Symmetric Infection of Rotavirus on Polarized Human Intestinal Epithelial (Caco-2) Cells

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When rotavirus infects the mature villus tip cells of the small intestine, it encounters a highly polarized epithelium. In order to understand this virus-cell interaction more completely, we utilized a cell culture-adapted rhesus rotavirus (RRV) to infect human intestinal (Caco-2) and Madin-Darby canine kidney (MDCK-1) polarized epithelial cells grown on a permeable support. Filter-grown Caco-2 cells and MDCK-1 cells, producing a transepithelial resistance of 300 to 500 and $>1,000 \Omega \cdot \text{cm}^2$, respectively, were infected from either the apical or basolateral domain with RRV or Semliki Forest virus. Whereas Semliki Forest virus infection only occurred when input virions had access to the basolateral domain of MDCK-1 or Caco-2 cells, RRV infected MDCK-1 and Caco-2 monolayers in a symmetric manner. The effect of rotavirus infection on monolayer permeability was analyzed by measuring the transepithelial electrical resistance. Rotavirus infection on filter-grown Caco-2 cells caused a transmembrane leak at 18 h postinfection, before the development of the cytopathic effect (CPE) and extensive virus release. Electrical resistance was completely abolished between 24 and 36 h postinfection. Although no CPE could be detected on RRV-infected MDCK cells, the infection caused a transmembrane leak that totally abolished the electrical resistance at 18 to 24 h postinfection. Cell viability and the CPE analysis together with immunohistochemistry and immunofluorescence data indicated that the abolishment of resistance across the monolayer was due not to an effect on the plasma membrane of the cells but to an effect on the paracellular pathway limited by tight junctions. Attachment and penetration of rotavirus onto Caco-2 cells caused no measurable transmembrane leak during the first hour of infection.

Viruses use a variety of routes to gain entry into susceptible hosts. Rotavirus, the leading cause of infantile gastroenteritis, uses one such route by passage through the host's polarized epithelial cell barrier in the small intestine. Polarized epithelial cells line all body cavities and occur in tissues that carry out transport functions, such as absorption and secretion. Polarized epithelial cells are characterized by the presence of two distinct plasma membrane domains, the apical, corresponding to the luminal surface, and the basolateral, corresponding to the serosal surface (19, 23). The two domains are separated by tight junctions which play a crucial role in maintaining the cell polarity and the unique protein-lipid composition of each domain (19, 23). The best-characterized experimental epithelial cell system for the study of polarized virus infection and maturation is that of the Madin-Darby canine kidney (MDCK) cell (8, 9, 20, 23). It provides a convenient model for the study of epithelial cell polarity because it retains in vitro many of the structural and functional properties of epithelia in vivo. Rodriguez-Boulan and Sabatini (20) reported that maturation of influenza, Sendai, and vesicular stomatitis viruses (VSV) occurs in a polar fashion in MDCK cells. Semliki Forest virus (SFV) and VSV have been shown to infect exclusively through the basolateral surface (8, 9), whereas influenza virus can infect from both the apical and the basolateral sides (8). Recently, it was reported that the nonenveloped simian virus 40 and

the canine parvovirus infect and bind from apical and basolateral domains, respectively (2, 3). The asymmetric pathway in virus entry is presumed to reflect the polarized expression of virus receptors and may, as such, play a role in viral pathogenesis.

Rotavirus infects mature villus enterocytes in the mid- and upper-villus epithelia (5) and can be detected in lamina propria macrophages (26) and goblet cells (26). In enterocytes, rotavirus is usually found in the expanded rough endoplasmic reticulum. Infected cells demonstrate shortened microvilli and are generally abnormal in appearance. Although rotavirus infection is usually limited to the small intestine, recently it has been shown to spread, under some circumstances, to the liver hepatocytes, another polarized epithelial cell type (23). Electron microscopy observations, as well as biochemical studies, suggest that direct plasma membrane penetration (14, 25) is the mechanism by which rotavirus enters cells. In this study, we have examined the site of rotavirus infection in a human polarized intestinal epithelial cell line (Caco-2) grown on permeable support. Caco-2 cells are derived from a relatively well differentiated human colon tumor and form, when grown on permeable supports, polarized monolayers with well-developed brush borders (17). The morphological, functional, and biochemical properties of Caco-2 cells resemble those of the small intestinal epithelium (17). Caco-2 cells also express disaccharidases and peptidases typically found in normal small intestinal villus cells, form domes on impermeable substrates, and transport ions and water toward the basolateral surface (17). Furthermore, monolayers of Caco-2 cells develop tight junctions which prevent the passage of macro-

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molecules on permeable filters (12, 17) and produce an electrical resistance typical of polarized epithelium cells (10, 12).

MATERIALS AND METHODS

Cell lines and growth of cells on permeable filters. Viral infection experiments require the use of virus-permissive, well-polarized epithelium cell lines, which form a tight monolayer. The human intestinal epithelial cell line Caco-2, established from a moderately well differentiated colon adenocarcinoma, fulfills these criteria (10, 12) and is of intestinal origin. Cells were grown in Dulbecco's modified Eagle's minimum essential medium (DMEM) with high glucose (4,500 mg/ml) at 37°C in 5% CO₂ atmosphere. The cells were further supplemented with 10% fetal bovine serum, 1 mM sodium pyruvate, penicillin, and streptomycin. Media were changed every 3 days. Cells were grown in 150-cm² flasks and were passaged once a week. A highly polarized subclone (MDCK-1) of the MDCK cell line was grown on filters essentially as described by Fuller et al. (8) and von Bonsdorff et al. (28). MDCK-1 cells were employed as an additional control for the viral polarity studies, as they represent a highly polarized epithelium in which virus infection and maturation have been extensively studied (2, 8, 9, 20, 23). The experiment also required a nonpolarized cell line (CV-1) as a negative control (3, 4). CV-1 and MDCK-1 cells were propagated in the same manner as Caco-2 cells.

Transparent and nontransparent (polycarbonate) 3.0- μ m-pore-size Transwell filters (Costar) were seeded with Caco-2 (200,000 cells per ml), CV-1 (200,000 cells per ml), and MDCK-1 (400,000 cells per ml) cells. The large, 24.5-mm (diameter) filters were seeded with 1.5 ml of cell suspension, and the 6.5-mm filters were seeded with 150 μ l in the inner chamber. Whereas the nonpolarized CV-1 cells reached confluency after 3 to 4 days, the Caco-2 and MDCK-1 cells became confluent after 6 to 7 and 3 to 4 days postseeding, respectively.

All virus infection experiments employed 5- to 6-day-old CV-1 cells, the point at which the number of cells on a 4.7-cm² filter (diameter, 24.5 mm) increased to 1×10^6 , and 5- to 6-day-old MDCK-1 cells (density, 1.2×10^6 cells). Caco-2 cells were 14 to 16 days old at the time of infection and had reached a cell density of 1.25×10^6 cells per filter.

Peroxidase and immunofluorescence microscopy. Cells grown on permeable filters were rinsed once with phosphate-buffered saline (PBS) and fixed with 2% paraformaldehyde in PBS. After fixation, cells were rinsed twice with PBS and permeabilized by 0.2% Triton X-100 in PBS for 20 min. After two subsequent PBS washes, cells were incubated with antibody appropriately diluted in PBS–0.5% bovine serum albumin (BSA) for 2 h at 37°C. After additional washes, peroxidase-labeled goat anti-rabbit or goat anti-guinea pig conjugate was added. The immune reaction was visualized by the use of 3-amino-9-ethylcarbazole (Sigma) at a concentration of 4 mg/ml. For immunofluorescence studies, the filters were fixed in cold ethanol for 15 min and washed twice with 0.2% Triton X-100 before inoculation with the appropriate antibody diluted in PBS–0.5% BSA. After a 30-min incubation at 37°C, the filters were washed again three times and incubated with the appropriate antibody conjugate. Filters were mounted in Eukitt (Vitromed, Basel, Switzerland) and inspected in a fluorescence microscope.

Transmission electron microscopy. Cells grown on polycarbonate Transwell filters were fixed with 2% glutaraldehyde in PBS. After being washed with PBS, monolayers were

postfixed for 90 min in 1% OsO₄ in PBS and processed essentially as described by Finlay et al. (6). Before dehydration through a graded series of ethanol, the cells were washed twice with cold PBS and stained with 0.25% uranyl acetate overnight. After dehydration, cells were embedded in firm Spur's plastic, sectioned, and stained with uranyl acetate-lead citrate before examination in a Philips electron microscope.

Transepithelial electrical resistance measurements. Polarized Caco-2 and MDCK-1 and nonpolarized CV-1 cells grown on Transwell filters were measured for transmonolayer electrical resistance by use of a Millicell-ERS resistance apparatus (Millipore). Electrical resistance values obtained in the absence of cells were considered background. The net resistance was calculated by subtracting the background and multiplying the resistance by the area of the filter. Values are given as ohms times square centimeters.

Depolarization of monolayers. Depolarization of Caco-2 and MDCK-1 cells by disruption of tight junctions was performed as described by Finlay et al. (6). Briefly, polarized Caco-2 monolayers were washed and incubated in Ca²⁺-free minimal essential medium with Spinner salts (GIBCO) in 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (pH 7.4). Disruption of tight junctions was monitored by measuring the fall in electrical resistance.

Viruses. Rhesus rotavirus (RRV) was Freon extracted and trypsin activated with 10 μ g of trypsin (Sigma T-8553) per ml for 30 min at 37°C. Trypsin was added to convert noninfectious virus into infectious virus via cleavage of vp4 (14). Before being used for infection, RRV was diluted and filtered through a 0.22- μ m-pore-size filter. The filtration step was used to exclude cell-associated and aggregated viruses from the suspension. SFV was kindly supplied by Henrik Garoff (Stockholm, Sweden).

Virus infection on filters. Transwell filters containing confluent monolayers of Caco-2, MDCK-1, and CV-1 cells were rinsed 2 times with DMEM prewarmed to 37°C and infected with virus suspension in the apical or basolateral chamber. After 1 h of incubation at 37°C in 5% CO₂, the filters were washed three times with DMEM and incubated in MEM with 1 μ g of trypsin per ml (RRV) or without trypsin (SFV) for the desired time. Cell-associated virus titers were obtained by freeze-thawing the filter-grown cells and determining the yield.

Cell viability staining and counting by hemocytometer. Attached cells were washed twice with PBS and incubated at 37°C with a trypsin-EDTA solution until the cells were released from the filters. Before being counted in a hemocytometer, the cells were resuspended in an equal volume of DMEM and stained for 8 min with 0.4% trypan blue (GIBCO). Monolayers were first washed twice with PBS containing Mg²⁺ and Ca²⁺ and then stained with trypan blue for 8 min. Cell counting (500 cells) was done in a Nikon TBS microscope.

Assay for virus infectivity. Monolayers of MA-104 cells in 96-well plates were inoculated with serially diluted RRV. After being washed, the cells were incubated for 18 h and fixed, and cells producing viral antigen were visualized by immunofluorescence staining. Virus titers were expressed as fluorescence-forming units (FFU) per milliliter.

RESULTS

RRV infects polarized epithelial cells in a symmetric manner. In order to determine whether the site of rotavirus entry is the apical or basolateral cell surface, rotavirus was applied

FIG. 1. Immunofluorescence studies of the entry of rotavirus into polarized epithelia grown on permeable filters. Caco-2 and MDCK-1 monolayers were grown on permeable 3.0- μ m-pore-size Transwell filters and infected with 5 PFU per cell. At 18 h postinfection, the monolayers were fixed and stained for viral antigen with a vp6-specific monoclonal antibody (255/60) followed by fluorescein-conjugated rabbit anti-mouse antibody and examined by immunofluorescence. Caco-2 monolayers were infected from the apical (A) and basolateral (B) surfaces; MDCK-1 monolayers were infected from the apical (C) and basolateral (D) surfaces. Counting of immunofluorescent foci disclosed a ratio of approximately 1:1 for apical versus basolateral infection for both cell types.

to either domain on Caco-2, MDCK-1, and CV-1 monolayers grown on permeable supports. The time for infection was chosen after confirming the monolayer integrity by morphologic observation and by measuring the transepithelial electrical resistance across the monolayer. The net electrical resistance value for nonpolarized CV-1 cells ranged from 4 to 23 $\Omega \cdot \text{cm}^2$. In contrast to the CV-1 cells, Caco-2 cells developed a transepithelial resistance of 300 to 500 $\Omega \cdot \text{cm}^2$. At this point, the filter-grown Caco-2 cells differed from the same cells grown on a plastic surface by the lack of domes. As reported previously (8, 28), MDCK-1 monolayers reached a net electrical resistance exceeding 1,000 $\Omega \cdot \text{cm}^2$.

The capacity of rotavirus to infect through the apical and/or basolateral surfaces was compared by using various amounts of input virus (1, 5, and 25 FFU per cell). The yield of progeny cell-associated virus from Caco-2 and CV-1 cells was determined by virus titration, and the number of infected cells was determined by immunofluorescence and immunohistochemistry. The input multiplicity did not affect the results. As seen in Fig. 1A and B, RRV was found to infect Caco-2 monolayers in a symmetric manner. MDCK-1 cells were also infected by RRV in a symmetric fashion (Fig. 1C and D). The nonpolarized CV-1 cells were also infected equally from both surfaces when tested by immunostaining

FIG. 2. Electron micrographs of Caco-2 cells. Cells were grown on permeable supports and inoculated from the apical domain with 5 FFU of RRV per cell followed by preparation for electron microscopy at 24 h postinfection. Note the developed brush border system in the uninfected cells (A and B) as well as the typical tight junctions and desmosomes. Extensive cytoplasmic vacuolization occurred after infection (C and D), including relocation of the nucleus towards the apical plasma membrane and the disappearance of tight junctions. Although microvillus atrophy was observed, plasma membranes appeared to be intact. Abbreviations: A, apical surface; T, tight junction; D, desmosome; V, vacuoli; N, nucleus.

(data not shown). The yield (titers) of progeny virus obtained after a single replication cycle with apically infected Caco-2 cells was only slightly higher than that obtained after basolateral inoculation (4.1×10^6 versus 1.8×10^5 FFU); the yield of progeny virus obtained with apically infected CV-1 cells was 5.2×10^7 versus that obtained by basolateral inoculation (7.3×10^6). The apical/basolateral (A/B) ratio of the virus yield from inoculated, nonpolarized CV-1 cells was very similar to that from Caco-2- and MDCK-1-inoculated cells. This would suggest either that the rotavirus receptor is

present on both sides of the epithelium or that the polarization of the monolayers is incomplete. To confirm the cell surface polarity of Caco-2 and MDCK-1 cells, SFV, which infects exclusively from the basolateral domain on MDCK cells (9), was also studied. Infection experiments with SFV on Caco-2 monolayers demonstrated a moderate degree of SFV receptor polarization. About five times more infected cells were observed by immunofluorescence after basolateral inoculation than after apical inoculation on Caco-2 cells (data not shown). This is in contrast to the A/B ratio of about

FIG. 3. Transepithelial electrical resistance of Caco-2 and MDCK-1 cell monolayers grown on permeable Transwell filters and apically or basolaterally inoculated with RRV (3 FFU per cell). Each value is an average of four experiments and is expressed as net ohms times square centimeters. The background (filter) resistance was subtracted from the total electrical resistance across the monolayer. pi, postinfection.

1:1,000 observed by using MDCK-1 cells (data not shown). The effective basolateral infection of MDCK-1 and Caco-2 cells with SFV demonstrated that there was no steric hindrance of the filter for basolateral infection. The number of RRV-infected MDCK-1 cells, as determined by immunofluorescence after a single growth cycle (Fig. 1), indicated that, in contrast to SFV, rotavirus infects Caco-2 and MDCK cells in a nonpolar fashion. The A/B ratio of infected cells was about 1:1 for both Caco-2 and MDCK cells (Fig. 1). Interpretation of these results in terms of receptor concentration is difficult, since the apical and basolateral surface areas for Caco-2 and CV-1 cells are unknown. For MDCK-1 cells, however, an A/B surface area ratio of 1 to 7 has been reported (28).

Transmission electron microscopic examination of the filter-grown Caco-2 monolayers fixed at various periods postinfection revealed no morphologic differences related to the route of infection. The virus caused pronounced vacuolization, which increased rapidly from 12 to 24 h postinfection. A relocation of the nucleus towards the apical domain was also observed. The clearly identified tight junctions in uninfected Caco-2 cells (Fig. 2) had partly disappeared at 12 h postinfection and had completely disappeared at 36 h

postinfection (Fig. 2). As seen in Fig. 2, the brush border with its microvillus system was also affected by the infection. The microvilli 24 h after infection were reduced in absolute number and also showed signs of atrophy that proceeded through the course of infection. Although the cells became highly vacuolized, plasma membranes appeared to be intact up to 36 h postinfection.

Rotavirus induces a transepithelial leak of filter-grown polarized epithelia before the CPE. Caco-2 cells and other polarized epithelium cells are held together by tight junctions that give the monolayers the properties of an effective barrier. VSV and influenza virus have been previously shown to abolish the electrical resistance across the monolayer upon infection of MDCK cells (15). VSV causes a decrease in electrical resistance 5 to 6 h postinfection, coincident with the rise of virus budding; influenza virus on the other hand abolishes the resistance only after the virus production has already ceased (15). We were interested in studying how the transepithelial resistance on Caco-2 and MDCK-1 cells would react upon rotavirus infection. This event in the progression of cell injury in polarized epithelia has not been studied during nonenveloped virus infection.

Filter-grown Caco-2 cells were inoculated from the apical

TABLE 1. Effect of rotavirus infection on the transepithelial resistance and cell viability of filter-grown Caco-2 cells

Treatment and time post-infection (h)	Avg electrical resistance (Ω/cm^2) ^a	% Cell viability		Infected cells ^b	CPE ^c
		On filter ^d	In suspension ^e		
Apical infection ^f					
0	510	>99	>99	ND	—
18	452	>90	93	73	—
24	197	87	81	87	+/-
36	0	73	76	93	+++
Control					
0	493	>99	>99	ND	—
18	529	>99	>99	ND	—
24	488	>99	>99	ND	—
36	530	>99	>99	ND	—

^a Net average electrical resistance from four separate filters.^b Number of infected cells on a filter identified by immunohistochemistry, as described in Materials and Methods. ND, not done.^c CPE was determined by inspection of nonstained monolayers at various times and rated on a - to ++++ basis. +/- indicates an uncertain result.^d Monolayers were washed twice with PBS containing Mg^{2+} and Ca^{2+} and incubated with 0.4% trypan blue (GIBCO) for 8 min. After two washes with PBS, the number of stained cells was determined by counting the total number of cells and stained cells in a Nikon TBS light microscope at 100 and 200 $\times$ magnification.^e Attached cells were trypsin activated, resuspended in equal volume of DMEM, and stained with 0.4% trypan blue for 8 min before cell counting in a hemocytometer.^f RRV infection with 10 FFU per cell and 1 μg of trypsin per ml in maintenance medium.

or basolateral side with 3 FFU per cell, and the monolayers were analyzed for transepithelial resistance. As early as 18 h postinfection, the resistance in the Caco-2 monolayer decreased, and by 24 h postinfection, the monolayer had completely lost its resistance (Fig. 3). The reason for the drop in resistance was of interest, since little or no discernible cytopathic effect (CPE) could be observed until 36 h postinfection. The absence of the CPE was in contrast to the immunohistochemistry and immunofluorescence data which indicated extensive viral replication in virtually all cells (Fig. 1). Although less permissive than Caco-2 cells, the MDCK-1 cells showed a similar response to RRV infection. The MDCK-1 monolayer did not develop any CPE even at 36 h postinfection, although the monolayer was depolarized and most of the cells were infected (Fig. 1 and 3).

Rotavirus abolishes transepithelial resistance by interfering with the paracellular pathway limited by tight junctions. It was not possible from the transepithelial resistance data alone to determine whether the monolayer leakiness was due to an effect on the plasma membrane itself or to the integrity of the tight junctions. We therefore undertook an additional series of experiments including a cell viability analysis to clarify the mechanism of monolayer permeability. Filter-grown Caco-2 cells were infected with 10 FFU of RRV per cell or were mock infected. Then the effect of infection on the CPE, cell viability, and transepithelial resistance was monitored (Table 1). As summarized in Table 1, the most striking feature was the absence of dead cells (defined by trypan blue exclusion) and the absence of the CPE in the epithelia, in spite of the fact that nearly all cells were infected and the Caco-2 cell monolayer had lost its electrical resistance. The CPE appeared late and was characterized by rounding and enlargement of the cells and by detachment of the cells from the substrate (Fig. 4). The changes were

similar to those seen when uninfected Caco-2 monolayers are depolarized by Ca^{2+} - and Mg^{2+} -depleted media. Rotavirus-induced depolarization and depolarization of uninfected Caco-2 cells by cation depletion were characterized by the abolishment of tight junctions. The early drop (18 to 24 h) in transmembrane resistance in infected epithelia occurred at a time when the great majority of cells were viable (trypan blue exclusion) and there was an absence of a visible CPE. This indicates that the drop in transmembrane resistance caused by rotavirus is probably due to an effect on the tight junctions rather than to damage of the plasma membrane.

Transepithelial resistance is unaffected by attachment and penetration of rotavirus. In several recent studies (14, 25), direct penetration was suggested as the mode of entry for rotavirus into MA-104 cells. Previous studies demonstrated that very large amounts of inoculated rotavirus induce plasma membrane alterations, as measured by the ^{51}Cr release from infected cells. We were interested to see if attachment and the subsequent penetration and uptake of rotavirus from the apical and basolateral domains on Caco-2 cells would affect the transepithelial resistance. Trypsin-activated virus was added to the apical or basolateral chamber of Caco-2 cells grown on permeable supports. The transepithelial resistance was determined prior to infection and every 10 min thereafter up to 60 min postinfection. The resistance of the monolayer remained constant, independent of the virus concentration (1 to 70 FFU per cell) and route of infection (apical or basolateral).

DISCUSSION

Rotavirus infection in humans and animals is primarily restricted to the polarized mucosal cells of the small intestine. Such restriction could depend on polarization of either the infection (i.e., the receptor) or the maturation and release of virus.

Studies on rotavirus infection of polarized epithelia require a well-defined cell culture system in which the state of cell polarization can be measured. MDCK cells were included in the study because they have been shown to form well-polarized epithelia and have been extensively utilized for viral and bacteriological studies (2, 6, 8, 9, 15, 20). Caco-2 cells were chosen because of their intestinal origin and their permissivity for RRV infection. A 3.0- μm -pore-size membrane, rather than a 0.45- μm pore size, was employed because preliminary studies indicated that monodispersed preparations of RRV did not freely diffuse through the 0.45- μm filters, even in the absence of a cell monolayer. High electrical resistance and a basolaterally restricted infection by SFV confirmed the tightness and intactness of the MDCK and Caco-2 monolayers. Furthermore, the disruption of tight junctions by the removal of Ca^{2+} from the medium abolished the electrical resistance. Caco-2 monolayers were found to be less polarized than MDCK cells when the electrical resistance and the A/B infection ratio of SFV were used as criteria.

The growth of rotavirus in MDCK-1 and Caco-2 epithelial monolayers on permeable supports allowed us to explore the early stages of rotavirus infection in detail. In the present study, we have shown for the first time that the nonenveloped rotavirus binds to and infects polarized kidney and human intestinal epithelium monolayers equally well from both the apical and basolateral surfaces. The observation suggests that a receptor for RRV is expressed on both the apical and basolateral surfaces in both MDCK-1 and Caco-2 monolayers. Most viruses studied so far, such as VSV, SFV,

FIG. 4. Microscopic picture of the CPE seen on a Caco-2 monolayer after apical infection with rotavirus (A) and uninfected cells (B). Note the cell rounding and the dilated paracellular spaces, a consistent feature seen late (>36 h postinfection) after rotavirus infection on Caco-2 cells grown on permeable supports. Also observe that there is a relatively low number of dead cells (trypan blue-stained cells) in relation to the number showing the CPE (A).

simian virus 40, and parvovirus, have been shown to enter polarized epithelia in an asymmetric fashion (2, 3, 8, 9). Influenza virus, however, enters from both the apical and basolateral surfaces (8) and shares this property with rotavirus. This observation is not surprising, particularly since influenza virus, simian rotavirus SA11, and RRV each utilize sialic acid as receptors to initiate infection (2a, 7). Since human rotaviruses, in contrast to animal strains, are not dependent upon sialic acid for cell binding (7), there is a possibility that human strains would enter polarized epithelia by a route different from animal strains. Preliminary results indicate, however, that the Wa strain of human rotavirus infects Caco-2 cells in a nonpolar fashion (data not shown). Prior studies with RRV on MA-104 monolayers demonstrated that entry of the virus into cells was associated with the release of intracellular ^{51}Cr . The current studies cannot be directly compared with these studies since (i) less virus was used, (ii) cells were grown on a permeable support, and (iii) transepithelial resistance rather than ^{51}Cr release was measured. However, in these studies we were unable to demonstrate a measurable effect on the resistance generated by the polarized epithelium during the early stages of viral binding and entry.

It is interesting to speculate to what extent and at what level in the pathogenesis of rotavirus infection the polarity of infection and release of virus play roles. The apically restricted release of influenza virus (8, 20) and parainfluenza virus (20) may limit the spread of infection to the respiratory epithelium. Growth of several nonenveloped viruses is restricted as well to specific epithelia, and some of these viruses fail to spread to the underlying tissues. Simian virus 40, which infects and matures on the apical domain of kidney tubular cells (3, 4), is characterized by persistent infection of

the rhesus monkey kidney combined with the absence of viremia but with the presence of virus in urine (1, 16). Rhinoviruses infect nasopharyngeal epithelia, with restricted spreading to underlying tissues (11). Reovirus, which causes systemic infection in mice, has been shown to bind to the basolateral domain on intestinal epithelial cells and may infect intestinal cells only after it spreads because of systemic circulation (21). Rotavirus in pigs has recently been shown to spread systemically (22) and to infect the livers of certain mice (27). The ability to infect liver cells is linked to gene 4, the gene coding for vp4, a putative virus cell attachment protein (18). Presumably, if rotavirus spreads to the liver through the circulation, it initiates hepatocyte infection from the basolateral rather than the apical domain.

Intestinal mucosal membranes play a vital role in mediating nutrient transport as well as maintaining barrier functions from the intestinal lumen to the circulatory system. Infection of the intestinal epithelium with rotavirus results in an impaired barrier function, causing an increased epithelial permeability. Of note, several investigators have reported that rotavirus infection increases intestinal permeability to a variety of macromolecules (13, 24). We studied the monolayer permeability of polarized epithelium cells by measuring the transepithelial electrical resistance. Tight junctional complexes which correlate with the electrical resistance values are assumed to provide the main restriction to molecular communication between the apical and basolateral sides of the epithelium. We found that rotavirus infection (from either the apical or basolateral domain) of Caco-2 and MDCK-1 monolayers caused a drop in electrical resistance within 1 day of infection, although the CPE was virtually absent. The observation by Simons and Fuller (23) that polarized epithelial cells grown on filters appear to be more

resistant to the CPE than cells grown on impermeable substrates was confirmed in this study. Filter-grown Caco-2 monolayers infected with rotavirus were surprisingly resistant to the CPE, compared with Caco-2 cells grown on impermeable supports (data not shown). MDCK cells were found to support only a restricted replication of rotavirus, although most cells became infected. In MDCK cells, the CPE had not developed, although the monolayer lost its electrical resistance completely within 24 h postinfection. The loss of resistance caused by VSV and influenza virus infection depends on an effect on the paracellular rather than the transcellular route (15). This appears to be the case with rotavirus as well. Cell viability studies together with electron microscopy observations suggest that rotavirus initially causes a leak in the monolayer by opening the tight junctions. It is possible that the delay of the CPE observed in the polarized monolayer is due to the fact that many infected cells are shed from the epithelium prior to the development of the CPE and leave viable cells behind. Shedding may be stimulated by the loss of tight junctions early in the viral replication cycle.

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