Measurement of Surface Antigen by Specific Bacterial Adherence and Scanning Electron Microscopy (SABA/SEM) in Cells Infected by Vesiculovirus \textit{ts} Mutants

\textit{(Accepted 11 November 1981)}

\textbf{SUMMARY}

Temperature-sensitive (\textit{ts}) mutants of the rhabdoviruses vesicular stomatitis virus and Chandipura virus have been used to measure the appearance of virus antigen on the surface of infected cells by the technique of surface analysis by bacterial adherence and scanning electron microscopy (SABA/SEM). The number of staphylococci specifically adhering to antiserum-treated infected PTK-2 or BSC-1 cells at permissive (31 °C) and restrictive (39 °C) temperatures was followed in time-course experiments and a close correspondence was observed between the proportion of staphylococci bound at 39 °C and the known phenotypic properties of the \textit{ts} mutants. Virus surface antigen was undetected in cells infected by transcription- and replication-defective \textit{ts} mutants with thermolabile L proteins under restrictive conditions up to an input multiplicity of infection of 50, and in cells infected by a replication-defective NS protein mutant. Some surface antigen was detected late in infection in PTK-2 cells infected by a replication-defective N protein mutant. Surface antigen accumulated normally in maturation-defective mutants with lesions in envelope proteins. These results establish the suitability of the SABA/SEM technique for quantitative estimation of virus antigen on the surface of infected cells.

The ability of the A protein of \textit{Staphylococcus aureus}, Cowan strain, to recognize the Fc portion of immunoglobulins has been exploited both for immunoprecipitation and for detection of virus antigen on the cell surface by the specific adherence of staphylococci to infected cells following exposure to virus antiserum (Huang & Okorie, 1978, 1979). We have shown previously that the technique of specific adherence of staphylococci can be used in conjunction with scanning electron microscopy to study the distribution of virus antigen on the surface of infected cells (Pringle & Parry, 1980). Staphylococcal cells have advantages as markers over latex beads and polymethyl methacrylate or polystyrene spheres by virtue of uniform diameter (about 1 μm) and ease of preparation and storage. Spatial localization of virus surface antigen was most marked in cells infected with pneumoviruses (human respiratory syncytial virus and murine pneumonia virus), where the staphylococci bound predominately to the filaments characteristically produced from the surface of the host cell following infection with these viruses (Parry \textit{et al.}, 1979). With many viruses, however, the staphylococci adhered uniformly over the surface of infected cells. Nonetheless, the accumulation of virus antigen at the cell surface could be followed by enumeration of the number of bound staphylococci up to a limiting time when the cell surface became saturated. Using vesicular stomatitis virus (VSV), antigen was detected on the surface of infected BSC-1 cells 1 h before the appearance of infectious virus, and could be followed up to 8 h after infection when the exponential phase of replication was over. The binding of staphylococci was highly specific and only occurred following exposure of infected cells to the homologous virus antiserum. It was essential, however, to assay the suitability of each preparation of \textit{S. aureus}, because there was variation in binding efficiency between batches.
Short communications

Table 1. Phenotypes of vesiculovirus ts mutants in relation to their staphylococcal binding properties

<table>
<thead>
<tr>
<th>Virus</th>
<th>Mutant</th>
<th>Complementation group</th>
<th>RNA phenotype*</th>
<th>Presumptive ts lesion*</th>
<th>Identified non-ts lesions†</th>
<th>Staph. binding 39/31°C x 10^-2 at 8 h post-infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>VSV-NJ</td>
<td>ts A1</td>
<td>A</td>
<td>+</td>
<td>N</td>
<td></td>
<td>11:3</td>
</tr>
<tr>
<td></td>
<td>ts B1</td>
<td>B</td>
<td>-</td>
<td>L</td>
<td></td>
<td>2:0</td>
</tr>
<tr>
<td></td>
<td>ts C1</td>
<td>C</td>
<td>+</td>
<td>M</td>
<td>G, N</td>
<td>81:2</td>
</tr>
<tr>
<td></td>
<td>ts D1</td>
<td>D</td>
<td>+</td>
<td>(G)</td>
<td>G, N, NS</td>
<td>87:6</td>
</tr>
<tr>
<td></td>
<td>ts E3</td>
<td>E</td>
<td>+</td>
<td>NS</td>
<td>G, M</td>
<td>2:0</td>
</tr>
<tr>
<td></td>
<td>ts F1</td>
<td>F</td>
<td>-</td>
<td>(?)</td>
<td></td>
<td>3:4</td>
</tr>
<tr>
<td>VSV-Ind</td>
<td>ts G114</td>
<td>I</td>
<td>-</td>
<td>L</td>
<td></td>
<td>2:5</td>
</tr>
<tr>
<td>CHV</td>
<td>ts Ch851</td>
<td>ChV</td>
<td>+</td>
<td>M</td>
<td></td>
<td>91:2</td>
</tr>
<tr>
<td></td>
<td>ts†</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>82:3</td>
</tr>
</tbody>
</table>

* From Lesnaw & Reichmann, 1975; Evans et al., 1979; Pringle & Szilagyi, 1980; J. F. Szilagyi, unpublished results. P.T., Primary transcription; S.T., secondary transcription.
† From Pringle et al., 1981.

The following experiments have been carried out to establish the sensitivity of the technique and to provide additional information concerning the phenotype of certain temperature-sensitive (ts) mutants of vesiculoviruses (Rhabdoviridae) with lesions in core and envelope proteins. Potoroo kidney cells (PTK-2) cells or African green monkey kidney (BSC-1) cells were grown on the surface of 13 mm diam. glass coverslips and infected as described previously (Pringle & Parry, 1980).

PTK-2 cells were used preferentially because of absence of surface features to confuse counting and absence of rounding-up during cell division. The mutants used are listed in Table 1 with details of their phenotype. Virus adsorption was carried out at +4 °C and multiplication initiated by addition of prewarmed medium. Replicate cultures were incubated at 31 °C or 39 °C and coverslips fixed at intervals. Samples of the culture fluid were collected at these times for infectivity assay. The coverslips were washed thoroughly with phosphate-buffered saline (PBS) to remove traces of serum before fixation for 10 to 15 min in 2.5% glutaraldehyde in PBS at room temperature. The fixed coverslip preparations were again washed thoroughly and stored in PBS at +4 °C or immediately treated with a 1/100 dilution of specific hyperimmune guinea-pig serum (generously provided by Dr F. Brown, Animal Virus Research Institute, Pirbright, Surrey, U.K.) for 1 h at 37 °C. The antiserum was then removed by thorough washing with PBS. A suspension of S. aureus, Cowan strain, prepared as described previously, was added to each coverslip and incubated at 37 °C for 5 min. The non-adhering staphylococci were removed by rapid washing in PBS. A control coverslip, virus-infected but not exposed to specific antiserum, was always included to monitor non-specific adherence. In most experiments, non-specific adherence was negligible. The coverslips were then post-fixed in 2% PBS-buffered osmium tetroxide for 60 min at room temperature before processing for scanning electron microscopy by critical point drying. The coverslips were then attached to aluminium stubs and gold-coated prior to examination at 25 kV in a Philips PSEM 500 scanning electron microscope. Areas of coverslips were photographed at a magnification of ×640 and the mean number of staphylococci bound per cell was obtained by counting two complete fields. Fig. 1(a) shows a typical field, in this case a monolayer of VSV-infected PTK-2 cells after 6 h at 31 °C showing extensive staphylococcal adherence to the surface of the infected cells prior to appreciable cytopathology.
Fig. 1. (a) Typical field of PTK-2 cells infected with ts mutant A1 (VSV New Jersey) with adhering staphylococci after treatment with specific antiserum and preparation for scanning electron microscopy; bar marker represents 25 μm. (b) Number of staphylococci bound per cell to PTK-2 cells infected with mutant ts G114 (VSV Indiana) and incubated at 31 °C (○) or 39 °C (▲). (c) Number of staphylococci bound per cell to PTK-2 cells infected with mutant ts Ch851 (CHV) and incubated at 31 °C (○) or 39 °C (▲). (d) Number of staphylococci bound per cell to PTK-2 cells infected with mutant ts E3 (VSV New Jersey) and incubated at 31 °C (○) or 39 °C (▲). Also shown is the amount of infectious virus present in the incubation medium at 31 °C (○) and 39 °C (▲) (right-hand ordinate). (e) Number of staphylococci bound per cell to PTK-2 cells infected with mutant ts A1 (VSV New Jersey) and incubated at 31 °C (○) and 39 °C (▲). Also shown is the amount of infectious virus present in the incubation medium at 31 °C (○) and 39 °C (▲) (right-hand ordinate). (f) Number of staphylococci bound per cell to PTK-2 cells infected with mutant ts D1 (VSV New Jersey) and incubated at 31 °C (○) and 39 °C (▲). Also shown is the amount of infectious virus present in the incubation medium at 31 °C (○) and 39 °C (▲) (right-hand ordinate).
Fig. 1(b) shows staphylococcal binding to PTK-2 cells infected at a low multiplicity of infection (0-1) with the ts G114 mutant of VSV Indiana, which has a thermolabile lesion of the L core protein preventing primary transcription at 39 °C (Szilagyi & Pringle, 1972; Hunt et al., 1976; Repik et al., 1976). There was no increase in adherence of staphylococci during 8 h of incubation at 39 °C. Increasing the multiplicity of infection up to 50 (not shown) caused a threefold increase in the number of staphylococci bound after 8 h at 31 °C without any corresponding increase at 39 °C. This experiment confirmed that staphylococcal binding measured new antigen synthesis and not extrusion of the inoculum. In subsequent experiments the multiplicity of infection was between 5 and 10. Similar results (see Table 1) were obtained with mutant ts B1 of VSV New Jersey (Pringle et al., 1971), another L protein mutant (Szilagyi & Pringle, 1979; Pringle & Szilagyi, 1980; J. F. Szilagyi, personal communication).

By contrast, Fig. 1(c) illustrates binding to cells infected with ts Ch851 of Chandipura virus (CHV), an M protein mutant (Gadkari & Pringle, 1980). Table 1 also indicates the high level of binding of staphylococci at 31 °C and 39 °C observed with another envelope protein mutant (mutant ts C1 of VSV New Jersey) and wild-type CHV. It was therefore concluded that these mutations of the M protein prevented virus maturation but not the appearance of antigen in the cell membrane.

The NS protein is also an essential component of the virion transcriptase (Emerson & Yu, 1975), and mutant ts E3 of VSV New Jersey possesses a ts lesion of the NS protein (Evans et al., 1979). Mutant ts E3 is defective for replication, but not for transcription (Pringle & Szilagyi, 1980). Fig. 1(d) shows that there was no increase in staphylococcal binding at 39 °C in PTK-2 cells infected with this mutant. A clone of ts E3 has been isolated with additional non-ts lesions of the M and G envelope proteins (Pringle et al., 1981) and this multiple mutant remained ts and unable to induce antigen synthesis as measured by staphylococcal binding at 39 °C (Table 1).

Mutant ts A1 of VSV New Jersey has a ts lesion in the N protein (J. F. Szilagyi, personal communication), the remaining protein component of the virion helical core. Secondary, but not primary, transcription was inhibited at 39 °C by this mutation (Lesnaw & Reichmann, 1975). Fig. 1(e) shows that some staphylococcal binding was observed late in infection although there was no corresponding increase in the amount of infectious virus recovered. These results showed that products of primary transcription reached the membrane of cells infected with the N-defective mutant ts A1 but not of cells infected with the NS-defective mutant ts E3.

Mutant ts D1 of VSV New Jersey is another ts mutant in which defined non-ts mutational lesions have been identified in addition to the primary ts lesion (Pringle et al., 1981). Fig. 1(f) shows that antigen accumulated at the cell surface normally at 39 °C, although maturation was inhibited. Non-ts lesions in core (N, NS) and envelope (G) proteins did not diminish staphylococcal binding significantly (Table 1). Thus, secondary non-ts mutations did not override the primary ts lesion in either mutant ts D1 or mutant ts E3.

These experiments establish that the SABA/SEM procedure is suitable for measurement of changes in the amount of virus antigen in the membrane of cells infected with vesiculoviruses, although the precise identity of the antigen was not discriminated. The use of monoclonal antibody in place of hyperimmune antiserum, however, may enable the appearance of individual virus proteins in the cell membrane to be followed.

Corresponding results (not shown) were obtained with some of these mutants in BSC-1 cells, and it is likely that this type of analysis can be applied to other virus–cell systems. The reported presence of Fc receptors on herpesvirus-infected cells (Westmoreland et al., 1976), however, emphasizes the necessity for careful monitoring of non-antibody-mediated adherence.
The assistance of Jim Aitken with electron microscopy and Valerie Devine and Margaret Wilkie with cell culture is gratefully acknowledged.

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(Received 3 August 1981)