prepared against PAGE-separated glycoproteins of region a, one was strictly type-specific (HSV-1, Powell & Watson, 1975) and the other was cross-reactive (HSV-2, R. J. Courtney, personal communication). Although the reasons for these differences are unknown, they may well reflect changes resulting from differences in purification or immunization procedures. Recent evidence indicates that region a may contain as many as four or five glycopeptide components (Spear, 1976; R. J. Courtney, personal communication). Separation and purification of these components in biologically active form should help to determine whether the region contains individual surface glycopeptides that are strictly type-specific or cross-reactive.

Considerable evidence has been amassed to show that HSV induces plasma membrane antigens with mol. wt. less than those found in region a (Roizman & Furlong, 1974; Pauli & Ludwig, 1977). There is some question, however, as to the specificity of the low mol. wt. antigens that we detected in PAGE regions b and c. Activity was detected in these regions not only when antisera to HSV was reacted with infected cells, but also when normal sera were substituted and, to a limited extent, when reactions involved uninfected cells. These reactions could be the result of non-specific precipitation, or possibly be due to the presence of naturally occurring antibody to HSV or cell-surface components. The former explanation is unlikely, however, as neither region b or c was detected when immunoglobulin-free sera were used (data not shown). The glycoproteins in regions b and c were precipitated to a larger extent with HSV-infected cells than with uninfected cells. This finding suggests that naturally occurring antibody to BHK-21 cells was not a major factor but still does not
Antibody to HSV surface antigens

eliminate the possibility that infection with HSV either uncovered or induced formation of additional antigens that reacted with natural antibody. It does, however, indicate that precipitation depended largely on interaction of antibody with surface components of membranes modified by infection.

An alternative explanation concerns the presence of receptors for the Fc portion of normal immunoglobulin of cells infected with HSV (Westmoreland & Watkins, 1974). The presence of these receptors might account for the limited ability of normal sera to sensitize infected cells (Tables 2 and 3) and to precipitate surface proteins. Recent findings by Sakuma et al. (1977) indicate that Fc receptors of cells infected with cytomegalovirus are associated with proteins of 42,000 daltons, which corresponds to our region c. Preliminary experiments in our laboratory indicate that BHK-21 cells infected with HSV form rosettes with red blood cells sensitized with normal immunoglobulin, suggesting the presence of Fc receptors on the infected cells.

Our data show that both neutralizing and cytolysyte antibody reacted with antigens in region a. In normal sera that contained no neutralizing antibody, some cytolysyte antibody was detected when regions b and c were evident in electropherograms of immune precipitates derived from the same sera. One could interpret these data as meaning that antigens in regions b and c can mediate cytolysis but are not involved in virus neutralization. The discrepancy, however, may merely represent disparities in sensitivity between the two types of assay.

Pauli & Ludwig (1977) reported that membrane glycoproteins corresponding to region b were the most important for neutralization of HSV, a conclusion that our data do not support. The major glycoprotein peak found by most investigators corresponds to region a (Roizman & Furlong, 1974; Courtney & Powell, 1975; Spear, 1975; Watson & Honess, 1977), thus supporting the likelihood that these larger peptides constitute the major molecular complexes on the membrane of infected cells or in infectious virus that react with specific antiviral antibody.

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