TRANSMEMBRANE COMMUNICATION IN CELLS
CHRONICALLY INFECTED WITH MEASLES VIRUS

DAVID L. J. TYRRELL and ANNEKA EHRNST

From the Division of Infectious Diseases, Department of Medicine, Edmonton, Alberta, Canada, the Department of Virology, Karolinska Institutet, and the Department of Immunology, Statens Bakteriologiska Laboratorium, S-105 21 Stockholm, Sweden

ABSTRACT
The transmembrane association of the measles virus hemagglutinin and hemolysin surface proteins with intracellular viral antigens was studied. Rabbit antisera monospecific for measles virus matrix and nucleocapsid proteins and a human antiserum containing specificities for both the hemagglutinin and hemolysin proteins were used to study the co-capping of these proteins in human Lu 106 cell-line, chronically infected with measles virus. Capping of the surface-associated envelope components was accompanied by co-capping of the matrix and nucleocapsid proteins, the latter being localized mainly within the inclusions. This demonstrated transmembrane communication between surface-associated envelope components and the intracellular measles virus matrix and nucleocapsid proteins. The results demonstrated the existence of a linkage between viral inclusions and viral proteins associated with cell membranes. In the presence of cytochalasin B (1–2 μg/ml), co-capping of the matrix protein was unchanged or slightly enhanced, whereas co-capping of the nucleocapsid protein decreased, indicating that actin filaments may mediate the communication between viral nucleocapsids and the cell membrane.

KEY WORDS transmembrane communication • measles • actin • matrix • nucleocapsid • virus assembly

The identification of actin in many enveloped viruses (2, 7, 10, 15) including measles virus (8, 14) raises the question of the role of actin in these viruses. There is morphological and biochemical data indicating that the actin in these viruses is an internal protein of the virion (2, 14). During virus budding, the nucleocapsids of paramyxoviruses move to an area of the cell membrane rich in viral matrix and glycoproteins before being enveloped (1). The mechanism of the movement of nucleocapsids to the site of budding remains unknown.

Acute infection of cells in tissue culture with paramyxoviruses changes the distribution of cellular microfilaments (6, 12). Microfilaments have been assigned a role in the appearance and spreading of measles virus envelope proteins on the cell surface (3, 4). Recently, Damsky et al. have suggested that actin interacts with virus nucleoids for the extrusion of murine mammary tumor virus from infected cells (2). Using monospecific antisera for viral matrix and nucleocapsid proteins, we present evidence for transmembrane communication between viral envelope components on the cell surface and the intracellular matrix and nucleocapsid proteins. The communication of nucleocapsid-containing inclusions with membrane-associated viral antigens was disrupted by treatment with cytochalasin B, indicating a possible
role for actin filaments in the movement of nucleocapsids to the cell membrane.

MATERIALS AND METHODS

Cells

A human heteroploid cell line, Lu 106 cells, and a carrier cell line of Lu 106 cells chronically infected with the Edmonston strain of measles virus (9) were used. The cells were grown in Eagle's minimal essential medium, supplemented with streptomycin, penicillin, and 5% calf serum at 33°C for 3-5 d to induce maximal expression of measles virus antigens in the carrier cells (9).

Sera

Rabbit monospecific antisera for matrix and nucleocapsid proteins were prepared using antigens sliced from large, preparative SDS slab gels of 2.5 mg of purified measles virus (14). The proteins in the acrylamide gel were used in complete Freund's adjuvant for intramuscular injection. After boosters at 3 and 4 wk, the animals were exsanguinated at 6 wk postimmunization. The monospecific antisera for matrix and nucleocapsid proteins were characterized by an absence of hemagglutination-inhibition (HI) and hemolysis-inhibition (HLI) activities, but had complement-fixing antibodies for matrix and nucleocapsid proteins, respectively. Normal rabbit sera were used as controls.

A human serum with an HI titre of 1:400 and a non-HI hemolysis-inhibition titre of 1:320 was used to detect measles virus envelope antigens present on the carrier cell surface. Absorption with measles virus reduced the HI titer to <20 and the non-HI hemolysis-inhibition titer to <80.

Combined Fluorescence Staining of Surface and Intracellular Antigens

After 1-2 min of treatment with 0.1% trypsin in 0.02% EDTA, the carrier cells and control noninfected Lu 106 cells were resuspended in Eagle's minimal essential medium supplemented with streptomycin, penicillin, and 5% calf serum. Cell viability was >95% as determined by trypan blue dye exclusion. Human serum containing antibodies to both measles virus envelope components, the hemagglutinin and the hemolysin, was diluted 1:10, and 0.2 ml was added to one million cells. The cells were evaluated in a Zeiss fluorescence microscope. The activation filters for TRITC staining were BG 38 and KP 490. The beamsplitter was FT 580 and barrier filter was LP 590. Activation filters for FITC staining were BG 38, KP 490 and LP 455. The beamsplitter was FT 510 and barrier filters were 51-58.

RESULTS AND DISCUSSION

Immunofluorescence Patterns of Antisera against Measles Matrix Protein and Measles Nucleocapsid Protein in Chronically Infected Cells

After acetone fixation of measles-infected carrier cells, the monospecific antiserum for matrix protein (Fig. 1A) showed staining of cytoplasmic viral inclusions and some membrane staining. The monospecific antiserum for nucleocapsid protein showed primarily staining of cytoplasmic inclusions, with only a few cells showing a little membrane staining (Fig. 1B). These rabbit monospecific antisera did not stain the surface of live or paraformaldehyde-fixed carrier cells, or the intracellular components of acetone-fixed, uninfected Lu 106 cells in the dilution range of 1:15 to 1:60. Therefore, the membrane immunofluorescent staining seen in the acetone-fixed cells with these antisera, diluted 1:50, must have been located on the cytoplasmic side of the membrane. Rabbit
FIGURE 1 Immunofluorescence of different measles virus components in chronically infected cells. Distribution of measles virus matrix (A) and nucleocapsid (B) antigens in acetone-fixed Lu 106 carrier cells as demonstrated by indirect immunofluorescence, using rabbit anti-matrix protein serum, 1:50 (A), or rabbit anti-nucleocapsid protein serum, 1:50 (B), and sheep anti-rabbit FITC conjugate, 1:10. The surface distribution of measles envelope antigens (C) in Lu 106 carrier cells fixed in 4% paraformaldehyde. The cells were stained by a human serum containing measles virus antibodies diluted 1:10, and a sheep anti-human FITC conjugate 1:10. Bar, 10 μm. × 750.

sera lacking measles virus antibodies did not react with either cell type.

The carrier cells and noninfected Lu 106 cells were fixed with 4% paraformaldehyde (11), which is a procedure that preserves both measles virus envelope components present on the carrier cell surface and excludes immunofluorescence staining of intracellular antigens (3). Measles virus envelope antigens on the surface of carrier cells were detected in immunofluorescence by a human serum, containing antibodies to both measles virus envelope components (Fig. 1 C).

Neither the matrix protein nor the nucleocapsid protein is exposed on the cell surface as measured by the sensitive, mixed hemadsorption technique. Therefore, the human serum, despite its multispecificity for measles virus antigens, may be used for capping and detection of envelope antigens on the surface of carrier cells under conditions which exclude staining of intracellular antigens. The human serum did not react with the surface of uninfected Lu 106 cells, and absorption with measles virus removed the reactivity with the carrier cell surface as evidence that its reactivity with the carrier cell surface was measles virus-specific. A control human serum, devoid of measles virus antibodies, did not react with the surface of carrier cells or uninfected Lu 106 cells.

Co-capping of the Intracellular Matrix and Nucleocapsid Proteins with Envelope Components

We thought that antibody-induced redistribution and capping of surface-associated components would lead to a co-capping of intracellular components if there was transmembrane communication between these entities. Capping and staining of live cells were performed in combination with staining for intracellular antigens of fixed cells by use of double immunofluorescence with separate fluorochromes; rhodamine for surface antigens and fluorescein for intracellular antigens (13).

Capping of the surface-associated envelope components (Fig. 2A) was accompanied by co-capping of the membrane-associated matrix protein (Fig. 2B) in ~90% of capped cells (Table I). This experiment demonstrated transmembrane communication between the matrix protein and

2 Tyrrell, D. L. J., Y. Thorbell, and E. Norrby. Manuscript in preparation.
Antibody-induced transmembrane co-capping of viral antigens in Lu 106 carrier cells. (A) Capping of viral envelope antigens as demonstrated by human anti-measles serum, 1:10, and rhodamine (TRITC)-conjugated sheep anti-human IgG absorbed by rabbit serum, 1:5, and (B) the co-capping of matrix protein in the same cell as demonstrated by rabbit anti-matrix protein serum, 1:50, and fluorescein (FITC)-conjugated sheep anti-rabbit IgG absorbed by human serum, 1:10. (C) Capping of envelope components as in Fig. 2A, and (D) the co-capping of nucleocapsid protein in the same cell as demonstrated by rabbit anti-nucleocapsid protein serum, 1:50. The fluorochromes used in Fig. 2C and D were the same as used in Fig. 2A and B, respectively. Bar, 10 μm. × 750.

Viral envelope antigens on the cell surface. In ~70% of capped cells, there was a complete co-capping of the matrix protein staining with no evidence of intracellular matrix protein distant from the cap. This indicated that inclusions, containing matrix protein, were in the capped region. Alternatively, capping might have resulted in a dissociation of matrix protein from some viral inclusions or from parts of inclusions. The nucleocapsid protein staining showed co-capping in 60% of capped cells and complete co-capping in 30% of capped cells. Since very little nucleocapsid protein staining is normally present adjacent to the cell membrane, inclusions, staining with the nucleocapsid protein antiserum and demonstrating co-capping, probably are linked to submembrane-associated viral proteins. Similar co-capping of nucleocapsid-containing inclusions has been demonstrated by electron microscopy (M. Oldstone, personal communication).
**TABLE I**

*Co-Capping* of Membrane-Associated and Inclusion-Associated Antigens: Effect of Cytochalasin B

| Treatment for 60 min at 37°C | Envelope components | Matrix protein | Nucleocapsid protein |
|-----------------------------|---------------------|----------------|----------------------|
| Medium                      | Homogenous ring     | Co-capping in 90% of capped cells | Co-capping in 60% of capped cells |
| Antibody-induced redistribu-| Caps                | Complete co-capping in 70% of capped cells | Complete co-capping in 30% of capped cells |
| tion                        |                     | No capping     | No capping           |
| 2 μg Cytochalasin B/ml of   | No capping          | Co-capping in 100% of capped cells | Co-capping in 30% of capped cells |
| medium                      |                     | Complete co-capping in 80% of capped cells | Complete co-capping in 8% of capped cells |

The percentage values of co-capping are derived from the mean of three experiments. 50 well-capped cells were evaluated for co-capping in each preparation.

* Co-capping indicates a redistribution of intracellular antigens to the area where surface-associated antigens had accumulated into a cap after reaction with antibodies of live cells and incubation at 37°C. Complete co-capping indicates that there was no staining of intracellular antigens distant from the cap.

---

**Interference by Cytochalasin B with the Co-Capping Phenomenon**

Cytochalasin B, a drug which alters microfilament function (16) was used to study the mechanism of co-capping of the matrix and nucleocapsid proteins with the envelope antigens (Fig. 3A-D). Cytochalasin B, 2 μg/ml, enhanced the capping of envelope components as previously described (13). The co-capping of matrix protein (Fig. 3A and B) was somewhat enhanced by 2 μg/ml of cytochalasin (Table I). However, 2 μg/ml of cytochalasin B markedly decreased the co-capping of inclusions stained by the nucleocapsid protein antiserum (Fig. 3C and D, and Table I). The difference in staining pattern obtained by the antisera against the matrix protein and the nucleocapsid protein in the presence of cytochalasin B shows that these antisera are directed against separate entities. We have summarized the finding from three experiments in Table I.

These experiments demonstrate a loss of communication between the membrane-associated viral antigens and the nucleocapsid-containing inclusions in the presence of cytochalasin B. This provides additional evidence for the existence of a connection between the inclusions and cell membrane. Since cytochalasin B alters microfilament function, our results suggest that microfilaments may mediate the communication between nucleocapsids and the membrane-associated viral proteins. However, we cannot exclude the possibility that cytochalasin B interferes indirectly with the association between inclusions and the membrane and not via alterations of microfilament function.

The discrepancy in the degree of co-capping of the matrix protein and the nucleocapsid protein in the presence of cytochalasin B is noteworthy. The similar physicochemical characteristics of the matrix protein and actin (both are filamentous and hydrophobic) may indicate that the possible matrix-actin association is stronger than the matrix-nucleocapsid protein association. An actin-nucleocapsid protein association may also exist. It is possible that cytochalasin B by its action on microfilament function induces a disassociation of the matrix protein from the nucleocapsids. Thereby the matrix protein may become more closely associated with the membrane and membrane-associated viral antigens whereas the nucleocapsid-containing inclusions may become disassociated from the membrane-associated viral antigens.

We conclude (a) that envelope components are in transmembrane communication with the matrix protein on the cytoplasmic side of the cell membrane, and (b) that there is a linkage between membrane-associated viral antigens and viral inclusions. The present study also indicates that actin filaments may mediate the connection between viral inclusions and the cell membrane. The transfer of nucleocapsids and matrix proteins from
The effect of cytochalasin B on the co-capping of viral antigens in Lu 106 carrier cells. (A) Capping of viral envelope antigens by the human antiserum in the presence of 2 μg/ml of cytochalasin B, and (B) the co-capping of membrane-associated matrix protein in the same cell. The antisera and fluorochromes used in Fig. 3A and B were the same as used in Fig. 2A and B. (C) Similar capping as shown in Fig. 3A, and (D) The failure of inclusion-associated nucleocapsid antigens in the same cell to co-cap with surface antigens in the presence of 2 μg/ml cytochalasin B. The antisera and fluorochromes in Fig. 3C and D were the same as used in Fig. 2C–D. Bar, 10 μm. × 750.

the cytoplasm to the site of budding at the cell membrane could possibly be mediated by microfilaments. On the basis of these and earlier findings (3, 4), we propose that the role of actin in paramyxoviruses is to participate in the assembly of these viruses.

The excellent technical assistance of Ms. Karin Winberg and Ms. Anita Bergström is acknowledged.

This research was supported by grants from the Swedish Medical Research Council, grant nr B18-16X-00116 and B76-16X-04774-01. D. Tyrrell was a Centennial Fellow of the Medical Research Council of Canada working in the Department of Virology, Karolinska Institutet during this study.

Received for publication 24 March 1978, and in revised form 13 November 1978.

REFERENCES

1. Chopin, P. W., and R. W. Courtaux. 1975. Reproduction of paramyxoviruses. In Comprehensive Virology. H. Fraenkel-Conrat and R. R. Wagner, editors. Plenum Press, New York. 4:95–197.
2. Daniel, C. H., J. B. Sheffield, G. P. Tuszynski, and L. Warren. 1977. Is there a role for actin in virus budding? J. Cell Biol. 75:595–605.
3. Emberton, A., and K. G. Sjökvist. 1975. Polar appearance and
nonligand induced spreading of measles virus hemagglutinin at the surface of chronically infected cells. **Cell.** 8:351-359.
4. **Erdmann, A., and K. G. Sundqvist.** 1976. Mechanisms of appearance of viral glycoproteins at the cell surface membrane. **Exp. Cell Biol.** 44:198-225.
5. **Fagard, A., K. Lieberman, and R. Norberg.** 1975. Indirect immunofluorescence staining of contractile proteins in smeared cells by smooth muscle antibodies: the influence of chelating agents. **Clin. Exp. Immunol.** 20:469-477.
6. **Fagard, A., D. L. J. Turnbull, R. Norberg, and E. Norrby.** 1978. Actin filaments in parvovirus-infected human fibroblasts studied by indirect immunofluorescence. **Arch. ViroL** 77:201-239.
7. **Fleischer, R., and E. Trees.** 1973. Chromatographic and electrophoretic analysis of viral proteins from hamster and chicken cells transformed with Rous sarcoma virus. **J. Virol.** 11:250-252.
8. **Mountcastle, W. E., and P. W. Chopin.** 1977. A comparison of the polypeptides of four measles virus strains. **Virology.** 78:463-474.
9. **Norrby, E.** 1967. A carrier cell line of measles virus in Lu 106 cells. **Arch. Gesamte Virusforsch.** 20:213-224.
10. **Orvell, C.** 1978. Structural polypeptides of mumps virus. **J. Gen. Virol.** 41:527-539.
11. **Robertson, J. D., T. S. Bodenreider, and D. E. Stahl.** 1963. The ultrastructure of Mauthner cell synapses and nodes in goldfish brains. **J. Cell Biol.** 19:159-199.
12. **Ruttner, G., and X. Wannenweller.** 1977. Alterations of actin-containing structures in BHK 21 cells infected with Newcastle disease virus and vesicular stomatitis virus. **J. Gen. Virol.** 37:233-242.
13. **Sundqvist, K. G., and A. Erdmann.** 1976. Cytoskeletal control of cell surface mobility. **Nature (Lond.)** 264:226-231.
14. **Turnbull, D. L. J., and E. Norrby.** 1978. Structural polypeptides of measles virus. **J. Gen. Virol.** 39:219-229.
15. **Wang, E., B. A. Wolf, R. A. Lamb, P. W. Chopin, and A. R. Goldberg.** 1975. The presence of actin in enveloped viruses. **J. Cell Biol.** 67(2, Pt. 2):445a. (Abstr.)
16. **Wesche, N. K., B. S. Spooner, F. Ash, M. D. Bradeley, M. A. Lindsay, E. L. Taylor, T. Wank, and K. M. Yamada.** 1971. Microfilaments in cellular and developmental processes. **Science (Wash. D. C.)** 171:135-143.