Immunoelectron Microscopic Studies on Haemagglutinin and Haemolysin of Measles Virus in Infected HEp2 Cells

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SUMMARY

The antigenic determinants of the haemagglutinin and haemolysin antigens of measles virus were located at the surface of HEp2 cells infected with measles virus and on measles virions released from these cells, using immunoelectron microscopy. Antisera specific for haemagglutinin or haemolysin antigen and peroxidase-conjugated antiglobulin were used. Treatment of the infected cells with trypsin removed the virus spikes and prevented binding by the anti-haemagglutinin serum, while the reaction with anti-haemolysin serum was unaltered. This suggests that the antigenic determinants for measles haemagglutinin reside on the spike, while the antigenic determinants for haemolysin reside on, or are close to, the virus membrane.

Measles virus, a member of the genus Morbillivirus of the family Paramyxoviridae (Fenner, 1976), exhibits haemagglutination and haemolysis with rhesus monkey red blood cells (Waterson, 1965). It differs from members of the genus paramyxovirus, such as Sendai, SV5 and Newcastle disease virus (NDV), in that it lacks neuraminidase activity (Choppin & Compans, 1975). In the paramyxoviruses, it has been shown that the haemagglutinin (HA) and neuraminidase activities reside on one morphologically distinct type of spike, while the fusion factor (or haemolysin) resides on another (Compans & Klenk, 1979). Measles virus HA and haemolysin (HL) are antigenically distinct (Norrby & Gollmar, 1975) and the HL is more sensitive than HA to thermal inactivation, treatment with formalin, ether, or Tween 80 ether (Norrby & Falksveden, 1964; Norrby & Gollmar, 1975). Biochemical evidence suggests that measles virus HA and HL are separate polypeptides (Tyrrell & Norrby, 1978; Hardwick & Bussell, 1976), but little is known of the structural arrangement of these antigens within the virion envelope. Trypsin treatment of purified measles virions has been shown to remove the spikes from the particles (Norrby & Gollmar, 1975) and antiserum prepared against purified haemagglutinin of measles virus failed to react with cells persistently infected with measles virus which had been treated with trypsin (Fraser et al., 1978, 1979).

In our studies we observed that treatment of measles virus-infected cells or measles virus with acetone destroyed haemolysin both antigenically and functionally, and this provided a method for preparing monospecific anti-HL serum distinct from anti-HA serum (Armstrong et al., 1979). Trypsin treatment of persistently infected cells did not affect the binding of this antiserum to haemolysin antigen (Fraser et al., 1979; Armstrong et al., 1979). Since antisera against haemagglutinin and haemolysin reacted differently to trypsin-treated infected cells, we decided to use this system to study the location of HA and HL antigens at an ultrastructural level by immunoelectron microscopy. We employed a cell system with a lytic measles infection in preference to either purified virions or a persistent infection because this allowed the study of the antigens both during maturation at the cell surface and also in measles virions released from these cells.

The specificity of each antiserum for the two structural proteins in question, was tested by radioimmunoprecipitation employing [35S]methionine-labelled measles virus-induced proteins (Fig. 1a). Anti-HA antibody (lane 1) precipitated one polypeptide, with a mol. wt. of 80000,
Fig. 1. (a) Immunoprecipitation of [35S]methionine-labelled measles virus-infected cells (Lamb et al., 1978; Hall et al., 1979) analysed by electrophoresis on 5 to 12% continuous SDS-polyacrylamide gels (Laemmli, 1970). Fluorography was performed according to the method of Bonner & Laskey (1974). Lane 1, anti-HA; lane 2, anti-HL; lane 3, SSPE antiserum. (b) Electron micrograph of a measles virion, from a 10-fold concentrate of supernatant medium from measles-infected cells, semi-purified in a 20 to 50% discontinuous sucrose gradient and stained with 2% phosphotungstic acid. (c) Electron micrograph of a measles virion from the same preparation as (b) treated with 0.004% trypsin (Norrby & Gollmar, 1975) and stained with 2% phosphotungstic acid. Bar markers represent 100 nm.
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previously identified as the HA glycoprotein (Mountcastle & Choppin, 1977; Tyrrell & Norrby, 1978). Anti-HL antibody did not precipitate any polypeptides (lane 2). Human anti-measles serum from a subacute sclerosing panencephalitis (SSPE) patient was used as a control (lane 3) and precipitated all of the major measles structural polypeptides. These results demonstrate that the anti-HA antiserum is monospecific, precipitating the HA polypeptide only. The anti-HL antiserum does not precipitate the HA polypeptide and is therefore binding to a separate surface antigen. As it does not precipitate any other measles structural polypeptides and does not combine with uninfected cells (negative fluorescence staining, Armstrong et al., 1979), we consider that the antibody is specific for measles haemolysin. In addition, all our data in previous experiments (not shown) gave identical titres for the fluorescent antibody staining reaction and the functional HL inhibition (HLI) test, both with the unabsorbed serum, and at each successive step of absorption. The possibility of a second antibody and an unrecognized antigen of measles virus being involved is remote. Absence of specific immune precipitates in measles has been shown with monoclonal antibodies (Giraudon & Wild, 1981); there have also been reports of technical difficulties in the fixation of certain glycoproteins after polyacrylamide gel electrophoretic analysis (Goldwasser, 1975).

Monolayers of HEp2 cells were infected with the Edmonston strain of measles virus, P9 (Shirodaria et al., 1976), at a multiplicity of 0-04. After 48 h incubation at 37 °C, the infected monolayers were washed twice with 0.1 M-Sørensen's phosphate buffer pH 7.4 (PB) and divided into two aliquots each containing approx. 1 \times 10^7 cells. One aliquot was incubated with 0.004% crystalline trypsin in PB for 90 min at 37 °C, and the other aliquot was incubated with PB alone for a similar period. Both aliquots were fixed in 4% paraformaldehyde in PB for 5 min at +4 °C and washed three times with PB. Using the indirect immunofluorescence technique, the criterion for the successful removal of HA antigen was a reduction from 40% positive specific HA staining of 500 cells counted to less than 1% after trypsin treatment; there was no reduction with the anti-HL serum. The untreated and trypsin-treated preparations were examined by electron microscopy. Negative staining of semi-purified measles virions showed mature particles with clearly defined external spikes and internal nucleocapsid (Fig. 1b). After trypsin treatment the external spikes were absent and measles virions were identified by the presence of internal nucleocapsid (Fig. 1c). An indirect labelling method was employed, using guinea-pig anti-HA or human anti-HL with peroxidase-conjugated antiglobulins, to determine the location of the HA and HL antigens at the membrane of the measles virion and infected cell. Uninfected HEp2 cells were included as a control. The conjugates were prepared according to the method of Miller et al. (1974) and were absorbed with acetone-treated HEp2 cells, Vero cells and trypsin-treated HEp2 cells. They were standardized by titration on measles virus-infected cells on coverslips, examined by light microscopy and used at optimal staining dilutions. The paraformaldehyde-fixed preparations were each incubated in suspension for 50 min at 37 °C with either anti-HA or anti-HL serum at a dilution 10-fold lower than the endpoint, washed four times with PB and incubated with the appropriate peroxidase-conjugated antiglobulin in a further 50 min at 37 °C. After washing again four times with PB, the preparations were incubated with a diaminobenzidine substrate (Graham & Karnovsky, 1966) for 10 min at room temperature and washed once with 0.05 M-tris-HCl buffer pH 7.6. The preparations were fixed with 3% glutaraldehyde for 30 min at +4 °C, treated with 2% osmic acid for 60 min at +4 °C, dehydrated and embedded in Spurr resin. Sections of 60 to 90 nm thick were cut and stained with Reynolds' lead citrate, and examined using a Philips 301 transmission electron microscope.

Examination of the sections of the infected cells showed mature virus particles with clearly defined external spikes and internal nucleocapsid similar to those in the negatively stained
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Fig. 2. Electron microscopy of measles virus-infected HEp2 cells. (a) Untreated infected cells. Arrows indicate spikes on virion envelope with underlying nucleocapsid. (b) Trypsin-treated infected cells. Arrows indicate absence of spikes on virion envelope with underlying nucleocapsid. Both (a) and (b) were stained with uranyl acetate and lead citrate. (c, d) Indirect immunoperoxidase labelling with guinea-pig anti-HA serum on (c) untreated infected cells and (d) trypsin-treated infected cells. (e, f) Indirect immunoperoxidase labelling with human anti-HL serum on (e) untreated infected cells and (f) trypsin-treated infected cells. Sections (c) to (f) stained with lead citrate only. Bar markers represent 100 nm.

preparations. The surface of the infected cell also showed altered cell membrane bearing spikes, and with underlying nucleocapsid (Fig. 2a). After trypsin treatment, virus particles and adjacent sites of virus budding could be recognized by the presence of nucleocapsid, although spikes were completely absent (Fig. 2b).

The immunoperoxidase labelling of infected cells with anti-HA serum showed an accumulation of electron-dense precipitate along the surface of the membrane, the budding virus and the released virions. Spikes were not distinguishable because of the accumulation of reaction product, and the labelling appeared discontinuous on both the infected-cell
membrane and the virion (Fig. 2c). After treatment with trypsin no label was found (Fig. 2d). Immunoperoxidase labelling of infected cells with anti-HL (Fig. 2e) showed a similar pattern of electron-dense precipitate to that obtained with anti-HA, but after trypsin treatment the pattern of labelling was unchanged (Fig. 2f). No labelling was observed in the uninfected control cells (not illustrated).

The results reported above suggest that the antigenic determinants of HA within the viral membrane complex are located on the spikes, whereas the antigenic determinants of haemolysin remain after the spikes are removed with trypsin. Current concepts of the cell membrane envisage a structure wherein proteins are embedded in or across a lipid bilayer by virtue of their hydrophobic regions allowing movement of these proteins within the plane of the membrane (Singer & Nicolson, 1972). As we have previously shown that acetone treatment destroys haemolysin antigen in both the virion and the infected cell (Armstrong et al., 1979), it is logical to assume that it is intimately connected with the lipid component of the viral envelope. Furthermore, as it is accessible to surface labelling by immunofluorescence on live infected cells (Fraser et al., 1978; Armstrong et al., 1979), this suggests that the antigenic determinants for the molecule are external to the lipid with their hydrophilic ends exposed at the outer surface of the membrane. Our studies here have shown that the antigenic determinants for measles HA are situated on the spikes, while the determinants for HL are on or near the surface of the viral membrane. However, it is possible, as has recently been shown for Sendai virus (Bowen & Lyles, 1981), that either or both are transmembrane proteins with a small non-active segment spanning the membrane.

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