Glycolipid Nature of the Complement-fixing Host Cell Antigen of Vesicular Stomatitis Virus

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Serological evidence for the presence of a host-cell component of vesicular stomatitis virus particles was provided by Cartwright & Pearce (1968). They showed by complement fixation reactions that purified virus particles prepared from virus grown in BHK 21 cells reacted with antiserum to BHK cell but not with antiserum to pig kidney cells. Conversely, virus grown in pig kidney cells fixed complement with antiserum to pig kidney cells but not with antiserum to BHK 21 cells. The amount of complement fixed with the cell antiserum was as great as that fixed with antiserum to virus.

It could be argued that the presence of host cell constituents in the purified virus preparations, possibly adhering to the surface of the particles, would account for these reactions. However, two observations suggest that the reactions are not due to the adventitious presence of cellular constituents. In the first place, treatment of the virus with Tween 80, which has a mild detergent action but does not affect the infectivity of the virus, has little effect on the complement-fixing activity of the virus with cell antiserum. Secondly, trypsin, which removes the glycoprotein projections from the surface of the virus with the consequent loss of complement-fixing activity with virus antiserum, has no effect on the complement-fixing activity with cell antiserum (Cartwright, Smale & Brown, 1969).

We expected that a host cell component which fixed complement would contain protein but found, in agreement with Wagner, Schnaitman & Snyder (1969) and Kang & Prevec (1969), that virus grown in cells pre-labelled with $^{14}$C-amino acids was not radioactive. Since this suggested that the host cell component was not a protein, we directed our attention to non-protein constituents of the virus.

We had shown previously that virus grown in BHK 21 cells pre-labelled with $^{32}$PO$_4$ was radioactive (Cartwright & Pearce, 1968). None of the $^{32}$P of virus labelled under these conditions was associated with the RNA, and polyacrylamide gel electrophoresis of the virus following disruption with sodium dodecyl sulphate under conditions we have described previously (Cartwright, Talbot & Brown, 1970) showed that the $^{32}$P was not associated with any of the virus proteins. Similarly, virus grown in cells pre-labelled with $^{14}$C-choline was also radioactive and the $^{14}$C migrated to a position in polyacrylamide gels well ahead of the area to which the virus proteins migrated. These observations suggested that phospholipid and lipid constituents of the host cell were closely associated with the virus and confirmed the analytical results of McSharry & Wagner (1971) which showed that some of the virus lipid is derived from the host cell.

Evidence that the complement-fixing activity with host cell antiserum was likely to be associated with the lipid fraction was obtained by using virus which was grown in the presence of $^3$H-amino acids in BHK 21 cells pre-labelled with $^{14}$C-choline. The virus was disrupted with 3% sodium dodecyl sulphate and passed through a column of Sephadex G-25. There was no separation of the $^{14}$C and $^3$H but the procedure served to remove the detergent. The peak fraction of radioactivity fixed complement with BHK 21 cell antiserum but only to a small extent with virus antiserum. Hydrolysis of the peak fraction with trypsin (250 μg./ml.) eliminated all the complement-fixing activity with virus antiserum but did not reduce the activity with cell antiserum (Table 1).
Table 1. *Complement-fixing activity of SDS-disrupted virus after trypsin hydrolysis and alcohol fractionation*

<table>
<thead>
<tr>
<th></th>
<th>Radioactivity (counts/min.) [(^3)H]</th>
<th>Complement-fixing activity in fraction with (Virus antiserum) BHK cell antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact virus</td>
<td>--</td>
<td>40 25</td>
</tr>
<tr>
<td>Sephadex filtrate</td>
<td>4750</td>
<td>3100 3 5 25</td>
</tr>
<tr>
<td>Sephadex filtrate + trypsin</td>
<td>--</td>
<td>0 25</td>
</tr>
<tr>
<td>Sephadex filtrate—alcohol precipitate</td>
<td>4720 3600</td>
<td>-- --</td>
</tr>
<tr>
<td>Sephadex filtrate—alcohol supernatant</td>
<td>0 3600</td>
<td>0 18</td>
</tr>
</tbody>
</table>

Virus labelled with [\(^3\)H]-amino acids and [\(^14\)C]-choline.

More direct evidence that protein was not involved in the reaction was obtained by fractionating the peak of radioactivity from the G-25 column with 90% alcohol. One drop of guinea pig serum to act as carrier was added to 0.5 ml. of the fraction and 4.5 ml. of alcohol then added. The precipitate obtained by centrifuging at 20,000 g for 30 min. after overnight storage at 4°C contained more than 99% of the protein (measured as [\(^3\)H]) and none of the [\(^14\)C] (Table 1). The supernatant was dialysed exhaustively against several changes of barbitone buffer and then tested for complement-fixing activity. About 75% of the activity found in the Sephadex G-25 filtrate was present (Table 1), thus providing evidence that the complement-fixing activity with cell antiserum was associated with material containing lipid.
Further evidence for this view was obtained by polyacrylamide gel electrophoresis. Virus which had been grown in the presence of [3H]-amino acids in BHK 21 cells pre-labelled with [14C]-choline was disrupted with 8 M-urea, 1% sodium dodecyl sulphate and 1% mercaptoethanol and examined by polyacrylamide gel electrophoresis in identical 10 cm. gel columns. One mm. slices from one gel were eluted in 0.5 ml. barbitone buffer and complement-fixing and radioactivity measurements made on the eluates (Fig. 1a). The distribution of these activities shows the coincidence of the complement-fixing activity with the [14C]. A second gel was stained with Schiff’s reagent before slicing for radioactive counting. Stained bands were obtained at the position of virus protein P2 (the surface projection) and also at the position of the [14C] (Fig. 1b). These results provide strong evidence that the host cell complement-fixing component is a glycolipid. This evidence also provides support for the suggestion made by Klenk & Choppin (1970) that cellular glycolipid incorporated into enveloped virus particles could act as a host antigen, with the carbohydrate moiety serving as the antigenic determinant.

Recently Hakomori & Murakami (1968) showed that BHK 21 cells contain one major glycolipid, namely, the glycosphingolipid hematoside, and Klenk & Choppin (1971) have since demonstrated that this component is incorporated into vesicular stomatitis virus particles grown in these cells. It seems probable from the evidence presented in this communication that hematoside is associated with the host cell complement-fixing component of this virus.

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REFERENCES


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