VIROLOGY 210, 91–99 (1995)

Entry and Release of Measles Virus Are Polarized in Epithelial Cells

DIANNA M. BLAU and RICHARD W. COMPANS[1]

Department of Microbiology and Immunology, Emory University School of Medicine, Rollins Research Center, 1510 Clifton Road, Atlanta, Georgia 30322

Received March 14, 1995; accepted March 31, 1995

The initial site of virus replication during measles infection is in the epithelial cells of the respiratory tract. We have investigated measles virus infection of two types of polarized epithelial cells to determine if entry and/or release of the virus is confined to either the apical or the basolateral plasma membrane. The Caco-2 line of human intestinal epithelial cells and the polarized Vero C1008 monkey kidney cell line were grown on permeable supports and inoculated either through the apical or basolateral surfaces. Cells exposed to virus in the apical medium showed high levels of synthesis of virus-specific proteins, whereas no synthesis of viral proteins was detected in cells inoculated at the basolateral surface. Virus titers derived from apically infected cells were found to be about 1000-fold greater than titers derived from cells infected at the basolateral surface. Indirect immunofluorescence results also demonstrated that expression of measles viral antigens occurs at high levels only when input virions are inoculated at the apical surface. To investigate the localization of CD46 and moesin, which are receptors for measles virus, Caco-2 cells were incubated with monoclonal antibodies against CD46 or moesin followed by ^{125}I-labeled anti-mouse Ig. The results indicate that CD46 is expressed preferentially on the apical membranes while moesin appears to be present at similar levels on both surfaces. Release of the virus was also examined and found to be polarized as well. Virus was released into the apical medium at up to 1000-fold higher titers than virus released into the basolateral medium. These results demonstrate that in two epithelial cell types measles virus preferentially enters and is released from epithelial cells in a polarized fashion through the apical plasma membrane.
© 1995 Academic Press, Inc.

INTRODUCTION

Measles virus is a member of the Morbillivirus genus in the *Paramyxoviridae* family. The Morbilliviruses are distinct from other paramyxoviruses because they do not bind to sialic acid-containing receptors or contain a detectable neuraminidase activity. Recently, evidence has been obtained that CD46 or membrane cofactor protein serves as a receptor for measles virus (Dörig *et al.*, 1993; Naniche *et al.*, 1993; Manchester *et al.*, 1994; Maisner *et al.*, 1994). CD46 is reported to be present on all human cells except erthryocytes and is involved in complement inactivation (for review see Liszewski *et al.*, 1991). Additionally, another cell surface protein, moesin, has been reported to be a measles virus receptor (Dunster *et al.*, 1994). Moesin is a member of the talin-protein 4.1-erzin family of proteins (Lankes and Furthmayr, 1991), and may act as a link between the cytoskeleton and cell membrane (Luna and Hitt, 1992; Sato *et al.*, 1992). *In vivo*, measles infection is initiated via aerosols and the initial site of infection is the respiratory tract (Fraser and Martin, 1978; Hall *et al.*, 1971; Katz and Enders, 1965; Robbins, 1962). The virus replicates locally in the respiratory mucosa and then spreads to the draining lymph nodes, perhaps carried by macrophages or other mobile cells. The virus then enters the bloodstream through leukocytes and produces the primary viremia that results in dissemination through the reticuloendothelial system. However, epithelial cells are most prominently involved during the period of early viral replication (Black, 1989).

Epithelial cells line various body cavities and serve as a primary protective barrier against entry of microorganisms. Epithelial cells also carry out specialized vectorial transport functions. Each epithelial cell is polarized and possesses two distinct plasma membranes, the apical domain which faces the external lumen and the basolateral domain which faces the internal milieu. These two plasma membrane domains contain different proteins and lipids and are separated by tight junctions which encircle the cell. Interactions of viruses with polarized epithelial cells have been extensively studied (for review see Tucker and Compans, 1993). Some viruses such as SV40 (Clayson and Compans, 1988) enter polarized cells through the apical surface while vesicular stomatitis virus (VSV) (Fuller *et al.*, 1984) and Semliki Forest virus (Fuller *et al.*, 1985) preferentially enter through the basolateral domain. In addition, due to the polarized sorting of viral proteins in epithelial cells, enveloped viruses are usually released preferentially by budding at either the apical or the basolateral plasma membrane. For example, influenza virus is released at the apical surfaces while VSV is released at the basolateral surfaces of polarized epithelial cells (Rodriguez-Boulan and Sabatini, 1978). These interactions of viruses with epithelial cells

[1] To whom reprint requests should be addressed.

0042-6822/95 $6.00
Copyright © 1995 by Academic Press, Inc.
All rights of reproduction in any form reserved.

may be determinants of aspects of viral pathogenesis. Viruses that enter and are released at the apical surface can spread laterally through the epithelial cell layer without entering the bloodstream, resulting in a localized infection. In contrast, a virus that enters apically but is released at the basal surface can infect underlying tissues and enter the bloodstream causing a systemic infection. This hypothesis is supported by results with a Sendai virus mutant that buds at both the apical and basolateral membranes, whereas the release of wild-type virus is restricted to the apical membrane. The mutant virus causes a pantropic or systemic infection, whereas the wild type is exclusively pneumotropic (Tashiro *et al.*, 1988, 1990a,b).

In this study we have investigated the interaction of measles virus with two polarized epithelial cell lines, Vero C1008, a monkey kidney cell line, and Caco-2, a human intestinal cell line. Both of these cell types have been shown to maintain their polarized phenotypes in culture (Srinivas *et al.*, 1986; Fogh *et al.*, 1977). The cells were grown on porous supports to provide access to both the apical and basolateral surfaces. Epithelial cells grown on porous supports show evidence of increased differentiation in comparison with cells grown on conventional solid surfaces (Handler *et al.*, 1984). The sites of entry and release of measles virus were investigated and compared to the distribution of CD46 and moesin which have been implicated as measles virus receptors.

MATERIAL AND METHODS

Cells and virus

Caco-2, Vero C1008, HeLa, Vero, and BHK-21 cells were grown in Dulbecco's modification of Eagle's minimal essential medium supplemented with 10% fetal bovine serum. For studies of virus entry and release, cells were grown on Falcon 0.45-μm PET membrane inserts (4.65 cm^2). The cells were seeded at 1.0×10^6 cells per filter, and the medium was changed every 2–3 days. The Edmonston strain of measles virus was kindly supplied by Dr. William Bellini and was grown and titered by plaque assay in Vero cells (Bellini *et al.*, 1979). The Indiana strain of vesicular stomatitis virus was grown and titered in BHK-21 cells (Roth and Compans, 1981). Parainfluenza virus type 3 (PI3) was grown and titered in Vero cells (Ray *et al.*, 1985).

Before infection of cells on filters, we confirmed that the cells had established a polarized monolayer by measuring the transepithelial electrical resistance using a Millicell-ERS resistance apparatus (Millipore Corp., Bedford, MA). The net resistance was calculated by subtracting the background and multiplying the resistance by the surface area of the filter. Caco-2 cells had a resistance of 700–900 ohms$\cdot cm^2$ at time of infection, while Vero C1008 exhibited a resistance of 50–70 ohms$\cdot cm^2$. For infection, the virus inoculum was added to either the apical chamber or to the basal chamber and allowed to absorb for 1 hr at 37°.

Reagents

Polyclonal antibody against measles virus was obtained from Cortex Biochemicals (San Leandro, CA). A monoclonal antibody TRA-2-10 which binds to the SCR-1 domain of CD46 was kindly provided by Dr. Michael Oldstone. A monoclonal antibody against measles virus nucleoprotein was kindly supplied by Dr. William Bellini. A monoclonal antibody 70/35 against human moesin was kindly provided by Dr. Jürgen Schneider-Schaulies. Fluorescein-conjugated anti-mouse antibody was purchased from Sigma (St. Louis, MO). Fluorescein-conjugated anti-rabbit antibody was obtained from Southern Biotechnology Associates (Birmingham, AL). Antibodies specific for CD46 (clone J4.48) and HLA-ABC class I were purchased from Amac Inc. (Westbrook, ME).

Immunofluorescence and radioimmunoassays

For intracellular immunofluorescence, cells grown on filters were rinsed twice with phosphate-buffered saline (PBS) and fixed with acetone:methanol (1:1) for 2 min. Nonspecific binding sites were blocked by incubating the cells for 2 hr at 4° with PBS containing 3% BSA and 1% goat serum. Cells were incubated with a 1:100 dilution of anti-measles NP for 30 min at 4° in a humidity chamber. The cells were washed and then incubated with fluorescein-conjugated anti-mouse antibody for 30 min at 4° in a humidity chamber. Filters were washed extensively, cut from the chambers, and mounted on a microscope slide. Filters were examined under a Nikon Optiphot microscope equipped with a modified B2 cube.

For cell surface radioimmunoassays, cells were treated as above but without fixing and, after the primary antibody incubation, the cells were washed and incubated with an ^{125}I-conjugated anti-mouse IgG for 30 min at 4° in a humidified chamber. Filters were washed extensively, cut from the chambers, and the cell associated radioactivity was counted in a Packard gamma counter.

Viral protein synthesis

For immunoprecipitation of intracellular viral proteins, the medium was removed from both chambers and replaced with Eagle's medium without cysteine or methionine. 100 μCi of [^{35}S]methionine cysteine cell labeling mix (Amersham Corp., Arlington Heights, IL) was added to the basal medium. After 6 hr of labeling the medium was removed and the cells were solubilized in 1 ml of lysis buffer. The lysates were cleared of cellular debris and incubated with antibodies specific for viral proteins. The immune complexes were precipitated by incubating with protein A–Sepharose (Pierce, Rockford, IL) and centrifugation. The precipitates were washed extensively with wash buffer, resuspended in sample buffer, boiled

for 2 min, and loaded on a SDS–10% polyacrylamide gel (Laemmli, 1970).

For antibody blocking studies, the filters were rinsed twice with PBS and then antibody TRA-2-10 diluted in PBS was added to one side and incubated for 2 hr at 4°. The cells were then rinsed with PBS and virus was added to the same side as the antibody and allowed to absorb for 1 hr at 37°. The cells were then labeled and immunoprecipitated as described above.

Electron microscopy

Caco-2 cells were grown to confluence on 35-mm plastic dishes, infected with measles virus, and incubated at 37° for varying time intervals. Samples were taken at 26, 35, 40, and 48 hpi and prepared for electron microscopy.

Samples were washed with PBS, fixed with 1% glutaraldehyde, mordanted with 0.01% tannic acid, stained with 1% osmium tetroxide, *en bloc* stained with 1% uranyl acetate, dehydrated with a graded series of ethanol, floated off the plasticware with propylene oxide, and embedded in EMBED 812 (Electron Microscopy Sciences, Fort Washington, PA). Sections (50 to 70 nm) were prepared on a Reichert Ultracut E microtome, mounted on 300 mesh copper grids, poststained with uranyl acetate and lead citrate, and then examined with a Philips CM10 electron microscope.

RESULTS

Measles virus enters epithelial cells at the apical surface

In order to determine whether measles virus entry occurs preferentially at the apical or basolateral surfaces, Caco-2, Vero C1008, or nonpolar Vero cells were grown on permeable filters, inoculated with measles virus via either the apical or basal medium, and the intracellular synthesis of viral proteins was examined. Figure 1 shows the results when the cells were labeled at 32 hpi. Both Caco-2 and Vero C1008 cells inoculated with measles virus (MV) in the apical medium (A) show synthesis of viral proteins while cells exposed to virus in the basal medium (B) show no detectable viral proteins. In nonpolarized Vero cells, measles virus protein synthesis is seen in cells inoculated from both the apical medium and the basolateral medium. It has been shown previously (Fuller *et al.*, 1984) that VSV preferentially enters polarized MDCK cells through the basolateral surfaces. In Fig. 1 VSV was used to show that Caco-2 and Vero C1008 cells are polarized, since VSV protein synthesis only was seen in cells inoculated at the basolateral surfaces. Unlike measles virus, PI3 does not utilize a specific cellular protein receptor but binds to sialic acids on the cell surface. As shown in Fig. 1, we observed that PI3-specific proteins are synthesized in cells inoculated via either the apical or basal surface, although in Caco-2 cells there appears to be a slight preference for entry through the apical surface. These results indicate that measles virus preferentially enters both Caco-2 and Vero C1008 at the apical surfaces, while PI3 can enter through either surface.

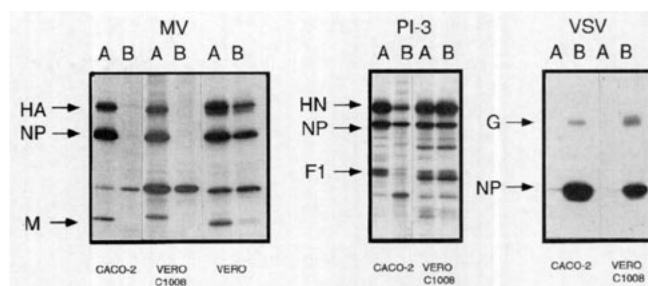

FIG. 1. Measles virus-specific polypeptide synthesis is detected in cell cultures inoculated via the apical membrane but not in cell cultures inoculated via the basolateral membrane. Radiolabeled measles virus-specific, PI 3-specific or VSV-specific polypeptides were immunoprecipitated from Caco-2, Vero C1008, or Vero cells grown on Falcon filters. The cells were infected with measles virus (m.o.i. of 1), PI3 (m.o.i. of 5), or VSV (m.o.i. of 5) via either the apical (A) or basolateral (B) domains. The MV-infected cells were labeled as described under Materials and Methods and immunoprecipitated with polyclonal antibody against MV. The VSV-infected cells were labeled at 8 hpi and the lysates were prepared at 12 hpi. PI3-infected cells were labeled at 12 hpi and lysates prepared at 16 hpi.

As another approach to investigate the ability of measles virus to establish an infection, the expression of viral antigens was examined by indirect immunofluorescence. The Caco-2 cell cultures inoculated with virus in the apical media showed a large number of cells expressing the NP antigen at 36 hpi (Fig. 2A). Similar results were obtained in Vero C1008 cells (Fig. 2D). In contrast, in both cell types only occasional cells inoculated by the basolateral route were found to express viral antigen (Figs. 2B and 2E). The signal in the basal-inoculated cultures (Figs. 2B and 2E) could be due to localized lesions in the polarity of the monolayers or presence of occasional nonpolarized cells at some sites. These results also support the conclusion that measles virus entry occurs selectively at the apical plasma membrane.

The virus yields from cells inoculated with measles virus at either plasma membrane domain are shown in Table 1. The cells were infected by adding virus to either the apical or basal medium and then at 48 hpi the cells were collected, the virus was harvested, and virus yields were titered. Table 1 shows that measles virus titers from apically infected cells are about 1000 fold higher than titers from cells inoculated with the virus by the basal surface. Assuming that a productively infected cell yields the same amount of virus irrespective of the cellular site at which infection was established, it can be calculated that only 0.20% of the cells are being infected when the virus is inoculated via the basolateral surface. In contrast, in nonpolarized Vero cells similar titers of virus were observed in cells inoculated at either surface, indicating a high efficiency of infection through the basolateral surface with 83% of cells being infected, thus demonstrating

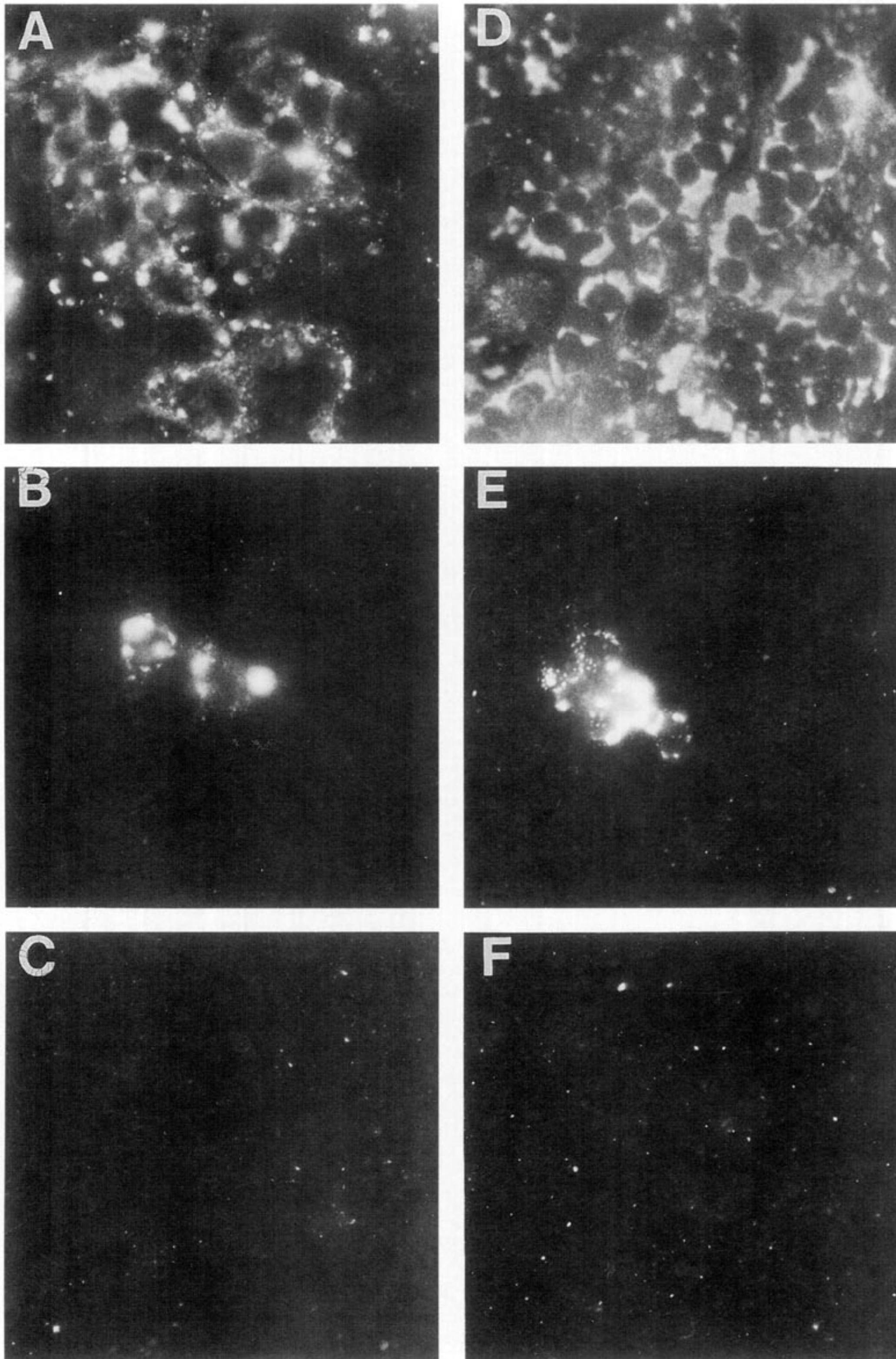

FIG. 2. Measles virus proteins are expressed in cells inoculated via the apical surface but not in cells inoculated via the basolateral surfaces. Caco-2 (A–C) and Vero C1008 (D–F) cells were grown on transparent Falcon filters and virus was added to either the apical media (A, D) or to the basal media (B, E). C and F represent mock-infected cells. The cells were fixed at 40 hpi and monoclonal antibody specific for measles virus nucleoprotein was added followed by FITC-conjugated secondary antibody.

TABLE 1

Polarity of Virus Entry[a]

Cell line	PFU/ml A	PFU/ml B	PFU/cell A	PFU/cell B	% Cells infected from basal chamber
Vero	9.6×10^6	8.0×10^6	4.36	3.63	83
Caco-2	8.2×10^6	1.6×10^4	4.32	0.008	0.20
Vero C1008	8.6×10^6	1.84×10^4	3.91	0.008	0.21

[a] The cells grown on filters were inoculated either through the apical (A) or basal (B) media. At 48 hpi the cells were harvested and the virus was collected by two rounds of freeze–thawing. The virus collected was titered by plaque assays on Vero cells.

that measles virus is able to penetrate the permeable support and initiate infection of this nonpolarized cell type. Therefore the results of viral protein production, viral antigen expression, and viral infectivity titers all support the conclusion that measles virus preferentially enters polarized epithelial cells through the apical surface.

Antibody to CD46 blocks measles virus infection at the apical surface

It has recently been reported (Dörig *et al.,* 1993; Naniche *et al.,* 1993; Manchester *et al.,* 1994) that CD46 or MCP serves as the measles virus receptor. Therefore it was of interest to investigate the site of CD46 expression in epithelial cells and its relationship to the site of measles virus entry. First we determined whether antibody against CD46 could block infection of Caco-2 cells by measles virus. Varying concentrations of antibody specific for CD46 were incubated with polarized Caco-2 cells prior to viral absorption. It was found (Fig. 3) that when higher concentrations of the antibody were used, measles virus infection at the apical surface was almost completely blocked. Therefore, the CD46 molecule plays an essential role in the entry of measles virus in Caco-2 cells.

CD46 is preferentially expressed on the apical surface

To determine whether CD46 is preferentially expressed on a specific plasma membrane domain in polarized cells, Caco-2 cells were assayed by radioimmunoassay to determine the levels of expression of CD46 on each surface. Figure 4A shows that apically labeled surfaces of Caco-2 cells had at least twofold higher levels of CD46 than the basolaterally labeled surfaces. In comparison, HeLa cells showed no significant differences in the level of CD46 expression on apical and basolateral surfaces. It can therefore be concluded from Fig. 4A that CD46 is preferentially located on the apical

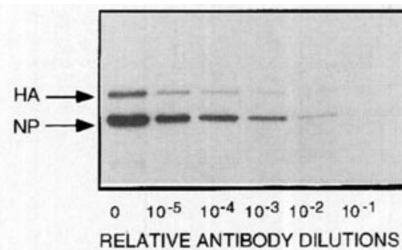

FIG. 3. Antibody against CD46 blocks measles virus infection. Monolayers of Caco-2 cells were grown on filter inserts and incubated with serial dilutions of TRA-2-10 antibody indicated prior to infection with measles virus (m.o.i. of 1) at the apical plasma membrane. Cells were radiolabeled at 36 hpi and immunoprecipitated at 42 hpi.

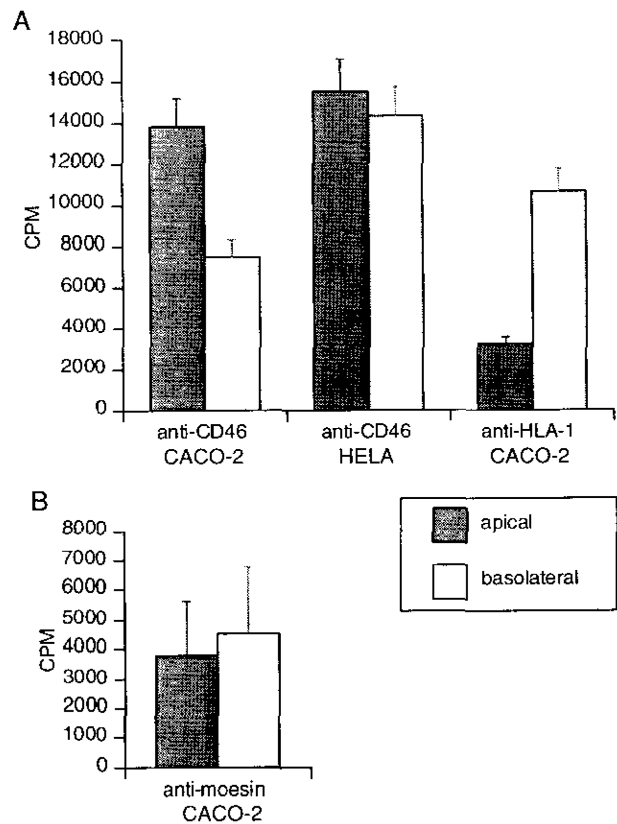

FIG. 4. CD46 is expressed predominantly on the apical surfaces of Caco-2 cells. 4A shows filter grown Caco-2 and HeLa cells incubated with antibodies specific for CD46 (J4.48) or class I MHC followed by ^{125}I-labeled secondary antibody. 4B represents Caco-2 cells incubated with antibodies specific for moesin (70/35). Values represent the ^{125}I-labeled antibody bound to cells after subtracting nonspecific binding and were derived from the mean values of triplicate samples for CD46 and class I and mean values of five samples for moesin.

surfaces of Caco-2 cells. It has previously been reported that in Caco-2 cells HLA class 1 antigen is predominantly expressed on the basolateral surfaces (LeBivic *et al.*, 1990). This is also shown in Fig. 4A for comparison, indicating that these cells have the characteristic polarized phenotype. These results are consistent with the idea that CD46 acts as a receptor for the preferential entry of measles virus through the apical surfaces of epithelial cells, although other components may be required to explain the strict polarity of virus entry.

Moesin expression in Caco-2 cells

Recently, it has been reported that there is another molecule, moesin, that plays a role as a measles virus receptor in addition to CD46 (Dunster *et al.*, 1994). This molecule is widely expressed in tissues and cells of different animal species, and is concentrated on cell surface protrusions (Furthmayr *et al.*, 1992). Figure 4B shows the results when the expression of moesin was investigated on Caco-2 cells by a cell surface radioimmunoassay like that used for CD46. In contrast to CD46, no significant difference in levels of moesin expression was seen between the apically labeled surfaces and the basolaterally labeled surfaces. These results indicate that moesin is found at similar levels on both surfaces of Caco-2 cells.

Measles virus is preferentially released at apical surfaces

To determine if the release of measles virus from these epithelial cells was polarized as well, the apical and basal media were examined for yields of progeny virus. The cell cultures were inoculated via the apical surfaces and at various time points postinfection the virus titers were determined in the apical and basolateral culture medium. Figure 5A shows a growth curve for the release of measles virus in the two culture supernatants of Caco-2 cells. The titers of apically released virus in Caco-2 cells are at least 1000-fold higher than basolaterally released virus at 48 hr postinfection. In comparison, measles virus is released at similar levels from both surfaces of nonpolar Vero cells (Fig. 5B), demonstrating that the virus is not impeded by the filter from being released into the basal medium. These results support the conclusion that measles virus is preferentially released into the apical medium in Caco-2 cells. In Vero C1008 cells, similar results were obtained (data not shown). It has been previously reported (Rodriguez-Boulan and Sabatini, 1978; Tashiro *et al.*, 1990a,b) that other paramyxoviruses are also released at apical surfaces from epithelial cells. In Fig. 5C, it can be seen that PI3, like measles virus, is preferentially released into the apical medium. The titers of apically released virus are at least 100-fold higher than basolaterally released virus.

The polarity of measles virus release was also observed by electron microscopy. Figure 6 represents

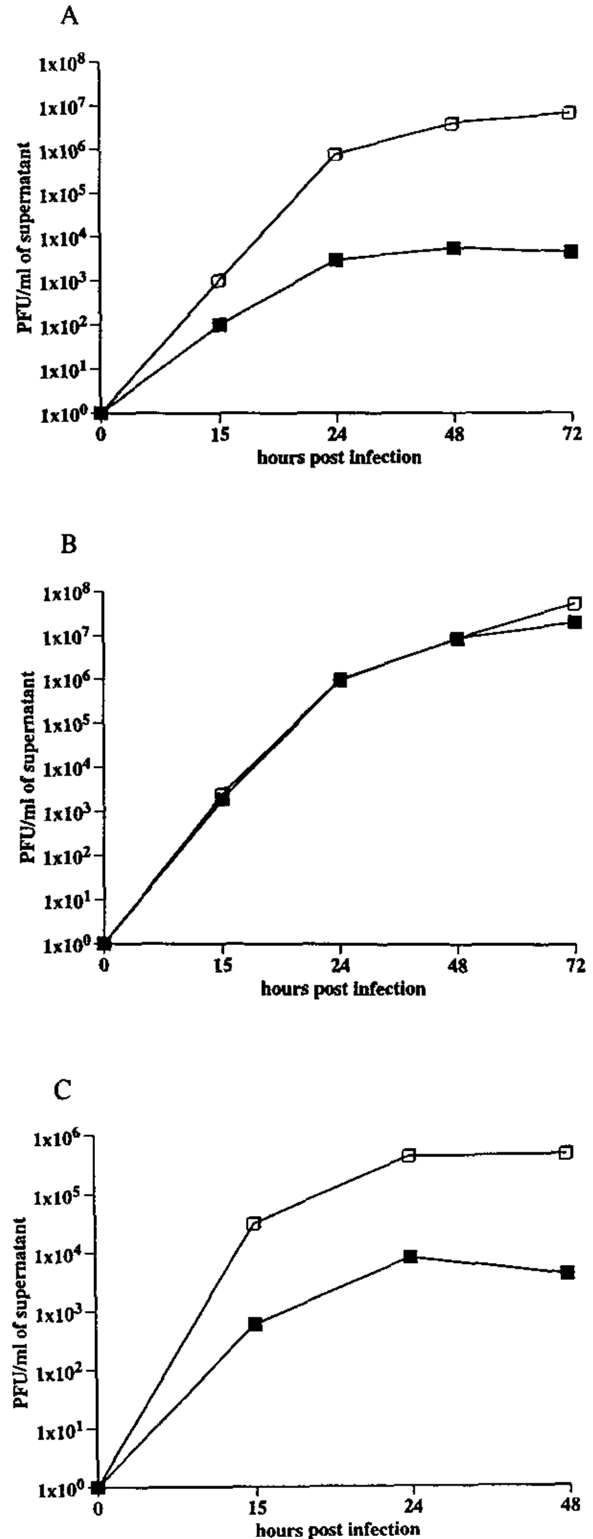

FIG. 5. Measles virus is released preferentially at the apical surfaces of Caco-2 cells. Monolayers of Caco-2 cells grown on Falcon filters were infected with either measles virus (m.o.i. of 1) (A) or parainfluenza type 3 virus (m.o.i. of 5) (C) via the apical surface. For comparison, monolayers of nonpolarized Vero cells grown on filters (B) were infected with MV (m.o.i. of 1). At the times indicated postinfection, the culture media was collected and the extracellular virus titers in apical (open symbols) and basolateral (closed symbols) media were determined by plaque assay.

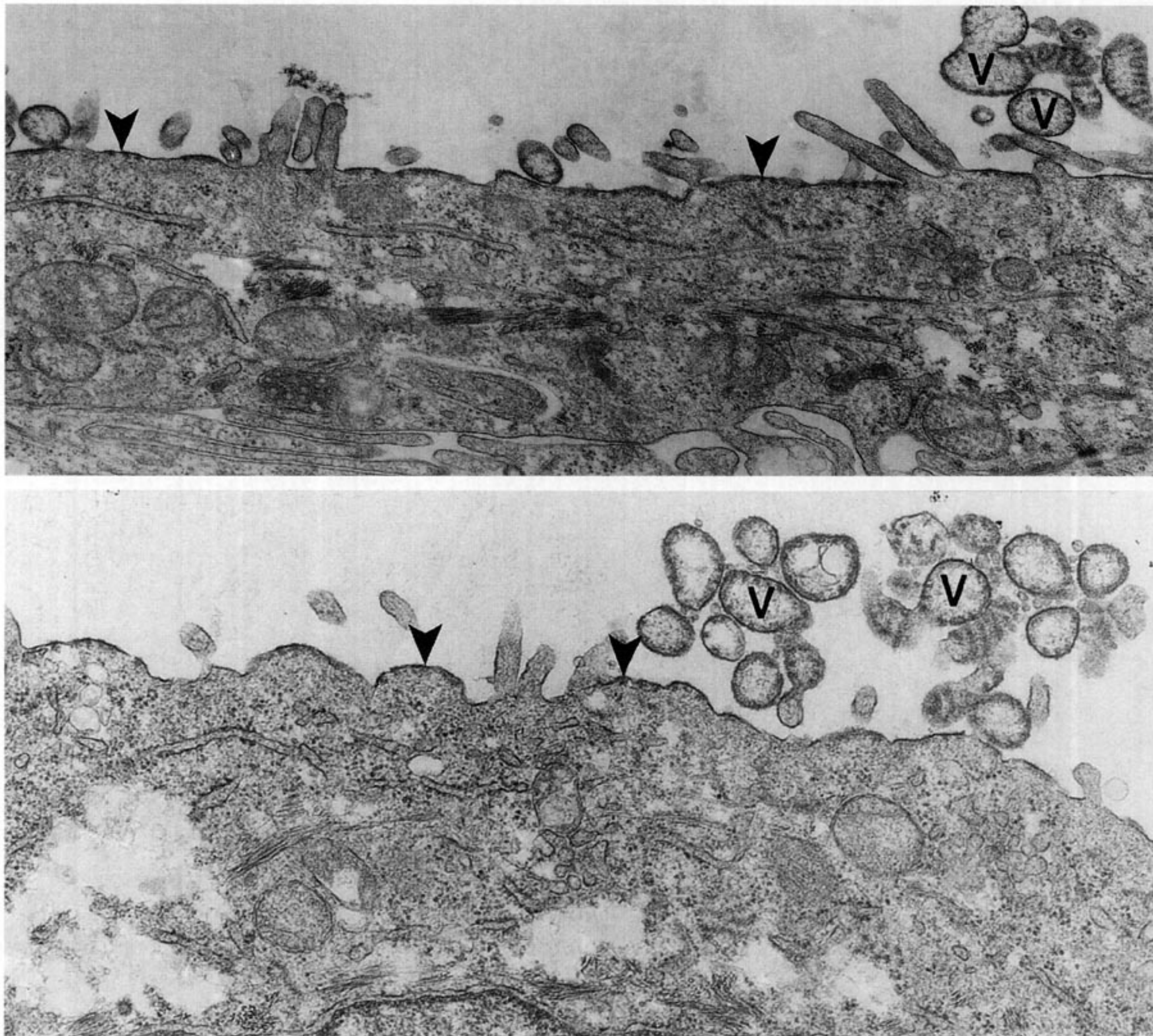

FIG. 6. Measles virus budding occurs predominantly at the apical plama membrane. Caco-2 cells were infected with measles virus (m.o.i. of 1) at the apical surface and samples were fixed and prepared for electron microscopy at 48 hpi. Magnification is ×11,500. Two apical surfaces are shown. In the top panel some lateral surfaces can be seen. Solid arrows indicate viral budding or nucleocapsids at the membrane. V indicates virions.

apical surfaces of Caco-2 cells at 48 hpi. Viral nucleocapsids can be seen aligned beneath the apical membrane and released virions were predominantly associated with apical plasma membranes. Possible virions were seen at the basolateral surface or between cells (data not shown) in only approximately 5% of the infected cells examined. These results support the conclusion that measles virus is preferentially released at the apical surfaces of polarized epithelial cells.

DISCUSSION

Morbilliviruses, such as measles virus, are distinct from other paramyxoviruses because they do not contain detectable neuraminidase activity and they bind to cells via a specific host cell protein receptor. Other paramyxoviruses that have neuraminidase activity, including PI3, bind to sialic acid residues which are present on apical as well as basolateral glycoproteins and glycolipids, and such viruses therefore can enter polarized cells through either the apical or basolateral plasma membranes. In contrast, we have shown that measles virus preferentially enters polarized Caco-2 and Vero C1008 cells at the apical surfaces. We have also shown that antibody against CD46 blocks measles virus infection at the apical surface. The data showing the apical entry of the virus would suggest that the receptor for measles virus is expressed predominantly on the apical surface, which we have observed for CD46 on Caco-2 cells. However, we observed only about a 2-fold difference between levels of expression of CD46 on apical and basolateral surfaces while there was almost a 1000-fold difference in the efficiency of entry of measles virus between the apical and basolateral plasma membranes. There are several possible explanations for these differences. It has been reported (Parton *et al.,* 1989; von Bonsdorff *et al.,* 1985) for

MDCK cells that the surface area on the apical plasma membrane is about 4-fold lower compared to that of the basolateral plasma membrane. If this is true for Caco-2 cells, the actual density of the CD46 molecule on the apical surface may be as much as 10-fold higher than on the basolateral surface. A critical receptor density may be required for measles virus entry, and this receptor density may only be present on the apical surface. Recently, it has been reported that in addition to CD46, moesin also acts as a measles virus receptor (Dunster *et al.,* 1994). These authors report that CD46 is not present on all cells susceptible to measles virus, suggesting that there is another receptor present. In our studies moesin appears to be present at similar levels on both membrane surfaces, so it is not as likely to be a determinant of the polarity in virus entry. However, the polarity of measles virus entry could also be explained by the possibility that there may be another cofactor involved in virus entry that restricts measles virus entry to the apical membrane.

Viruses that enter and are released from epithelial cells at the apical membrane may be restricted to epithelial surfaces and produce a localized infection. It has been reported that other paramyxoviruses such as Sendai virus and simian virus 5 (Rodriguez-Boulan and Sabatini, 1978) are released predominantly at the apical surface. However, in contrast to these viruses, measles virus produces a systemic infection *in vivo.* It is likely, therefore, that there are other determinants of measles virus pathogenesis, such that its apical release into the lumen of the respiratory tract does not restrict it to causing a localized infection. Certain avian influenza subtypes, H7 and H5, also produce a systemic infection, although they are released at the apical surface of epithelial cells in culture (Basak *et al.,* 1983). Polarized measles virus release has not yet been shown in respiratory tract epithelial cells which are important in the primary replication of the virus, because of the lack of an appropriate cell line. In addition, replication of measles virus leads to disruption of the cellular cytoskeleton (Fagraeus *et al.,* 1978). This phenomenon possibly could cause a temporary loss of epithelial polarity *in vivo,* thus enabling the virus to breach the barrier presented by the respiratory epithelium. Another likely mechanism by which measles virus can traverse the epithelial barrier leading to a systemic infection involves infection of immune cells. Measles virus can infect cells of the immune system (Berg and Rosenthal, 1961), including lymphocytes in the mucosal immune system. These lymphocytes are interspersed between the epithelial cells and may traverse the epithelial barrier and could introduce the virus to underlying tissues or to the bloodstream.

A key step in viral budding is the accumulation of viral envelope proteins at the site of maturation. HIV is preferentially released at the basolateral membrane surface (Owens, *et al.,* 1991; Fantini *et al.,* 1991), and evidence has been obtained that the site of expression of the envelope glycoprotein determines the site of virus maturation at the basolateral membrane domain (Owens *et al.,* 1991). As mentioned above, other paramyxoviruses that have been examined are released preferentially at the apical membranes of polarized epithelial cells. It is therefore likely that the envelope proteins of paramyxoviruses such as simian virus 5 and Sendai virus share common sorting signals with measles virus glycoproteins for transport to the apical membrane. The cloning and sequencing of paramyxoviruses glycoproteins has shown that there is homology between the members glycoproteins (for review see Morrison and Portner, 1991). The measles virus H protein sequence shows some sequence conservation with the HN protein of PI3 virus (30%) and Sendai virus (37%). The F protein of the measles virus has 48% sequence conservation of the Sendai F protein and 43% of PI3 virus. It will be of interest to determine which measles virus protein, HA or F, and which portion of the protein is responsible for determining the site of virus release in polarized epithelial cells.

ACKNOWLEDGMENTS

The authors thank Lawrence Melsen for expert assistance with electron microscopy and photography. This study was supported by Research Grants AI 12680 and CA 18611 from the National Institutes of Health.

REFERENCES

Basak, S., Melsen, L. R., Alonso-Caplen, F. V., and Compans, R. W. (1983). Maturation sites of human and avian influenza viruses in polarized epithelial cells. *In* "The Origin of Pandemic Influenza Viruses" (W. G. Laver, Ed.), pp. 139–145. Elsevier, New York.

Bellini, W. J., Trudgett, A., and McFarlin, D. E. (1979). Purification of measles virus with preservation of infectivity and antigenicity. *J. Gen. Virol.* **43,** 633–639.

Berg, R. B., and Rosenthal, M. S. (1961). Propagation of measles in suspension of human and monkey leukocytes. *Proc. Soc. Exp. Biol. Med.* **106,** 581–585.

Black, F. L. (1989). Measles. *In* "Viral Infections of Humans: Epidemiology and Control" (A. S. Evans, Ed), 3rd ed. pp. 451–469. Plenum, New York.

Choppin, P. W., and Scheid, A. (1980). The role of viral glycoprotein in adsorption, penetration, and pathogenicity of viruses. *Rev. Infect. Dis.* **2,** 40–61.

Choppin, E. T., and Compans, R. W. (1988). Entry of simian virus 40 is restricted to apical surfaces of polarized epithelial cells. *Mol. Cell Biol.* **8,** 3391–3396.

Clayson, E. T., and Compans, R. W. (1988). Entry of simian virus 40 is restricted to apical surfaces of polarized epithelial cells. *Mol. Cell Biol.* **8,** 3391–3396.

Dörig, E., Marcil, A., Chopra, A., and Richardson, C. D. (1993). The human CD46 molecule is a receptor for measles virus (Edmonston strain). *Cell* **75,** 295–305.

Dunster, L. M., Schneider-Schaulies, J., Löffler, S., Lankes, W., Schwartz-Albiez, R., Lottspeich, F., and Ter Meulen, V. (1994). Moesin: A cell membrane protein linked with susceptibility to measles virus infection. *Virology* **198,** 265–274.

Fagraeus, A., Tyrrell, D. L. J., Norberg, R., and Norrby, E. (1978). Actin filaments in paramyxovirus-infected human fibroblasts studied by indirect immunofluorescence. *Arch. Virol.* **57,** 291–296.

Fantini, J., Baghidigvian, S., Yahi, N., and Chermann, J.-C. (1991). Selected human immunodeficiency virus replicates preferentially

through the basolateral surface of differentiated human colon epithelial cells. *Virology* **185**, 904–907.

Fogh, J., Fogh, J. M., and Orfeo, T. (1977). One hundred and seventy-seven cultured human tumor cell lines producing tumors in nude mice. *J. Natl. Cancer Inst.* **59**, 221–226.

Fraser, K. B., and Martin, S. J. (1978) "Measles Virus and its Biology" (K. B. Fraser, and S. J. Martin, Eds.) Academic Press, London.

Fuller, S. D., von Bonsdorff, C.-H., and Simons, K. (1984). Vesicular stomatitis virus infects and matures only through the basolateral surface of the polarized epithelial cell line, MDCK. *Cell* **38**, 65–77.

Fuller, S. D., von Bonsdorff, C.-H., and Simons, K. (1985). Cell surface influenza haemagglutinin can mediate infection by other animal viruses. *EMBO J.* **4**, 2475–2485.

Furthmayr, H., Lankes, W., and Amieva, M. (1992). Moesin, a new cytoskeletal protein and constituent of filopodia: Its role in cellular functions. *Kidney Int.* **41**, 665–670.

Hall, W. C., Kovatch, R. M., Herman, P. H., and Fox, J. C. (1971). Pathology of measles in Rhesus monkeys. *Vet. Pathol.* **8**, 307–319.

Handler, J. S., Preston, A. S., and Steele, R. E. (1984). Factors affecting the differentiation of epithelial transport and responsiveness to hormones. *Fed. Proc.* **43**, 2221–2224.

Katz, S. L., and Enders, J. F. (1965). Measles Virus *In* "Viral and Rickettsial Infections of Man" (F. L. Horstall, T. M. Rivers, and I. E. Tamm, Eds.) J. B. Lippincott, Philadelphia.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* **277**, 680–685.

Lankes, W. T., and Furthmayr, H. (1991). Moesin: A member of the protein 4.1-talinezrin family of proteins. *Proc. Natl. Acad. Sci. USA* **88**, 8297.

LeBivic, A., Quaroni, A., Nichols, B., and Rodriguez-Boulan, E. (1990). Biogenic pathways of plasma membrane proteins in Caco-2, a human intestinal epithelial cell line. *J. Cell Biol.* **111**, 1351–1361.

Liszewski, M. K., Post, T. W., and Atkinson, J. P. (1991). Membrane cofactor protein (MCP or CD46): Newest member of the regulators of complement activation gene cluster. *Annu. Rev. Immunol.* **9**, 431–455.

Luna, E. J., and Hitt, A. L. (1992). Cytoskeleton-membrane interactions. *Science* **258**, 955–964.

Maisner, A., Schneider-Schaulies, J., Liszewski, M. K., Atkinson, J. P., and Herrler, G. (1994). Binding of measles virus to membrane cofactor protein (CD46): Importance of disulfide bonds and N-glycans for the receptor function. *J. Virol.* **68**, 6299–6304.

Manchester, M., Liszewski, M. K., Atkinson, J. P., and Oldstone, M. B. (1994). Multiple isoforms of CD46 (membrane cofactor protein) serve as receptors for measles virus. *Proc. Natl. Acad. Sci. USA* **91**, 2161–2165.

Morrison T., and Portner, A. (1991). Structure, function, and intracellular processing of the glycoproteins of *Paramyxoviridae*. *In* "The Paramyxoviruses" (D. W. Kingsbury, Ed.), pp. 347–382. Plenum, New York.

Naniche, D., Varior-Krishnan, G., Cervoni, F., Wild, T. F., Rossi, B., Rabourdin-Combe, C., and Gerlier, D. (1993). Human membrane cofactor protein (CD46) acts as a cellular receptor for measles virus. *J. Virol.* **67**, 6025–6032.

Owens, R. J., Dubay, J. W., Hunter, E., and Compans, R. W. (1991). The human immunodeficiency virus envelope protein determines the site of virus release in polarized epithelial cells. *Proc. Natl. Acad. Sci. USA* **88**, 3987–3991.

Parton, R. G., Prydz, K., Bomsel, M., Simons, K., and Griffiths, G. (1989). Meeting of the apical and basolateral endocytic pathways of the Madin-Darby canine kidney cell in late endosomes. *J. Cell Biol.* **109**, 3259–3272.

Ray, R., Brown, V. E., and Compans, R. W. (1985). Glycoproteins of human parainfluenza virus type 3: Characterization and evaluation as a subunit vaccine. *J. Infect. Dis.* **152**, 1219–1230.

Robbins, F. C. (1962). Measles: Clinical features. Pathogenesis, pathology, and complications. *Am. J. Dis. Child.* **103**, 266–273.

Rodriguez-Boulan, E., and Sabatini, D. D. (1978). Asymmetric budding of viruses in epithelial monolayers: A model system for study of epithelial polarity. *Proc. Natl. Acad. Sci. USA* **75**, 5071–5075.

Roth, M. G., and Compans, R. W. (1981). Delayed appearance of pseudotypes between vesicular stomatitis virus and influenza virus during mixed infection of MDCK cells. *J. Virol.* **40**, 848–860.

Sato, N., Funayama, N., Nagafuchi, A., Yonemura, S., Tsukita, S., and Tsukita, S. (1992). A gene family consisting of ezrin, radixin and moesin: its specific localization at actin filament/plasma membrane association sites. *J. Cell Sci.* **103**, 131–143.

Srinivas, R. V., Balachandran, N., Alonso-Caplen, F. V., and Compans, R. W. (1986). Expression of herpes simplex virus glycoproteins in polarized epithelial cells. *J. Virol.* **58**, 689–693.

Tashiro, M., Pritzer, E., Knoshnan, M. A., Yamakawa, M., Kuroda, K., Klenk, H.-D., Rott, R., and Seto, J. T. (1988). Characterization of a pantropic variant of Sendai virus derived from a host-range mutant. *Virology* **165**, 577–583.

Tashiro, M., Yamakawa, M., Tobita, K., Seto, J. T., Klenk, H.-D., and Rott, R. (1990a). Altered budding site of a pantropic mutant of Sendai virus, F1-R in polarized epithelial cells. *J. Virol.* **64**, 4672–4677.

Tashiro, M., Yamakawa, M., Tobita, K., Klenk, H.-D., Rott, R., and Seto, J. T. (1990b). Organ tropism of Sendai virus in mice: Proteolytic activation of the fusion glycoprotein in mouse organs and budding site at the bronchial epithelium. *J. Virol.* **64**, 3627–3634.

Tucker, S. P., and Compans, R. W. (1993). Virus infection of polarized epithelial cells. *Adv. Virus Res.* **42**, 187–247.

von Bonsdorff, C.-H., Fuller, S. D., and Simons, K. (1985). Apical and basolateral endocytosis in Madin-Darby canine kidney (MDCK) cells grown on nitrocellulose filters. *EMBO J.* **4**, 2781–2792.