POLARIZED ENTRY OF BOVINE CORONAVIRUS IN EPITHELIAL CELLS

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ABSTRACT

Epithelial cells are highly polarized cells divided into an apical and a basolateral plasma membrane. The two domains are composed of a distinct set of proteins and lipids. Concerning virus infection of epithelial cells, the polarity of host cell receptor distribution defines the domain from which infection may be mediated. We were interested to analyze the infection of polarized cells by bovine coronavirus (BCV). The entry of BCV into MDCK I cells was investigated by growing the cells on a permeable support. Cells were infected with BCV from either the apical or basolateral domain. The efficiency of infection was determined by measuring the hemagglutinating activity of the virus released into the apical compartment. Virus replication was only detectable after inoculation from the apical surface. Therefore, infection of MDCK I cells with BCV is restricted to the apical side.

INTRODUCTION

Epithelial cells line the body cavities of higher eukaryotes and, therefore, represent the primary barrier to a number of viruses infecting a host. The polarized organization of these cells involves the division of the plasma membrane into an apical and a basolateral portion that are separated by tight junctions. As the two membrane domains differ in the protein and lipid composition, they may also differ in the content of receptors for an infecting virus¹. The presence of suitable receptors determines whether epithelial cells are infectable from the luminal (apical) or from the serosal (basolateral) surface.

We were interested to find out which way bovine coronavirus (BCV) uses to enter polarized cells. This virus may initially infect the respiratory tract, but the disease caused by BCV is due to infection of cells of the gastrointestinal tract resulting in a severe diarrhea in newborn calves. For our studies we used MDCK I cells a well characterized polarized epithelial cell line². Cells grown on a filter membrane were infected with BCV from either the apical or the basolateral side. The efficiency of infection was judged by the yield of virus

released into the apical medium, measured by hemagglutination titration. Infection of MDCK cells with BCV was restricted to the apical side. To rule out that the restriction was due to difficulties in penetrating through the pores of the filter membrane, MDCK cells were also infected with influenza C virus. This virus was able to infect cells from both sides indicating that the filter itself is no hindrance for virus to reach the basolateral plasma membrane.

MATERIALS AND METHODS

Cells. MDCK I cells, a subline of Madin-Darby canine kidney cells, were grown in minimum essential medium containing 10% fetal calf serum. Polycarbonate membrane filters (pore size 0.4 μm , diameter 24.5mm) were purchased from Costar. Cells were grown for 3-4 days replacing the apical and basal media at daily intervals. Resistance measurements were made by using a Millicell ERS apparatus. Only cell monolayers with a resistance higher than 1000 Ωcm^2 were used for experiments.

Virus. Strain Johannesburg/1/66 of influenza C virus was grown in 8-day-old embryonated eggs by allantoic inoculation. The allantoic fluid was harvested after incubation of the eggs for 3 days at 33°C³. Strain L-9 of BCV was grown in MDCK I cells as described previously⁴.

Virus infection. Cells grown on a filter membrane with a resistance higher than 1000 Ωcm^2 were infected with virus. After an adsorption time of 60 min at 37°C, cells were washed with PBS and incubated at 37°C with MEM. The efficiency of infection was judged by the yield of virus released into the medium as indicated by the hemagglutinating activity of the cell supernatant.

Hemagglutination assay. Hemagglutination titration was performed as described previously⁵.

RESULTS AND DISCUSSION

The initial event in the life cycle of a virus is the attachment to specific receptors on the host cell. Epithelial cells which line the external surface of body cavities represent the primary barrier to a number of viruses during infection. Since epithelial cells are highly polarized and express distinct sets of lipids and proteins on their apical and basolateral surface, some viruses are restricted to a particular plasma membrane domain. We were interested to find out which way bovine coronavirus (BCV) uses to enter polarized epithelial cells.

For this purpose we used a filter system (Figure 1). The filter is composed of an apical chamber and a basolateral compartment separated by a microporous membrane. Cells were placed into the apical chamber and allowed to attach to the filter membrane to grow to confluency and to form a tight monolayer. Medium added to the apical and the basal compartment was replaced at daily intervals. Like in the natural environment, cells were fed from the basolateral side. After three to four days, the tightness of the monolayer was determined by measuring the electrical resistance between the apical and the basolateral compartment using two electrodes and the Millicell ERS apparatus. The extent of resistance is dependant not only on the tightness of the monolayer but also on the cell type and the number of passages. MDCK I cells usually reach an electrical resistance of more than 1000 Ωcm^2 which is very high compared to other cell types. The tight monolayer forms a barrier between the apical and the basolateral compartment, so that substances and virus

Figure 1. Model of the transwell membrane filter system.

added to one side of the cell monolayer cannot reach the other side. Therefore, cells can be infected selectively from either the apical or basolateral surface.

MDCK I cells grown on a filter membrane were infected with BCV and influenza C virus from either the apical or basolateral surface, respectively (Table 1). The efficiency of infection was judged by the yield of virus released into the apical medium, measured by hemagglutination titration. Infection of MDCK I cells with BCV from the apical surface resulted in a high yield of virus, whereas no virus production was detectable after infection from the basolateral side. On the other hand, influenza C virus was able to infect filtergrown cells from both sides. This indicates that the filter membrane itself is no hindrance for the virus to reach the basolateral plasma membrane for infecting the cell. From these results we conclude that infection of epithelial cells with BCV occurs preferentially from the apical side.

Binding sites for viruses are a crucial component for the infectibility of a cell. The presence of suitable receptors on polarized epithelial cells may determine whether a virus can enter the cell via the apical and/or the basolateral domain of the plasma membrane. For example, SV 40 virus has been shown to infect polarized cells only from the apical side (Figure 2)⁶. This restriction correlates with the ability of the virus to bind to the apical, but not to the basolateral domain of the plasma membrane. The receptor for SV 40 has not been identified, but it appeared to be present only on the apical surface. N-acetylneuraminic acid the receptor for influenza A and B virus is distributed on both sides of the epithelial cell; therefore, virus entry occurs from both the apical and the basolateral domain. For other viruses, e.g. vesicular stomatitis virus, canine parvovirus and vaccinia virus it has been shown that infection of epithelial cells occurs preferentially from the basolateral side¹. As

Table 1. Infection of polarized MDCK I cells with BCV and influenza C virus from either the apical or basolateral domain. The yield of virus was determined by measuring the hemagglutinating activity of the supernatant in the apical chamber.

Virus	Infection from	Hemagglutination-titer (HA units/ml)
BCV	apical	256
	basolateral	<2
Influenza C	apical	128
	basolateral	32

Figure 2. Entry of polarized epithelial cells by different viruses

far as BCoV is concerned, the restriction of virus entry to the apical surface of epithelial cells is consistent with the natural infection, i.e. the localized infection of the intestinal epithelium

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