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Bovine Respiratory Syncytial Virus Infection Enhances *Pasteurella multocida* Adherence on Respiratory Epithelial Cells

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INTRODUCTION

Bovine respiratory syncytial virus (BRSV) is a single negative-stranded RNA virus belonging to the Paramyxoviridae family and shows a close genetic relationship with human respiratory syncytial virus (HRSV). BRSV is the primary etiological agent of respiratory disease in calves aged up to 12 months [1]; beef and dairy cattle worldwide [2, 3]. Initial infection by BRSV alter bovine immune system and facilitates secondary infection of the lower respiratory tract by bacteria [1, 4]. Therefore, BRSV is considered to be a causative agent of bovine respiratory disease complex, which results in economic losses to farmers because of the morbidity and mortality in cattle [2, 3]. According to our preliminary findings based on the gene detection from respiratory samples, paired virus and bacteria were detected; *Pasteurella multocida* (PM) was the most common bacterial agent (unpublished data). PM is common in the nasopharynx of cattle [5, 6], although PM appears to be part of the normal flora, it can contribute to pneumonia when cattle stressed and/or infected by a respiratory virus [7]. However, the interactions between multiple agents associated with BRDC are not clear. Therefore, the aim of this study was to investigate the effect of BRSV infection on PM adherence to respiratory epithelial cells.

MATERIALS AND METHODS

Cell culture, virus and bacteria. A549, HEp-2, MDBK cells were used. Cells were seeded at 1×10^5 cells/ml and cultured as a monolayer in DMEM, 10% FBS; 100 U/ml penicillin and 100 µg/ml streptomycin, maintained at 37°C/5% CO_2. BRSV RS-52 strain, propagated in HEp-2 cells and virus titer was checked in a plaque assay using MDBK cells. BRSV was inactivated by UV treatment for 1 h and confirmed in a plaque assay. PM 2368 strain, capsular type B isolated, was grown at 37°C in Brucella broth. BRSV and PM were aliquoted, and kept at –80°C until use.

Adherence assay. A549, HEp-2, and MDBK cells were seeded, >80% confluence, inoculated with BRSV (MOI = 0.1 or 1), UV-inactivated BRSV (MOI = 1), and culture medium as a negative control. Inoculation time was 2 h and replaced with culture medium with 2% FBS. After 24, 48 and 72 h post infection (hpi), BRSV-infected and uninfected cells were exposed with PM suspension for 2 h. Cells were dissociated by trypsinization and collected by centrifugation. Dissociated resulting bacteria plated on Brucella agar. Adherence of bacteria per cell was calculated from total counting of CFU with the total number of monolayer cell.

qRT-PCR of mRNA encoding cytokines and chemokines. Cellular RNA was extracted from the cell lysates and performed using a One Step SYBR PrimeScript plus RT-PCR kit; primer sets and amplification conditions for of IL-1β, IL-6, IL-8, MCP-1, and RANTES mRNA were described previously [8]. Amplification was carried out in a LightCycler 96 system. Data normalized with GAPDH and calculated using the $2^{-\Delta\Delta C}$T method and expressed as a -fold change.

RESULT AND DISCUSSION

BRSV infection led to a significant increase in the number of PM cells adhering to respiratory and/or bovine epithelial cells: adherence was both time- and MOI-dependent (Fig. 1 and Fig. 2). Increased adhesion may be related to virus-induced changes in expression of receptors for bacteria depend on types of cell. Here, we found that epithelial cells exposed to PM showed increased expression of genes encoding IL-1β, IL-6, IL-8, MCP-1, and RANTES (Fig. 3); however, expression increased further upon co-infection with BRSV. We assume that upregulation of these

cytokines depends on the number of PM cells adhering to the respiratory epithelial cells. These cytokines play important roles in BRDC-related pneumonia.

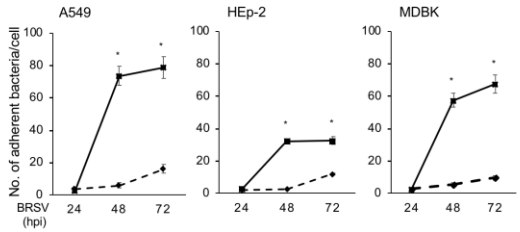

Fig. 1: The number of PM cells adhering to BRSV-infected A549, HEp-2 and MDBK at 48 and 72 hpi (straight line) was significantly higher than the number adhering to uninfected cells (dash line) at the same times.
* $p < 0.01$.

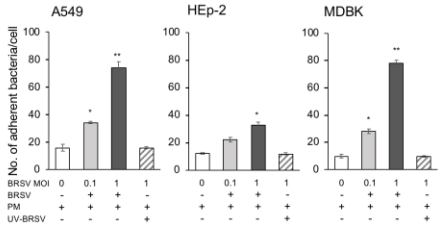

Fig. 2: Adherence of *Pasteurella multocida* (PM) to bovine respiratory syncytial virus (BRSV)-infected epithelial cells is MOI-dependent (closed bars). This was not the case for uninfected cells (open bars) and cells infected with UV-inactivated BRSV (UV-BRSV; patterned bars).
* $p < 0.05$ and ** $p < 0.01$.

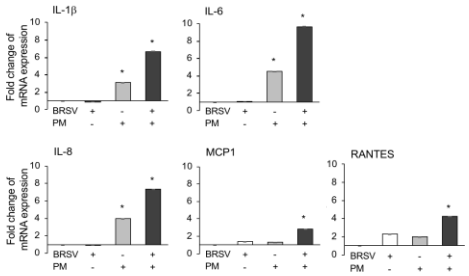

Fig. 3: Expression of IL-1b, IL-6, and IL-8 by PM-infected or BRSV plus PM-infected A549 cells is shown. * $p < 0.01$.

CONCLUSION

We showed here that BRSV infection of respiratory epithelial cells increases adherence of PM, leading to increased expression of genes encoding proinflammatory cytokines. The *in vitro* data suggest that initial infection by BRSV increases PM adherence, which in turn induces a local acute inflammatory response during the early stages of BRDC

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