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Infection of differentiated airway epithelial cells from caprine lungs by viruses of the bovine respiratory disease complex

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ABSTRACT

Bovine respiratory syncytial virus (BRSV), bovine parainfluenza virus type 3 (BPIV3) and bovine herpesvirus type 1 (BHV-1) are important pathogens associated with the bovine respiratory disease complex (BRDC). Non-bovine ruminants such as goats may also be infected and serve as a virus reservoir to be considered in the development of control strategies. To evaluate the susceptibility of caprine airway epithelial cells to infection by viruses of BRDC, we established a culture system for differentiated caprine epithelial cells. For this purpose, we generated precision-cut lung slices (PCLS), in which cells are retained in their original structural configuration and remain viable for more than a week. The three bovine viruses were found to preferentially infect different cell types. Ciliated epithelial cells were the major target cells of BPIV3, whereas BHV-1 preferred basal cells. Cells infected by BRSV were detected in submucosal cell layers. This spectrum of susceptible cells is the same as that reported recently for infected bovine PCLS. While infection of caprine cells by BRSV and BPIV3 was as efficient as that reported for bovine cells, infection of caprine cells by BHV-1 required a tenfold higher dose of infectious virus as compared to infection of bovine airway cells. These results support the notion that non-bovine ruminants may serve as a reservoir for viruses of BRDC and introduce a culture system to analyze virus infection of differentiated airway epithelial cells from the caprine lung.

1. Introduction

Bovine respiratory disease (BRD) is a multifactorial disease which has been associated with bovine respiratory syncytial virus (BRSV), bovine parainfluenza virus type 3 (BPIV3) and bovine herpesvirus type 1 (BHV-1). It has been assumed that the primary virus infection increases susceptibility to subsequent bacterial infection, e.g. by *Mannheimia haemolytica* or *Pasteurella multocida* which is responsible for the severity of disease (Ackermann and Brodgen, 2000; Ellis, 2009). BRSV and BPIV3 are both members of the family *Paramyxoviridae* but use different receptors for entry of target cells. BRSV binds to glycosaminoglycans and a specific protein receptor (reviewed in Valarcher and Taylor, 2007), BPIV3 attaches to sialic acid-containing cellular glycoconjugates (Chanock et al., 2001). BHV-1, an alphaherpesvirus, uses heparan sulfate structures for primary attachment and a defined protein, such as nectin-1, for close interaction (reviewed in Jones and Chowdhury, 2007). Viruses of the BRDC may also infect other ruminants like goats and sheep. Evidence is based on serological analyses and experimental infections (Lehmkuhl and Cutlip, 1979; Fulton et al., 1982; Elazhary et al., 1984; Engels et al., 1992; Meehan et al., 1994; Six et al., 2001). Therefore, these ruminants may serve as a

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reservoir for viruses of BRD (Van der Poel et al., 1995; Thiry et al., 2006).

Here we report the first culture system for caprine differentiated respiratory epithelial cells, precision-cut lung slices (PCLS). These slices exhibit relevant features of airway epithelial cells, e.g. ciliated and mucus-producing cells that are maintained in their original setting. Furthermore, slices can be generated in large amounts and cells are viable for at least one week. Recently we have reported that different viruses of the BRDC have developed different strategies to infect differentiated bovine respiratory epithelial cells (Kirchhoff et al., 2014). Here, we analyzed these viruses for their ability to infect differentiated caprine airway cells and thus to cross the species barrier.

2. Materials and methods

2.1. Virus

Recombinant BRSV expressing green fluorescent protein (BRSV-GFP) was provided by Karl-Klaus Conzelmann (BRSV ATue51908, Max-von-Pettenkofer-Institut, Munich). Stocks were prepared in MDBK cells. After grown in culture flasks till 80% confluence, cells were inoculated with BRSV-GFP (MOI ~ 0.1) for 2.5 h. After 6–7 days, supernatants were harvested, centrifuged by low speed centrifugation, shock frosted and stored at −80 °C. BPIV3 was provided by Friedrich-Loeffler-Institut (Insel Riems, Germany). BHV1-GFP was generated from the strain BHV1/Aus12 as described previously (Keil, 2000). Both were propagated in KOP-R cells for 1–3 days and the following procedure was the same as for BRSV-GFP (Goris et al., 2009).

2.2. PCLS

PCLS were obtained from the lungs of 2–7 months old goat kids (7 in total) originating from one organic goat dairy farm and housed in the Clinic for Swine and Small Ruminants and Forensic Medicine at the University of Veterinary Medicine, Hannover. Goats were vaccinated twice against Pasteurellosis and Clostridiosis by Heptavac® P Plus (Intervet Deutschland GmbH, Unterschleißheim). Goats did not show any signs of respiratory or systemic disease. After euthanasia or slaughter the *lobus accessorius* was removed, filled with low-melting-point agarose and cut into 250 μm thick slices by using a Krumdick tissue slicer as previously described (Goris et al., 2009). PCLS were incubated in 1 mL of RPMI 1640 medium in 24-well plates at 37 °C and 5% CO_2. Medium was changed several times to remove the agarose.

The viability of the cells in PCLS was verified by different criteria: (i) monitoring the ciliary activity using a light microscope during the whole experiment (Zeiss Axiovert 35); (ii) in selected slices, by addition of 100 μM methacholine (acetyl-β-methylcholine chloride, Sigma–Aldrich) to induce bronchoconstriction and (iii) determining the integrity of the cells by staining with a live/dead viability/cytotoxicity assay kit (Fluo Probes, FP-BE4710). To evaluate ciliary activity, the bronchus was virtually divided into portions and for each of them the presence of ciliary activity was examined by light microscopy. The slices were rinsed in phosphate-buffered saline (PBS) and then incubated for 30 min with Calcein AM (1 μM) and ethidium bromide (EthD-1; 2 μM). After further washing steps with PBS, slices were embedded in Mowiol resin and photomicrographs were taken by using a Leica TCS SP5 AOBS confocal laser scanning microscope.

2.3. Infection

PCLS were washed extensively with PBS to remove mucus and then infected with 500 μl of diluted virus stocks (10^5 or 10^6 foci-forming units per mL (FFU/mL)) in RPMI medium. With BPIV3 and BHV-1-GFP, the inoculum was removed after 2 h; in the case of BRSV-GFP after 3 h. After infection, slices were rinsed twice in PBS and then 1 mL of RPMI was added. Infected slices were incubated for 1–5 days at 37 °C and 5% CO_2. To open tight junctions, PCLS were incubated with Ca^{2+}/Mg^{2+}-free PBS containing 100 mM EGTA (AppliChem, Darmstadt) at 37 °C for 30 min. Before and after the procedure, the slices were washed three times with Ca^{2+}/Mg^{2+}-free PBS.

2.4. Immunofluorescence analysis of PCLS

PCLS were fixed with 3% paraformaldehyde in PBS for 20 min. For permeabilization, cells were treated with 0.2% Triton-X-100 diluted in PBS for 10 min. Primary and secondary antibodies were diluted in PBS with 1% bovine serum albumin and added for 1 h at room temperature. Between each step, slices were rinsed 3 times in PBS. The slices were embedded in Mowiol resin and analyzed by confocal microscopy as described above. BPIV3-infected cells were visualized using an anti-bovine parainfluenza 3 monoclonal antibody (Bio 290) followed by FITC-labeled anti-mouse IgG (Sigma–Aldrich). Beta-tubulin staining was used to identify ciliated cells (Sigma–Aldrich); mucin was stained with anti-muc5ac antibody (gastric, Acris). Nuclei were visualized by staining of cells with DAPI (4′,6′-diamidino-2-phenylindole), for 10 min followed by three washing steps.

3. Results

3.1. Analysis of PCLS

To analyze the infection of differentiated caprine airway epithelial cells by viruses of the bovine respiratory disease complex, we established a culture model for differentiated epithelial cells from the caprine lung. Precision-cut lung slices were generated from the *lobus accessorius* of 2–7 months old animals. To verify the viability of the cells different criteria were applied. The ciliary activity was determined by light microscopy at daily intervals. Only slices which have retained the full ciliary activity were used for infection experiments. Furthermore, we analyzed the vitality of the slices by applying a live/dead viability/cytotoxicity assay. Examination by confocal microscopy at days 1, 3 (not shown) and 7 revealed that the cells of the respiratory epithelium lining the bronchioles