Bovine Herpesvirus 1 Productive Infection Stimulates Inflammosome Formation and Caspase 1 Activity

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Bovine herpesvirus 1 (BoHV-1) is an alpha-herpesvirinae subfamily member that causes significant economic losses to the cattle industry. Infection of cattle with BoHV-1 can lead to conjunctivitis, genital disorders, abortions and bovine respiratory disease complex, a life threatening respiratory tract infection, reviewed by (Jones, 2009; Jones and Chowdhury, 2007). The ability of BoHV-1 to induce immune suppression in cattle is important for its pathogenic potential, reviewed in (Jones, 2009). For example, infection inhibits cell-mediated immunity (Carter et al., 1989; Griebel et al., 1987a, b, 1990), CD8+ T cell recognition of infected cells (Harihara et al., 1993; Hinkley et al., 1998; Koppers-Lalic et al., 2005; Nataraj et al., 1997), and induces apoptosis in CD4+ T cells (Eskra and Splitter, 1997; Winkler et al., 1999). Furthermore, two viral regulatory proteins, bICP0 and bICP27, inhibit interferon dependent transcription (da Silva and Jones, 2012; Henderson et al., 2005; Jones, 2009; Saira et al., 2007; Saira and Jones, 2009). Finally, infection erodes mucosal surfaces within the upper respiratory tract, and consequently promotes establishment of bacterial pathogens in the lower respiratory tract (Highlander et al., 2000; Highlander, 2001; Zecchinon and Desmecht, 2005).

The focus of these studies was to examine the effect that BoHV-1 had on inflammasome formation. The rationale for these studies stem from observations demonstrating that acute infection of calves leads to inflammation in affected tissue during acute infection. Consequently, we tested whether productively infected bovine cells stimulate inflammasome formation. Expression of two components required for inflammasome formation, the DNA sensor IFI16 (gamma-interferon-inducible protein 16) and NLRP3 (NOD-like receptor family, pyrin domain containing 3), were induced in bovine kidney cells by eight hours after infection. IFI16 was detected in punctate granules localized to the cytoplasm and nucleus. During productive infection, more than ten times more cells were caspase 1 positive, which is activated following inflammasome formation. Two caspase 1 inhibitors had no effect on productive infection. Conversely, another caspase 1 inhibitor, glyburide, significantly inhibited virus infection suggesting it had off-target effects on related enzymes or interfered with infection via non-enzymatic mechanisms. Collectively, these studies demonstrated that BoHV-1 infection stimulated inflammasome formation, which we predict is important for clinical symptoms in cattle.

Keywords: BoHV-1, Inflammasome, DNA sensor IFI16, NLRP3, Caspase 1
A subset of bICP0 was detected in the cytoplasm; however, most of the bICP0 protein was detected in the nuclear fraction. Evidence is accumulating for a functional role of IFI16 in controlling HSV-1 infection because IFI16 requires an intact nuclear localization signal to bind to HSV-1 DNA in infected cells and activate IFN-β expression (Li et al., 2012). An independent study demonstrated that IFI16 sensing of HSV-1 DNA is nuclear in human foreskin fibroblast cells (Orzalli et al., 2012). It will be of interest to determine whether IFI16 interacts with BoHV-1 DNA during productive infection and influences viral replication.

Caspase 1 cleavage and activation is the hallmark of inflammasome formation, reviewed by (Grant and Dixit, 2013; Latz et al., 2013; Stutz et al., 2009). Therefore, it was of interest to determine whether caspase 1 was cleaved and activated following infection. We initially tested whether caspase 1 was cleaved using polyclonal antibodies. These antibodies did not yield reliable results: perhaps because they do not recognize bovine caspase 1 (data not shown). Consequently, we measured caspase 1 activity in infected cells using a fluorescent peptide (Immunochemistry Technologies, Bloomington, Minnesota) that specifically and covalently binds activated caspase 1. Approximately 10 times more fluorescent positive cells were observed at 16 h after infection when compared to uninfected cells (Figure 3A). To confirm these studies, flow cytometry was used to quantify the number of caspase 1-activated cells after infection relative to mock-infected cells. In agreement with studies in Figure 3A, there was a significant difference between the number of caspase

Figure 1. BoHV-1 infection induces IFI16 and NLRP3 protein expression. Panel A: CRIB cells were infected with BoHV-1 (1 plaque forming unit/cell of the Cooper Strain) for 1, 2, 4, or 8 h after infection. Total cell lysate was prepared as previously described (Workman et al., 2012; Workman and Jones, 2011). As a control, cell lysate from mock-infected cells was used (lane M). Panel B: CRIB cells were infected as described in panel A for 8, 16, 24, or 32 h after infection. As controls, cell lysate from mock-infected cells that were cultured for 8 or 24 h were examined (Mock lane). Proteins from each sample in Panels A and B (100 μg protein) were separated on a SDS-polyacrylamide gel and proteins subsequently transferred to a membrane. Western blot analysis was performed using antibodies directed against IFI16 (sc-6050, Santa Cruz Biotechnology), NLRP3 (ab4207, Abcam), bICP0 (peptide specific antibody), and β-actin as previously described (Workman et al., 2012; Workman and Jones, 2010, 2011). The results in panels A and B are consistent with 5 independent experiments.
for these studies: (1) glyburide, a sulfonylurea drug (Lamkaniet al., 2009), (2) YVDA-CHO a peptide based inhibitor (ENZO Life Sciences), and VX-765 (Cellagen Technology) (Gastaldello et al., 2013; Lamkani et al., 2009). YVDA-CHO and VX-765 had little or no effect on virus yield in CRIB cells (Figure 3C). In contrast,
the respective drugs, no major effects on cell growth or morphology were observed (data not shown). The concentrations used for the respective drugs are in the range known to inhibit caspase 1 activity (Gastaldello et al., 2013; Lamkani et al., 2009). In keeping with this published study, we found that treating infected cells with 25 μM YVDA-CHO, 40 μM VX-765, or 400 μM glyburide reduced the number of caspase 1 activated cells (data not shown).

In conclusion, these studies provided evidence that BoHV-1 stimulates inflammasome formation and consequently caspase 1 activation. Although caspase 1 had no obvious effect on productive infection in CRIB cells, we predict that inflammasome formation mediates certain aspects of BoHV-1 induced pathogenesis following infection of calves. Studies designed to address the effect of the inflammasome in cattle and identification...
of viral factors that regulate inflammasome formation is underway.

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References

da Silva, L.F., Jones, C., 2012. The IC32 protein encoded by bovine herpesvi-

ruses (bIC32) interferes with promoter activity of the bovine genes encoding beta interferon 1 (IFN-β) and IFN-γ. Virus Res. 169: 162-168.


Gariano, G.R., Dell’Oste, V., Bronzini, M., Catt, D., Lugani, A., De Andream, M., Gribaudo, G., Gariglio, M., Landollo, S., 2012. The intracel-


action between bovine herpesvirus type 1 and activated bovine T lym-


Nataraj, C., Eidmann, S., Hariharan, M.J., Sur, J.H., Perry, G.A., Srikum-


Peres, S., Inman, M., Doster, A., Jones, C., 2005. Latency-related gene en-


tab. 88: 528-530.

Saira, K., Zhou, Y., Jones, C., 2007. The infected cell protein 0 encoded by bovine herpesvirus 1 (bICP0) induces degradation of interferon re-

sponse factor 3 (IRF3), and consequently inhibits beta interferon pro-


Saira, K., Jones, C., 2009. The infected cell protein 0 encoded by bovine herpesvirus 1 (bICP0) associates with interferon regulatory factor 7 (IRF7), and consequently inhibits beta interferon promoter activity. J. Virol. 83: 3,977-3,981.


Sinani, D., Jones, C., 2011. Localization of sequences in a protein encoded by the latency related gene of bovine herpesvirus 1 (ORF2) that inhibits apoptosis and interferes with Notch mediated trans-activation of the bICP0 promoter. J. Virol. 85: 12,124-12,133.

Szewczyc, K., Mettenleiter, T.C., Rijsewijk, F.A.M., Tampe, R., Neefes, J. E. J. H., Wiertz, J., 2005. Varicello viruses avoid T cell recognition by UL40.5-mediated inactivation of the transporter associated with anti-
