Antibody-Induced Capping of Measles Virus Antigens on Plasma Membrane Studied by Electron Microscopy

PETER W. LAMPERT, BARRY S. JOSEPH, AND MICHAEL B. A. OLDSTONE

Department of Immunopathology, Scripps Clinic and Research Foundation,* and Department of Pathology, University of California, San Diego School of Medicine, La Jolla, California 92037

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Antibodies specific for measles virus could redistribute ("cap") virus antigens on infected HeLa cells as shown by transmission and scanning electron microscopy. Using an indirect immunoperoxidase technique, infected cells showed diffuse, circumferential distribution of virus antigens over the cell surface when mixed with antibody at 4°C. At 37°C, virus-coated microvilli concentrated on one pole of the cell, leaving the remainder of the plasma membrane devoid of both viral antigens and microvillus projections. Whereas extreme polar displacement of virus-antibody complexes frequently occurred, endocytosis was rarely seen. The findings indicate that antiviral antibodies can move and cluster virus on plasma membranes and suggest that virus-antibody complexes are stripped and shed from the cell surface.

According to the Singer-Nicolson hypothesis, most cell membranes have a fluid mosaic structure; their surface proteins are free to diffuse in a lipid matrix and assume a random distribution over the cell's surface (11). In support of this concept, it has been shown that immunoglobulin determinants (6, 12, 14) and receptors for concanavalin A (Con A) (2, 10, 14) are randomly distributed on lymphocytes and other cells. Furthermore, exogenous agents can induce surface elements to assume a nonrandom distribution; e.g., multivalent antibody to immunoglobulin induces "capping" of surface immunoglobulin on lymphocytes and Con A causes focal clustering of Con A binding sites on transformed cells and lymphocytes (2, 6, 10, 12, 14). This redistribution of receptor sites is followed primarily by endocytosis and partly by shedding of the capped complexes, leaving the cell's surface temporarily free of binding sites (reviewed in 13).

Recently we reported that antibody specific for measles virus could redistribute (cap) measles virus antigens on infected cells in culture as judged by immunofluorescence microscopy (3). To extend these findings, we studied the capping of measles virus antigens by using transmission, scanning, and immunoelectron microscopy. Here we report that the distribution of measles virus antigens visualized by electron microscopy is essentially diffuse, but after exposure to antiviral antibody at 37°C the antigens become discrete, forming large unipolar aggregates on the cell's surface. This process is rarely associated with evidence of interiorization of viral antigen-antibody complexes.

**MATERIALS AND METHODS**

**Virus.** Wild-type Edmonston strain measles virus (American Type Culture Collection, Rockville, Md.) was grown in HeLa cells. Supernatant fluid from cells showing cytopathology was freed of cellular debris by centrifugation at 700 × g, divided into small aliquots, and frozen at −70°C until use. This pool titered 5 × 10^6 mean tissue culture infective dose units per ml.

**Cells and culturing techniques.** HeLa cells were infected with measles virus at a multiplicity of infection of 0.2 and grown in Eagle minimal essential medium supplemented with glutamine, antibiotics, and 10% heat-inactivated fetal calf serum (growth medium) in T75 plastic culture flasks. Four days after infection, cells were washed with phosphate-buffered saline (PBS) and then treated at room temperature with 0.05% trypsin-EDTA solution (Grand Island Biological Co.) for 2 to 5 min with gentle mechanical agitation. Cells were collected, washed four times in growth medium, and maintained at 4°C until experimental manipulation. The viability of these cells exceeded 95% as judged by trypan blue dye exclusion, and all cells expressed measles virus antigens on their surface in a random distribution when assayed by direct fluorescence microscopy (3).

**Antibody.** Procedures for assaying the specificity of antibody to measles virus antigens and conjugating it to fluorescein isothiocyanate have been reported (3). The antimeasles virus antibody purified as an immunoglobulin G (IgG) fraction was obtained from a human convalescent serum having a hemagglutinin
inhibition titer of 1:64. All the antimeasles virus activity could be absorbed by a concentrated preparation of measles virus. Techniques used to determine hemagglutinin inhibition titer, prepare the IgG fraction from serum, and absorb the fraction with measles virus have been published (3; B. S. Joseph, N. R. Cooper, and M. B. A. Oldstone, submitted for publication).

Electron microscopy studies. Washed cells (5 × 10⁶) infected with measles virus were mixed with 100 μl of antibody in glass tubes and incubated for 60 min at 4 or 37 C. Concurrently a similar sample was mixed with fluorescence-conjugated antiviral antibody (Fig. 3). For controls, uninfected HeLa cells were treated with antibody in an analogous manner; also, infected cells were incubated with human serum devoid of antimeasles virus antibodies. Thereafter each preparation was washed three times in growth medium, fixed in 2.5% phosphate-buffered glutaraldehyde, and processed for electron microscopy.

For immunoperoxidase electron microscopy, cells were first mixed with antibody for 40 min, washed four times, and then incubated with 100 μl of the peroxidase-conjugated IgG fraction of rabbit antihuman IgG (Cappel Laboratories, Inc., Downington, Pa.). After an additional 40-min incubation, cells were washed three times in PBS, fixed in 1% glutaraldehyde for 30 min, left overnight in PBS, washed three times in Tris buffer (pH 7.6), and then incubated for 2 h at room temperature with diaminobenzidine (0.5 mg/ml). After repeated washings in PBS, the cells were initially placed in 2.5% glutaraldehyde and then in phosphate-buffered 1% osmium tetroxide. These osmium-fixed cells were dehydrated, embedded in Araldite, cut with an LKB microtome, stained with uranyl acetate and lead citrate, and examined by a Siemens Elmscope 101 operating at 100 kV.

For scanning electron microscopy, the glutaraldehyde fixed cells were dehydrated in graded alcohol series, continued through Freon for critical-point drying, coated with 40.0 nm of gold, and examined on an ETEC U-1 scanning electron microscope with an accelerated voltage of 30 kV and a specimen tilt of 30 or 45°.

RESULTS

Uninfected HeLa cells. Uninfected HeLa cells displayed stubby microvilli that were uniformly distributed over the cell surface as seen by transmission microscopy. The villi varied in length and width. Microtubules were seen within the villi. Pleomorphic mitochondria, lysosomes, endoplasmic reticula, and ribosomes were randomly scattered throughout the cytoplasm. No virus particles were detected in uninfected cells. Scanning electron microscopy also revealed an even distribution of numerous microvilli arising from the surface membrane, akin to that recently described by Porter et al. (9).

Diffuse and random distribution of virus antigens on the surface of infected cells. Abundant, closely packed, slender microvillus projections covered the surface of acutely infected cells (Fig. 1a). Virus particles budding from the plasma membranes of these microvilli were seen throughout (Fig. 1b). Numerous areas of osmiophilic thickening of the plasma membrane’s inner surface were associated with a fuzzy coating on the outer surface in the absence of underlying tubular nucleocapsids. Apart from viral buds, the infected cells also contained aggregates of tubular nucleocapsids in both cytoplasm and nuclei. In sections of cells incubated at 4 C with antibodies and studied by immunoelectron microscopy, viral antigens were uniformly distributed over the entire cell surface (Fig. 2).

Capping after antibody cross-linking at 37 C. Infected cells exposed to antibody at 37 C had striking conglomerations of microvilli at one pole (Fig. 3). Budding virus was still visible at the surface of such compacted villi but no longer apparent over the remaining smooth surface of the cell. Various stages of capping were noted in cells from the same pellet. Several sections through these pellets confirmed that the observed polar redistribution was a true phenomenon and did not represent an unusual plane of sectioning. Extreme capping of cells suggested the release or shedding of microvillus virus-antibody aggregates from the cell (Fig. 4).

Immunoelectron microscopy using peroxidase-conjugated antibody confirmed the focal redistribution of measles virus antigens over one pole of the cell (Fig. 5). Pinocytosis of peroxidase or peroxidase-conjugated molecules was rarely seen within intracellular vesicles.

Scanning electron microscopy dramatically affirmed the redistribution of microvilli on infected cells. The abundant fine microvilli randomly placed over the surface of HeLa cells were redistributed after the addition of antibody at 37 C (Fig. 6). Viewed under scanning electron microscopy, uninfected HeLa cells had numerous surface projections, but these were shorter, thicker, and less abundant than those on infected cells. Microvilli on uninfected cells were not redistributed by measles antibody; infected cells incubated with human serum devoid of measles antibody likewise failed to exhibit microvillus aggregation.

DISCUSSION

These experiments provide further evidence that multivalent antibody can redistribute measles virus antigens on the surface of infected HeLa cells in culture. With a virus pathogenic
FIG. 1. HeLa cell acutely infected with measles virus. (A) Note random circumferential distribution of abundant microvilli over the cell surface. ×12,000. (B) Enlargement of microvilli shows that they contain budding measles virus (arrows). Note fuzzy outer coat of the plasma membrane also in areas that do not reveal an underlying tubular nucleocapsid. ×110,000.
for man, a human cell line, and a human antibody source, capping occurred consistently at 37 C. Extreme capping suggested that the formed caps were being extruded into the fluid phase rather than being interiorized. Preliminary studies in our laboratory (L. H. Perrin, B. S. Joseph, and M. B. A. Oldstone, unpublished observations) with radiolabeling techniques indicate that the majority of surface viral antigens are shed into the fluid phase after complexing with antiviral antibody. In the absence of antibody, viral antigens are distributed randomly and diffusely on the cell membrane.

Various stages of capping were noted in cells from the same pellet, suggesting an ongoing dynamic process. On the basis of these experiments, we could not determine whether capping resulted from (i) a discrete movement of viral antigenic molecules along the cell surface with concomitant collapse of "evacuated" microvilli or (ii) direct cross-linking of antigen-coated cell protrusions with active transport of these clusters to one end of the cell. Our earlier studies indicated that capping was an enzymatically dependent phenomenon and required energy production. In addition we noted that both microfilaments and microtubules were involved in the capping process in HeLa cells infected with measles virus antigens since capping was inhabitable with vinblastine sulfate, cytochalasin B, and colchicine (3).

These observations provide further evidence that surface proteins can move freely in plasma membrane, lending additional support to the "fluid mosaic" model (11). Hence, in addition to surface immunoglobulin determinants (6, 12, 14), transplantation alloantigens (4), receptors for plant lectins (2, 10, 14), and receptors for immune complexes (7), viral antigens can also be capped at the cell surface. We have no reason to doubt that capping may occur in other infections in which viral antigens are expressed in the plasma membrane at sufficient density to be cross-linked by antiviral antibodies. Preliminary observations in this laboratory (5) and by others (1, 8) suggest that capping of surface viral antigens can be elicited in other systems where viral maturation occurs through a budding process. Antibody-induced capping and shedding of viral antigens from the cell's surface...
Fig. 3. Advanced capping (arrows) of virus-coated microvilli of a HeLa cell exposed to antibodies to measles virus at 37 C for 30 min. x8,000.

Fig. 4. Extreme capping of microvilli suggesting detachment of the villous virus-coated plasma membrane. Cell incubated with antibodies to measles virus at 37 C for 30 min. x15,000.
Fig. 5. Infected HeLa cell first exposed to human serum containing antibodies to measles virus at 37 C for 40 min, washed, and then incubated with rabbit antibody to human IgG conjugated to peroxidase for 40 min at 37 C. (A) Unipolar redistribution (capping) of measles virus antigens noted by indirect immunoperoxidase light microscopy. x3,000. (B) Capping of measles virus antigens noted by indirect immunoperoxidase electron microscopy. There is no visible pinocytosis of peroxidase and no evidence of peroxidase-conjugated molecules within intracellular vesicles. x8,000.
Fig. 6. Scanning electron micrographs of HeLa cells acutely infected with measles virus. ×4,000. (A) Cell not treated with antibodies to measles virus. (B) Cell treated with antibodies to measles virus. Note the redistribution of microvilli over one pole of the cell. (C) Advanced capping after incubation with antiviral antibody.

may play an important role in establishing chronic infections and preventing immune components (sensitized cells, antibody, and complement) from destroying virus-infected cells.

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CAPPING OF MEASLES VIRUS ANTIGENS

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LITERATURE