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Human bocavirus 1 infects commercially available primary human airway epithelium cultures productively

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Human bocavirus 1 (HBoV1), a human parvovirus, belongs to the genus *Bocavirus* of the *Parvoviridae* family. It causes wheezing in young children with acute respiratory tract infections. HBoV1 has been shown to infect polarized human airway epithelium (HAE) made in house, and induces airway epithelial damage. In this study, two commercially available HAE cultures, EpiAirway and MucilAir HAE, were examined for HBoV1 infection. Both HAE cultures support fully productive HBoV1 infection. Infected EpiAirway and MucilAir HAE cultures showed loss of cilia, disruption of the tight junction barrier, and a significant decrease in transepithelial electrical resistance. Notably, HBoV1 persistent infection was demonstrated by maintaining HBoV1-infected EpiAirway HAE for as long as 50 days. After 2 days post-infection, progeny virus was produced consistently daily at a level of over 2×10^8 viral genome copies per culture (0.6 cm^2). This study is the first to use commercial sources of HAE cultures for HBoV1 infection. The availability of these cultures will enable a wide range of laboratories to study HBoV1 infection.

1. Introduction

Human bocavirus 1 (HBoV1) was discovered in 2005 by large scale sequencing in nasopharyngeal aspirates from young children (Allander et al., 2005). It is a respiratory virus associated with acute respiratory tract infections in young children (Allander et al., 2007; Brodzinski and Ruddy, 2009; Don et al., 2010; Garcia-Garcia et al., 2010; Gendrel et al., 2007; Jartti et al., 2011; Kahn, 2008; Proenca-Modena et al., 2011; Schildgen et al., 2008). Acute respiratory tract infection is one of the leading causes of hospitalization of young children in developed countries (Brodzinski and Ruddy, 2009; Lopez et al., 2006; Shay et al., 1999). HBoV1 infection shows frequently persistence and co-infections with other respiratory viruses (Jartti et al., 2011; Kesebir et al., 2006; Manning et al., 2006; Wang et al., 2010). However, acute HBoV1 infection, diagnosed by a high virus load in respiratory samples, viremia, detection of HBoV1-specific IgM, or an increase in the levels of HBoV1-specific IgG antibodies, and detection of HBoV1 mRNA in nasopharyngeal aspirates, results in respiratory illness (Allander et al., 2007; Christensen et al., 2010, 2013; Deng et al., 2012; Don et al., 2010; Kantola et al., 2008; Nascimento-Carvalho et al., 2012; Soderlund-Venermo et al., 2009; Wang et al., 2010). Life-threatening HBoV1 infections in pediatric patients, which were associated with high virus loads or diagnostic HBoV1-specific antibodies (Edner et al., 2011; Korner et al., 2011; Ursic et al., 2011), have also been described. Moreover, a recent longitudinal study in children (from infants to puberty) documented a clear association between acute primary HBoV1 infection and respiratory symptoms (Meriluoto et al., 2012).

The virus was tentatively classified as a member of genus *Bocavirus* in the subfamily *Parvovirinae* of the *Parvoviridae* family (Tijssen et al., 2012). Parvoviruses are small, non-enveloped icosahedral viruses with linear single-stranded DNA genomes. The genus *Bocavirus* includes bovine parvovirus type 1 (BPV1) (Chen et al., 1986), minute virus of canines (MVC) (Sun et al., 2009), and other tentative members that are closely related to HBoV1, including porcine bocavirus (Zhai et al., 2010), gorilla bocavirus (Kapoor et al., 2010a) and HBoV genotypes 2–4 (Arthur et al., 2009; Kapoor et al., 2009, 2010b)

A pseudostratified and well-differentiated primary human airway epithelium (HAE) culture model has been used widely to infect respiratory RNA viruses from the apical surface, e.g., influenza viruses (Ilyushina et al., 2012; Matrosovich et al., 2004; Zeng et al., 2011), parainfluenza virus (Zhang et al., 2005, 2011), respiratory syncytial virus (Villenave et al., 2012; Zhang et al., 2002), human coronaviruses (Pyrc et al., 2010; Sims et al., 2005; Wang et al., 2000), and human rhinovirus type C (Hao et al., 2012). Although respiratory DNA viruses have been reported to infect HAE, they infect HAE

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only from the basolateral surface, e.g., adenovirus (Zabner et al., 1997). In 2008, Dijkman et al. demonstrated that HBoV1 infects apically and replicates in HAE made in house (Dijkman et al., 2009). In 2012, an infectious clone of HBoV1 was established, which generates HBoV1 progeny virions from HEK293 cells transfected with this clone. Moreover, these HBoV1 virions infected HAE made in house productively, from the apical surface (Huang et al., 2012) as well as from the basolateral surface (Deng et al., 2013), which leads to airway epithelial damage.

In this report, two commercially available HAE cultures, i.e., EpiAirway and MucilAir HAE purchased from MatTek Co. (MA, USA) and Epithelix SàRL (Geneva, Switzerland), respectively, were tested for HBoV1 infection. Both HAE cultures can be infected by HBoV1 and caused hallmarks of airway epithelial damage.

2. Materials and methods

2.1. Polarized primary HAE cultures

EpiAirway HAE, which was purchased from MatTek (Ashland, MA, USA), was cultured in a Millicell insert of 0.6 cm^2 (Millipore, Billerica, MA, USA). MucilAir HAE was obtained from Epithelix SàRL (Geneva, Switzerland), and was maintained in a Costar Transwell insert of 0.33 cm^2 (Corning, NY, USA). Both HAE cultures were derived from healthy human primary tracheobronchial epithelial cells (derived from individual donors) cultured in an air-liquid interphase (ALI) with their respective property media.

2.2. Virus and infection

HBoV1 virions were collected from apical washes of HBoV1-infected primary B-HAE (Huang et al., 2012), and were used for infection as described previously (Deng et al., 2013).

For apical infection of EpiAirway HAE, HBoV1 virions were diluted in 200 μl of EpiAirway medium to achieve an MOI of 100 viral genomic copy numbers (gc)/cell, and were applied to the apical chamber. For apical infection of MucilAir HAE, HBoV1 was diluted in 100 μl of MucilAir medium to reach an MOI of 100 gc/cell. After incubation for 2 h, the apical chamber of the infected HAE ALI was washed three times with 400 μl and 200 μl of PBS for EpiAirway and MucilAir HAE, respectively. The cultures were maintained continuously at an ALI. To determine virus release kinetics, 200 μl and 150 μl aliquots of PBS, for EpiAirway and MucilAir HAE, respectively, were added to the apical chamber of the HAE culture at various time points, incubated for 15 min at 37 °C, and were harvested as apical washes. Meanwhile, an aliquot of 100 μl of medium from the basolateral chamber of the HAE culture was collected, followed by replacement with 100 μl of fresh medium. All the harvested aliquots were stored at 4 °C for quantification of viral DNA as gc.

For basolateral infection, HBoV1 virions were diluted in the ALI medium (1 ml and 0.5 ml for EpiAirway and MucilAir, respectively) in the basolateral chamber of the HAE cultures. The cultures were incubated at 37 °C and 5% CO_2 for 2 h, and then the basolateral inoculums were removed and washed three times with PBS (1 ml and 0.5 ml for EpiAirway and MucilAir, respectively), followed by supplementation of fresh media. Progeny virion release was monitored daily by quantification of viral gc in samples collected from the basolateral chamber at a volume of 100 μl.

2.3. Real time quantitative PCR (qPCR) analysis of HBoV1 genome copy numbers

Aliquots of 100 μl of apical sample and 50 μl of basolateral sample, respectively, were incubated with 25 units of benzonase (Sigma, St. Louis, MO, USA) for 2 h at 37 °C. The treated samples were digested with 20 μl of proteinase K (15 mg/ml; Amresco, Solon, OH, USA) at 56 °C for 10 min. Viral DNA was extracted using the QIAamp blood mini kit (Qiagen, Valencia, CA, USA), and eluted in 100 μl and 50 μl of deionized H_2O for apical and basolateral samples, respectively. The extracted DNA samples were quantified with respect to the numbers of HBoV1 gc by the qPCR method described previously (Deng et al., 2013; Huang et al., 2012; Lin et al., 2007).

2.4. Immunofluorescence analysis

Immunofluorescence analysis of HBoV1-infected HAE was performed as described previously (Deng et al., 2013; Huang et al., 2012). A rat anti-HBoV1 NS1 polyclonal antibody (Chen et al., 2010) was developed previously in-house (Chen et al., 2010). Anti-ZO-1 (Invitrogen, Grand Island, NY, USA) and anti-β-tubulin IV (Sigma, St. Louis, MO, USA) antibodies were used for detecting the tight junction and cilia, respectively.

2.5. Measurement of the transepithelial electrical resistance (TEER)

The TEER of both mock- and HBoV1-infected HAE cultures was measured using an epithelial volt-ohm meter (Millipore, Billerica, MA, USA) at the indicated days p.i. as described previously (Deng et al., 2013; Huang et al., 2012).

3. Results

3.1. HBoV1 infects EpiAirway HAE persistently

EpiAirway HAE was first infected with the apically washed HBoV1 virions at a multiplicity of infection (MOI) of 100 gc/cell. Progeny virions released from the apical surface at 1×10^6 gc/μl at 3 days post-infection (p.i.), and remained as a plateau along with the infection for 50 days (Fig. 1A). Virus was not detected in mock-infected EpiAirway HAE. Since 200 μl of phosphate buffered saline (pH 7.4; PBS) was used to wash the progeny virions from the apical surface every day, the total virus release from one HAE culture was approximately 2×10^8 gc per day. Virus was also released from the basolateral surface, but at a level of ~1–2 log lower than that from the apical surface (Fig. 1A).

Consistent with the virus release kinetics, the transepithelial electrical resistance (TEER) of HBoV1-infected HAE started to drop off at 4 days p.i., gradually lowering to a level of 390 Ω cm^2 at 12 days p.i., 306 Ω cm^2 at 28 days p.i, and to a level close to 200 Ω cm^2 at 48 days p.i. (Fig. 1B). Compared with the mock-infected counterpart, the TEER decreased ~3.5-fold by the end of infection (at 48 days p.i., Fig. 1B).

Of note, a few of the epithelial cells were washed off in the apical chamber of HBoV1-infected HAE, and many of which were NS1-positive (Fig. 1C), suggesting that HBoV1 infection induces epithelial cell death. By the end of the infection (at 50 days p.i.), β-tubulin IV (a marker of cilia (Matrosovich et al., 2004; Villenave et al., 2012)), the tight junction protein zona occludens-1 (ZO-1) (Gonzalez-Mariscal et al., 2003), as well as HBoV1 NS1 expression, was detected. Although only one third of the cells in the HBoV1-infected EpiAirway HAE expressed NS1, the infected HAE showed no expression of β-tubulin IV and a disassociation of the ZO-1 (Fig. 1D).

In addition, EpiAirway HAE was infected at the basolateral surface. A gradual virus release from both the apical and basolateral surface was observed from 1 to 11 days p.i. Virus release from apical infection reached a plateau (~2×10^6 gc/μl) at 12 days p.i.; however, there was ~1–1.5 log less virus released from the basolateral surface during infection between 10 and 26 days p.i. (Fig. 2A).