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Polarized rotavirus entry and release from differentiated small intestinal cells

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ABSTRACT

Rotaviruses infect mature enterocytes from small intestine, however most data about their cellular entry are from studies carried out in non-intestinal polarized or non-polarized cell lines. In this work the entry of porcine rotavirus YM strain into small intestinal cell line IPEC-J2 was studied. It was found that YM and the human rotavirus Wa strain infect preferentially from the basolateral cell surface. Cell infection from the apical and basolateral surfaces was dependent on the presence of cholesterol. The treatment with neuraminidase, sucrose, and bafilomycin suggests that there are differences in the receptor usage and entry mechanism of the virus from the apical and basolateral surface. While cell entry is more efficient from basolateral surface, the viruses egressed mainly from the apical cell side.

1. Introduction

Rotaviruses are an important cause of children gastroenteritis. As member of the family *Reoviridae*, they are non-enveloped viruses formed by three concentric layers of protein. The outermost layer is formed by VP4 and VP7 proteins, which are responsible for receptor binding and cell penetration (Lopez and Arias, 2006).

In vivo, rotaviruses infect mature enterocytes of the small intestine, however the majority of the studies regarding their cell entry have been performed in non-differentiated MA104 cells (epithelial monkey kidney cells). Several cell surface molecules have been associated with the entry of the virus into the cell, including sialic acids (SA), several integrins, and the heat shock cognate protein 70 (hsc70) (Coulson et al., 1997; Guerrero et al., 2002, 2000a; Lopez and Arias, 2004). The ability of neuraminidase (NA), an enzyme that cleaves terminal SA, to decrease the infectivity of some rotavirus strains from animal origin, have led to the description of these viruses as being NA-sensitive, in contrast to human and other animal rotavirus isolates whose infectivity is not affected by NA treatment, and as such they are known as NA-resistant (Isa et al., 2006). Recently, histo-blood group antigens, gangliosides, and the tight junction proteins JAM-A, occludin, and ZO-1 have been also reported to be involved in binding and cell entry of some rotavirus strains (Hu et al., 2012; Martinez et al., 2013; Torres-Flores et al., 2015).

Most rotavirus strains, isolated either from humans (Wa, DS-1, and WI69) or animals (YM, UK, and SA11-4S), enter MA104 cells by clathrin-mediated endocytosis (Diaz-Salinas et al., 2013; Gutierrez et al., 2010). In contrast, the simian RRV strain follows an atypical endocytic pathway that is independent of clathrin and caveolin, but depends on the presence of dynamin 2, the small GTPase RhoA, cdc42, actinin-4, and cholesterol (Diaz-Salinas et al., 2013, 2014; Gutierrez et al., 2010). Some of these molecules are not exclusive for infection of RRV, but are also needed by rotavirus strains that use clathrin-mediated endocytosis (Diaz-Salinas et al., 2014). Independently of the receptors and entry route, all rotavirus strains seem to reach early endosomes; some strains leave the endocytic pathway at this point, while others continue to reach late endosomes (Arias et al., 2015).

Rotavirus entry into polarized cell lines has been less studied. There are only a few works on this subject, and they show discrepancies (Ciarlet et al., 2001; Svensson et al., 1991). An early study suggested that the NA-sensitive strain RRV enters polarized human colon adenocarcinoma (Caco-2) cells with the same efficiency from both the apical and basolateral surfaces (Svensson et al., 1991), while in another study, carried out in six different cell lines, and using distinct rotavirus strains, it was concluded that NA-sensitive strains infect efficiently only from the apical side, while NA-resistant strains infect equally well both cells surfaces (Ciarlet et al., 2001). The differences observed could be explained,

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at least in part, by the pore size of the permeable filters employed in these studies. Svensson et al. (1991) used filters with 3.0 μm pores, while in the study of Ciarlet et al. (2001) filters with 0.4 μm pores were used. In the latter study it was shown that Caco-2 cells could pass through 3.0 μm pores, forming apical surfaces on both sides of the permeable filter (Ciarlet et al., 2001), thus explaining the equal susceptibility to virus infection of apical and "basolateral" cell surfaces' reported in that work (Svensson et al., 1991). A more recent study in polarized MDCK II cells with rotavirus strains having distinct receptors requirements showed that all rotavirus strains were able to infect from both cell surfaces, with preference for the basolateral side (Realpe et al., 2010).

It has been described that the receptors/co-receptors involved in rotavirus entry into differentiated cells can be different in the basolateral and apical sides of the cell monolayer. SA was found to be involved in the entry of RRV into polarized Caco-2 cells from the apical, but not from the basolateral surface (Ciarlet et al., 2001). The requirement of SA for the cell entry of RRV and its NA-resistant mutant nar3 was further investigated by Realpe et al. (2010) in MDCK II cells, observing the need for the wild type virus but not for its mutant counterpart to interact with terminal SA from both, basolateral and apical surfaces. The entry route of RRV into polarized MDCK cells was also characterized by the means of different pharmacological treatments. Thus, the inhibition of vacuolar-type H^+-ATPase decreased viral infectivity, while the inhibition of dynamin by dynasore, the removal of cholesterol by metyl-β-cyclodextrin, and treatment of the cells with chloroquine or ammonium chloride did not affect rotavirus cell entry (Wolf et al., 2012). The lack of inhibition of virus infectivity by dynasore and metyl-β-cyclodextrin was not expected, as in MA104 cells dominant negative mutants of dynamin 2 decreased cell entry of several rotavirus strains (Gutierrez et al., 2010), and so did the removal of cholesterol from the cell membrane with metyl-β-cyclodextrin (Gutierrez et al., 2010).

With respect to rotavirus egress from infected cells, studies made in MA104 cells suggested that rotavirus progeny exit cells after cell lysis. However, latter studies using differentiated Caco-2 cells found that viral release was actin-dependent (Gardet et al., 2007) and polarized, occurring almost exclusively from the apical cell surface, before any cell lysis occurred (Ciarlet et al., 2001; Jourdan et al., 1997).

To date, the rotavirus cell entry into polarized cells of small intestinal origin has not been reported. In this work we tested the cell entry and release of porcine rotavirus YM strain using the differentiated porcine small intestine IPEC-J2 cell line. We found that the YM virus enters polarized cells more efficiently from the basolateral surface. Cell entry from the apical surface was dependent on the presence of terminal SA, as it was sensitive to NA treatment, while infection from the basolateral surface was only partially NA-sensitive. Viral binding was also more effective to the basolateral surface, while cell egress was principally observed from the apical surface of the cell. Our data suggest that there are differences in rotavirus entry into polarized intestinal cells, and that IPEC-J2 cells represent a good model system to study rotavirus replication in a small intestine cell system.

2. Material and methods

2.1. Cell lines and viruses

The non-transformed cell line IPEC-J2, derived from jejunal epithelia of unsuckled piglets (Berschneider, 1989) was kindly provided by A. Blikslager (College of Veterinary Medicine, North Carolina State University, USA). The IPEC-J2 cell used in this work were grown in Dulbecco's Modified Eagle Medium Ham's Nutrient Mixture F12 (DMEM-F12) (Gibco, Thermo Fisher Scientific, USA) supplemented with 5% fetal bovine serum (FBS), L-glutamine (2 mM), insulin (5 μg/ml), transferrin (5 μg/ml), selenium (5 ng/ml) (Sigma Aldrich, St. Louis, USA), penicillin (100 U/ml) and streptomycin (100 μg/ml). The cells were used between passages 30 and 45. Cell culture medium was changed every 2–3 days and cells were split every 10–14 days. The rhesus monkey epithelial cell line MA104 was grown in advanced DMEM supplemented with 2% FBS. Both cell lines were maintained in a humidified atmosphere, with 5% CO_2 at 37 °C. The human rotavirus strain Wa was obtained from H. B. Greenberg (Stanford University, USA) and the porcine virus strain YM was isolated and characterized in our laboratory (Ruiz et al., 1988). Both rotavirus strains were propagated in MA104. The virus lysates were activated prior to infection with trypsin (25 μg/ml) for 30 min at 37 °C, followed by inactivation with soybean trypsin inhibitor (25 μg/ml). When necessary viruses were purified by cesium chloride density gradients as described previously (Zarate et al., 2000).

2.2. IPEC-J2 differentiation

To induce differentiation, IPEC-J2 cells were seeded on collagen-coated transwell inserts (diameter 6.5 mm, pore size 3.0 μm) (Corning, Sigma Aldrich, St. Louis, USA) at 2×10^6 cell per ml. Cells were grown in DMEM-F12 supplemented as described above, and the trans-epithelial electrical resistance (TEER) was measured daily starting on day 3 after seeding, using epithelial voltmeter EVOMX (World Precision Instruments, Sarasota, USA) until they reached a TEER of $> 300\ \Omega\ cm^2$. At this point, the medium was replaced with DMEM-F12 medium supplemented as described above, without FBS, and containing 10^{-7} M of water-soluble dexamethasone (Sigma Aldrich, St. Louis, USA). The differentiation was monitored daily by measuring the TEER, and cells with at least $500\ \Omega\ cm^2$ were considered polarized and used in the assays described below. TEER was monitored before and after all experimental treatments, before viral infection, to ensure cell monolayer polarity and integrity.

2.3. Electron microscopy

Formation of polarized epithelia was analyzed by electron microscopy. To this end, IPEC-J2 cells grown and differentiated as described above, were washed with pre-warmed (37 °C) Hank's balanced salt solution (HBSS) and then fixed with 2.5% glutaraldehyde in 100 mM cacodylate buffer pH 7.2–7.4 during 1 h at room temperature. This was followed by two 5 min washes with cacodylate buffer, adjusted to 300 mili-osmoles with 6.84% sucrose. Afterwards, cells were post-fixed in 1% osmium tetroxide in 100 mM cacodylate buffer containing 100 mM sucrose at pH 7.2–7.4 during 1 h at 4 °C in the dark. Subsequently, cells were washed with cacodylate-sucrose buffer for 20 min at room temperature, and then the cell monolayers were dehydrated by serial 10 min incubations with increasing concentrations of ethanol (35%, 50%, 70%, 95%, and twice 100%). Cells were later embedded in LR white resin (Sigma Aldrich, St. Louis, USA) by successive incubations of 30 min with increasing concentrations of resin in ethanol (25%, 50%, and 75%). Finally cells were incubated with 100% resin for 16 h. Then cell monolayer was mounted on an aluminum disc and incubated at 60 °C for 72 h. Ultra thin sections were prepared on microtome and they were observed on a Zeiss 900 electron microscope.

2.4. Infectivity assay

Polarized IPEC-J2 cells grown in 6.5 mm transwell inserts were washed twice with DMEM-F12 supplemented with