Veterinary Microbiology 195 (2016) 104–114

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Induction of functional interferon alpha and gamma responses during acute infection of cattle with non-cytopathic bovine viral diarrhea virus

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ARTICLE INFO

Article history:
Received 18 May 2016
Received in revised form 19 September 2016
Accepted 20 September 2016

Keywords:
Bovine viral diarrhea virus
Kinome
Interferon
Host-pathogen interaction

ABSTRACT

As a part of their pathogenic mechanism, many pathogens causing persistent infections, including bovine viral diarrhea virus (BVDV), immunosuppress their hosts, often by limiting the ability to either produce, or respond to, interferon. The objective of this study was to quantify the extent to which an acute infection of cattle with a non-cytopathic strain of BVDV induces interferon responses and to establish the functionality of these responses. Functionality of responses was investigated using a bovine specific peptide array to monitor kinase-mediated signal transduction activity within peripheral blood mononuclear cells (PBMCs) at time points corresponding to the interferon gamma (IFN-γ) and alpha (IFN-α) responsive phases of acute BVDV infection. Further, with an appreciation of diverse mechanisms and levels at which pathogens modulate host cell defences, patterns of expression of IFN-γ and -α responsive genes were also quantified within PBMCs. Infection of cows with ncpBVDV2-1373 induced significant increases in levels of serum IFN-γ and IFN-α. Within the PBMCs of the infected animals, distinct patterns of kinase-mediated signal transduction activity, in particular with respect to activation of classic IFN-activated signalling pathways, such as Jak-Stat, as well as induced expression of IFN-γ and IFN-α regulated genes, support the functionality of the host interferon response.

1. Introduction

As a main contributor to loss and poor reproductive performance, bovine viral diarrhea virus (BVDV) is one of the most economically devastating pathogens in the global cattle industry (Smirnova et al., 2008). Genotypically, strains of BVDV are divided into two categories – BVDV1 and BVDV2 – and each of these genotypes are further sub-divided into two phenotypic biotypes – cytopathic (cp) and non-cytopathic (ncp) – based on their lytic activity to tissue culture epithelial cells (Smirnova et al., 2008). Acute infections with BVDV are typically subclinical, transient, and self-limiting although highly virulent BVDV2 strains, such as 1373, often result in severe clinical disease with a high mortality rate (Liebler-Tenorio et al., 2003b; Ellis et al., 1998). The pathological implications of acute BVDV infections are typically associated with the ability of the virus to potentiate infections by other bacterial and viral pathogens. This increased susceptibility to secondary infections contributes to a clinical illness known as bovine respiratory disease complex, which is among the most significant infectious disease challenges to commercial feedlot production. The contribution of acute BVDV infections to bovine respiratory disease reflects the abilities of the virus to immunosuppress the infected host, synergize and enhance the pathogenesis of other infectious agents, and impair the efficacy of various treatments (Kapil et al., 2005).

There are a number of mechanisms by which BVDV impairs the immune capabilities of the infected host. These include, particularly among high virulence BVDV strains, reducing lymphocyte numbers and depleting lymphoid tissue (Liebler-Tenorio et al., 2003a,b). BVDV also impairs the functional capabilities of leukocytes, including bacterial killing, systemic innate immune sensory responses, and chemotaxis (Baigent et al., 2004; Charleston et al., 2001; Schweizer et al., 2006).

Classically, host responses to viral pathogens are centralized in interferon (IFN); in particular, type I (IFN-α- and -β) IFN-mediated

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http://dx.doi.org/10.1016/j.vetmic.2016.09.015
0378-1135/

defenses. There is considerable debate on the influence of BVDV on the IFN defense system. Specifically, while there is overall consensus on the tendency of cpBVDV strains to activate IFN responses, there are conflicting reports on whether similar activation of IFN-mediated responses occurs during infection by ncp strains. Early *in vitro* infection models indicated that ncpBVDV isolates did not induce IFN α/β (Baigent et al., 2004; Schweizer et al., 2006; Diderholm and Dinter, 1966) or block the induction of IFN by double-stranded RNA or other viruses (Baigent et al., 2004; Rossi and Kiesel, 1980; Schweizer and Peterhans, 2001). Further, it was demonstrated that fetal challenge with cpBVDV results in type I IFN production, whereas ncpBVDV does not, suggesting the ability of ncpBVDV to inhibit the induction of type I IFN in order to enable the virus to establish persistent infection during early fetal development (Charleston et al., 2001). In contrast, *in vitro* infection (Weiner et al., 2012) and acute *in vivo* infection (Smirnova et al., 2008; Brackenbury et al., 2005; Charleston et al., 2002; Müller-Doblies et al., 2004; Palomares et al., 2013, 2014) with ncpBVDV induced a type I IFN response (Charleston et al., 2002).

Type II IFN (IFN-γ) also plays a role in the anti-viral response by activating and enhancing macrophage activity, as well as promoting adaptive cell-mediated immunity (Schroder et al., 2004). The same *in vivo* study that demonstrated induction of type I IFN in gnotobiotic animals during acute BVDV infection also reported increased expression of IFN-γ (Charleston et al., 2002). Further, ncpBVDV has been shown to promote IFN-γ expression in pregnant cattle in both early and late stages of pregnancy (Smirnova et al., 2014; Waldvogel et al., 2000).

In this study, the responses of cows experimentally infected with ncpBVDV2-1373 were investigated with a priority on defining the induction and consequences of IFN-α and -γ in the context of acute BVDV infection. While BVDV-mediated induction of serum IFN-α and -γ has been described in gnotobiotic calves (Charleston et al., 2002), the current investigation, involving healthy cattle of normal immune status, provides a more biologically relevant context for evaluation of responses to BVDV. As many pathogens enable infection by limiting the sensitivity of host cells to endogenous immune signals, it is important to address the potential for functional disconnect between the induction and action of IFN-mediated responses. Specifically, as pathogen-mediated disruption of host responsiveness can occur at a number of points between receptor activation and the ensuing phenotype, there is value in defining cellular responses at a number of biological levels. In the current investigation, acute infection of cows with ncpBVDV2-1373 resulted in significant increases of serum IFN-γ and -α with concomitant induction of IFN-regulated gene expression and phosphorylation-mediated signal transduction within peripheral blood mononuclear cells (PBMCs), confirming the ability of these animals to mount functional IFN responses to the pathogen.

2. Materials and methods

2.1. BVDV challenge and clinical evaluation

Two groups of Angus-Aberdeen cross-bred heifers, identified as Group A (n = 8) and Group B (n = 14), were screened via ear notch extract and ELISA (Idexx) to confirm negative results for BVDV, bovine herpes virus, and bovine respiratory syncytial virus. The heifers were inseminated at one-year-old and confirmed pregnant via ultrasound. At Day 80 of pregnancy, Group B cows were intranasally infected with ncpBVDV2-1373 ($TCID_{50} = 10^{7.2}$). The BVDV type 2 strain, 1373, was originally collected by the USDA Animal and Plant Health Inspection Service (APHIS) and has been previously characterized by our group (Liang et al., 2008). The pregnancy of the animals was not a priority aspect of this investigation. All experimental protocols were performed according to the guidelines approved by the Canadian Council on Animal Care.

2.2. Body weight and temperature

Body weight and temperature measurements during the challenge period were taken daily between 9 A.M. and 12 P.M. by the Animal Care staff of VIDO-InterVac.

2.3. Serum and peripheral blood mononuclear cell isolation

From each animal, 50 mL of blood was extracted using a 60-mL syringe that contained 2 mL of 7.5% EDTA (Sigma-Aldrich) in PBSA (Mg^{2+}-free PBS). The blood was transferred to a 50-mL polypropylene tube and centrifuged at 1400 × *g*, at 20 °C, for 20 min. The PBMC layer from the centrifuged sample was collected and combined with room temperature 0.1% EDTA in PBSA up to a volume of 35 mL. This volume was layered onto 15 mL of 54% Percoll (GE Healthcare) in PBS and centrifuged at 2000 × *g*, at 20 °C, for 20 min. The PBMC layer was collected and combined with cold PBS. The cells were centrifuged at 300 × *g*, at 4 °C, for 8 min; after which, the supernatant removed, and the cells resuspended. This step was repeated twice, with the third centrifugation being run at 150 × *g*. Before the third centrifugation, the pellet was resuspended in 10 mL of PBS, 100 μL of which was removed for cell counting. After the third centrifugation, the supernatant was removed and 5×10^6 cells were resuspended in 250 μL of PBS. This was combined with 750 μL of Trizol LS (Life Technologies) and frozen at −80 °C for gene expression analyses. For kinome analysis, the pellet is resuspended in 100 μL of lysis as described later. After centrifugation at 1400 × *g* within the previous steps, 800 μL of serum was transferred to a 96 deep-well plate, which was stored at −20 °C for ELISA analysis.

2.4. White blood cell (WBC) isolation

For each animal, 5 mL of blood was combined with 9.5 mL of a lysis buffer (124 mM NH_4Cl (Sigma-Aldrich) and 34.6 mM Tris (Sigma-Aldrich), pH 7.2). The red blood cells were lysed for three minutes before the samples were centrifuged. After removing the supernatant, the pellet was resuspended with 10 mL of PBS and centrifuged. The supernatant was poured off and the pellet was resuspended in 1 mL of 1% HEPES in minimum essential medium (MEM) (Sigma-Aldrich, Life Technologies). All centrifugations were set at 300 × *g*, at 4 °C, for 10 min. The samples were stored at −80 °C for future analyses. WBCs were used for quantification of BVDV loads.

2.5. Viral titration

The presence of BVDV was assessed in nasal and WBC samples on days 3, 7, and 10 post-challenge, as well as day -1 pre-challenge. Madin-Darby Bovine Kidney (MDBK) cells were grown in 10% fetal bovine serum (FBS) in MEM (Life Technologies, Sigma Aldrich) within T150 flasks, counted using a haemocytometer, and diluted to a suspension of 2.5×10^5 cells mL^{-1}. 100 μL of cells was then added to each well of a 96-well flat-bottomed plate at 2.5×10^4 cells per well. The cells were grown overnight at 37 °C. Serial 2-fold dilutions of the nasal and WBC samples were made in a separate plate. Dilutions of pure virus stock served as positive controls. The medium was removed from the cells and replaced with 100 μL of sample from the corresponding wells of the dilution plates. The now-infected cells were incubated at 37 °C for 1.5 h; after which, 100 μL of 2% FBS in MEM was added to each well followed by incubation at 37 °C for 96 h.