JOURNAL OF VIROLOGY, Apr. 2011, p. 3695–3697 Vol. 85, No. 7
0022-538X/11/$12.00 doi:10.1128/JVI.02626-10

Letter to the Editor

Bovine Viral Diarrhea Virus Strain- and Cell Type-Specific Inhibition of Type I Interferon Pathways▽

The interaction of noncytopathogenic bovine viral diarrhea virus type 1 (BVDV-1ncp) with antigen-presenting cell (APC) subsets is of great interest due to the recent increase in severe acute BVDV outbreaks attributed to this genotype (1, 12, 13). We report the effect of a BVDV-1ncp strain (Ho916ncp [13]) causing severe acute infection on *in vitro* type I interferon (alpha/beta interferon [IFN-α/β]) production compared to that of a mild-acute strain (Ky1203ncp) (15). Ho916ncp but not Ky1203ncp induced IFN-α/β in only lineage-negative (LIN^-) cells, a recently described enriched IFN-α/β-producing cell population in cattle (A. Gibson, S. Miah, P. Griebel, J. Brownlie, and D. Werling, submitted for publication). In agreement with published data, monocytes and monocyte-derived dendritic cells (moDC) did not produce IFN-α/β in response to either strain (4, 11, 17).

Experimental infection of calves with BVDVncp results in the detection of IFN-α/β and the IFN-α/β-inducible gene *Mx* (5, 20) as well as serum IFN-α/β activity in pregnant dams and the fetus (22). The identification of IFN-α/β-producing cells within the lymph nodes of BVDVncp-infected calves (4) and of enriched circulating IFN-α/β-producing cells (Gibson et al., submitted) points to an APC subset as a cellular source of IFN-α/β during *in vivo* BVDVncp infections. In the present study, monocytes, moDC, and LIN^- cells, generated from the same animal (11, 24–26, 28; Gibson et al., submitted), were exposed to either strain at a multiplicity of infection (MOI) of 0.1 and IFN-α/β was assessed after 48 h, using a previously described Mx-CAT (chloramphenicol acetyltransferase) reporter assay (4–6, 9). LIN^- cells, but not monocytes or moDC, produced significant amounts of IFN-α/β in response to Ho916ncp but not to Ky1203ncp or mock-infected control (Fig. 1). To assess whether these observed differences in IFN-α/β production were due to differences in viral replication, the presence of BVDV was determined by immunoperoxidase staining using a bovine hyperimmune serum, similar to what was described previously (11). Astonishingly, no clear differences in abilities to infect the different cell types were seen for the two BVDVncp strains (Fig. 2). As differences in viral replication did not explain differences in IFN-α/β production, we next assessed the potential effects of both BVDVncp strains on interferon response factors (IRFs). BVDVncp is known to inhibit IFN-α/β production through viral proteins N^{pro} (2, 3, 10, 14) and E^{rns} (16, 18, 19), which block IFN-α/β production by targeting IRF-3 for degradation by polyubiquitination (7, 14) or by degrading viral RNA (19). Translocation of IRF-3 occurs during infection but not binding to DNA (2). In contrast, IRF-7 is neither activated nor translocated to the nucleus (2).

We assessed the impact of both strains on IFN-α/β signaling components IRF-3 and IRF-7 by Western blotting,

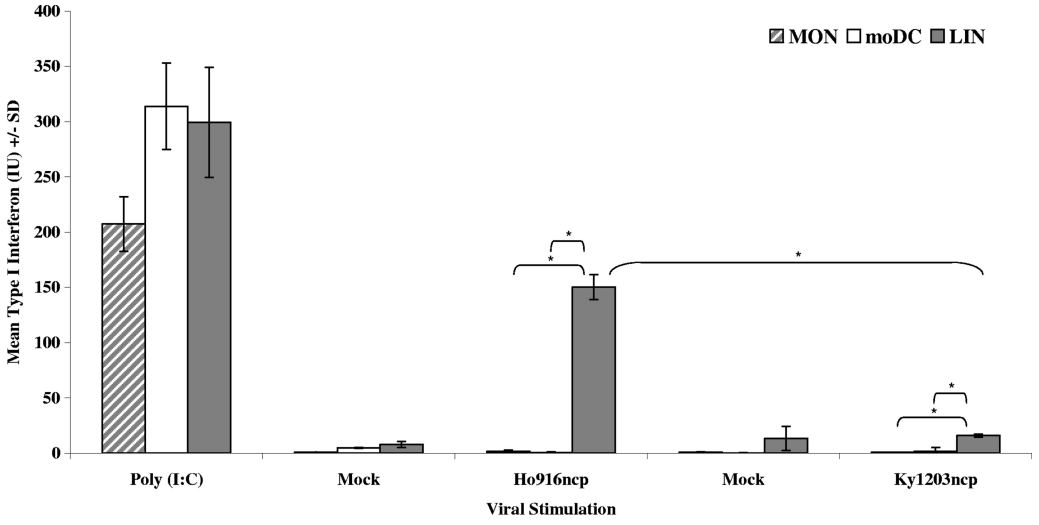

FIG. 1. Type I interferon response to BVDVncp. Monocytes (MON), moDC, and LIN^- cells (LIN) were isolated from the same animal and stimulated with 50 μg ml^{-1} of poly(I:C), BVDVncp strains Ho916ncp and Ky1203ncp at an MOI of 0.1, and corresponding volumes of mock-infected supernatant for 48 h. Supernatants were harvested for the detection of IFN-α/β by an Mx-CAT reporter assay where $n = 4$. *, P value of <0.05 at a 95% confidence interval.

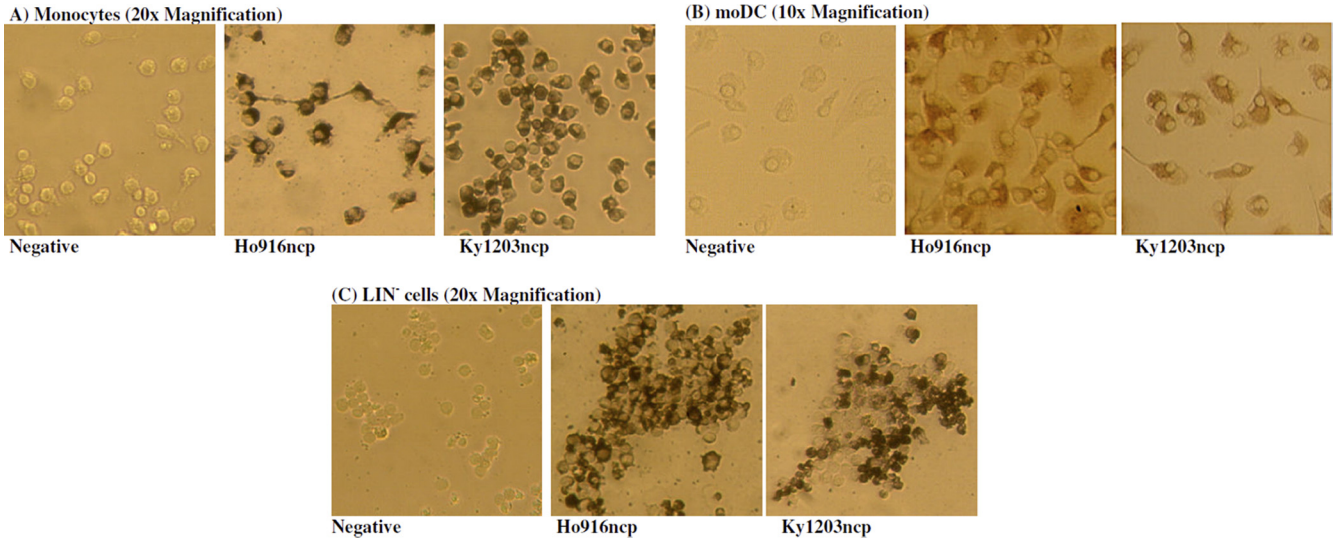

FIG. 2. Antigen-presenting cell susceptibility to BVDVncp. Monocytes (A), moDC (B), and LIN$^-$ cells (C) were infected with BVDVncp strains Ho916ncp and Ky1203ncp for 48 h (MOI, 0.1). Cells were fixed and stained for the presence of BVDV antigen using a bovine hyperimmune serum, and the antigen was detected either with anti-bovine alkaline phosphatase-conjugated secondary antibody (monocytes and LIN$^-$ cells) or with anti-bovine horseradish peroxidase (HRP)-conjugated secondary antibody (moDC). Dark blue/gray cytoplasmic staining or red/brown cytoplasmic staining indicates the presence of BVDV. Light microscopy images were captured with a MiniVID lens camera (LW Scientific Inc.) and further analyzed with Scope Photo image software version 2.0.4. The left panels display mock-infected cells, the center panels display Ho916ncp-infected cells, and the right panels display Ky1203ncp-infected cells. Displayed data are representative of 4 individual animals for monocytes and LIN$^-$ cells and 2 individual animals for moDC.

which showed that IRF-3 and IRF-7 were increased in all three cell types infected with Ho916ncp compared to mock-infected controls. In contrast, Ky1203ncp induced a reduction of IRF-7 expression in monocytes and moDC and a reduction in IRF-3 expression in all three cell types. IRF-7 remained unchanged in LIN$^-$ cells, potentially indicating cell type-specific degradation in monocytes and moDC for Ky1203ncp (Fig. 3). Ho916ncp-induced IFN-α/β production by LIN$^-$ cells appears to be independent of IRF-3 or IRF-7 modulation; however, LIN$^-$ cells, unlike monocytes or moDC, express TLR7 (27; Gibson et al., submitted), which has been recently implicated in the recognition of other pestiviruses, such as West Nile virus and dengue virus (8, 21, 23). Ho916ncp, as a severe acute BVDV-1ncp strain could be more readily accessible to the endosomal compartment within LIN$^-$ cells, thus producing cell type-restricted IFN-α/β through TLR7. Our data show for the first time differences in BVDV-1ncp strains with regard to a cell-specific

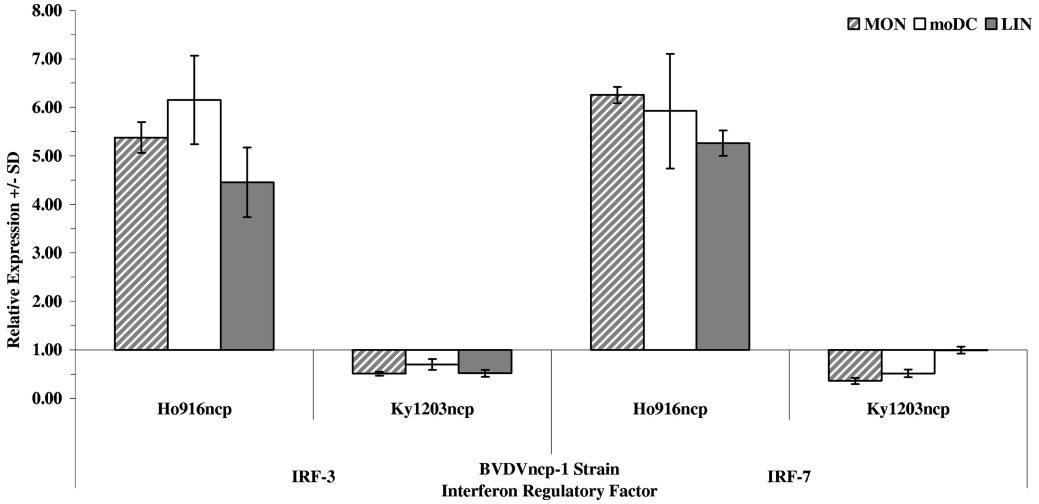

FIG. 3. Relative expression of IRF-3 and IRF-7 in response to BVDVncp. Monocytes (MON), moDC, and LIN$^-$ (LIN) cells were isolated from the same animal and stimulated with Ho916ncp and Ky1203ncp alongside mock-infected controls for 48 h. Protein lysates were prepared with protease inhibitors and analyzed by Western blotting for the expression of IRF-3 and IRF-7, detected by enhanced chemiluminescence and further analyzed using Quantity One densitometry software version 4.6.6 (Bio-Rad Laboratories, United Kingdom). Relative expression of IRF-3 and IRF-7 is displayed normalized to β-actin and mock-infected control for each viral supernatant, where $n = 3$.

IFN-α/β response and offer some suggestions for this modulation.

We thank B. Charleston (IAH Compton) for providing MDBKt2 cells.

This work was suppported through DEFRA grant SE0777 to J.B. and D.W.

The manuscript represents publication P/PID/000117 of the RVC.

REFERENCES

1. **Adair, B. M., S. J. McCullough, S. Kennedy, and M. Spagnuolo.** 1992. Isolation and characterization of a "high-virulence" bovine virus diarrhoea virus from calves, p. 111. *In* Proceedings of the Second Symposium on Ruminant Pestiviruses, Annecy, France, 3 October 1992.
2. **Baigent, S. J., S. Goodbourn, and J. W. McCauley.** 2004. Differential activation of interferon regulatory factors-3 and -7 by non-cytopathogenic and cytopathogenic bovine viral diarrhoea virus. Vet. Immunol. Immunopathol. **100:**135–144.
3. **Baigent, S. J., et al.** 2002. Inhibition of beta interferon transcription by noncytopathogenic bovine viral diarrhea virus is through an interferon regulatory factor 3-dependent mechanism. J. Virol. **76:**8979–8988.
4. **Brackenbury, L. S., et al.** 2005. Identification of a cell population that produces alpha/beta interferon in vitro and in vivo in response to noncytopathic bovine viral diarrhea virus. J. Virol. **79:**7738–7744.
5. **Charleston, B., et al.** 2002. Alpha/beta and gamma interferons are induced by infection with noncytopathic bovine viral diarrhea virus in vivo. J. Virol. **76:**923–927.
6. **Charleston, B., M. D. Fray, S. Baigent, B. V. Carr, and W. I. Morrison.** 2001. Establishment of persistent infection with non-cytopathic bovine viral diarrhoea virus in cattle is associated with a failure to induce type I interferon. J. Gen. Virol. **82:**1893–1897.
7. **Chen, Z., et al.** 2007. Ubiquitination and proteasomal degradation of interferon regulatory factor-3 induced by Npro from a cytopathic bovine viral diarrhea virus. Virology **366:**277–292.
8. **Daffis, S., et al.** 2008. Interferon regulatory factor IRF-7 induces the antiviral alpha interferon response and protects against lethal West Nile virus infection. J. Virol. **82:**8465–8475.
9. **Fray, M. D., G. E. Mann, and B. Charleston.** 2001. Validation of an Mx/CAT reporter gene assay for the quantification of bovine type-I interferon. J. Immunol. Methods **249:**235–244.
10. **Gil, L. H., et al.** 2006. The amino-terminal domain of bovine viral diarrhea virus Npro protein is necessary for alpha/beta interferon antagonism. J. Virol. **80:**900–911.
11. **Glew, E. J., et al.** 2003. Differential effects of bovine viral diarrhoea virus on monocytes and dendritic cells. J. Gen. Virol. **84:**1771–1780.
12. **Hamers, C., et al.** 2000. Differences in experimental virulence of bovine viral diarrhoea viral strains isolated from haemorrhagic syndromes. Vet. J. **160:** 250–258.
13. **Hibberd, R. C., A. Turkington, and J. Brownlie.** 1993. Fatal bovine viral diarrhoea virus infection of adult cattle. Vet. Rec. **132:**227.
14. **Hilton, L., et al.** 2006. The NPro product of bovine viral diarrhea virus inhibits DNA binding by interferon regulatory factor 3 and targets it for proteasomal degradation. J. Virol. **80:**11723–11732.
15. **Howard, C. J., J. Brownlie, and M. C. Clarke.** 1987. Comparison by the neutralisation assay of pairs of non-cytopathogenic and cytopathogenic strains of bovine virus diarrhoea virus isolated from cases of mucosal disease. Vet. Microbiol. **13:**361–369.
16. **Iqbal, M., E. Poole, S. Goodbourn, and J. W. McCauley.** 2004. Role for bovine viral diarrhea virus Erns glycoprotein in the control of activation of beta interferon by double-stranded RNA. J. Virol. **78:**136–145.
17. **Lee, S. R., G. T. Pharr, B. L. Boyd, and L. M. Pinchuk.** 2008. Bovine viral diarrhea viruses modulate toll-like receptors, cytokines and co-stimulatory molecules genes expression in bovine peripheral blood monocytes. Comp. Immunol. Microbiol. Infect. Dis. **31:**403–418.
18. **Magkouras, I., P. Matzener, T. Rumenapf, E. Peterhans, and M. Schweizer.** 2008. RNase-dependent inhibition of extracellular, but not intracellular, dsRNA-induced interferon synthesis by Erns of pestiviruses. J. Gen. Virol. **89:**2501–2506.
19. **Matzener, P., I. Magkouras, T. Rumenapf, E. Peterhans, and M. Schweizer.** 2009. The viral RNase E(rns) prevents IFN type-I triggering by pestiviral single- and double-stranded RNAs. Virus Res. **140:**15–23.
20. **Muller-Doblies, D., et al.** 2004. Innate immune responses of calves during transient infection with a noncytopathic strain of bovine viral diarrhea virus. Clin. Diagn. Lab. Immunol. **11:**302–312.
21. **Silva, M. C., A. Guerrero-Plata, F. D. Gilfoy, R. P. Garofalo, and P. W. Mason.** 2007. Differential activation of human monocyte-derived and plasmacytoid dendritic cells by West Nile virus generated in different host cells. J. Virol. **81:**13640–13648.
22. **Smirnova, N. P., et al.** 2008. Acute non-cytopathic bovine viral diarrhea virus infection induces pronounced type I interferon response in pregnant cows and fetuses. Virus Res. **132:**49–58.
23. **Wang, J. P., et al.** 2006. Flavivirus activation of plasmacytoid dendritic cells delineates key elements of TLR7 signaling beyond endosomal recognition. J. Immunol. **177:**7114–7121.
24. **Werling, D., et al.** 1999. Involvement of caveolae in the uptake of respiratory syncytial virus antigen by dendritic cells. J. Leukoc. Biol. **66:**50–58.
25. **Werling, D., J. C. Hope, C. J. Howard, and T. W. Jungi.** 2004. Differential production of cytokines, reactive oxygen and nitrogen by bovine macrophages and dendritic cells stimulated with Toll-like receptor agonists. Immunology **111:**41–52.
26. **Werling, D., et al.** 2002. Role of bovine chemokines produced by dendritic cells in respiratory syncytial virus-induced T cell proliferation. Vet. Immunol. Immunopathol. **87:**225–233.
27. **Werling, D., J. Piercy, and T. J. Coffey.** 2006. Expression of TOLL-like receptors (TLR) by bovine antigen-presenting cells-potential role in pathogen discrimination? Vet. Immunol. Immunopathol. **112:**2–11.
28. **Yamakawa, Y., et al.** 2008. Identification and functional characterization of a bovine orthologue to DC-SIGN. J. Leukoc. Biol. **83:**1396–1403.

Amanda Gibson†
Jenny Larsson
Michaela Bateman
Joe Brownlie
Dirk Werling*
Department of Pathology and Infectious Diseases
Royal Veterinary College
North Mymms, Hertfordshire AL9 7TA, United Kingdom

*Phone: 44 01 707 666358
Fax: 44 01 707 652090
E-mail: dwerling@rvc.ac.uk

† Present address: Health Protection Agency, National Mycobacterium Reference Laboratory (NMRL), Abernethy Building, 2 Newark Street, London E1 2AT, United Kingdom.

▽ Published ahead of print on 26 January 2011.